ECONOMICS COLLEGE IN STALOWA WOLA

MEDICAL SCIENCES: DEVELOPMENT PROSPECTS IN COUNTRIES OF EUROPE AT THE BEGINNING OF THE THIRD MILLENNIUM

Collective monograph

Stalowa Wola, Poland
2018

Recommended for publication
by the Academic Council of Economics College in Stalowa Wola

Responsible for release: dr Małgorzata Korecka, rector
(Economics College in Stalowa Wola)

Medical sciences: development prospects in countries of Europe at the beginning of the third millennium: Collective monograph. Riga : Izdevnieciba "Baltija Publishing", 2018. 288 p.

ISBN 978-9934-571-60-2

CONTENTS

LIFE QUALITY OF PATIENTS, WITH CHRONIC KIDNEY DISEASE VD STAGE THAT ARE TREATED WITH HELP OF HEMODIALYSIS, WITH SKIN ITCHING

Abrahamovych K. J., Kurban M. M.

INTRODUCTION

At the present stage of development of medicine, is a topical issue of quality of life (LQ) of patients treated with hemodialysis (HD)[1]. The quality of life, as defined by the WHO, is a characteristic of the physical, mental, emotional and social status of a person based on subjective assessment. The evaluation of LQ is the main criterion for the individualization of treatment and psychosocial rehabilitation, it allows to direct corrections to the area of life that suffers most[2].

Itching of skin (SI) is a symptom, of not only allergic or dermatological diseases, pruritus can reduce LQ: it affects sleep, working capacity, causes depression, sometimes suicidal thoughts[3]. This is one of the most common factors that worsen LQ in patients with chronic kidney disease (CKD) treated with HD, since it occurs in 50,0–60,0% of patients[4].

Until today, there are no common views on the pathophysiological mechanisms of the pruritus occurrence. The most probable hypothesis is the involvement of several etiological factors and pathogenetic mechanisms. One is the “immune hypothesis”, which explains the appearance of itching in violation of the balance of the immune system with the accumulation of proinflammatory cytokines (PC) and other one is “histamine hypothesis”,

---

[1] Дудар І.О., Гончар Ю.І. Якість життя пацієнтів із хронічними хворобами нирок, яких лікують з використанням методів ниркової замісної терапії. *Therapia*. 2007. № 11. С. 75–80.

Дудар І.О., Гончар Ю.І., Абрагамович Х.Я. Свербіж шкіри та якість життя у хворих на гемодіалізі. *Український журнал нефрології та діалізу*. 2007. № 3(15). С. 33–35.

[2] Дудар І.О., Абрагамович Т.Н., Абрагамович Х.Я. Свербіж шкіри як загальне поняття. Свербіж шкіри при хронічній нирковій недостатності. *Український журнал нефрології та діалізу*. 2008. № 1(17). С. 67–78.

Pisoni R.L., Wikström B., Elder S.J. et al. Pruritus in haemodialysis patients: international results from the Dialysis Outcomes and Practice Patterns Study (DOPPS). *Nephrology Dialysis Transplantation*. 2006. № 12. P. 3495–3505.

[3] Kosmadakis G.C., Zerefos N. Uremic pruritus: a revie. *Hemodial lnt*. 2005. № 9. P. 180–188.

[4] Дудар І.О., Абрагамович Т.Н., Абрагамович Х.Я., Гончар Ю.І. Свербіж у хворих на гемодіалізі та його лікування. Ефект лікування ультрафіолетовим випромінюванням. *Український журнал нефрології та діалізу*. 2011. № 1(29). С. 12–20.

which suggests that itching occurs due to the excessive release of histamine by mast cells (MC) in the skin[5].

Nephrologists, not so long ago, were interested in the problem of skin itching, and today the mechanisms of pruritus in patients with CKD VD stage, are actively discussed[6]. There are single reports about reducing itching under the influence of ultraviolet irradiation (UVI)[7]. It is claimed, that as a result of irradiation, in the skin decreases the amount of MC, inhibited proliferation of MC and release of histamine, that is the modulating effect of UVI on the differentiation of T-helper (Th) 1 and Th2 lymphocytes and inhibition of Th1 activity. Is not excluded possibility of occurrence, under influence of ultraviolet irradiation, ultrastructural changes in nerve fibers or increase threshold of sensitivity of nerve endings. Suggested assumption, that this influence, has a systemic character[8].

Existing methods of treating itching of the skin (systemic administration of antihistamines and their skin application, correction of secondary hyperparathyroidism) are effective only in 20,0–40,0% of patients, that is why searching and developing methods, that would improve the effectiveness of its treatment, remains relevant. The use of ultraviolet radiation (UVR), seems appropriate, but a little studied method of treatment of the above mentioned category of patients[9].

---

[5] Дудар І.О., Абрагамович Т.Н., Абрагамович Х.Я. Запалення та свербіж. *Український журнал нефрології та діалізу*. 2011. № 4(32). С. 80–89.

[6] Дудар І.О., Бісярін Ю.В., Абрагамович Т.Н., Абрагамович Х.Я. Опасиста клітина. Роль опасистої клітини у свербіжі шкіри хворих хронічною нирковою недостатністю, котрі лікуються програмним гемодіалізом. *Український журнал нефрології та діалізу*. 2009. № 4(24). С. 3–7.

[7] Дудар І.О., Абрагамович Т.Н., Абрагамович Х.Я., Гончар Ю.І. Свербіж у хворих на гемодіалізі та його лікування. Ефект лікування ультрафіолетовим випромінюванням. *Український журнал нефрології та діалізу*. 2011. № 1(29). С. 12–20.

[8] Дудар І.О., Абрагамович Т.Н., Абрагамович Х.Я. Запалення та свербіж. *Український журнал нефрології та діалізу*. 2011. № 4(32). С. 80–89.

Дудар І.О., Абрагамович Т.Н., Абрагамович Х.Я., Гончар Ю.І. Свербіж у хворих на гемодіалізі та його лікування. Ефект лікування ультрафіолетовим випромінюванням. *Український журнал нефрології та діалізу*. 2011. № 1(29). С. 12–20.

[9] Дудар І.О., Гончар Ю.І. Якість життя пацієнтів із хронічними хворобами нирок, яких лікують з використанням методів ниркової замісної терапії. *Therapia*. 2007. № 11. С. 75–80.

Дудар І.О., Гончар Ю.І., Абрагамович Х.Я. Свербіж шкіри та якість життя у хворих на гемодіалізі. *Український журнал нефрології та діалізу*. 2007. № 3(15). С. 33–35.

Дудар І.О., Абрагамович Т.Н., Абрагамович Х.Я. Свербіж шкіри як загальне поняття. Свербіж шкіри при хронічній нирковій недостатності. *Український журнал нефрології та діалізу*. 2008. № 1(17). С. 67–78.

1. Purpose, materials, methods and methodology of research

The aim of the study. To increase the efficiency of treatment of patients with CKD VD which are treated with hemodialysis, with skin itching, by applying ultraviolet radiation, on the basis of the study, of the effect of skin itch on the quality of their life, the condition of mast cells and proinflammatory cytokines. In accordance with the set goal, the following tasks were defined:

1. To find out the frequency of itching of the skin, its clinical features and the impact on life quality of patients with CKD VD stage, which are on hemodialysis treatment.
2. To study levels of proinflammatory cytokines in thematic patients with and without skin itching.
3. To investigate the presence and activity of mast cells in thematic patients with and without skin itching.
4. To compare immunological and morphological indicators, to determine the correlation between them in patients with skin itch and compare with patients without it.
5. To determine the clinical effectiveness of exposure of ultraviolet radiation on skin itch in patients with CKD VD stages, which are treated with hemodialysis.
6. To study the influence of ultraviolet radiation on levels of proinflammatory cytokines in the group of thematic patients with severe skin itching.
7. To investigate the influence of ultraviolet radiation on the quality of life of the thematic patients.

To the study, were involved, 109 patients with CKD VD stages, treated with HD in the Lviv Regional Clinical Hospital and the Kyiv City Scientific and Practical Center of Nephrology and Dialysis.

Criterias for inclusion of patients to the study: informed consent of the patient to take part in the study; presence of CKD VD stage and hemodialysis treatment for at least three months; absence of secondary hyperparathyroidism; absence of severe anemia; absence of acute or exacerbation of chronic liver diseases; estimated life expectancy of patients over 6 months; not aging people and not pregnant women.

The examination of patients included a general clinical examination which consisted of inspection, objective examination (including monitoring of blood pressure of patients according to the approved diary – monitoring of

BP in outpatient settings, that was performed according to the European Society of Hypertension recommendations (ESH, 2008)) of the patient, laboratory (comlete blood count and urine analysis, biochemical blood test with determination of urea, creatinine, total protein, albumin, electrolytes, total cholesterol, ALT, AST, serum bilirubin, determination of PTH level, calcium, serum phosphorus, screening of patients on hepatitis B and C) and instrumental examination methods (electrocardiographic examination, echocardiography, x-ray examination of the chest, ultrasound examination of the abdominal cavity).

The age of the examined patients ranged from 23 to 70 years and on average was 44,60±1,10 years. Among the examined patients, were n=56 (51,4%) men and n=53 (48,6%) women. Depending on the main reason that led to the CKD VD stage, prevailed patients with glomerular kidney damage (without diabetes) – n=61 (56,30%). Depending on the duration of HD treatment, the most patients were in the group from one to six years of dialysis treatment, namely n=51 (46,5%). In n=98 (90,0%) of patients was Kt/V≥1,2.

Finding out the quality of life was conducted using the questionnaire SF-36 (T.S. Ospanova, G.D. Semydotska and N.J. Kotulevych "Method of evaluation of quality of life of patients treated with program hemodialysis" Application for registration No. 2003119917 dated 04.10.03 year). Itching of the skin was assessed by questionnaire Skindex-16. The questionnaire was developed at the University of California at San Francisco Department of Dermatology.

The questionnaire SF-36 contained 36 questions of the main module, supplemented by multi-point scales, aimed specifically at patients with CKD who are being treated with hemodialysis. Each question was assessed from 0,00 to 100,00 points. The results were evaluated on the following scales: symptom / problems "Symptoms of kidney disease" (12 points: № 17–28); "Influence of disease on everyday life" (8 points: № 29–36); "The burden of kidney disease" (4 points: № 13–16); "Patient's subjective evaluation of general health status (SF-12)" – short form of general health assessment (№ 1–12). Also was calculated the following total components: "Physical total component" (№ 1–5, 8), "Mental total component" (№ 6–7, 9–12), "Total Quality of Life score" – total amount of points.

The Skindex-16 questionnaire described the condition of the skin during the past week. It consisted of 16 questions. The answers to each question

were evaluated from 0,00 to 100,00 points with the subsequent calculation of the average of three scales ("Symptoms", "Emotions", and "Activity") and the average for all 16 questions – "Total score". "Symptoms" is a total index that includes the intensity of itching, skin irritation, burning of the skin and wounds on the skin from scratching. "Emotions" – the total index of the effect of itching on the psychological state of the patient. "Activity" – the total index of the effect of itching on the daily life of the patient.

Determination of the level of proinflammatory cytokines (IL-1β, IL-17, MCP-1) in serum, was carried out, using the immune enzyme method on the analyzer STAT-FaxPlus-303 (USA); was used a test system "Interleukin-IFA-BEST" (JSC "Vector-Best", Russian Federation) in accordance with the instructions of the manufacturer.

The study of MC in the skin of patients was as follows: after conducting by surgeons skin biopsy from the shoulder, of the right arm, size 1,0×1,0 cm^2, a pathomorphological study of the skin was made. With the help of histochemical research, the presence and activity of MC in these samples of the skin was determined.

Ultraviolet radiation (UVR) was performed using a mercury-quartz illuminator designed for general and local irradiation. For each patient, ten procedures of UVR were performed, once every two days, usually three times a week. The first procedure lasted 5 minutes, the second – 6 minutes, the third – 7 minutes, the fourth – 8 minutes, the fifth – 9 minutes, the following five procedures lasted for 10 minutes. The study of the effect of UVR on skin itching was carried out by questionnaires. The questionnaires-Skindex-16 were distributed after ten sessions of the UVR, one, six, and twelve months after the completion of the UVR treatment, with the subsequent scoring points of all scales and comparing these results with the baseline. We evaluated the clinical efficacy of UVR treatment for itching of the skin as follows: "good" (absence of all symptoms of itching after the treatment of UVR or increased intensity of symptoms of skin itching on the scale "Total Score" to 1,00 points); "satisfactory" (an increase in the intensity of symptoms of itching on the scale "Total Score" from 1,01 to 25,00 points); "no effect" (points of the "Total Score" scale for skin itching, were higher than 25,01, or returned to the initial indexes of itching intensity, that were installed before UVR treatment).

The effect of UVR at the level of proinflammatory cytokines was evaluated by measuring the levels of PC in the peripheral blood. Blood

collection was carried out after one UVR session and two weeks after the completion of the treatment course. We compared these results with data before UVR.

The study of the effect of UVR on the LQ of patients, was evaluated with the help of above mentioned questionnaire SF-36. Questionnaires were distributed after ten sessions of the UVR, one, six, and twelve months after the completion of the course of treatment with the UVR, with the subsequent scoring points of all scales, and comparing these results with the data set at the beginning of the study.

As a result of research, groups of patients were clearly defined, depending on the presence/absence of skin itch. The intensity of manifestations of skin itch, was estimated, using the "Total Score" scale of the questionnaire, from 0,00 to 100,00 points. Absence of skin itching symptoms were set in the range from 0,00 to 1,00 points, mild itchy symptoms were set in the range from 1,01 to 25,00 points, moderate itching – from 25,01 to 49,99 points (patients with mild and moderate symptoms of itching, we combined into one group due to the very small number of patients with mild skin itching), the severe manifestations of SI were set in the range of 50,00 to 100,0 points. Consequently, the following groups were formed: Group I – patients with CKD VD stage treated with HD without SI, Group II – patients with CKD VD stage treated with HD, with symptoms of SI, of these, subgroup I – with mild and moderate severity of SI and subgroup II – with severe manifestations of SI.

Statistical analysis of the results of the study was carried out with the help of Microsoft Office Excel 2007 and a packet STATISTICA for Windows 6.0 (Statsoft Inc., USA).

2. Study of skin itching and its pathophysiological links

Doing the *first task,* we obtained the following results. Analysis of the questionnaire of skin itching, showed, that from 109 patients who were on HD in 61 (56,0%) was SI, respectively, 48 (44,0%) had no symptoms of itching. Scoring points of all scales of the Skindex-16 questionnaire, revealed significant difference between the group without SI manifestations in comparison with group with SI manifestations, namely on the "Symptoms" scale 0,93±1,64 in group without SI and 34,77±3,71 in group with SI ($p<0,001$), on the scale of "Emotions" 0,00±0,00 in group without SI and 48,00±4,26 in group with SI ($p<0,001$), on the scale of "Functions"

0,00±0,00 and 34,49±4,69 (p<0,001) and on the "Total Score" scale 0,23±0,41 and 40,38±3,79 (p<0,001). In the group with SI was detected n=40 (36,7%) patients with mild and moderate itching – subgroup I, and n=21 (19,3%) with severe symptoms of itching – subgroup II.

Detailed analysis of the questionnaire Skindex-16, in the group of patients with SI, detected: scale "Symptoms" – dominated patients with SI in combination with irritation from scratching 37 (60,7%), scale "Emotions" – dominated patients which SI mentally depletes 54 (88,5%), scale "Functions" – dominated patients suffering because SI affects thier being in society, at work, in an unfamiliar community 40 (65,6%) (table 1).

Table 1

Distribution of patients by signs and manifestations of skin itching

	Skin itching	**n**	**%**
	Scale "Symptoms"		
1	Skin itching	61	100,00%
2	Combined with burning of the skin	25	41,00%
3	Combined with wounds on the skin from scratching	24	39,30%
4	Combined with irritation of the skin from scratching	37	60,70%
	Scale "Emotions"		
5	Was anxious about the constancy of the above mentioned symptoms	35	57,40%
6	These symptoms were the cause of emotional anxiety	43	70,50%
7	Was anxious about the appearance of the skin	47	77,00%
8	There was fear because of possible consequences of itching	47	77,00%
9	Shyness due to itching	43	70,50%
10	Skin itching mentally depletes	54	88,50%
11	Itching of the skin depresses	45	73,80%
	Scale "Function"		
12	Itching affects communication with friends and family	36	59,00%
13	Itching affects being in a society, at work, in an unfamiliar community (not close people)	40	65,60%
14	Itching causes difficulty in the manifestations of love and friendliness	35	57,40%
15	Itching affects everyday activities	34	55,70%
16	Itching complicates the possibility of doing things you love	35	57,40%

Comparison of groups with and without SI, according to general indicators, revealed a significant difference between a group without SI and a subgroup with severe SI manifestations by duration of hemodialysis treatment ($p<0,05$), which confirms the idea of occurrence or a certain increase in the intensity of the SI with longer treatment of HD, namely frequency of SI increases with an increase in the duration of hemodialysis treatment. Correlation analysis confirmed the results, and it was discovered, reliable direct correlation between the duration of HD treatment and SI ($p<0,05$; $r=0,41$).

According to our task, we conducted an analysis of LQ questionnaires. The primary analysis showed, that the most numerous, was the group of patients, with "Total Quality of Life score" from 25,01 to 50,00 points, namely 49 (45,0%) patients, and this shows, that the majority had a "bad" LQ (table 2).

Table 2

Distribution of patients by scale "Total Quality of Life score"

"Total Quality of Life score"	**n**	**%**
Very bad (0,00–25,00 points)	1	0,92
Bad (25,01–50,00 points)	49	44,95
Not bad (50,01–75,00 points)	47	43,12
Good (75,01–100,00 points)	12	11,01

A comparative analysis of the questionnaires based on the results of the evaluation of LQ of 48 patients with no manifestations of SI and 61 patients with SI showed a statistically significant difference between the groups on all scales for assessing the quality of life ($p<0,001$). In patients with SI, levels of LQ indicators reliably lower, than in patients without SI symptoms (table 3).

Table 3

Data of life quality of patients without skin itch and with signs of skin itch

Scales SF-36	**Patients without skin itch n=48 (points)**	**Patients with skin itch n=61(points)**
"Total Quality of Life score"	62,31±1,89	44,17±1,56*
"Symptoms of kidney disease"	79,87±1,39	65,30±1,90*
"Influence of disease on everyday life"	72,59±2,29	51,12±2,26*
"The burden of kidney disease"	29,42±3,82	15,67±1,66*
"Patient's subjective evaluation of general health status" SF-12	48,85±2,95	27,90±1,83*
"Physical total component"	44,96±3,56	23,27±2,21*
"Mental total component"	52,74±3,25	32,52±2,02*

*Note: * – significant differences in comparison with patients without skin itch, p <0,001*

We also conducted a more detailed comparison between SI and LQ by analyzing the quality of life in groups with varying intensity of manifestations of SI, namely between subgroup I (mild and moderate symptoms of itching) and subgroup II (severe manifestations of SI). The analysis showed significant difference between subgroups, practically, of all LQ indicators: “Total Quality of Life score” ($p<0,01$), “Symptoms of kidney disease” ($p<0,01$), “Influence of disease on everyday life” ($p<0,01$), “Patient’s subjective evaluation of general health status – SF-12” ($p<0,01$) and “Mental total component” ($p<0,01$). A correlation analysis between LQ and SI has revealed, that the SI is inversely correlated with most indicators of LQ, especially SI affects “Total Quality of Life score” ($p<0,001$; $r=-0,64$), “Symptoms of kidney disease” ($p<0,001$; $r=-0,52$), “Influence of disease on everyday life” ($p<0,001$; $r=-0,61$), “The burden of kidney disease” ($p<0,001$; $r=-0,29$), “Patient’s subjective evaluation of general health status (SF-12)” ($p<0,001$; $r=-0,61$), and also affects the physical activity of patients – “Physical total component” ($p<0,001$; $r=-0,39$) and plays a negative role in the mental field – “Mental total component” ($p<0,001$; $r=-0,48$). The obtained results showed, that SI with high probability, worsens the LQ.

Then we began to study the pathophysiological links of skin itching, which we realized as follows. Our first step (*second task*), was, to study of PC levels. Investigated groups included, patients with SI n=48 (group 3) and patients without SI n=48 (group 2), and their results was compared with the data of the control group n=20 (group 1 – practically healthy persons). A statistically significant difference was found between all the groups under study, by the levels of proinflammatory cytokines. By levels of IL-1β, IL-17 and MCP-1 (pg/ml) was detected, a significant difference between the groups 3 and 2 ($p<0,001$), 2 and 1 ($p<0,001$), and also 3 and 1 ($p<0,001$). We believe, that discovered higher PC levels in the groups of patients with CKD VD stage in comparison with practically healthy persons, obviously, are signs of kidney disease, but significantly higher levels of proinflammatory cytokines in the group of patients with CKD VD stages with SI, in comparison with patients without SI, in our opinion, is a sign of the participation of proinflammatory cytokines in the pathogenesis of itching.

Levels of PC in the group with SI were raised not evenly, namely 58,3% (n=28, further subgroup I) of patients, had PC levels fluctuated within indicators of the group without SI, and the rest 41,7% (n=20, further

subgroup II) patients, had PC levels much more higher, than in group without SI ($p<0,001$).

Received result was unexpected, so we decided to establish, is there a correlation between the increase in the SI symptoms and the growth of PC levels, or maybe SI appearance and intensity does not depend from growth of PC levels, which we study. We compared points of all Skindex-16 scales between group 2 (without SI) and subgroups I, II (with SI sings).

A significant difference was found between the subgroups with SI and the group without SI of all SI scales ($p<0,001$). Although, the indexes of itching in subgroup II were slightly higher than in subgroup I, significant difference between subgroups, was not found ($p>0,05$).

Consequently, given that, in some patients with SI, PC levels are higher than those in a group without SI, and SI manifestations in the groups that statistically differ by PC levels, can be both mild and severe, we can assumed, that only in some patients SI can be explained by high PC levels.

In subgroup II, were was established statistically significantly higher PC levels compared to non-itchy groups, we also conducted a correlation analysis between PC levels and scale “Total Score” Skindex-16 questionnaire. Discovered direct correlation connection between all PC levels and SI manifestations: IL-1β pg/ml and scale “Total Score” – $p<0,05$, $r=0,49$, IL-17 pg/ml and scale “Total Score” – $p<0,05$, $r=0,61$, MCP-1 pg/ml and scale “Total Score” – $p<0,05$, $r=0,62$. This suggests, that PC, are associated with the onset of SI in patients treated with HD.

The second step, on the pathway to the study of the pathogenesis of the SI (*third task*), was devoted to the study of the state of the MC in the skin. To this study, we got involved 20 patients who were in subgroup II with SI and statistically higher levels of PC (group 1), and 20 patients from group 2 without SI. The control group consisted of 20 practically healthy persons (group 3).

Given the lack of scientifically established information regarding the status of the MC and their relation to the SI, we determined the repetition frequency of the detected signs and established the following patterns. Common features for all, were the presence of MC in all investigated cases, and their placement in the surface layers of the dermis. The difference between the groups was based on signs such as the mass distribution, or more precisely the amount of MC, and the presence of signs of their degranulation. Then we conditionally assigned, to each sign of evaluation

criteria, points. If all characteristics signs of MC in one case was present, the maximum number of points was 100,00.

Having scored points and conducted a comparative analysis between the groups, we found, that the indicators of MC were significantly higher in the groups of patients with CKD VD stage, which are treated with HD (group 1 – with SI and group 2 – without SI) in comparison with the control group 3 ($p<0,05$), and also discovered reliable excess of points in group 1 with SI in comparison with group 2 without SI ($p<0,01$).

In the group 1 (with SI), a correlation study was conducted between points of MC signs and SI scales, and discovered between them reliable direct correlations: "Symptoms" – $p<0,05$, $r=0,59$; "Emotions" – $p<0,05$, $r=0,47$; "Functions" – $p<0,05$, $r=0,51$; "Total Score" – $p<0,001$, $r=0,64$.

Performing the *fourth task*, namely the comparison of immunological and morphological indicators, we found a statistically significant difference between all studied groups according to the PC levels, depending on the points of MC signs ($p<0,001$). The highest PC levels, were in the group, with SI and the points of MC characteristic signs from 70,00 to 100,00, namely: IL-1β pg/ml – 172,08±4,20; IL-17 pg/ml – 792,54±28,07; MCP-1 pg/ml – 639,66±7,80.

Next, a correlation analysis was performed between the points of MC characteristic signs and the levels of PC in each group. The following results was obtained: in the group of healthy persons (group 3) correlation links was not found; in the group 1 with SI, a direct correlation was discovered between the levels of all the studied PC and the points of MC characteristic signs (IL-1β – $p<0,001$, $r=0,75$; IL-17 – $p<0,05$, $r=0,55$; MCP-1 – $p<0,01$, $r=0,69$), which indicates the dependence of PC levels from the increase in the number of MC in the skin and their degranulation.

3. The effectiveness of treatment with ultraviolet irradiation

The third stage, was to find out, the effectiveness of treatment with UVR in patients with CKD VD stage on the HD. These sessions were conducted for 48 patients with SI. The first step at this stage (*fifth task*), was to trace the clinical effectiveness of UVR within one year (twelve months), comparing the results of SI questionnaire (Skindex-16) (table 4).

Table 4

Dynamics of skin itch sings, during the year after ultraviolet radiation treatment, in comparison with the data before treatment

Scale Skindex-16	Before treatment	Right after the treatment	One month after treatment	Six months after treatment	One year after treatment
1	38,82±4,30	0,62±0,20*	11,04±2,30*	10,80±2,10*	12,50±2,50*
2	56,19±4,70	0,00±0,00*	14,22±3,40*	15,10±3,10*	19,04±3,80*
3	40,50±5,50	0,00±0,00*	6,37±2,40*	8,33±2,50*	12,31±3,60*
4	46,82±4,30	0,15±0,05*	10,85±2,40*	11,79±2,30*	15,18±3,00*

*Note: * (p<0,001) – significant difference in comparison with the data before UVR treatment. Legend: 1 – "Symptoms", 2 – "Emotions", 3 – "Functions", 4 – "Total Score"*

A significant difference, was found, between SI manifestations before and after UVR treatment (p<0,001). After the course of UVR treatment, in all patients with SI, its manifestations disappeared. Clinical efficacy of the UVR on the SI we estimated as "good", in view of the, absence of all SI symptoms.

A month after the UVR treatment, in 26 (54,1%) patients SI did not recover. In 22 (45,9%) patients its appeared again. In 19 (39,6%) patients was SI<50,00 points, and in three (6,3%) SI>50,00 points. There was a reliable difference in points on all scales of SI in comparison with data before UVR (p<0,001). Points were significantly lower. The clinical effectiveness of influents of UVR treatment on the SI manifestations after one month, we estimated as "good" in 26 (54,1%) patients, "satisfactory" in 15 (31,2%) patients and "no effect" in 7 (14,6%) patients.

Six months after the course of UVR treatment in 24 (50,0%) patients, sustained a steady positive effect, the SI manifestations did not recovered. In 24 (50,0%) patients SI has appeared again: in 21 (43,8%) patients SI was<50,00 points, and in three (6,3%) patients>50,00 points. Detected a significant difference, between SI data before and six months later, after UVR treatment (p<0,001). We evaluated the clinical efficacy of UVR on SI manifestations, after six months of treatment, as "good" in 24 (50,0%) patients, "satisfactory" in 15 (31,2%) patients and "no effect" in 9 (18,8%) patients.

One year after the course of treatment, the clinically stable positive effect of UVR stayed in 22 (45,8%) patients. In 26 (54,2%) SI recovered, of them in 22 (45,8%) patients SI<50,00 points, and in four (8,4%) patients

SI>50,00 points. Detected a significant difference between SI data before and twelve months later after UVR treatment ($p<0,001$). We evaluated the clinical efficacy of the UVR on SI manifestations as “good” in 22 (45,8%) patients, “satisfactory” in 15 (31,2%) patients and “no effect” in 11 (23,0%) patients.

Even taking into account, the fact, that in some patients SI recovered again, its intensity was less than before treatment, so we can assume that, UVR is an effective method of sanation of patients with CKD VD stage with SI.

The second step at this stage of research (*sixth task*), was to trace the dynamics of PC levels in patients with SI (n=48). In the comparison group, there were patients without SI (n=48). After the first session of the UVR, there was a tendency to increase the PC levels in all patients with SI, that was not accompanied by a deterioration in the course of the disease and itching, and therefore there was no reason to stop the treatment. In our opinion, this increase could be the result of an increase in the activity and degranulation of MC.

Despite such effect, after the first session of the UVR, the course of treatment was accompanied by a significant decrease in PC levels: IL-1β pg/ml – $p<0,001$; IL-17 pg/ml – $p<0,001$; MCP-1 pg/ml – $p<0,001$.

Next, we conducted a differentiated assessment, of the dynamics of PC levels, before and after treatment, depending on their baseline levels, in the groups of patients with SI. In group, were was significantly higher PC levels before UVR treatment in comparison with patients without SI, the course of treatment was accompanied by a significant decrease of these levels: IL-1β pg/ml before and after treatment with UVR ($p<0,001$); IL-17 pg/ml before and after treatment with UVR ($p<0,001$); MCP-1 pg/ml before and after treatment with UVR ($p<0,001$). This may indicate the effect of UVR on MC and the activity of monocyte-macrophage immunity, concerning the production of the PC. UVR treatment effect, was prolonged, and most likely was due to morphological changes in the skin.

The third step at this stage (*seventh task*), was devoted to the clarification of the clinical efficacy of UVR effect on LQ within one year. After a course of UVR treatment in all patients with SI, who participated in the study, a significant difference, was found between the points of LQ before and after two weeks of UVR treatment (42,70±1,80 and 65,50±1,90 respectively; $p<0,001$ “Total Quality of Life Score” scale). LQ was improved. Exploring LQ after one month of UVR treatment, we found a significant difference of

LQ point in comparison with the data before UVR treatment (42,70±1,80 and 60,10±2,20 respectively; p<0,001 “Total Quality of Life Score” scale). LQ was higher. Six months later, a significant difference was detected between the LQ indicators in the group with SI in comparison with the data before UVR treatment (42,70±1,80 and 58,80±2,30 respectively; p<0,001 “Total Quality of Life Score” scale). One year after the course of UVR treatment, there clinically stable positive effect of the UVR on the LQ, resisted in 22 (45,8%) patients. Points of all LQ scales were significantly higher in comparison with data before UVR treatment (42,70±1,80 and 57,50±1,90 respectively; p<0,001 “Total Quality of Life Score” scale) (tables 5, 6).

Table 5

Dynamics of quality of life data, during the year after patient’s treatment with ultraviolet radiation, in comparison with the data before treatment

Scales SF-36	Before treatment	Right after the treatment	One month after treatment	Six months after treatment	One year after treatment
1	42,70±1,80	65,50±1,90*	60,10±2,20*	58,80±2,30*	57,50±1,90*
2	63,40±2,20	80,90±1,40*	77,30±1,60*	76,90±1,80*	74,80±1,80*
3	49,90±2,60	76,60±2,10*	70,70±2,60*	69,10±2,50*	67,40±2,60*
4	13,90±1,80	34,50±3,90*	30,10±3,40*	28,60±3,60*	25,30±2,70*
5	26,80±2,10	53,20±2,80*	45,90±2,90*	43,90±3,10*	44,40±2,90*
6	22,60±2,60	53,00±3,50*	45,80±3,60*	42,90±3,90*	40,10±3,40*
7	31,10±2,20	53,30±2,90*	46,00±3,00*	45,10±3,10*	48,70±3,30*

*Note: * – significant difference in comparison with the data before treatment (p<0,001). Legend: 1 – “Total Quality of Life score”, 2 – “Symptoms of kidney disease”, 3 – “Influence of disease on everyday life”, 4 – “The burden of kidney disease”, 5 – “Patient’s subjective evaluation of general health status”, 6 – “Physical total component”, 7 – “Mental total component”*

Table 6

Dynamics of patients Quality of Life, during the year after UVR treatment

"Total Quality of Life score"	**n**	**%**
Right after the treatment		
Very bad (0,00–25,00 points)	0	0,00
Bad (25,01–50,00 points)	1	2,08
Not bad (50,01–75,00 points)	36	75,00
Good (75,01–100,00 points)	11	22,92
One month after treatment		
Very bad (0,00–25,00 points)	0	0,00
Bad (25,01–50,00 points)	12	25,00
Not bad (50,01–75,00 points)	26	54,17
Good (75,01–100,00 points)	10	20,83
Six months after treatment		
Very bad (0,00–25,00 points)	0	0,00
Bad (25,01–50,00 points)	14	29,17
Not bad (50,01–75,00 points)	24	50,00
Good (75,01–100,00 points)	10	20,83
One year after treatment		
Very bad (0,00–25,00 points)	0	0,00
Bad (25,01–50,00 points)	14	29,17
Not bad (50,01–75,00 points)	28	58,33
Good (75,01–100,00 points)	6	12,50

Consequently, in patients with CKD VD stage, treated with HD and with SI manifestations, after the course of UVR treatment revealed, a significant improvement in LQ indicators in comparison with the indicators before treatment, which was maintained during the year.

CONCLUSIONS

Investigating the effect of skin itching on the quality of life of patients with CKD VD stage, which are treated by hemodialysis, having studied the connections between the manifestations of skin itching and the levels of proinflammatory cytokines, the activity of mast cells, having substantiated the expediency of using ultraviolet radiation to treat this category of patients, can be done the following conclusions.

It has been established that the constant itching of the skin, of different intensity, have 56,0% of the examined patients with CKD VD stage, who are treated with hemodialysis. Itching of skin is combined with burning of the

skin in 41,0% of patients, with wounds on the skin from scratching in 39,3%, with irritation of the skin from scratching in 60,7% of patients.

Itching inversely correlated with most life quality indicators: "Total Quality of Life score" ($p<0,001$; $r=-0,64$), "Symptoms of kidney disease" ($p<0,001$; $r=-0,52$), "Influence of disease on everyday life" ($p<0,001$; $r=-0,61$), "The burden of kidney disease" ($p<0,001$; $r=-0,29$), "Patient's subjective evaluation of general health status SF-12" ($p<0,001$; $r=-0,61$). Itching of the skin also affects the physical activity of the patients – "Physical total component" ($p<0,001$; $r=-0,39$) and plays a negative role in the mental field – "Mental total component" ($p<0,001$; $r=-0,48$).

Regardless of the presence or absence of itching of the skin in patients with CKD VD stages, which are treated with hemodialysis, the levels of the studied proinflammatory cytokines were significantly higher in comparison with practically healthy persons ($p<0,05$) and in the presence of itching skin, are higher than in patients without pruritus with CKD VD stages, which are treated with hemodialysis, namely IL-1β ($p<0,001$), IL-17 ($p<0,001$), MCP-1 ($p<0,001$).

Mast cells manifestations, were significantly higher, in the groups of patients with CKD VD stage, treated with hemodialysis, in comparison with the group of practically healthy persons ($p<0,05$), and in the presence of itching of the skin revealed, that their number and activity was higher in comparison with patients with CKD VD stage without pruritus ($p<0,01$).

In the group of patients with CKD VD stage, treated with hemodialysis, with pruritus, where levels of proinflammatory cytokines was significantly higher, in comparison with patients without itching ($p<0.001$), was established a direct correlation between the studied cytokines and mast cells (IL-1β – $p<0,001$, $r=0,75$; IL-17 – $p<0,01$, $r=0,55$; MCP-1 – $p<0,001$, $r=0,69$).

The course of treatment with ultraviolet radiation helped to eliminate the manifestations of itching of the skin in 100,0% of patients with the CKD VD stage, which are treated with hemodialysis. The steady, positive clinical effect of treatment with ultraviolet radiation was maintained in 50,0% of patients within six months and in 45,8% of patients during the year.

The course of treatment with ultraviolet radiation, significantly reduces the levels of IL-1β, IL-17, MCP-1 ($p<0,001$) in patients with CKD VD stage, that are treated with hemodialysis and have itching of the skin.

Treatment with ultraviolet radiation contributed to a significant improvement of life quality, in comparison with data before treatment ($p<0,01$) of all patients with CKD VD stage, which are treated with hemodialysis with skin itch.

SUMMARY

The performed work allows to give the following practical recommendations.

It is advisable to perform diagnostics of skin itching, for patients with CKD VD stages treated with hemodialysis, using questionnaires Skindex-16 – assessment of skin itch and SF-36 – assessment of quality of life, once every six months. In the presence of pruritus manifestations in patients undergoing CKD VD, that are treated with hemodialysis, it is advisable to investigate the morphological changes in the skin (the number and activity of mast cells) and IL-1β, IL-17, MCP-1 levels as additional markers of diagnosis and effectiveness of treatment.

In the presence of skin itching in patients undergoing hemodialysis, it is expedient to use treatment with ultraviolet radiation, with wavelengths from 290 to 320 nm (middle range), two to three times a week, the course of treatment for ten sessions. The effect of treatment lasts an average of six to twelve months.

REFERENCES

1. Дудар І.О., Гончар Ю.І. Якість життя пацієнтів із хронічними хворобами нирок, яких лікують з використанням методів ниркової замісної терапії. *Therapia*. 2007. № 11. С. 75–80.

2. Дудар І.О., Абрагамович Т.Н., Абрагамович Х.Я. Запалення та свербіж. *Український журнал нефрології та діалізу*. 2011. № 4(32). С. 80–89.

3. Дудар І.О., Гончар Ю.І., Абрагамович Х.Я. Свербіж шкіри та якість життя у хворих на гемодіалізі. *Український журнал нефрології та діалізу*. 2007. № 3(15). С. 33–35.

4. Дудар І.О., Абрагамович Т.Н., Абрагамович Х.Я. Свербіж шкіри як загальне поняття. Свербіж шкіри при хронічній нирковій недостатності. *Український журнал нефрології та діалізу*. 2008. № 1(17). С. 67–78.

5. Дудар І.О., Бісярін Ю.В., Абрагамович Т.Н., Абрагамович Х.Я. Опасиста клітина. Роль опасистої клітини у свербіжі шкіри хворих хронічною нирковою недостатністю, котрі лікуються програмним гемодіалізом. *Український журнал нефрології та діалізу*. 2009. № 4(24). С. 3–7.

6. Дудар І.О., Абрагамович Т.Н., Абрагамович Х.Я., Гончар Ю.І. Свербіж у хворих на гемодіалізі та його лікування. Ефект лікування ультрафіолетовим випроміненням. *Український журнал нефрології та діалізу*. 2011. № 1(29). С. 12–20.

7. Kosmadakis G.C., Zerefos N. Uremic pruritus: a revie. *Hemodial lnt*. 2005. № 9. P. 180–188.

8. Pisoni R.L., Wikström B., Elder S.J. et al. Pruritus in haemodialysis patients: international results from the Dialysis Outcomes and Practice Patterns Study (DOPPS). *Nephrology Dialysis Transplantation*. 2006. № 12. P. 3495–3505.

9. Zucker I., Yosipovitch G., David M. et al. Prevalence and characterization of uremic pruritus in patients undergoing hemodialysis: Uremic pruritus is still a major problem for patients with end-stage renal disease. *J Am Acad Dermatol*. 2003. Vol. 49. № 5. P. 842–846.

Information about authors:
Abrahamovych K. J.,
Candidate of Medical Sciences,
Associate Professor at the Department of Propaedeutics
to Internal Medicine № 1
Danylo Halytsky Lviv National Medical University
22, Konovaltsia st., Lviv, 79010, Ukraine
Kurban M. M.,
Candidate of Medical Sciences,
Assistant Professor at the Department of Propaedeutics
to Internal Medicine № 1
Danylo Halytsky Lviv National Medical University
22, Konovaltsia st., Lviv, 79010, Ukraine

ACCUMULATION OF FLAVONOIDS IN THE *CARDUUS ACANTHOIDES L.* HERBAL RAW MATERIAL AND THEIR PHARMACOLOGY ACTIVITY

Balanchuk T. I., Mazulin A. V.

INTRODUCTION

Asteraceae is a very large and widespread family of flowering plants. The family currently has 32,913 accepted species names, in 1,911 genera and 13 subfamilies, in terms of number of species, the *Asteraceae* revaled only by the *Orchidaceae*. Many members have composite flowers in the form of flower heads surrounded by involucral bracts, when viewed from a distance, each capitulum may have the appearance of being a single flower.

Asteraceae may represent as much as 10% of autochtonous flora in many regions of the world. Traditionally, two subfamilies were recognized, *Asteroideae* and *Cichorioideae*, the latter has been shown to be extensively paraphyletic, and has now been divided into 12 subfamilies, but the former still stands[1].

Carduus is a genus of flowering plants in the *Asteraceae* family, and the tribe *Cynareae*, one of two genera considered to be true thistles, the other being *Cirsium*. Plants of the genus are commonly as plumeless thistles.The *Carduus L.* genus counts up to 120 species in native flora of Europe, Asia, North Africa, North America and Canada, China, Argentina, Australia, New Zealand and other countries[2].

Up to 30 species of *Carduus L.* genus are grows in Ukraine. The most wide spread species it is been *Carduus acanthoides L.* and *Carduus nutans L.* They sprout as ruderal plants in steppes of east and western Ukraine up to left-bank of Polesya (Palsy), in Crimea, South and central regions[3].

And several are known elsewhere as introduced species and this genus is noted for its disproportionately high number of noxious weeds compared to

---

[1] Черепанов С.К. Сосудистые растения России и сопредельных государств. СПб.: Мир и семья, 1998. 990 с.

[2] Rauschert E.S.J., Shea K., Bjornstad O.N. Coexistence patterns of two invasive species, *Carduus nutans* and C. *acanthoides*, at three spatial scales. *Biol. Invasions*. 2012. Vol. 14(1). P. 151–164.

[3] Кортиков В.Н., Кортиков А.В. Полная энциклопедия лекарственных растений. Ростов-на-Дону: Феникс, 2008. 797 с.

other flowering plant genefa. It is also related to the card, which as a noun means a device for aligning and cleaning fihers[4].

These are usually annual or biennial herbs, sometimes perennial, species often grow 2 meters in height but are known to reach 4 meters. The erect stems are winged and spiny, and usually have woolly hairs, the leaf blades are hairy to hairless and entire or divided into lobes, and they have spine-toothed edges.

The flower heads are solitary or borne in inflorescences of up to 20, the head is spherical to cylindrical and covered in several layers of spreading or curving spine-tipped phyllaries.

Species such as *Carduus nutans, C. acanthoides, C. pycnocephalus L., C. crispus* is allelopathic, producing compounds that inhibit the growth and development of other plants[5].

The *Carduus* genus species herbal raw materials in modern medicine can be used as means with anti-inflammatory and antioxidant activities.

The aim of the work was to study the flavonoids accumulation in herbal raw materials and hepatoprotective activity of *Carduus acanthoides L.* herbal extracts.

1. Study of the flavonoids accumulation in *Carduus acanthoides L.* herbal raw materials

Carduus acanthoides L., known as the spiny plumeless thistle, welted thistle, and plumeness thistle, is a biennial plant species of thistle in the *Asteraceae* – sunflower family. The plant is native from France, Italy, western Turkey; through Russia and Kazakhstan to China. *Carduus acanthoides L.* is well known in many other parts of the world, including parts of North and South America, New Zealand, and Australia, as a noxious weed. It is an invasive species in many regions of Canada and the United States, including California and West Virginia. *Carduus acanthoides L.* presents multiple factors in growth and reproduction that negatively influence native species. This invasive species has spread easily across different regions due to the fact that it spreads through its seeds, and each plant itself creates around 1 000 seeds alone. In its native range the plant is

[4] Определитель высших растений Украины / Д.Н. Доброчаева и др.; под ред. Ю.Н. Прокудина. К.: Наукова думка, 1987. 548 с.

[5] Dunn P.H. Distribution of *Carduus nutans L., C. acanthoides, C. pycnocephalus* and *C. crispus,* in the United States. *Weed Science.* 1976. Vol. 24(5). P. 518–524.

found in open grassland sand distrurbed areas, and in non-native ranges it is a weed of annual grasslands, roadsides, felds and pastures, and disturbed areas. *Carduus acanthoides L.* may exceed 2 metres in height and can form weedy monotypic stands. It contains long, tubular disc florets in shades of white, pink, the fruit is a cypsela tipped with a pappus of barbed bristles or scales. At the top of each branch of the stem is an inflorescence of one to several flower heads, each rounded, covered in spinyphyllaries, and bearing many threadlike purple disc florets (Fig. 1). The stem and foliage are spiny and sometimes wooly. The plant stars from a flat basal rosette and then bolts an erect stem with occasional toothed, wrinkled, spiny leaves (Fig. 2). They mainly grows on open muddy laces, pastures, sides of roads, steppe hills, lime stones, black earth soils. The achenes are 2–3 mm long, with faint longwise stripes. Pappus bristles are 11–133 mm long[6].

And several are known elsewhere as introduced species and this genus is noted for its disproportionately high number of noxious weeds compared to other flowering plant genefa.

It is also related to the card, which as a noun means a device for aligning and cleaning fihers.

Fig. 1. The flowers of *Carduus acanthoides L.*

[6] Определитель высших растений Украины / Д.Н. Доброчаева и др.; под ред. Ю.Н. Прокудина. К.: Наукова думка, 1987. 548 с.

Fig. 2. The leaves of *Carduus acanthoides L.*

These are usually annual or biennial herbs, sometimes perennial, species often grow 2 meters in height but are known to reach 4 meters.

The erect stems are winged and spiny, and usually have woolly hairs, the leaf blades are hairy to hairless and entire or divided into lobes, and they have spine-toothed edges.

The flower heads are solitary or borne in inflorescences of up to 20, the head is spherical to cylindrical and covered in several layers of spreading or curving spine-tipped phyllaries. Species such as *Carduus acanthoides L.*, *C nutans L.*, *C. pycnocephalus L.* is allelopathic, producing compounds that inhibit the growth and development of other plants.

The *Carduus L.* genus species herbal raw materials in modern medicine can be used as means with hepatoprotective, anti-inflammatory and antioxidant activities[7].

For the first time a chemical composition of flowers and leaves of *Carduus acanthoides L.* had been studies: flavonoids, hydroxycinnamic acids, organic acids, amino acids, coumarins, mineral elements, essential oils, sesquiterpenoids lactones have been quantified[8].

[7] Aktay G., Deliorman D., Ergun E. et al. Hepatoprotective effects of Turkish folk remedies on experimental liver injuri. *Journal of Ethnopharmacology*. 2000. Vol. 73(1–2). P. 121–129.

[8] Dimitrova-Dyalgerova I., Zheley I., Mihaylova D. Phenolic profile and *in vitro* antioxidant activity of endemic Bulgarian *Carduus* species. *Pharmacognosy Magazine*. 2015. Vol. 11(4). P. 575–579.

Fourteen compounds were isolated from wholeplants of *Carduus acanthoides L.* by various chromatographic techniques including column chromatography over HP-20 macroporous resin, MCI gel, silica gel, Sephradex LH-20 and ODS and reversed-phase HPLC.

Their structures were identified as salidroside (2-(3,4-dihydroxyphenyl-ethyl-O-β-D-glucopyranoside), 3,5-di-hydroxyphenethyl alcohol-3-O-β-D-glucopyra-noside, p-coumaric acid, 3-hydro-1-(4-hydroxy-3,5-dimethoxyphenyl) propan-1-one, syringin, p-hydroxybenzaldehyde, salicylic acid, tachioside, vanillic acid-4-O-β-D-glucopyranoside, syringic aldehyde, 2,6-dymethoxy-4-hydroxyphenol-1-O-β-D-glucopyranoside, 2,6-dimethoxy-p-hydroquinone-4-O-P-D-glucopyranoside on the basis of spectroscopic data analysis. All compounds were isolated from the *Carduus L.* genus for the first time[9].

The study of promising plant raw materials during the growing period and development of effective modern methods of standartization are great theoretical and practical significance.

The standartization of herbal raw materials species *Carduus L.* genus must set contains for biological active flavonoids compounds.

A research object the flowers and leaves procured in the different regions of Ukraine in the period of flowering (july – august)[10].

Drying was conducted in a drying chamber "Termolab СНОЛ 24/350" (t=60ºC) during 12 hours.

An analysis was conducted by physical-chemical methods. A detection was conducted by chemical reactions, paper chromatography (PC), thin layer chromatography (TLC) on "Aluminium oxide 150 F 254 (0.20 mm) (MERCK, Germany)" plates in the systems: benzol : ethyl-acetate : acetic acid : formamide (70:30:2:1), ethyl-acetate : methane acid : acetic acid : water distilled (100:11:11:27), chloroform : methanol : acetic acid : water distilled (6:2:0,1:0,1).

By methods of PC, TLC were contented the 8 flavonoids (luteolin-7-O-β-D-glucopyranoside, campherol-3-O-β-D-glucopyranoside, campherol-3-O-β-D-ramnoglucoside, apigenin-7-O-β-D-glucopyranoside, apigenin-5-O-β-

[9] Dimitrova-Dyalgerova I., Zheley I., Mihaylova D. Phenolic profile and *in vitro* antioxidant activity of endemic Bulgarian *Carduus* species. *Pharmacognosy Magazine*. 2015. Vol. 11(4). P. 575–579.

[10] Державна Фармакопея України. Доповнення 2 / ДП «Науково-експертний фармакопейний центр». 1-е вид. Х.: Державне підприємство «Науково-експертний фармакопейний центр», 2008. 620 с.

D-gluco-pyranoside,c ampherol, luteolin, apigenin) and 3 hydroxycinnamic acids (caftaric, protocatechic, chlorogenic).

The identification of flavonoids and their quantitative content was conducted on a spectrophotometer “Specord-200 Analytic Jena UV-vis” (λ=330 nm). Prevailing content there were flavonoids derivates of apigenine.

The spectrophotometry method revealed of *Carduus acanthoides L.* herbal raw materials standardization by content of the apigenine-7-O-β-D-glycoside was proposed.

Method: about 1,0 g (exact weight) of herbal raw materials was introduced into a 100 ml flask, poured with 30 ml ethanol, heated on a water heater “ВБ-4 micromed” (t=100°C) for 30 min. with thorough mixing.

The operation was performed 2 more times with new portions of the ethanol (96%). The solutions were combined, cooled 30 min., filtered, adjusted to the mark. 2 ml of the solution was introduced into a 50 ml volumetric flask and made up to mark with the same solvent.

The optical density was measured on a spectrophotometer “Specord-200 Analytic Jena UV-vis” (λ=330 nm) in a cuvete with a layer thickness of 10 mm. The reference solution is ethanol in parallel we determined the optical drusity of the working standard solution apigenine-7-O-β-D-glycoside under the same conditions.

Calculation of the quantitative content of the amount of the flavonoids was performed in terms of the largest component of apigenine-7-O-β-D-glycoside.

The specters of 96% ethanol extracts from inflorescences and leaves of *Carduus nutans L.* are presented in the Fig. 1, 2.

The results of quantitative contens of flavonoids in herbal raw materials of *Carduus acanthoides L.* are presented in the Table 1.

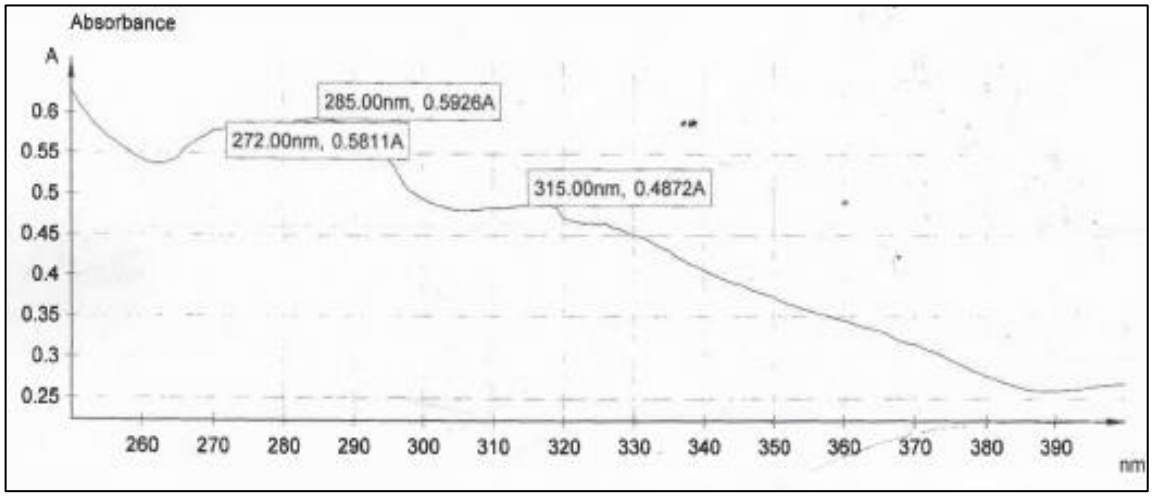


Fig. 1. The UF-specter of 0,04% ethanol extract from *Carduus acanthoides L.* inflorescens

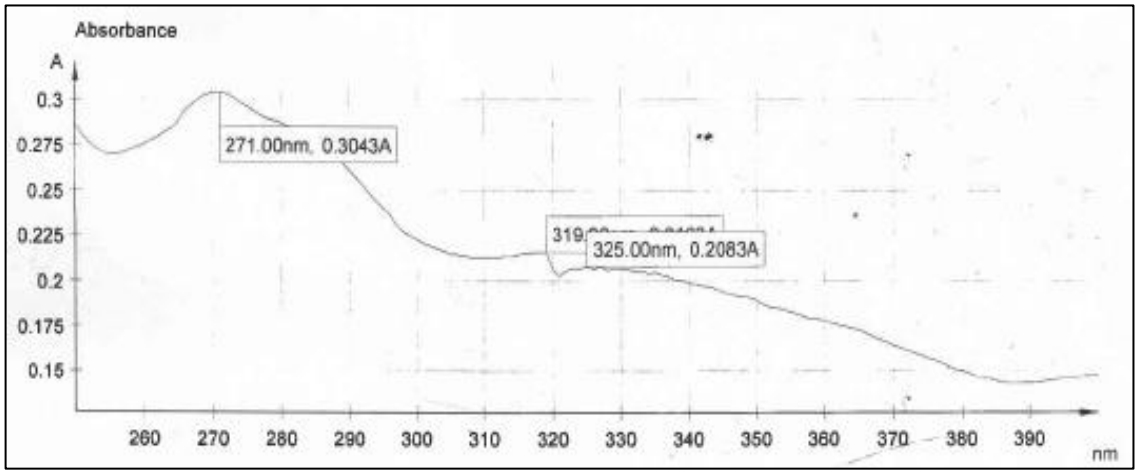


Fig. 2.The UF-specter of 0,04% ethanol extract from *Carduus acanthoides L.* leaves

The results were treated by the method of mathematical statistics with the use of license program "Statistica 6.0 for Windows" (Stat. Soft. Inc., № AXXR712D833214FANS).

The quantitative contents of flavonoids in herbal raw materials of *Carduus acanthoides L.* to 5,41±0,52% (flowers) and 3,12±0,30% (leaves) has been determined.

Authenticity of differences sizes of concentrations was estimated on the t-criterion of Student (p>95%)[11].

The accumulation of flavonoids in the flowers of *Carduus acanthoides L.* were higher than in leaves.

Table 1

The quantitative contents of flavonoids in herbal raw materials of *Carduus acanthoides L.*, ($\bar{x}\pm\Delta\bar{x}$), % μ=6, (july – august, 2014–2015)

№	The botanical name	Content of flavonoids	
		flowers	Leaves
1	*Carduus acanthoides L.*, Donetsk region., s. Druzcovka	5,11±0,50	3,12±0,30
2	*Carduus acanthoides L.*, Zaporizhzhia region., s. Tokmak	5,20±0,51	3,20±0,31
3	*Carduus acanthoides L.*, Donetsk region., s. Kramatorsk	5,10±0,49	3,19±0,32
4	*Carduus acanthoides L.*, Zaporizhzhia region., s. Melitopol	5,11±0,50	3,22±0,31
5	*Carduus acanthoides L.*, Kherson region., s. Nova Kachovka	5,41±0,52	3,33±0,30

2. Obtaining a lyophilic extract from *Carduus acanthoides L.* herbs

Phytochemical and pharmacological studies undertaken, that lyophilized water extracts from the flowers and leaves of *Carduus acanthoides L.* on the laboratory setting "Chris Alpha 1-2 LD plus (Germany)" expose an hepatoprotective and antioxidant actions.

Method: about 10,0 g (exact weight) of herbal raw materials (d=0,3 mm), was introduced into a 100 ml flask, poured with 50 ml ethanol, heated on a water heater "ВБ-4 micromed" (t=100°C) for 30 min. with thorough mixing. 50 ml of purified water was extracted on a ultrasonic unit "УЗДН-А1200Т" with an operating frequency of 50 Hz.

The solution was filtered, freeze drying was performed on the laboratory setting "Chris Alpha 1-2 LD plus (Germany)" in epindorfs (V=1,5 ml).

Contributed to the freezing chamber (t=-50°C) for 1 hour, hermetically sealed and turned on the vacuum device.

[11] Державна Фармакопея України. Доповнення 2 / ДП «Науково-експертний фармакопейний центр». 1-е вид. Х.: Державне підприємство «Науково-експертний фармакопейний центр», 2008. 620 с.

When the pressure in the sublimator decreases by 4 Pa, the temperature decreases (t=-50°C).

The duration of the process was 9 hours. Technological scheme for obtaining lyophilized extract from *Carduus acanthoides L.* herbs is shown in the Fig. 3.

The quantitative contents of flavonoids in lyophilized water extract from *Carduus acanthoides L*. herbs is shown in the Table 2.

Table 2

The quantitative contents of flavonoid compounds in lyophilized water extract of *Carduus acanthoides L.*, ($\bar{x}\pm\Delta\bar{x}$)), % μ=6, (july – august, 2014–2015)

№	The botanical name	Contents of flavonoids
1	*Carduus acanthoides L.*, Donetsk region., s. Druzcovka	10,33±1,11
2	*Carduus acanthoides L.*, Zaporizhzhia region., s. Tokmak	12,11±1,20
3	*Carduus acanthoides L.*, Donetsk region., s. Kramatorsk	10,19±1,09
4	*Carduus acanthoides L.*, Zaporizhzhia region., s. Melitopol	11,22±1,12
5	*Carduus acanthoides L.*, Kherson region., s. Nova Kachovka	10,22±1,21

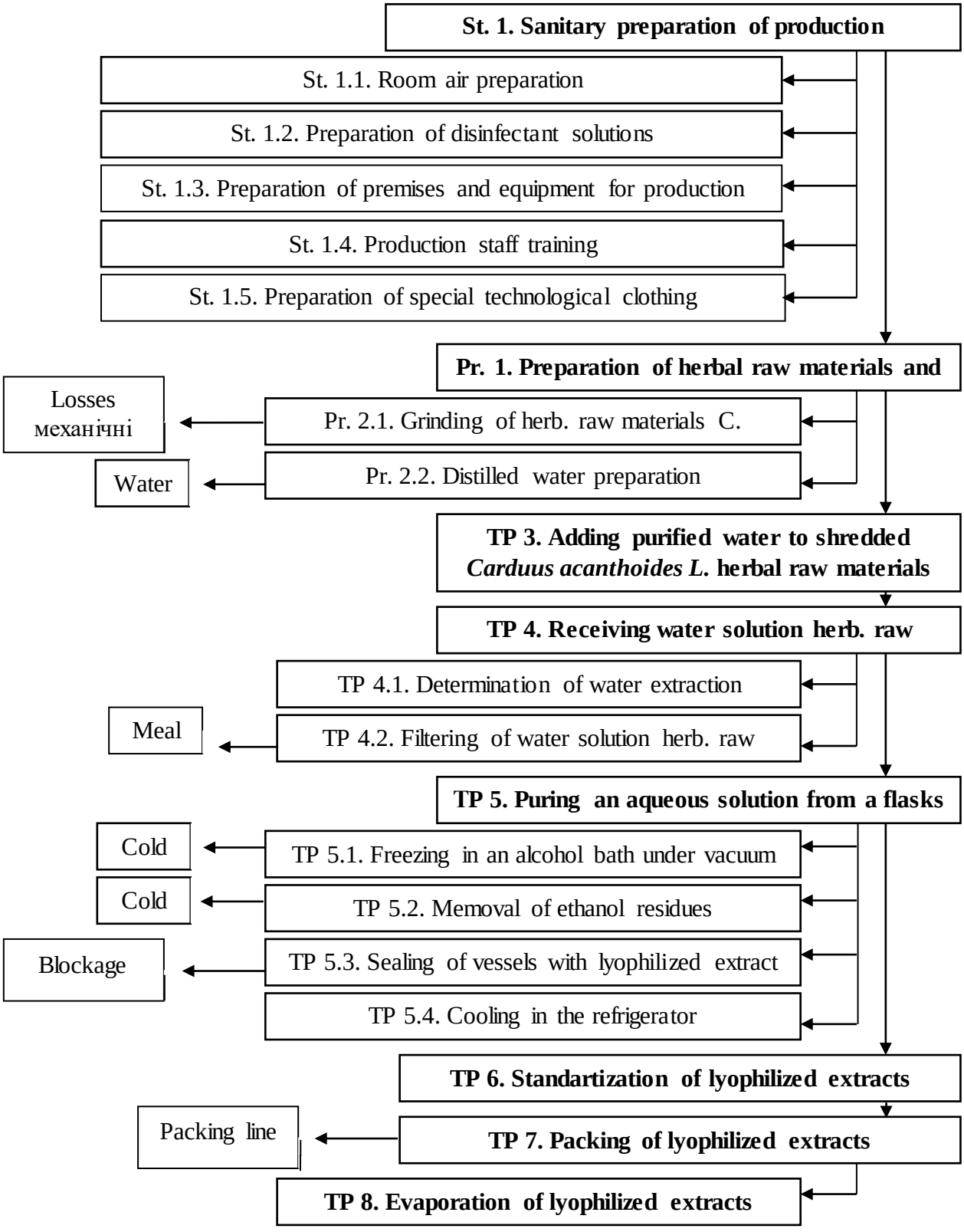


Fig. 3. Technological scheme for obtaining lyophilized herb extract from *Carduus acanthoides L.*

The quantitative contents of flavonoids in lyophilized water extracts of *Carduus acanthoides L.* from 10,19±1,09% and to 12,11±1,20% has been determined. Authenticity of differences sizes of concentrations was estimated on the t-criterion of Student (p>95%).

The species of *Carduus L.* genus sprout in unfavorable terms and accumulate toxic pollutants from an environment.

The standartization of lyophilized water extracts should include determination of the content of nitrates, inorganic elements and general ash.

A research object the herbs procured in the different regions of Ukraine in the period of flowering (july – august). Drying was conducted in a drying chamber "Termolab СНОЛ 24/350" (t=60ºС) during 12 hours[12].

By ionometric and atomic emission spectroscopy methods has been used for research. The contents of nitrates and 14 inorganic elements has been determined in lyophilized extract from *Carduus acanthoides L.* herbs.

An analysis was conducted by physical and physical-chemical methods.

Method: the nitrates identified on a reaction with a diphenylamine in the concentrated sulphuric acid. The quantitave contain of nitrates was conducted by a ionometric method on a device EV-74 (Belarus) with nitrate-selective electrode of type EI-NO_3^- (an electrode of comparison is a chlorine-silver).

The quantities contain of nitrates was expected on the gouge chart of standard solution of potassium of nitrate in a solution 1% of $KAl(SO_4)_2 \times 12H_2O$.

Hinge-plate of the herbal raw materials (lyophilized water extracts) 10,0 g was dispergated, carried in a retort on 100 ml, added 50 ml solution 1% of $KAl(SO_4)_2 \times 12H_2O$, mixed carefully 3 min., measured potential of electrode (mV).

Authentication and quantitative determination of element composition contain of the investigated herbal raw materials (lyophilized water extracts) was conducted by the method of atomic-emission analysis on the spectrometer of DFC-8 with the sprayer of IBC-28 at characteristic long waves[13].

---

[12] Державна Фармакопея України. Доповнення 2 / ДП «Науково-експертний фармакопейний центр». 1-е вид. Х.: Державне підприємство «Науково-експертний фармакопейний центр», 2008. 620 с.

[13] Arpadjan S., Çelik G., Taşkesen S., Güçer Ş. Arsenic, cadmium and lead in medicinal herbs and their fractionation. *Food and Chemical Toxicology*. 2008. Vol. 46(8). P. 2871–2875.

Authentication and quantitative determination of element composition contain of the investigated herbal raw materials (lyophilized water extracts) was conducted by the method of atomic-emission analysis on the spectrometer of DFC-8 with the sprayer of IBC-28 at characteristic long waves.

Hinge-plate of the herbal raw materials (lyophilized water extracts) 0,3 g was dispergated, was entered in a quartz cups, added 10 ml solution of sulphuric acid 5%, dried out (t=105°C) to permanent mass.

To increase the objectivity and accuracy of analyses the herbs was previously washed with purified water and the dust was mevoved from the surface.

The quartz cups was entered in a muffle stove up to 5 hours (t=500°C), cooled, weighed. Was dissolved in the diluted sulphuric acid, entered in the electro termical analyzer of device.

The atomization of tests was conducted on the graphite electrodes of device in the digit of arc of altemating current (I=16 A, U=220 V, t=60 sec.).

The spectrums were registered on a spectrograph DFC-8 (diffraction grate 600 st./mm at the three-lens system of illumination of crack).

Intensity of lines in spectrums was fixed by a micrometer MF-4 (λ=196-706,5 nm). Measuring temperature (t=23–25°C).

In parallel was conducted measuring of solution of comparing to the analogical reagents.

The method of quantitative determination of inorganic elements is quite reliable and objective.

The results were treated by the method of mathematical statistics with the use of license program “Statistica 6.0 for Windows” (Stat. Soft. Inc., № AXXR712D833214FANS).

Authenticity of differences sizes of concentrations was estimated on the t-criterion of Student (p>95%).

The results of researches of inorganic elements is driven to the tables 3, 4.

Authenticity of differences sizes of concentrations was estimated on the t-criterion of Student (p>95%). The results of researches of inorganic elements is driven to the tables 3, 4.

Table 3

The quantitative contents of nitrates in lyophilized water extract of *Carduus acanthoides L.*, ($\bar{x}\pm\Delta\bar{x}$), % μ=6, (july – august, 2014–2015)

№	The botanical name	Contents of nitrates
1	*Carduus acanthoides L.*, Donetsk region., s. Druzcovka	10,33±1,11
2	*Carduus acanthoides L.*, Zaporizhzhia region., s. Tokmak	12,11±1,20
3	*Carduus acanthoides L.*, Donetsk region., s. Kramatorsk	10,19±1,09
4	*Carduus acanthoides L.*, Zaporizhzhia region., s. Melitopol	11,22±1,12
5	*Carduus acanthoides L.*, Kherson region., s. Nova Kachovka	10,22±1,21

Table 4

The quantitative contents of inorganic elements in lyophilized water extracts from *Carduus acanthoides L.*, ($\bar{x}\pm\Delta\bar{x}$) mg/100 g, μ=6 (may – july 2015)

№	The name of inorganic elements	(λ, нм)	Contents of inorganic elements
1	Zinc (Zn)	213,9	0,45±0,05
2	Nickel (Ni)	232,0	0,01±0,001
3	Iron (Fe)	248,3	3,03±0,31
4	Silicon (Si)	251,6	5,47±0,55
5	Manganese (Mn)	279,5	0,03±0,002
6	Magnesium (Mg)	285,2	18,02±1,90
7	Aluminum (Al)	309,3	0,20±0,02
8	Molybdenum (Mo)	313,3	0,01±0,001
9	Copper (Cu)	324,7	0,08±0,001
10	Phosphorus (P)	357,9	5,62±0,55
11	Calcium (Ca)	422,6	45,46±4,66
12	Strontium (Sr)	460,0	0,15±0,02
13	Natrium (Na)	589,0	3,23±0,34
14	Potassium (K)	706,5	16,51±1,79
Mass of general ash (%)			0,73±0,08

The flowers, leaves and lyophilized water extracts from *Carduus acanthoides L.* herbs contented the nitrates level more than toxically concentrations (up to 300–370 mg/kg).

The concentrations of nitrates in lyophilized water extracts from *Carduus acanthoides L.* herbs from 10,19±1,09 and to 12,11±1,20 mg/kg has been determined[14].

The lyophilized water extracts from *Carduus acanthoides L.* herbs contented 14 inorganic elements, from that essential macro- and micro-inorganic elements (Ca, Cu, Fe, K, Mg, Mn, Zn, Co, P, Mo) in sufficiently high concentrations. In high concentrations was contented macro-inorganic elements (mg/100g): Ca (45,46±4,66), Mg (18,02±1,90), K (16,51±1,79), Na (3,23±0,34), Fe (3,03±0,31); micro- inorganic elements (mg/100 g): Zn (0,45±0,05), Al (0,20±0,02), Sr (0,15±0,02), Cu (0,08±0,001), Mn (0,03±0,002), Mo (0,01±0,001); Ni (0,01±0,001). The accumulation of toxically inorganic elements (Pb, Cd, Co, Hg, As,) were contented (>0,01 mg/100 g)[15].

Formation of general ask is insignificant higher in the lyophilized water extracts of *Carduus acanthoides L.* herbs (mg/100 g) (to 0,73±0,08), that is may be related to the different level of accumulation of inorganic elements.

3. Study of the pharmacological activity of *Carduus acanthoides L.* lyophilized water herbal extracts

The infusions (1:10) of *Carduus L.* genus species in modern medicine of many countries is applied. Infusion is appointed for treatment of diseases of gastrointestinal tract, hepatoprotective and antioxidant medicinal means. Also a water extract with roots shows scolative, antioxidant and anti epilepsy actions. The normalizing operating is set on functioning of liver, especially at viral, chemical and mechanical damages. For treatment and prophylaxis of diseases of liver apply a phylogenetic near type *Carduus marianus L.* of *Carduoideae Kitam.* subfamily and drugs from the herbal raw materials. They shows the expressed antioxidant, detoxical, immune modulating, membrano-proliferative activities, stimulated the biosynthesis

[14] Сирий М.М., Кулєшов М.М., Гаджиєва Н.М. та ін. Біохімія рослин: навч. посібник. Х.: ХНАУ ім. В.В. Докучаєва, 2006. 175 с.

[15] Сирий М.М., Кулєшов М.М., Гаджиєва Н.М. та ін. Біохімія рослин: навч. посібник. Х.: ХНАУ ім. В.В. Докучаєва, 2006. 175 с.

of proteins and phosphotides[16] .The value of the index of acute toxicity of lyophilized water extracts from *Carduus acanthoides L.* herbs were determined on white rats of the same sex in 6 animals per group. The lyophilized water extracts were administered inside the gastro-metal probe in increasing concentrations at intervals of 30 minutes for 2–3 hours. When intragastric lyophilized water extracts were administered in a dose of 15 000 mg/kg, no effects on the behavior of animals, significant violations of the general condition, or local irritation on the intact eye mucosa were found. The studied lyophilized water extracts belong to the 6th class of toxicity, including relatively non-toxic substances[17]. When applying concentrated solutions of lyophilized water extracts to the skin of white rats, no allergic reactions were observed. The study of hepatoprotective, antioxidant, detoxification activity of lyophilized water extracts were carried out on 50 male rats of the Institute of Pharmacology and Toxicology of the Academy of Medical Sciences of Ukraine. The model of toxic hepatitis was caused by the introduction of a 50% solution of dichloroethane in sunflower oil, which the rats were injected inside with a gastro-metallic probe. Dosage 500 mg 1 time per week for 4 weeks. When reaching 5 weeks of the experiment, the introduction of dichloroethane was stopped. During for 10 days, the test animals were administered intragastrically 1 time per day: lyophilized water extracts from herbs of *Carduus acanthoides L.* and Carsil® AT "Sopharma" preparation in doses of 100 mg. The study of the biological activity of lyophilized water extracts and the biochemical parameters of the blood of animals on a model of experimental hepatitis has been established that they possess a disturbed hepatoprotective activity identical to the drug Carsil® AT "Sopharma". The introduction of lyophilized extracts to animals leads to inhibition of the synthesis of oxide modification of rat liver protein (a decrease in CPG, APG). The drug Carsil® AT "Sopharma" has a significantly less pronounced antioxidant activity.

CONCLUSIONS

Carduus acanthoides L. is native plant from France, Italy, western Turkey; through Russia and Kazakhstan to China. *Carduus acanthoides L.* is

[16] Черепанов С.К. Сосудистые растения России и сопредельных государств. СПб.: Мир и семья, 1998. 990 с.

[17] Arpadjan S., Çelik G., Taşkesen S., Güçer Ş. Arsenic, cadmium and lead in medicinal herbs and their fractionation. *Food and Chemical Toxicology*. 2008. Vol. 46(8). P. 2871–2875.

well known in many other parts of the world, including parts of North and South America, New Zealand, and Australia, as a noxious weed. It is an invasive species in many regions of Canada and the United States, including California and West Virginia.

By methods of PC, TLC were contented the 8 flavonoids (luteolin-7-O-β-D-glucopyranoside, campherol-3-O-β-D-glucopyranoside, campherol-3-O-β-D-ramnoglucoside, apigenin-7-O-β-D-glucopyranoside, apigenin-5-O-β-D-glucopyranoside, campherol, luteolin, apigenin) and 3 hydroxycinnamic acids (caftaric, protocatechic, chlorogenic).

The spectrophotometry method revealed of *Carduus acanthoides L.* herbal raw materials standardization by content of the apigenine-7-O-β-D-glycoside was proposed. The infusions (1:10) of *Carduus L.* genus species in modern medicine of many countries is applied. Infusion is appointed for treatment of diseases of gastrointestinal tract, hepatoprotective and antioxidant medicinal means. Phytochemical and pharmacological studies undertaken, that lyophilized water extracts from the flowers and leaves of *Carduus acanthoides L.* on the laboratory setting "Chris Alpha 1-2 LD plus (Germany)" expose an hepatoprotective action. The quantitative contents of flavonoids in lyophilized water extracts of *Carduus acanthoides L.* from 10,19±1,09% and to 12,11±1,20% has been determined.

The standartization of lyophilized water extracts should include determination of the content of nitrates, inorganic elements and general ash.

Pharmacological studies undertaken, that lyophilized water extracts of the *Carduus acanthoides L.* herbs possess significant hepatoprotective and antioxidant actions. The drug Carsil® AT "Sopharma" has a significantly less pronounced antioxidant activity.

SUMMARY

The *Carduus L.* genus species herbal raw materials is perspective in modern medicine as means with significant activity. Phytochemical and pharmacological studies undertaken, that lyophilized water extracts from the *Carduus acanthoides L.* herbs on the laboratory setting "Chris Alpha 1-2 LD plus (Germany)" expose an hepatoprotective and antioxidant actions. The study of the biological activity of lyophilized water extracts and the biochemical parameters of the blood of animals on a model of experimental hepatitis has been established that they possess a disturbed hepatoprotective and antioxidant activity identical to the drug Carsil® AT "Sopharma".

REFERENCES

1. Сирий М.М., Кулєшов М.М., Гаджиєва Н.М. та ін. Біохімія рослин: навч. посібник. Х.: ХНАУ ім. В.В. Докучаєва, 2006. 175 с.

2. Державна Фармакопея України. Доповнення 2 / ДП «Науково-експертний фармакопейний центр». 1-е вид. Х.: Державне підприємство «Науково-експертний фармакопейний центр», 2008. 620 с.

3. Кортиков В.Н., Кортиков А.В. Полная энциклопедия лекарственных растений. Ростов-на-Дону: Феникс, 2008. 797 с.

4. Определитель высших растений Украины / Д.Н. Доброчаева и др.; под ред. Ю.Н. Прокудина. К.: Наукова думка, 1987. 548 с.

5. Черепанов С.К. Сосудистые растения России и сопредельных государств. СПб.: Мир и семья, 1998. 990 с.

6. Arpadjan S., Ģelik G., Taşkesen S., Güçer Ş. Arsenic, cadmium and lead in medicinal herbs and their fractionation. *Food and Chemical Toxicology*. 2008. Vol. 46(8). P. 2871–2875.

7. Dimitrova-Dyalgerova I., Zheley I., Mihaylova D. Phenolic profile and *in vitro* antioxidant activity of endemic Bulgarian *Carduus* species. *Pharmacognosy Magazine*. 2015. Vol. 11(4). P. 575–579.

8. Dunn P.H. Distribution of *Carduus nutans L., C. acanthoides, C. pycnocephalus* and *C. crispus*, in the United States. *Weed Science.* 1976. Vol. 24(5). P. 518–524.

9. Aktay G., Deliorman D., Ergun E. et al. Hepatoprotective effects of Turkish folk remedies on experimental liver injuri. *Journal of Ethnopharmacology*. 2000. Vol. 73(1–2). P. 121–129.

10. Rauschert E.S.J., Shea K., Bjornstad O.N. Coexistence patterns of two invasive species, *Carduus nutans* and C. *acanthoides*, at three spatial scales. *Biol. Invasions*. 2012. Vol. 14(1). P. 151–164.

Information about authors:

Balanchuk T. I.,
Assistant at the Department of Pharmacy
National Pirogov Memorial Medical University
56, Pirogov Ave., Vinnytsya, 21018, Ukraine

Mazulin A. V.,
Doctor of Pharmaceutical Sciences,
Profesor at the Departament of Pharmacognosy, Pharmaceutical Chemistry and Medicinal Preparations Technology
Zaporizhzhia State Medical University
26, Maiakovskyi Ave., Zaporizhzhia, 69035, Ukraine

ON THE DISTRIBUTION OF CEREBROVASCULAR DISEASES IN UKRAINE AND POLAND AND ACCURACY OF CREATION OF NEW DRUGS FOR THEIR TREATMENT AND PREVENTION

Vyshnevska L. I., Romas K. P.

INTRODUCTION

For many years now, cardiovascular diseases rank first in prevalence not only in Ukraine but throughout the world. Moreover, they are the number one cause of disability and mortality of the population. Pathological conditions associated with lesions of the vessels of the brain are also associated to this significant group of diseases. They received the name of cerebrovascular disease (CVD). The problem of prevention and treatment of central nervous system in the world and in Ukraine is taking a leading role in combating with a decrease in mortality of the population.

CVDs are among the most common and frequent diseases of the nervous system, and their most dangerous form is acute violation of cerebral circulation (AVCC, stroke) is more common than acute myocardial infarction. The statistics show that there are more than 15 million strokes registered each year in the world. They account for 10% of all deaths (almost 6 million a year), making the stroke the cause of death number 2 on the planet. However, in most cases, the result of AVCC is not death, but limitation of life, because strokes lead to disability and, consequently, a significant decline in the quality of life among older people in the group.

As statistics show, 60% of patients after a stroke suffer from severe disorders of the nervous system that interfere with their daily activities[1]. Strokes often lead to movement disorders, speech and vision disorders, pelvic dysfunction, swallowing[2] and alimentary deficiency disorders, emotional (depression), and cognitive (to degree of dementia) disorders.

This pathological condition tends to rejuvenate: if earlier the stroke was considered a problem exclusively of elderly people, to date, cases of

[1] Волошин П.В., Міщенко Т.С., Здесенко І.В. Епідеміологія мозкового інсульту в Україні. *Діагностика, лікування, профілактика гострих та хронічних порушень мозкового кровообігу: матеріали наук.-практ. конф.* (Харків, 5–7 жовтня 2005 р.). Х., 2005. С. 74–75.

[2] Кочуева М.Н. , Кочуев Г.И., Корж А.Н. Современные возможности метаболической терапии – в фокусе Актовегин. *Східноєвропейський журнал внутрішньої та сімейної медицини.* 2015. № 1. С. 101–108.

cerebrovascular accident are more often registered in patients in the age of 30 to 40 years.

During the therapy of neurological diseases, preference is given to neurotropic agents (drugs from the group of nootropics) that are successfully used for the treatment and pharmacological prophylaxis of pathological conditions associated with brain activity.

1. General characteristics of CVD, their classification and risk factors that contribute to the occurrence of these pathologies

Cerebrovascular diseases are a group of diseases of the brain that are caused by pathological changes of cerebral vessels with impaired cerebrovascular circulation. The most common causes of cerebrovascular diseases are atherosclerosis and arterial hypertension, which lead to a narrowing of the lumen of the brain vessels and a decrease in cerebral blood flow[3]. Often such diseases are associated with diabetes, smoking, and coronary heart disease. There are transient, acute and chronic progressive disorders of the cerebral circulation. Brain circulation disorder is the second leading cause of mortality in the cardiovascular diseases group after coronary heart disease[4].

In accordance with the International Classification of Diseases of the tenth revision, cerebrovascular diseases are allocated to the block of headings with the codes I60–I69 (in the class "disease of the circulatory system"). The detailed content of these sections is given in Table 1.

The risk factors for developing cerebral circulation disorders include physiological, behavioral, and environmental factors that increase the risk of vascular diseases of the nervous system.

Risk factors are not the cause of the disease, they only reflect the connection with the etiological factor in the development of the disease. There are many causes that lead to cerebrovascular disorder. But the most common causes are the following three:

---

[3] Бурчинский С.Г. Нейропротекция при цереброваскулярной патологии: системные аспекты. *Український неврологічний журнал.* 2015. № 1. С. 103–108.

[4] Гандзюк В.А. Динаміка захворюваності та поширеності хвороб системи кровообігу серед населення України на сучасному етапі: національний та регіональний аспекти *Вісник соціальної гігієни та організації охорони здоров'я України*. 2014. № 2(60). С. 74–78.

Інформаційно-аналітична довідка про економічний стан в Україні (2016). URL: http://ukrexport.gov.ua/ukr/prom/ukr/26.html (дата звернення: 25.10.2017).

– cerebral atherosclerosis and general atherosclerosis are the cause of 75% of all acute cerebrovascular abnormalities;

– hypertonic disease – the frequency of arterial hypertension during stroke is 72%;

– combination of atherosclerosis with hypertension.

Table 1

Classification of the CVD according to the ICD-10

I00–I99 CLASS IX Diseases of the circulatory system		
I60–I69 Cerebrovascular diseases		
I60	Subarachnoid hemorrhage	
I61	Intracerebral hemorrhage	
I62	Another nontraumatic intracranial hemorrhage	
I63	Brain infarction	
	I63.9	Brain infarction unspecified
I64	A stroke that is not specified as a hemorrhage or a heart attack	
I65	Blockage and stenosis of precerebral arteries, which do not lead to brain infarction	
I66	Clogging and stenosis of cerebral arteries, which do not lead to brain infarction	
	I66.9	Brain artery blockage and stenosis unspecified
I67	Other cerebrovascular diseases	
	I67.2	Cerebral atherosclerosis
	I67.3	Progressive vascular leukoencephalopathy
	I67.4	Hypertensive encephalopathy
	I67.8	Other specified lesions of the brain vessels
	I67.9	Cerebrovascular disease unspecified
I68	Brain vessels lesions in diseases classified elsewhere	
I69	Consequences of cerebrovascular diseases	
	I69.0	Consequences of subarachnoid hemorrhage
	I69.1	Consequences of intracranial hemorrhage
	I69.3	Consequences of cerebral infarction
	I69.4	Consequences of a stroke that is not specified as a hemorrhage or a cerebral infarction
	I69.8	Consequences of other and unspecified cerebrovascular diseases

Apart from these main reasons, it is necessary to name others that can lead to certain disorders of the cerebral circulation:

– symptomatic arterial hypertension (for example, when kidney disease);

– heart disease, congenital and acquired defects, rhythm disturbance, coronary artery disease, cardiosclerosis, angina pectoris, transient myocardial infarction, etc.;

– infectious and infectious-allergic vasculitis (when collagenosis, rheumatism, etc.);

– arterial hypotension;

– vasomotor dystonia;

– blood diseases (leukemia, hemophilia, etc.);

– kidney disease;

– diseases of the endocrine glands (thyroid, pancreas, adrenal glands);

– diabetes mellitus;

– toxic lesions of vessels in exogenous and endogenous intoxications (for example, with acute and chronic renal or hepatic insufficiency, alcohol intoxication, toxicosis of the second half of pregnancy);

– traumatic vascular lesions in hemorrhages (subdural, epidural, parenchymal, ventricular);

– compression of arteries and veins, especially in the cervical spine (with osteochondrosis);

– anomalies (congenital and acquired) of the structure of the Vilisius circle (occlusions and stenoses of the major arteries of the head and neck, congenital defects of the structure of the brain vessels, such as aneurysms, loops, narrowing, underdevelopment);

– brain tumors.

According to the results of 20 years of prospective observation among people with four risk factors, the mortality rate for all causes is three times higher than among those who did not have any of the risk factors during the period of examination[5]. With their number, the proportion of deaths from cardiovascular diseases increases: in the absence of risk factors for this pathology, every fourth is dying, while in the presence of four risk factors for the circulatory system, death occurs in three of the four deaths[6].

---

[5] Зінченко О.М., Голубчиков М.В., Міщенко Т.С. Стан неврологічної служби України в 2014 році: статистично-аналітичний довідник. Х., 2015. 24 с.

[6] Зозуля А.И., Слабкий Г.А. Усовершенствование системы оказания медицинской помощи населению при ЦВЗ – составляющей общей реформы здравоохранения в Украине. *Збірник наукових праць співробітників Національної медичної академії післядипломної освіти імені П.Л. Шупика.* 2013. № 3. С. 14–19.

Zozulya I.S., Zozulya A.I. Epidemiology of cerebrovascular diseases in Ukraine. *Ukrainian medical chronicle.* 2011. № 5. P. 38–41.

2. The prevalence of cerebrovascular diseases in Poland and in Ukraine

In the official statistics of the World Health Organization, two indicators are used to describe the state of neurological pathology: they are an indicator of the prevalence and incidence of nerve diseases, which include diseases of the peripheral nervous system, inflammatory diseases of the nervous system, epilepsy, Parkinson's disease, multiple sclerosis, damage to the nerves and nerve roots, vegetative vascular dystonia, and an indicator of the prevalence and morbidity of cerebrovascular disease[7].

CVDs are prevalent in the third place among nosological forms of circulatory system diseases and account for 16% of the structure of the primary morbidity of the population of our planet[8].

Cerebrovascular pathology has the largest share in the causes of primary disability in the working age population due to nerve diseases – 44,2%. Among the vascular diseases of the brain, the most widespread and grave consequence is a cerebral stroke, which leads to disability in 60% of cases, 10% of patients are able to recover fully, and almost 30% of strokes are fatal – these are the statistics of the consequences of stroke among the Ukrainian population. In Western Europe, these figures are somewhat different: 45%, 35% and 20%, respectively, which is substantiated by the unquestionably higher level of social and economic development of the country, more stable social protection of the population, which in turn directly affects the state of the nervous system and the likelihood of the emergence of CVD.

In the mortality structure in developed countries, stroke takes place No. 3 after ischemic heart disease (IHD) and oncological diseases. In Poland, mortality rates for the year make up about 4–7% of the total number of fatal cases.

In comparison, in Ukraine, according to official statistics, the CVD, mainly stroke, is the cause of mortality No. 2 (100–110 thousand deaths, about 14% of all deaths), every year 100–110 thousand strokes are registered (more than a third of them – in people of working age), 30–40% of stroke patients die within the first 30 days and up to 50% – within 1 year of the onset of the disease, 20–40% of surviving patients become dependent

[7] Волошин П.В., Міщенко Т.С., Здесенко І.В. Епідеміологія мозкового інсульту в Україні. *Діагностика, лікування, профілактика гострих та хронічних порушень мозкового кровообігу: матеріали наук.-практ. конф.* (Харків, 5–7 жовтня 2005 р.). Х., 2005. С. 74–75.

[8] Гандзюк В.А. Динаміка захворюваності та поширеності хвороб системи кровообігу серед населення України на сучасному етапі: національний та регіональний аспекти *Вісник соціальної гігієни та організації охорони здоров'я України*. 2014. № 2(60). С. 74–78.

on out-patient care (12,5% primary disability), and only about 10% return to full-fledged life[9].

In the last decade, according to statistical data, there is a significant increase in the prevalence of cerebrovascular diseases, including cerebral stroke, in the world. The frequency of their occurrence has increased almost 1,2 times [5].

It is necessary to note that almost 50% of registered strokes account for Ukrainians who have not reached the retirement age. The number of stroke cases in able-bodied patients continues to grow. The proof of this is the statistics for the last 7 years, which recorded the incidence of this pathology in patients aged 30 to 40 years. In Poland, this percentage is somewhat lower, which is due and justified by the availability of social guarantees, a number of youth social and economic development programs (mortgage lending, loyalty programs, etc.). Fig. 1 shows comparative data on the frequency of registration of stroke cases in patients aged 30 to 40 years in Ukraine and Poland in the period of 2010–2016 years.

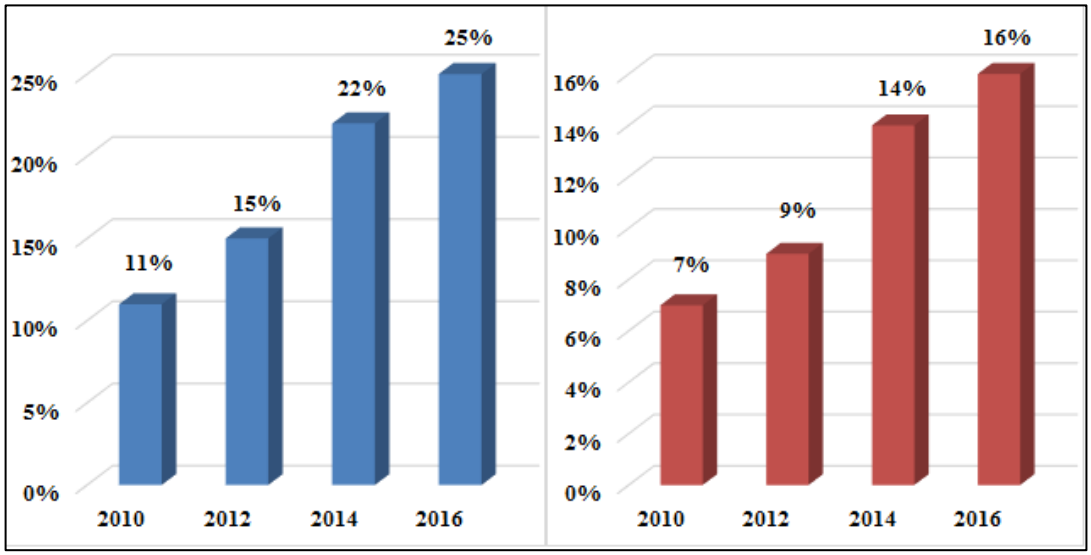


Fig. 1. Comparison of the frequency of registration of stroke cases in patients aged 30 to 40 years in Ukraine and Poland

[9] Куркин В.А. Основы фитотерапии: учеб. пособие для студентов фармацевтических вузов. Самара: ООО «Офорт»; ГБОУ ВПО «СамГМУ Росздрава», 2009. 963 с.

Analyzing the data of Fig. 1, it can be observed a rather sharp increase in the incidence of stroke at the same time in both countries due to the 2014 social and economic crisis.

That is why the choice of rational pharmacotherapy in treating the described conditions and improving the quality of life of patients who have suffered from disorders related to brain activity is of great importance and of primary importance.

3. Features of pharmacotherapy of cerebrovascular diseases in European countries

A leading factor in the preservation of neuroglial structures under conditions of ischemia and hypoxia is maintaining stable cerebral circulation, ensuring adequate oxygen supply and creating conditions for the activation of oxygen and glucose utilization in order to ensure the functioning of the Krebs cycle. Compliance with these provisions should be considered the true neuroprotection[10].

Strategies for rational neuroprotection are based on the activation of mechanisms that allow cerebral structures to properly function in the absence of oxygen and consist of restoring adequate tissue perfusion and eliminating the toxic effects of hypoxia. Contemporary concepts of the treatment of acute and chronic vascular cerebral disorders in Ukraine and Western Europe are based on the integrated usage of several groups of drugs:

1) infusion correctors of systemic and cerebral perfusion, which allow stabilizing the state of cerebral and systemic blood flow;

2) drugs that provide a stable level of blood pressure (now, in the acute period of a stroke it is considered appropriate to correct the blood pressure to figures not lower than 170/90 mm hg, which involves the use of mild hypotensive agents);

3) anticoagulants, activating mechanisms of blood flow; reoprotectors and vasoactivators, which improve blood supply;

4) drugs that allow for the early (in acute period of ischemic injury) and long-term use to stabilize cerebral energy metabolism;

[10] Піщиков В.А., Ященко Ю.Б., Кондратюк Н.Ю. Основні підходи до профілактики хвороб системи кровообігу. *Український медичний часопис.* 2014. № 6(104). С. 45–48.

5) drugs for substitution therapy (neurotransmitter), the conduct of which must be started with the stabilization of life-supporting functions of the body[11].

After a stroke, cognitive impairment occurs quite often (almost 25% of patients). The primary task is the selection of optimal neurotrophic therapy. Means of choice for cognitive, as well as linguistic post-stroke disorders are nootropic drugs[12].

So, based on the above information, it can be concluded that nootropic drugs are an integral part of pharmacotherapy and the prevention of diseases associated with damage to the vessels of the brain.

Initially, nootropics were created for the treatment of early stages of Parkinson's and Alzheimer's diseases, narcolepsy, depression, attention deficit disorder syndrome and ADHD (attention deficit hyperactivity disorder syndrome).

In the modern world, such drugs have become widely used. According to WHO statistics, one third of the population in Europe accepts drugs from this group. The peculiarity of the use of nootropics is the possibility of appointing them not only to patients, but also healthy individuals in stressful situations when reducing the overall level of human viability, which arise from a variety of diseases and extreme influences[13].

The evidence of high efficiency of this group of drugs is numerical research, in which a large number of patients participated. As a result, the positive influence of nootropic drugs on cognitive processes was proved and positive dynamics of functioning of cerebral structures was noted.

These drugs include, in particular, cyticoline – a drug from the group of nootropics, which has a proven positive effect on the indicators of cognitive function (Fioravanty M. et al., 2005). The confirmation of the neuroprotective effect when used in patients with acute ischemic stroke, chronic CVD was given in scientific publications. The accumulated data,

---

[11] Профілактика цереброваскулярних захворювань у пацієнтів з артеріальною гіпертензією. *Міжнародний неврологічний журнал.* 2013. № 8(62). С. 112–116.

Перелік ноотропних препаратів, дозволених до застосування в Україні. *Ліки України*. 2011. № 4(150). С. 132–135. URL: http://nbuv.gov.ua/j-pdf/likukr_2011_4_26.pdf (дата звернення: 20.09.2018).

[12] Профілактика цереброваскулярних захворювань у пацієнтів з артеріальною гіпертензією. *Міжнародний неврологічний журнал.* 2013. № 8(62). С. 112–116.

[13] Волошин П.В., Міщенко Т.С., Здесенко І.В. Епідеміологія мозкового інсульту в Україні. *Діагностика, лікування, профілактика гострих та хронічних порушень мозкового кровообігу: матеріали наук.-практ. конф*. (Харків, 5–7 жовтня 2005 р.). Х., 2005. С. 74–75.

according to the authors of the article, are sufficient grounds for the use of cyticoline in the treatment of patients with vascular cognitive dysfunction, vascular dementia, chronic cerebrovascular disorders. At the same time, it is necessary to note the very high interest, and because of this, a significant increase in the frequency of appointments of cyticoline-based drugs in violation of cerebral circulation not only in Ukraine, but also in the countries of the Near Europe, in particular, Poland[14].

Therapy aimed at improving microcirculation and cellular metabolism is an integral part of the management of patients with cognitive impairment or high risk of their development. A drug that has demonstrated efficacy in the treatment of such patients is Actovegin.

The use of Actovegin during 4 weeks in a prospective, double-blind, placebo-controlled study contributed to a significant improvement in the cognitive function (collecting and processing of information) and general well-being, as well as diminishing the symptoms of dementia and reducing the need for external care in hospitalized elderly patients with diagnosed bronchial asthma, or multiinfarction dementia[15].

4. Analysis of drugs with nootropic action in the pharmaceutical market of Ukraine and Poland

To date, nootropic drugs constitute a significant segment of the modern pharmaceutical market of these countries. The great demand for drugs of this group in the current clinical practice is explained by several reasons. One of them is a significant increase in the frequency of neurological and psychiatric pathology, including dementia of different genesis, various forms of encephalopathy, especially in advanced age.

Active pharmaceutical ingredients that exhibit neuroprotective effects and are actively used as a means of choice in the treatment and prevention of CVD include: nootropic and psychostimulant substance, an intermediate compound in the transformation of phosphatidylcholine from choline – cyticoline; derivative of pyrrolidone – piracetam; derivative of pyridoxine –

[14] Бурчинский С.Г. Нейропротекция при цереброваскулярной патологии: системные аспекты. *Український неврологічний журнал.* 2015. № 1. С. 103–108.

Mahomoodaly M.F. Complementary and Alternative medicines used against neurodegenerative diseases in Pharmacy. *Daly*. 2013. № 1. P. 103–123.

[15] Кочуева М.Н. , Кочуев Г.И., Корж А.Н. Современные возможности метаболической терапии – в фокусе Актовегин. *Східноєвропейський журнал внутрішньої та сімейної медицини.* 2015. № 1. С. 101–108.

pyrithinol; neuro-amino acid drugs – gamma-aminobutyric acid (GABA), derivatives of GABA – phenybut; substance that strengthens cerebrovascular circulation, microcirculation and metabolism – vinpocetine; drugs that contain the extract Ginkgo biloba[16].

These drugs have a wide and diverse range of pharmacological effects, but they all have a positive effect on brain activity, that is, they perform neuroprotective function.

The nomenclature of the modern range of medicinal products of nootropic action of plant and synthetic origin in the European pharmaceutical market is rather wide. We carried out a detailed analysis of the active pharmaceutical ingredients of the N06B X group (according to the ATC classification), "Other psychostimulants and nootropic drugs" in percentage shares, which are often the means of choice in the treatment and/or prevention of various forms of CVD in Ukraine and in Poland (Fig. 2).

A significant share of the Ukrainian pharmaceutical market belongs to herbal medicinal products based on the Ginkgo biloba leaves extract (24%), cyticoline (16%), phenibut and vinpocetine (12%), and the share of other drugs accounts for less than 5%.

It should be noted that the pharmaceutical market for nootropic drugs in Poland is mainly based on herbal drugs – 40% (24% are drugs based on Ginkgo biloba extract and 16% are drugs based on other types of ‚medicinal plant raw material), cyticoline is 26%, GABA – 17%, piracetam, vinpocetine and phenibut – by 7%, 6%, 4%, respectively.

The obtained result showed that in Poland and other countries of Western Europe (Austria, Belgium, Germany, etc.) the advantages in the treatment of various types of CVD are provided with prophylactic measures and prevention of significant changes in cerebrovascular circulation, and as preventive means, medicinal products of plant origin are used.

[16] Державний реєстр лікарських засобів (2017). URL: http://www.drlz.com.ua (дата звернення: 15.04.2016).

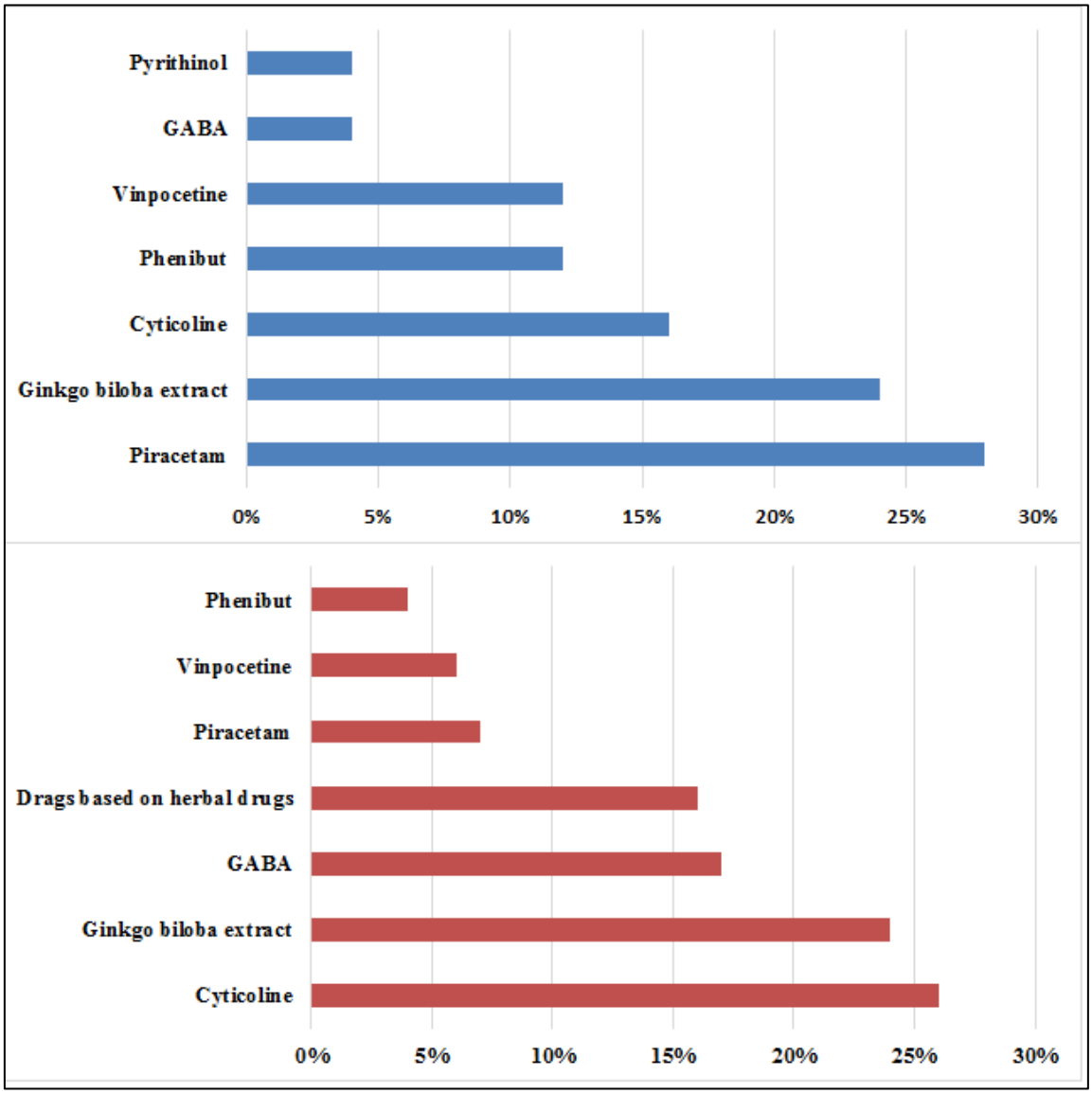


Fig. 2. Analysis of nootropic agents presented on the pharmaceutical market of Ukraine and Poland by the active substance

The studied group of drugs is represented by a variety of medicinal forms: tablets, injection solutions, capsules, oral drops (Fig. 3), which ensures ease of use and rational dosage for different categories of patients.

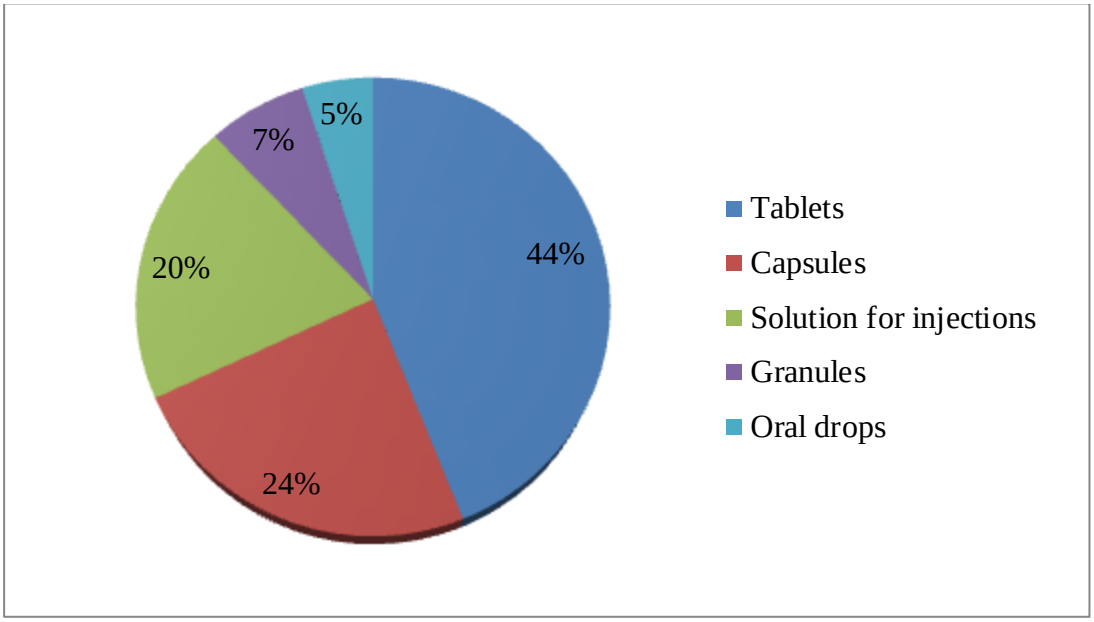


Fig. 3. Analysis of the nootropic drugs market presented in the European pharmaceutical market by the dosage form

Analysis of data in Fig. 3 shows that tablets are the most common dosage form (44%), which is explained by the ease of use, the precision of dosage, the masking of unpleasant organoleptic properties.

It is evident that granular nootropic drugs occupy a small part of the modern European pharmaceutical market, but it is necessary to take into account the advantages of the indicated dosage form, namely: a technological process such as granulation can achieve stability of active substances in the granular state, the convenience of dosing, the speed of onset of the pharmacological effect when using the drug in the form of granules, the simplicity of the technological process of producing the granular drug. In addition, granulation can combine active substances, avoiding their interaction and, as a consequence, prevent the occurrence of unwanted processes associated with their incompatibility[17].

[17] Современные направления в технологии твердых лекарственных средств: учеб. пособие для студентов высш. фармац. учеб. учрежд. / Е.А. Рубан, Л.Н. Хохлова, Л.А. Бобрицкая, С.В. Спиридонов; под ред. Е.А. Рубан. Х.: НФаУ, 2016. 88 с.

Neale C., Camfield D., Reay J., Stough C., Scholey A. Cognitive effects of two nutraceuticals Ginseng and Bacopa benchmarked against modafinil: a review and comparison of effect sizes. *Br J Clin Pharmacol*. 2013. № 75(3). P. 728–737.

Reay J.L., Scholey A.B., Kennedy D.O. Panax ginseng improves aspects of working memory performance and subjective ratings of calmness in healthy young adults. *Hum. Psychopharmacol.* 2010. № 25. P. 462–471.

With regard to the classification of nootropic drugs by origin, the largest share of the pharmaceutical market of Ukraine belongs to synthetic drugs (76%), while natural medicines have only 24% (Fig. 4).

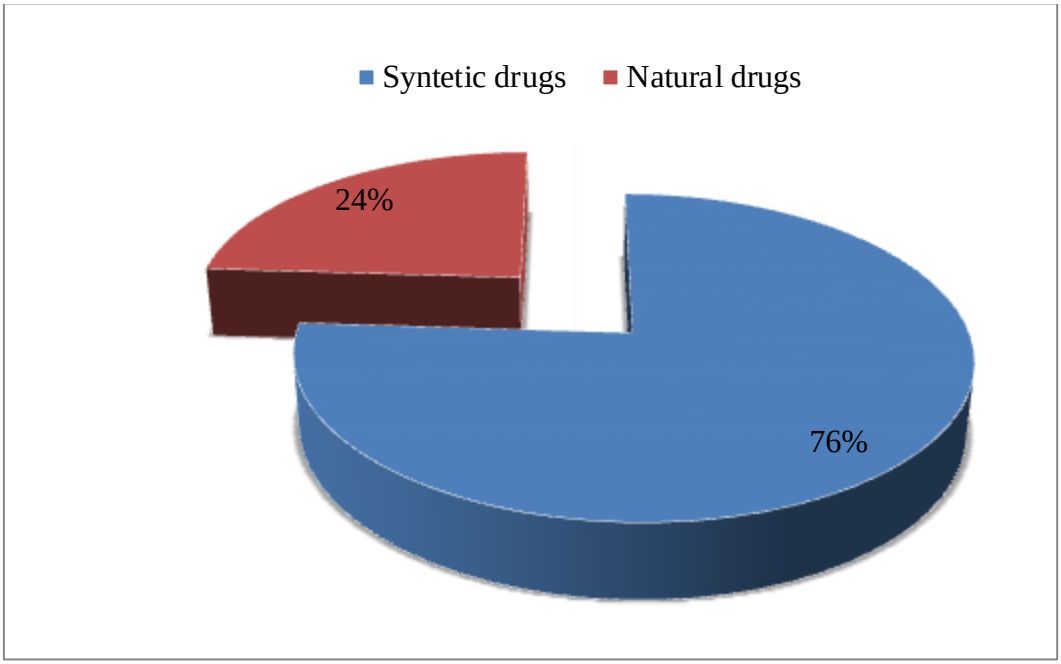


Fig. 4. Market analysis of nootropic drugs presented on the pharmaceutical market of Ukraine by origin

An important point in choosing the means of pharmacotherapy for central nervous system is the presence of a large number of side effects in nootropic preparations of synthetic origin (Table 2).

The obtained statistical data testify to the expediency of the creation of new drugs of domestic production of nootropic action, which have components of plant origin in their composition.

There is no doubt that there are a number of advantages of herbal drugs:

– reduction of toxic effects on the organism;

– the possibility of prolonged use without manifestations of adverse reactions;

– economic availability and a large raw material base;

– availability of a wide range of pharmacological effects, which allows the successful use of herbal medicinal products for the purpose of pharmacotherapy and pharmacoprophylaxis of many pathological conditions.

Table 2

The most common side effects of drugs with nootropic action

APhI	Side effects from different organs and systems						
	CNS	CVS	GIT	Immune system	Dermatology	Urogenital system	Other
Gamma-aminobutyric acid	+		+	+	+		+
Phenibut	+	+	+	+	+		
Dry extract of Ginkgo biloba leaves			+	+	+		+
Vinpocetine	+	+	+	+	+		+
Pyrithinol	+	+	+	+			
Cyticoline	+	+	+	+	+		+
Piracetam	+	+	+	+		+	+

Creation of drugs of nootropic action on the basis of herbal medicinal substance contributes to the increase in the frequency of their appointment and use for prophylactic purposes, which will reduce the prevalence of CVD in Ukraine and get closer to similar indicators in Poland.

5. Prospects for the use of medicinal plant raw material, namely ginseng (Panax ginseng) as part of a new drug of nootropic action of domestic production

It is known that CVD, to varying degrees, cause damage to neurons due to oxidative reactions and lead to the formation of amyloid plaques. Natural compounds with antioxidant properties can suppress development of the described degenerative processes. They are found in many plants. One of such plants is ginseng (Panax ginseng) of the family of Aralia (Araliaceae).

The bioactive components contained in the roots of ginseng usually account more than 60 varieties of ginsenosides. Each of them manifests an individual effect on the human body. Hydrogen and alcohol extracts,

ginsenosides Rb1, Rb2, Re and Rd, protopanaxatriols Re and Rg have neuroprotective and antioxidant properties, ginsenosides Rg1 and Rb1 show nootropic effect. The listed biologically active compounds have the ability to stimulate the main activating neurotransmitter systems of the brain – adreno-, dopamine- and glutamatergical systems by influencing biosynthesis and receptor binding of these neurotransmitters[18].

The ability of ginsenosides to increase the receptor binding of acetylcholine, the main neurotransmitter of the cognitive field in different regions of the central nervous system, which may be associated with improved memory, attention, and the ability to learn when taking ginseng preparations, is highly important. Alkaloids and flavonoids that are part of ginseng also have antihypoxic properties, the ability to increase the biosynthesis of ATP and to inhibit the development of free radical reactions leading to neurons damage[19].

Clinical studies have confirmed the positive effect of ginseng on cognitive performance in patients with Alzheimer's disease[20].

Consequently, ginseng exhibits toning and stimulating properties in mental and physical exhaustion, weakness, contributes to increased immune defense and overall improvement of the human body, sharp increase in efficiency, and reduced fatigue. Also dose-dependent ability of this plant to normalize blood pressure and cause excitation/inhibition of the cerebral cortex was confirmed[21]. That is why the development of a nootropic drug based on ginseng is a topical issue of modern medical and pharmaceutical science.

CONCLUSIONS

As a result of this work, the statistics on the distribution and frequency of cerebrovascular diseases, in particular stroke, among the population of

[18] Куркин В.А. Основы фитотерапии: учеб. пособие для студентов фармацевтических вузов. Самара: ООО «Офорт»; ГБОУ ВПО «СамГМУ Росздрава», 2009. 963 с.

Хімія рослинної сировини. 2013. № 2. С. 177–182.

[19] Heo J.H., Lee S.T., Chu K. Heat-processed ginseng enhances the cognitive function in patients with moderately severe Alzheimer's disease. *Nutr Neurosci*. 2012. № 15(6). P. 134.

Im W., Chung J.Y., Bhan J. Sun ginseng protects endothelial progenitor cells from senescence associated apoptosis. *J Ginseng Res*. 2012. № 36(1). P. 78–85.

[20] Lee S.T., Chu K., Sim J.Y. Panax ginseng enhances cognitive performance in Alzheimer disease. *Alzheimer Dis Assoc Disord*. 2008. № 22(3). P. 222–226.

[21] Куркин В.А. Основы фитотерапии: учеб. пособие для студентов фармацевтических вузов. Самара: ООО «Офорт»; ГБОУ ВПО «СамГМУ Росздрава», 2009. 963 с.

Ukraine and Poland were processed. The marketing researches of the modern pharmaceutical market of nootropic drugs in the mentioned countries used in the treatment of cerebrovascular diseases are conducted. It has been established that most of the medicines used in the treatment and prophylaxis of central nervous system in Poland and other Western European countries are related to herbal medicinal products.

It is proved that at the present stage of development of the pharmaceutical industry it is important to create new medicinal drugs of plant origin, which will allow them to be used for the treatment and prevention of pathological conditions associated with the disruption of the cerebral structures functioning. The prospects of using ginseng as APhI in the creation of a new nootropic agent due to a wide range of pharmacological actions are substantiated. The expediency of creation of a new nootropic preparation in the form of granules in the conditions of pharmacies is substantiated.

SUMMARY

Given the high prevalence of cerebrovascular diseases in Ukraine and in Western Europe, as well as the large number of severe and irreversible consequences of these pathologies, the development of new drugs of nootropic action that contribute to improved cerebrovascular circulation is relevant. Creation of drugs with nootropic action using components of plant origin allows the usage of these agents for pharmacoprophylaxis of CVD, as the emergence of a number of possible adverse effects in their application is minimized.

In view of this, it is important to use the ginseng extract as an active pharmaceutical ingredient (APhI) as part of a new drug with declared activity.

REFERENCES

1. Бурчинский С.Г. Нейропротекция при цереброваскулярной патологии: системные аспекты. *Український неврологічний журнал.* 2015. № 1. С. 103–108.

2. Волошин П.В., Міщенко Т.С., Здесенко І.В. Епідеміологія мозкового інсульту в Україні. *Діагностика, лікування, профілактика гострих та хронічних порушень мозкового кровообігу: матеріали наук.-практ. конф*. (Харків, 5–7 жовтня 2005 р.). Х., 2005. С. 74–75.

3. Гандзюк В.А. Динаміка захворюваності та поширеності хвороб системи кровообігу серед населення України на сучасному етапі:

національний та регіональний аспекти *Вісник соціальної гігієни та організації охорони здоров'я України*. 2014. № 2(60). С. 74–78.

4. Державний реєстр лікарських засобів (2017). URL: http://www.drlz.com.ua (дата звернення: 15.04.2016).

5. Зінченко О.М., Голубчиков М.В., Міщенко Т.С. Стан неврологічної служби України в 2014 році: статистично-аналітичний довідник. Х., 2015. 24 с.

6. Зозуля А.И., Слабкий Г.А. Усовершенствование системы оказания медицинской помощи населению при ЦВЗ – составляющей общей реформы здравоохранения в Украине. *Збірник наукових праць співробітників Національної медичної академії післядипломної освіти імені П.Л. Шупика.* 2013. № 3. С. 14–19.

7. Інформаційно-аналітична довідка про економічний стан в Україні (2016). URL: http://ukrexport.gov.ua/ukr/prom/ukr/26.html (дата звернення: 25.10.2017).

8. Кваша О.О. Популяційна оцінка внеску факторів ризику серцево-судинних захворювань в смертність за даними 20-річного проспективного дослідження: автореф. дис. … докт. мед. наук: 14.01.11. К., 2008. 40 с.

9. Кочуева М.Н., Кочуев Г.И., Корж А.Н. Современные возможности метаболической терапии – в фокусе Актовегин. *Східноєвропейський журнал внутрішньої та сімейної медицини.* 2015. № 1. С. 101–108.

10. Куркин В.А. Основы фитотерапии: учеб. пособие для студентов фармацевтических вузов. Самара: ООО «Офорт»; ГБОУ ВПО «СамГМУ Росздрава», 2009. 963 с.

11. Піщиков В.А., Ященко Ю.Б., Кондратюк Н.Ю. Основні підходи до профілактики хвороб системи кровообігу. *Український медичний часопис.* 2014. № 6(104). С. 45–48.

12. Профілактика цереброваскулярних захворювань у пацієнтів з артеріальною гіпертензією. *Міжнародний неврологічний журнал.* 2013. № 8(62). С. 112–116.

13. Перелік ноотропних препаратів, дозволених до застосування в Україні. *Ліки України*. 2011. № 4(150). С. 132–135. URL: http://nbuv.gov.ua/j-pdf/likukr_2011_4_26.pdf (дата звернення: 20.09.2018).

14. Современные направления в технологии твердых лекарственных средств: учеб. пособие для студентов высш. фармац. учеб. учрежд. /

Е.А. Рубан, Л.Н. Хохлова, Л.А. Бобрицкая, С.В. Спиридонов; под ред. Е.А. Рубан. Х.: НФаУ, 2016. 88 с.

15. Хімія рослинної сировини. 2013. № 2. С. 177–182.

16. Heo J.H., Lee S.T., Chu K. Heat-processed ginseng enhances the cognitive function in patients with moderately severe Alzheimer's disease. *Nutr Neurosci*. 2012. № 15(6). P. 134.

17. Im W., Chung J.Y., Bhan J. Sun ginseng protects endothelial progenitor cells from senescence associated apoptosis. *J Ginseng Res*. 2012. № 36(1). P. 78–85.

18. Lee S.T., Chu K., Sim J.Y. Panax ginseng enhances cognitive performance in Alzheimer disease. *Alzheimer Dis Assoc Disord*. 2008. № 22(3). P. 222–226.

19. Mahomoodaly M.F. Complementary and Alternative medicines used against neurodegenerative diseases in Pharmacy. *Daly*. 2013. № 1. P. 103–123.

20. Neale C., Camfield D., Reay J., Stough C., Scholey A. Cognitive effects of two nutraceuticals Ginseng and Bacopa benchmarked against modafinil: a review and comparison of effect sizes. *Br J Clin Pharmacol*. 2013. № 75(3). P. 728–737.

21. Reay J.L., Scholey A.B., Kennedy D.O. Panax ginseng improves aspects of working memory performance and subjective ratings of calmness in healthy young adults. *Hum. Psychopharmacol*. 2010. № 25. P. 462–471.

22. Zozulya I.S., Zozulya A.I. Epidemiology of cerebrovascular diseases in Ukraine. *Ukrainian medical chronicle*. 2011. № 5. P. 38–41.

Information about authors:

Vyshnevska L. I.,
Doctor of Pharmaceutical Sciences,
Head of the Pharmaceutical Technology of Drugs Department
of the National University of Pharmacy
4, Valentynska st., Kharkiv, 61002, Ukraine

Romas K. P.,
Candidate of Pharmaceutical Sciences,
Senior Lecturer of the Pharmaceutical Technology of Drugs Department
of the National University of Pharmacy
4, Valentynska st., Kharkiv, 61002, Ukraine

STRUCTURAL RECONSTRUCTION OF SPLEEN AFTER EXPERIMENTAL OBESITY

Harapko T. V.

INTRODUCTION

This study is a part of complex projects: "Features of the lymphoid organs and vascular bed structural organization in the normal ontogenesis and their alteration regularities under the effect of antigens, chemical and physical factors on the organism" – state registration number 0115U003903 and "Structure of organs and their bloodstream in the ontogenesis, under the influence of laser irradiation and pharmaceuticals, with blood supply disorders, reconstructive operations and diabetes mellitus"– state registration number 0110U001854.

Obesity is a chronic disease that develops as a result of metabolic disorders and eating behavior, has a recurrent nature and is characterized by accumulation of fatty tissues in the body[1].

Obesity is a pro-inflammatory condition in which hypertrophied adipocytes and resistant immune cells (primarily lymphocytes and macrophages) contribute to an increase in the level of circulating proinflammatory cytokines[2]. An obesity-related condition of chronic systemic inflammation, called "metabolic inflammation", is considered to be the starting point in the pathogenesis of resistance to insulin and type 2 diabetes in experiments conducted on animals[3].

---

[1] Власенко М.В., Семенюк І.В., Слободянюк Г.Г. Цукровий діабет і ожиріння – епідемія XXI століття: сучасний підхід до проблеми. *Український терапевтичний журнал*. 2011. № 2. С. 50.

Про затвердження протоколів надання медичної допомоги дітям за спеціальністю «Дитяча ендокринологія»: Наказ Міністерства охорони здоров'я України від 27 квітня 2006 р. № 254 / Міністерство охорони здоров'я України. URL: http://ukraine.uapravo.net/data/akt266/page8.htm.

[2] Gregor M.F., Hotamisligil G.S. Inflammatory mechanisms in obesity. *Annual Review of Immunology*. 2011. Vol. 29. P. 416.

[3] Makki K., Froguel P., Wolowczuk I. Adipose Tissue in Obesity-Related Inflammation and Insulin Resistance: Cells, Cytokines, and Chemokines. *International Scholarly Research Notices*. 2013. Vol. 2013. P. 457. URL: http://dx.doi.org/10.1155/2013/139239.

Hotamisligil G.S., Erbay E. Nutrient sensing and inflammation in metabolic diseases. *Nature Reviews Immunology*. 2008. Vol. 8. № 12. P. 925.

Excessive body weight and obesity during pregnancy are associated with frequent perinatal and maternal risk[4].

Authors demonstrated independent effects of maternal fat feeding during pregnancy and suckling because blood pressure, endothelial function, and plasma insulin were abnormal not only in offspring whose dams were exposed to the maternal fat-rich diet during pregnancy but also when the fat-rich diet was confined to the suckling period[5]. Obesity in women of reproductive age leads to numerous general-somatic and reproductive problems, which, in turn, lead to a decrease in fertility. This may be due to the fact that fatty tissue is the site of peripheral synthesis of many hormones. She actively participates in the work of the vascular system and the formation of the immune response. Its excessive content is accompanied by metabolic, hormonal, vascular and proinflammatory disorders. Scientific literature has data on the risk of complications and the deterioration of certain diseases in obesity (hypertension, hypothyroidism, chronic pancreatitis, diabetes, etc.)[6].

Structural changes of the pancreas typical for pancreatitis develop in patients with chronic pancreatitis on the background of obesity: an increase in the whole organ or its part, uneven contours, changes in echogenesis, calcification of the pancreas, expansion of the main pancreatic duct, pseudocyst[7].

The authors describe that the feeding with a high-fat diet (30%) causes obesity, hepatomegaly, and histopathological changes in the liver in female

---

[4] Коритко О.О. Вплив надмірної маси тіла та ожиріння на фертильність і виношування вагітності. *Международный эндокринологический журнал*. 2016. № 7(79). С. 23.

[5] Khan I.Y., Dekou V., Douglas G. A high-fat diet during rat pregnancy or suckling induces cardiovascular dysfunction in adult offspring. *Am. J. Physiol.* 2005. Vol. 288. P. 130.

[6] Якубцова І.В., Хілько Т.Д., Савицька І.М., Конопельнюк В.В., Преображенська Т.Д., Макай Ш. Вплив Trigonella foenum graecum L. на стан імунокомпетентних органів за умов дієтіндукованого ожиріння у щурів. *Scientific Journal "ScienceRise: Biological Science"*. 2016. № 3(3). P. 54.

Sankhla M., Sharma T.K., Mathur K., Rathor J.S., Butolia V., Gadhok A.K. et al. Relationship of oxidative stress with obesity and its role in obesity induced metabolic syndrome. *Clinical Laboratory*. 2012. Vol. 58(5–6). P. 385.

Wang H.J., Si Q.J., Shan Z.L., Guo Y.T., Lin K., Zhao X.N. et al. Effects of body mass index on risks for ischemic stroke, thromboembolism, and mortality in Chinese atrial fibrillation patients: a single-center experience. *PLoS One*. 2015. Vol. 10(4). P. 233.

[7] Губергриц Н.Б., Бондаренко О.А. Показатели ультразвуковой гистографии поджелудочной железы в динамике лечения больных с хроническим панкреатитом на фоне ожирения. *Гастроентерологія*. 2013. Вип. 2(48). С. 232.

rats. In spite of hepatomegaly, a decrease in the number of hepatocytes in the high-fat diet group was found. In this situation, hepatomegaly in obese rats may be caused by sinusoidal dilatation (increase in volume of sinusoids), microvesicular steatosis, or fibrosis resulting from an increase in connective tissue following hepatocellular necrosis. In this point decreasing nuclear volume sign that cell death, as light microscopically shown[8].

Authors was found that the increase in the body mass index was negative affects the course of hypertension, which is an increase moderate levels of blood pressure, as well as adverse changes in parameters left ventricular myocardial geometry[9].

The question of the effect of obesity on the lymphoid (immune) organs, in particular the spleen, remains insufficiently studied. The spleen is a secondary lymphoid organ in which antigen-dependent proliferation and differentiation of T- and B-lymphocytes are carried out, as well as the elimination of red blood cells and platelets[10]. B cells are emerging players in innate and adaptive immuneresponses associated with metabolic diseases, includingobesity, type II diabetes and cardiovascular disease[11].

The purpose of the work was to study histological changes of rat spleen structural components changes through different terms of experimental obesity.

Materials and methods. We carried out the study on 66 white rats of reproductive age (2,5–3,5 months) weighing 150–180 g.

Microanatomy of the spleen structural components in white rats under conditions of physiological norm was studied on 10 intact animals. Experimental animals were divided into 4 groups: the first group (10 animals), being fed a high-calorie diet for one week; the second group (10 animals), fed high-calorie diet for two weeks; the third group (10 animals), fed high-calorie diet for three weeks; the fourth group (10 animals), fed the same diet for four weeks.

---

[8] Altunkaynak B.Z., Ozbek E. Overweight and structural alterations of the liver in female rats fed a high-fat diet: a stereological and histological study. *Turk J Gastroenterol.* 2009. Vol. 20(2). P. 101.

[9] Кожем'яка Г.В., Ковальова О.М., Ащеулова Т.В., Гончарь О.В. Вплив ожиріння на формування змін геометрії міокарда у хворих на гіпертонічну хворобу. *Проблеми безперервної медичної освіти та науки*. 2016. № 4. С. 33.

[10] Elmore S.A. Enhanced histopathology of the immune system: a review and update. *Toxicologic Pathology*. 2012. Vol. 40(2). P. 150.

[11] Shaikh S.R., Haas K.M., Beck M.A., Teague H. The effects of diet-induced obesity on B cell function. *Clinical & Experimental Immunology*. 2015. Vol. 179(1). P. 90.

Each group included 5 male and 5 female rats. High-calorie diet was achieved due to the fact that glutamate sodium was added into food in a dose of 0,07 g/kg of rat body weight, and 20% fructose solution was added into water.

Control was provided by 16 white rats, fed a standard diet of vivarium instead of a high-calorie diet.

All experimental animals were kept under the vivarium of Danylo Halytsky Lviv National Medical University. The study was performed in accordance with the provisions of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986), Council of Europe Directives 86/609/EEC (1986), Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty", the general ethical principles of experiments on animals adopted by the First National Congress of Ukraine on Bioethics (2001).

Images from the histological preparations of the spleen in the computer monitor were displayed from the MICROmed SEO SCAN microscope by means of the Vision CCD Camera. The studies were carried out within the established schedule of the trial in samples stained with hematoxylin, eosin and azane.

To control the state of the liver, blood vessels, capillaries and heart, a biochemical analysis of the white male and female rats' blood was performed to determine the glucose, total protein, ALT and AST content throughout the experiment.

1. Results of the study and their discussion

As the results of the study showed, in animals of the intact and control groups, according to our histological studies, the structure of the spleen was consistent with the species norm. The morphological structure of the spleen in the control group of male and female rats was normal for the animal at that age, and at the end of the study, deviations from the norm were not observed. Outside, the spleen is surrounded by a connective tissue capsule, from which inside the body germinate the septum – trabecula.

The area of the spleen is occupied with white and red pulp. White pulp is formed by lymphocytes, plasmacytes, macrophages, dendritic and interdigital cells. The stroma for them is a reticular tissue. The accumulation of these cells is called lymph nodules or follicles (Fig. 1). The red pulp is

represented by an aggregate of formed blood elements that are either surrounded by reticular cells or in the system of vascular sinuses.

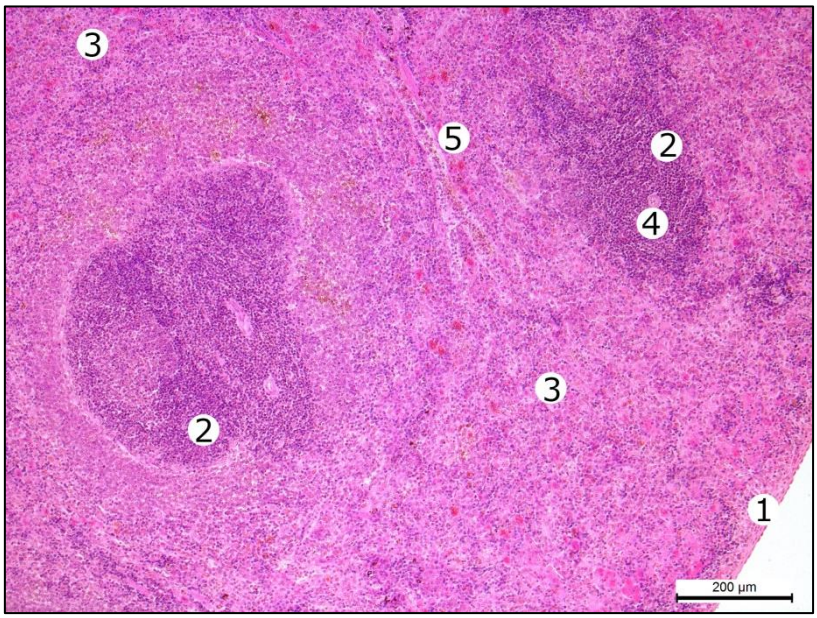


Fig. 1. A fragment of a spleen of an intact white rat male. Azane stained. Magnif: obj. × 10, ocul. × 10. Designation: 1 – capsule; 2 – white pulp; 3 – red pulp; 4 – central artery; 5 – transversal section of the artery

After one week of the experiment, the overall structure of the spleen corresponds to the intact group of animals. The amount of erythrocytes and thrombocytes in the lumen of the venous sinus of the red spleen is increasing somewhat both in male rats and in female rats (Fig. 2). The folicles are clearly separated from each other and from the red pulp.

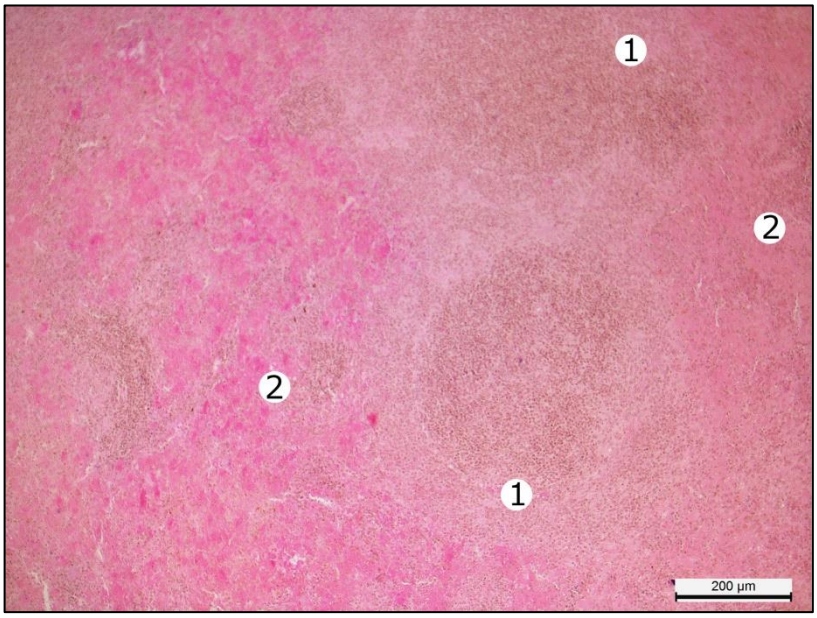


Fig. 2. A fragment of a spleen of a white female rat after one week of the experiment. Stained with hematoxylin and eosin. Magnif.: obj. × 10, ocul. × 10. Designations: 1 – lymphoid follicle; 2 – expanded and full-blooded venous lymph sinus in red pulp

The level of glucose in the blood of white male rats grows to its maximum after two weeks of the experiment by 72,3%, then gradually reduces and after four weeks it exceeds the intact animals' indices by 16,7%. The level of glucose in the blood of white female rats grows to its maximum after one week of the experiment by 67,7%, then gradually reduces and four weeks after it is lower by 13,7% than in the intact animals (table 1).

Table 1

Indices of blood glucose levels in white rats, mmol/l (M±m)

Group name	White male rat	White female rat
Intact animals	5,9±0,09	6,0±0,08
Group I	6,14±0,1	10,06±0,11
Group II	10,58±0,12	9,56±0,1
Group III	7,9±0,08	5,76±0,05
Group IV	7,08±0,12	5,18±0,09

The level of total protein in the blood of white male rats grows to its maximum after one week of the experiment by 11,9%, then gradually reduces and after four weeks it exceeds the intact animals' indices by only 3,5%. The level of total protein in the blood of white female rats during the experiment it fluctuates, and four weeks after it is lower by 11,3% than in the intact animals (table 2).

Table 2

Indices of blood total protein levels in white rats, gram/l (M±m)

Group name	White male rat	White female rat
Intact animals	57,3±0,09	67,5±0,12
Group I	64,1±0,11	67,1±0,1
Group II	59,6±0,1	61,5±0,09
Group III	59,3±0,17	67,9±0,08
Group IV	60,2±0,12	59,9±0,08

The ALT level in the blood of white male and female rats grows to its maximum after two weeks of experiment, which exceeds the rate of intact animals by 57,3% and 40,4% respectively. During the experiment, it gradually reduces and four weeks after it is by 18,2% and 11,6% less than that of intact animals (table 2).

The AST level in the blood of white male rats grows to its maximum after one week of the experiment by 44,2% and 40,4% respectively, exceeding that of intact animals. During the experiment it fluctuates, and four weeks after it exceeds by 8,0% the intact animals' index. The AST level in the blood of white female rats does not change significantly during the experiment (table 3).

Table 3

Indices of ALT and AST levels in the blood of white rats, U/L (M±m)

Group name	White male rat	White female rat	White male rat	White female rat
	AJT		ACT	
Intact animals	76,5±0,2	67,5±0,18	175,7±0,35	168±0,49
Group I	93,4±0,3	58,9±0,21	253,4±0,39	148,7±0,7
Group II	120,3±0,31	94,8±0,29	172,4±0,33	167,8±0,67
Group III	96,7±0,4	61,3±0,22	240,8±0,77	172±0,56
Group IV	62,8±0,08	59,7±0,09	189,7±0,45	168,2±0,44

The level of cholesterol in the blood of white male rats and white feline rats increases as much as two weeks of the experiment, which is 2,5 and 2,9 times correspondingly higher than the rate of intact animals. During the experiment, it decreases somewhat and in four weeks it is 56,7% and 82,8% higher than the rate of intact animals (table 3).

The level of triglycerides in the blood of white male rats at the beginning of the experiment decreases, and from the second week begins to increase and in four weeks it is 11,6% less than the rate of intact animals. The level of triglycerides in the blood of white female rats increases as much as 2,9 times in two weeks of the experiment, then gradually decreases and returns to the level of intact animals four weeks later (table 4).

Table 4

Indices of cholesterol (mmol/l) and triglycerides (mmol/l) in blood of white rats (M±m)

Group name	White male rat	White female rat	White male rat	White female rat
	cholesterol		triglycerides	
Intact animals	0,9±0,01	0,87±0,02	0,69±0,009	0,67±0,01
Group I	2,22±0,011	2,54±0,011	0,28±0,006	0,96±0,01
Group II	1,77±0,013	1,16±0,01	0,29±0,09	1,92±0,011
Group III	1,95±0,01	1,63±0,013	0,53±0,008	0,62±0,009
Group IV	1,41±0,01	1,59±0,011	0,61±0,007	0,68±0,01

After two weeks of experiment, both in male and female rats, the number of lymphoid nodules in a white pulp with enlightened germinal centers increases, trabecula slightly thickens, and the blood-filled venous sinuses of

the red pulp increases (Fig. 3). There are arteries with a thickened wall, full-blooded, veins slightly deformed, expanded and full-blooded.

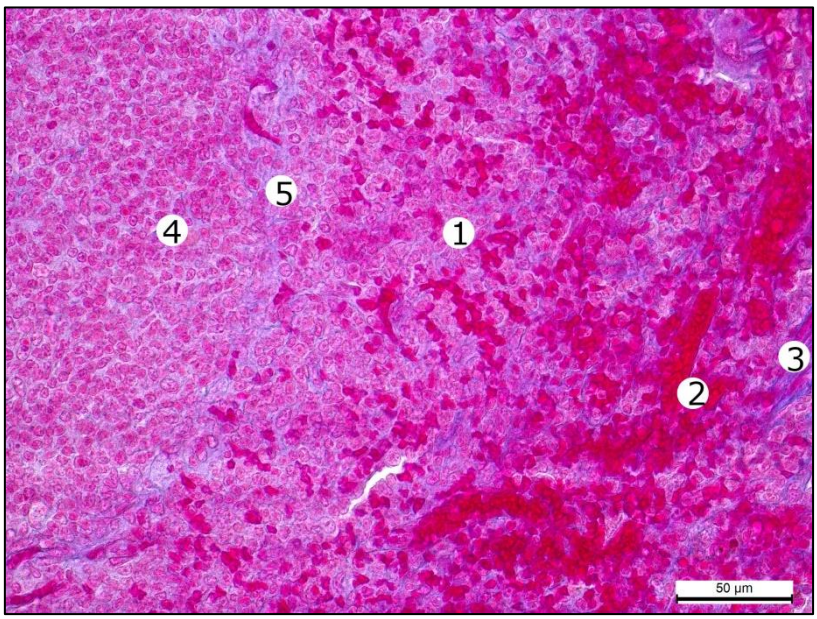


Fig. 3. A fragment of a spleen of a white male rat after two weeks of the experiment. Azane stained. Magnif.: obj. × 40, ocul. × 10. Designations: 1 – full-blooded venous lymph sinus in red pulp; 2 – compilation of erythrocytes; 3 – the cords of Billroth; 4 – lymphoid nodule; 5 – marginal zone

After three weeks of experiment, both male and female rats have signs of antigenic stimulation, which is confirmed by the opinion that is common among morphologists that obesity is a pro-inflammatory condition. It manifests itself in the hyperplasia of the lymphoid component of the spleen, the intensity of the formation of the germinal centers, the amplification of the processes of proliferation and differentiation of lymphocytic cells (Fig. 4). In studying spleen slices, many macrophages and necrotic figures were defined. Also, sinusoidal dilatation and hemosiderin deposits were observed and we found macrophages, filled with hemosiderin droplets. In some sections, especially around small vessels, eosinophilic aggregations and lipid accumulations in dilated sinusoids were detected (fig. 5).

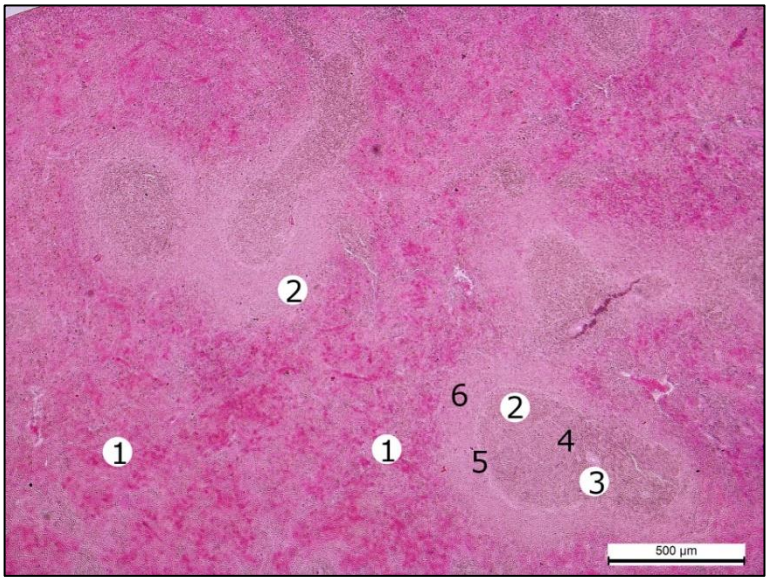


Fig. 4. A fragment of a spleen of a white female rat after three weeks of the experiment. Stained with hematoxylin and eosin. Magnif.: obj. × 5, ocul. × 10. Designations: 1 – full-blooded venous lymph sinus in red pulp; 2 – lymphoid nodules; 3 – central artery; 4 – germinal center of the lymphoid nodules; 5 – mantle zone; 6 – marginal zone

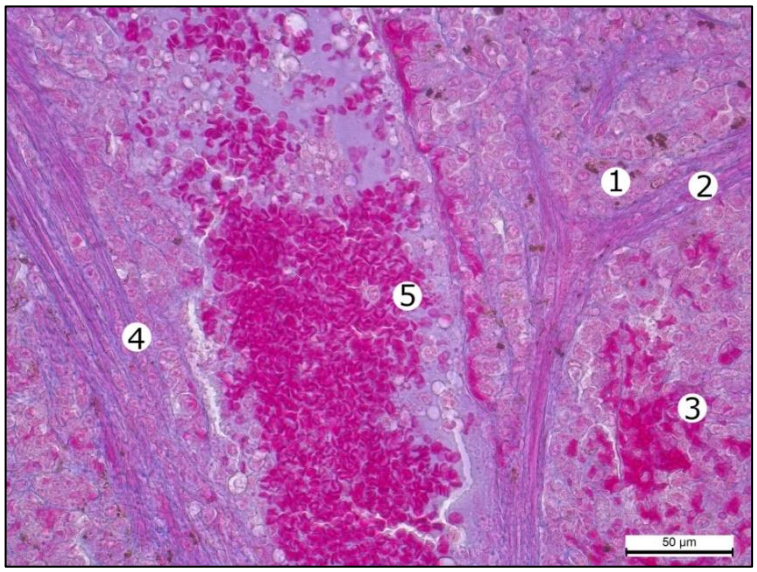


Fig. 5. A fragment of a spleen of a white male rat after three weeks of the experiment. Azane stained. Magnif.: obj. × 40, ocul. × 10. Designations: 1 – aggregations and lipid accumulations; 2 – trabecula; 3 – red pulp; 4 – thickened artery wall; 5 – compilation of erythrocytes and thrombocytes in the lumen of the artery

Four weeks of the experiment, both in male and female rats, there was an immuno-inducing effect with enhanced proliferation of activated lymphocytes and their further differentiation into plasma cells. This is precisely the morphological prerequisite for increasing the synthesis of immunoglobulins. Arteries with a thickened wall, full-blooded. Perivascularly degenerated erythrocytes (Fig. 6). Veins are deformed, enlarged, full-blooded. Many macrophages and necrotic figures were defined, sinusoidal dilatation and hemosiderin deposits were observed (Fig. 7).

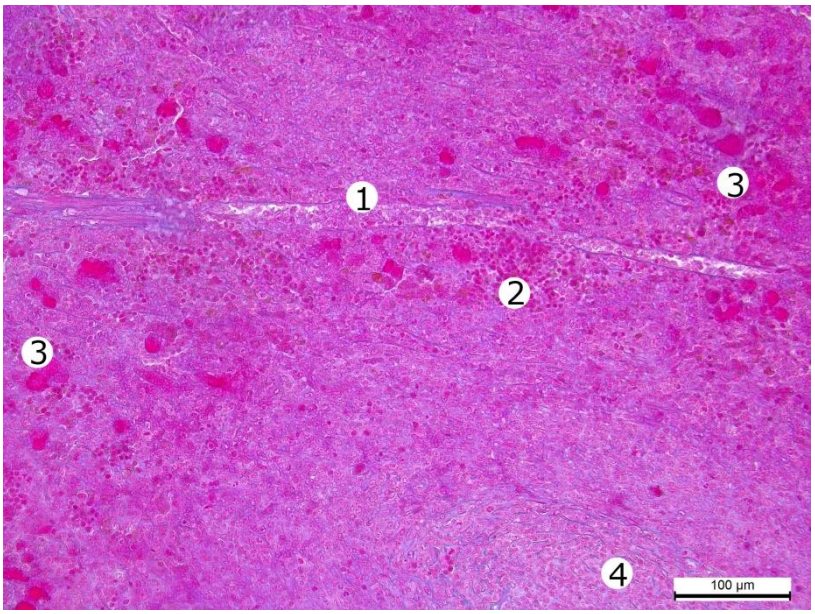


Fig. 6. A fragment of a spleen of a white female rat after four weeks of the experiment. Azane stained. Magnif.: obj. × 20, ocul. × 10. Designations: 1 – longitudinal section of artery; 2 – perivascular, degenerated erythrocytes; 3 – full-blooded venous lymph sinus in red pulp; 4 – white pulp

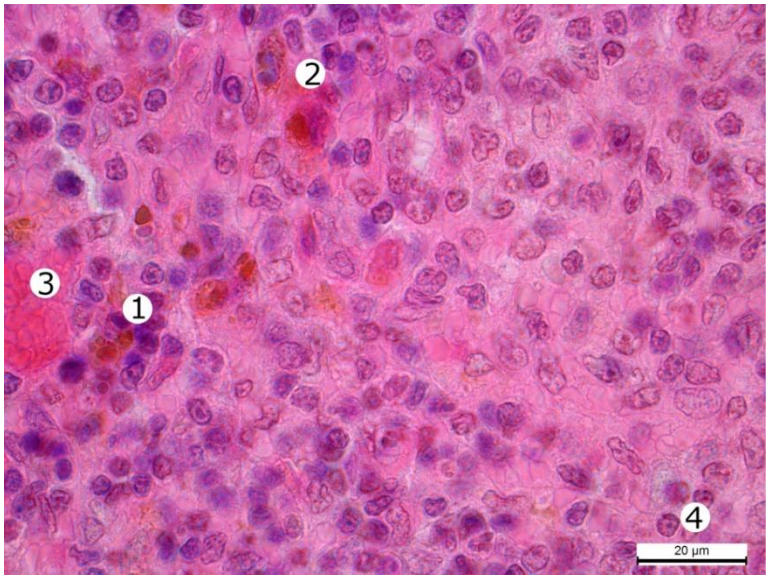


Fig. 7. A fragment of a spleen of a white female rat after four weeks of the experiment. Stained with hematoxylin and eosin. Magnif.: obj. × 100, ocul. × 10. Designations: 1 – macrophages, filled with hemosiderin droplets; 2 – necrotic lymphoid cells; 3 – erythrocytes and thrombocytes in red pulp; 4 – lymphocytes

CONCLUSIONS

As a result of a study conducted in male rats and female rats, we found that the number of lymphoid nodules in the white pulp of the spleen during the experiment increases. Arteries with a thickened wall, full-blooded, veins deformed, expanded and full-blooded. Trabeculae leaving the capsule expressed, thickened. After three and four weeks of experiment, many macrophages and necrotic figures were detected. Also, there were enlargements and sinuses of red pulp, hemosiderin in the lumen of venous sinuses, as well as macrophages filled with drops of hemosiderin. Around some vessels, eosinophilic aggregations and lipid accumulation in the extended sinusoid were detected. There are signs of constant immune activity.

Prospects for further research in this direction are related to the further study of morphometric and electron-microscopic changes in the structural components of the spleen of rats through different terms of experimental obesity.

SUMMARY

Obesity is a pro-inflammatory condition in which hypertrophied adipocytes and resistant immune cells (primarily lymphocytes and macrophages) contribute to an increase in the level of circulating proinflammatory cytokines. An obesity-related condition of chronic systemic inflammation, called "metabolic inflammation", is considered to be the starting point in the pathogenesis of resistance to insulin and type 2 diabetes in experiments conducted on animals.

After three and four weeks of experiment, many macrophages and necrotic figures were detected. Also, there were enlargements and sinuses of red pulp, hemosiderin in the lumen of venous sinuses, as well as macrophages filled with drops of hemosiderin. Around some vessels, eosinophilic aggregations and lipid accumulation in the extended sinusoid were detected. There are signs of constant immune activity.

As a result of a study conducted in male rats and female rats, we found that the number of lymphoid nodules in the white pulp of the spleen during the experiment increases. Arteries with a thickened wall, full-blooded, veins deformed, expanded and full-blooded. Trabeculae leaving the capsule expressed, thickened. With an increase in the duration of the experiment, the pathological changes are deepened

REFERENCES

1. Власенко М.В., Семенюк І.В., Слободянюк Г.Г. Цукровий діабет і ожиріння – епідемія XXI століття: сучасний підхід до проблеми. *Український терапевтичний журнал*. 2011. № 2. С. 50–55.

2. Про затвердження протоколів надання медичної допомоги дітям за спеціальністю «Дитяча ендокринологія»: Наказ Міністерства охорони здоров'я України від 27 квітня 2006 р. № 254 / Міністерство охорони здоров'я України. URL: http://ukraine.uapravo.net/data/akt266/page8.htm.

3. Gregor M.F., Hotamisligil G.S. Inflammatory mechanisms in obesity. *Annual Review of Immunology*. 2011. Vol. 29. P. 415–445.

4. Makki K., Froguel P., Wolowczuk I. Adipose Tissue in Obesity-Related Inflammation and Insulin Resistance: Cells, Cytokines, and Chemokines. *International Scholarly Research Notices*. 2013. Vol. 2013. P. 456–468. URL: http://dx.doi.org/10.1155/2013/139239.

5. Hotamisligil G.S., Erbay E. Nutrient sensing and inflammation in metabolic diseases. *Nature Reviews Immunology*. 2008. Vol. 8. № 12. P. 923–934.

6. Коритко О.О. Вплив надмірної маси тіла та ожиріння на фертильність і виношування вагітності. *Международный эндокринологический журнал.* 2016. № 7(79). С. 22–26.

7. Khan I.Y., Dekou V., Douglas G. A high-fat diet during rat pregnancy or suckling induces cardiovascular dysfunction in adult offspring. *Am. J. Physiol*. 2005. Vol. 288. P. 127–133.

8. Якубцова І.В., Хілько Т.Д., Савицька І.М., Конопельнюк В.В., Преображенська Т.Д., Макай Ш. Вплив Trigonella foenum graecum L. на стан імунокомпетентних органів за умов дієтіндукованого ожиріння у щурів. *Scientific Journal "ScienceRise: Biological Science"*. 2016. № 3(3). P. 53–60.

9. Sankhla M., Sharma T.K., Mathur K., Rathor J.S., Butolia V., Gadhok A.K. et al. Relationship of oxidative stress with obesity and its role in obesity induced metabolic syndrome. *Clinical Laboratory*. 2012. Vol. 58(5–6). P. 385–392.

10. Wang H.J., Si Q.J., Shan Z.L., Guo Y.T., Lin K., Zhao X.N. et al. Effects of body mass index on risks for ischemic stroke, thromboembolism, and mortality in Chinese atrial fibrillation patients: a single-center experience. *PLoS One*. 2015. Vol. 10(4). P. 231–242.

11. Губергриц Н.Б., Бондаренко О.А. Показатели ультразвуковой гистографии поджелудочной железы в динамике лечения больных с хроническим панкреатитом на фоне ожирения. *Гастроентерологія*. 2013. Вип. 2(48). С. 228–233.

12. Altunkaynak B.Z., Ozbek E. Overweight and structural alterations of the liver in female rats fed a high-fat diet: a stereological and histological study. *Turk J Gastroenterol*. 2009. Vol. 20(2). P. 93–103.

13. Кожем'яка Г.В., Ковальова О.М., Ащеулова Т.В., Гончарь О.В. Вплив ожиріння на формування змін геометрії міокарда у хворих на гіпертонічну хворобу. *Проблеми безперервної медичної освіти та науки*. 2016. № 4. С. 29–34.

14. Elmore S.A. Enhanced histopathology of the immune system: a review and update. *Toxicologic Pathology*. 2012. Vol. 40(2). P. 148–156.

15. Shaikh S.R., Haas K.M., Beck M.A., Teague H. The effects of diet-induced obesity on B cell function. *Clinical & Experimental Immunology*. 2015. Vol. 179(1). P. 90–95.

Information about author:
Harapko T. V.,
Candidate of Medical Sciences,
Docent at the Department of Human Anatomy
and Histology Medical Faculty
Uzhhorod National University
22, Kapushanska Str., Uzhhorod, 88000, Ukraine

THE TREATMENT AND REHABILITATION FUNCTIONS OF SANATORIUM „AVANGARD" (NEMYRIV CITY, VINNITSKA REGION, UKRAINE)

Hladkyi O. V., Kilivnik V. S.

INTRODUCTION

Rehabilitation is a branch of medicine that aims to enhance and restore functional ability to those with physical impairments or disabilities. It consist in physical therapy, that use mechanical force and movement, psychiatric rehabilitation, deals with restoration of mental health, vision rehabilitation, that improve vision, vocational rehabilitation, that include process which enables persons with impairments or disabilities to maintain or return to employment or occupation. Also rehabilitation involves into neuropsychological therapy that regain or improve neurocognitive function that has been lost or diminished. At last, rehabilitation includes medical or psychotherapeutic treatment for dependency on substances such as alcohol and drugs. Most of these types of rehabilitation are presented in sanatorium "Avangard" located in Nemyriv, Vinnitska region of Ukraine.

Therefore, treatment and rehabilitation functions of sanatorium "Avangard" play an important role in medical service of Vinnitska region. There are different methods of treatment and rehabilitation in clinical sanatorium "Avangard" used for burn patients, for women with pregnancy pathologies, for patients with chronic non-specific lung diseases as well as for patients with endocrinology pathologies. There are a lot of scientific approaches for treatment of circulatory system diseases, respiratory system diseases, loco-motor system diseases, endocrine system diseases as well as of organs of vision diseases used in sanatorium. Also, sanatorium has a lot of modern treatment facilities that available for treatment and rehabilitation procedures.

1. Historical background of sanatorium „Avangard" creation and development

Clinical sanatorium "Avangard" was established in one of the famous dendrologic parks in Vinnitska region that have a long favorable history. Far back in 1781 the owner of Nemyriv city the Earl Vicenty Pototsky established a landscape park with the area of 75 hectares. Later the owner

Boleslav Pototsky added rare kinds of trees and bushes to the park and arranged the buildings. In 1840 he starts to build a palace which was ruined. In 1894 the new owner of the estate – the Earl's granddaughter Maria Shcherbatova started to build a new palace. The building was managed by Czech architect Irzhi Stibral. The construction continued till 1920 when the duchess died[1].

At the beginning of the century when the Soviet Revolution came to Ukraine the palace was plundered. In 1921 the People's Committee Commission on "Ownerless Property" (so they called the palace) was created. The head of the Commission was Hustav Brilling – a former citizen of Nemyriv, a lawyer, a collector, the founder and the first director of Vinnitsa Museum of Local Studies. Due to him the palace and its pieces of art were saved and now they are stored in the museums of Vinnytsa and Kyiv[2]. In 1921 it became the first sanatorium in Ukraine. In the whole USSR there were only 4 establishments like it. It hosted 150 persons and 70 children every month. During a year nearly 1000 visited the rest house.

During the Second World War there was a military hospital with a sanatorium department. After the war it again became a sanatorium. Nowadays Branch Department Sanatorium "Avangard" of CS "Ukrprofozdorovnytsia" is a multifield treatment & rehabilitation all-year-round establishment with good and rich traditions[3].

The city of Nemyriv has a great historical heritage that influence on its treatment and rehabilitation functions development. The area where the district center Nemyriv is located has been inhabited for a long time. In the tract of Gorodische, which is located not far from Nemyriv, the remains of the Trypillian culture settlement have been found. In the same tract, Gorodische is one of the largest (150 hectares) Scythian sites of the VII – VI centuries B.C. It is surrounded by powerful shafts – 32 meters wide, 9 meters high and 5,5 km long.

In the X – XI centuries on this place there was an ancient Russian settlement. According to the legend, on the site of this settlement there was a city of Worlds, which during the Tatar invasion was completely destroyed.

At the end of the XIV century a new city of Nemyriv appeared. The first written mention of the city falls on 1506 (in the label of Khan Mengli-

[1] Clinical sanatorium "Avangard": official web-site. URL: http://sanatoriy-avangard.com/ua_en.
[2] Clinical sanatorium "Avangard": official web-site. URL: http://sanatoriy-avangard.com/ua_en.
[3] Clinical sanatorium "Avangard": official web-site. URL: http://sanatoriy-avangard.com/ua_en.

Giray). In the era of the Polish-Lithuanian Commonwealth Nemyriv became the city of Bratslavsky province.

Nemyriv was one of the centers of struggle against the oppressive oppression and cruel support. In the years of the peasant-Cossack uprising led by Severin Nalivaiko, the population of Nemyriv and the surrounding villages acted in his detachments. It participated in anti-ghetto uprisings and unrest in 1607, 1612, 1614 and subsequent years, as well as in the war against social and national oppression in 1648–1654. The Jewish population of the city was badly damaged on May 31, 1648, when the Cossacks entered the city of Nemyriv without a fight, under the guise of a Polish detachment, where thousands of Jews, including refugees from surrounding villages and towns, who were hiding in a Nemyriv fortress, were brutally murdered.

Since the 1640's Nemyriv belonged to Prince I. Vishnevetsky; during the War with Poland (1648–1654) with interruptions was the hundredth place of the Kalnicki and Bratslav Regiments. From 1672 to 1699, Nemyriv was under the rule of the Turks (part of the Sarmatian principality of the Ottoman Empire, being its capital for some time). The city was densely populated by Jews who enjoyed the protection of the Turkish authorities, which became especially necessary since the sultan appointed Yuri Khmelnytsky a hetman over the right-bank Ukraine, with a residence in Nemyriv (1677–1679): the hetman subjected the Jews to death for the slightest offense[4].

At the beginning of the 18th century, the place became the property of Potocki, who rebuilt the palace (former castle), where in 1737 Jozef Potocki received diplomats from Russia, Austria and Turkey who unsuccessfully tried to reach a peaceful compromise and end the Russian-Turkish war. At the beginning of the XIX century in Nemyriv was the residence of the Braslav Hasidim.

After the Sections of the Polish-Lithuanian Commonwealth – a place of the Bratslav district of the Podolsky province of the Russian Empire.

Many famous people are associated with the city. In Nemyriv, the famous Russian poet N.A. Nekrasov and writer N. Trublaini were born, the Jewish poet Motl Gruvman and the poet Alla Eisensharf lived and worked here, the prominent American geneticist Theodosius Dobzhansky was born, the famous Ukrainian writer Marco Vovchoklived and worked here, one of the first programmers Ida Rhodes (Hadassah Itzkowitz) was born.

[4] Clinical sanatorium "Avangard": official web-site. URL: http://sanatoriy-avangard.com/ua_en.

Bogdan Khmelnitsky and Yuri Khmelnitsky visited Nemyriv (in 1677–1679 Nemyriv was the capital of the hetman). Danilo Nechai, Polish kings Stanislav August Poniatowski and Jan III Sobieski, Russian Emperor Peter I, commander Alexander Suvorov, academicians Vladimir Filatov, Nikolai Pirogov, French writer Honore de Balzac, composers Ludwig van Beethoven, Ferenc Liszt and Claude Debussy, artist Alexander Vertinski, Russian general and traveler L.K. Artamonov, playwright Z.P. Moroz visited Nemyriv also.

All the sights that have saved to this day appeared in a place in the XIX – XX centuries, since in 1803 and 1811 Nemyriv burnt to the ground. Among them: male and female corps (1815) of the former gymnasium; Holy Trinity Church (1876–1881); the building of the former Diocesan School (1881); Mill (the second half of the XIX century, now Mukhovetsky store); The Shcherbatov Palace (Maria Scherbatova – the granddaughter of B. Potocki), now the main building of sanatorium "Avangard"; a landscape park (1885, 85 hectares); water tower, and building of the power plant (1903–1905, architect Pehler); the church of Joseph the Betrothed (1801); house (and a monument – 1974) of Marko Vovchok; a monument to the poet N. Nekrasov (1971); Nemyriv ancient settlement of VII – VI centuries. BC. etc.

In the city there are two distillery and alcohol production company "Nemiroff". However, the magnificent Shcherbatov's palace (late XIX century) is Nemyriv's visiting card. Today the "Avangard" sanatorium is located here. Nemyriv is a great place to relax and there is an opportunity to get better. Nature around Nemyriv strikes with its beauty and pristine purity, and the air with freshness and unique aroma.

One of the main attractions Nemyriv is sanatorium "Avangard", located in the former estate of Princess Scherbatova. There was a palace in the style of classicism, a park and several structures, including a unique powerhouse at that time on the Southern Bug.

In the 1787, the ex-owner of the city of Nemyriv, Count Vincentius Pototsky, established a landscaped park with an area of 75 hectares. A little later the new owner Boleslav Pototsky replenishes the park with rare species of trees and bushes, arranges its architecture. Since 1840, he built a palace in the center of the park, which in 1885 was demolished, and in 1894 a new owner of the estate, the granddaughter of the Count – Princess Maria Grigorievna Scherbatova began construction of a new one. The palace was designed by the Czech architect Jiri Stibral, and was built in conjunction

with Kramarzh. The construction was completed in 1900, and some minor construction work continued until the death of the princess in 1920.

After the final establishment of Soviet power in Nemyriv, the question arose of preserving the plundered palace of Princess M. G. Scherbatova and the cultural values that still remained in it. Gustav Brilling, a former Nemyriv, a graduate of the Nemyriv grammar school, a lawyer, a collector of antiquities and art, the founder and the first director of the Vinnitsa Museum of Local History, had a great work in this matter. It was he who, at the beginning of February 1921, created and headed the commission of the People's Commissariat for the Conservation of "no-man's property" (this is what the palace christened the Soviet power). Thanks to him, some of the works of art were saved, and now they are stored in the museums of Vinnitsa and Kiev.

The palace was given to the agronomy school (the western part of the palace), a museum was also created there; the remaining rooms of the palace were given to the people's theater, which gave the first performances on February 24 and March 10, 1921.

In May 1921, the first sanatorium in Ukraine for resting the inhabitants of the Podolsky province was created on the basis of the estate of M.G. Scherbatova. It is worth to note that in the Soviet Union there were only four such institutions. The sanatorium received the all-Union significance and was under the Republican subordination.

In 1925 the sanatorium owned 70 acres of a private land, 32 acres of arable land, 7 acres of ponds and 17 acres of pine forest. At this time the sanatorium organized 3 polyclinics with six offices and consultation for pregnant women. For every six weeks, the sanatorium was serviced by an average of 150 adults and 70 children. During 1925 year, 900–1000 people were recovered. Subsequently, the sanatorium was transformed into a recreation center "Avangard".

Since March 1944, the palace was reconstructed into a military hospital with a sanatorium, which was headed by Captain Shchukarev. When the hospital was closed, the sanatorium was returned to the institution. Since 1947 this sanatorium became a rest house, which worked until 1989, when the health resort for the third time became a sanatorium. Almost 40 years the health center was headed and developed by the Honorable citizen of the city of Nemyriv – Mikhail Naumovich Mostovoi. Today, the Subsidiary clinical sanatorium "Avangard" CJSC "Ukrainian professional health resort" is a

multidisciplinary medical and rehabilitation institution operating year-round with extremely rich and good traditions.

Therefore, sanatorium “Avangard” became a main attraction of Nemyriv city. There were located a palace in the style of classicism, a park and several structures, including a unique power plant at that bank on the Southern Bug. Maria Scherbatova was known for her charity work. In 1914, she gave the palace to the needs of a hospital for wounded soldiers, and she worked as a nurse there. A daughter of Peter Stolypin – Helen, daughter-in-law of the princess, who specially arrived to Nemyriv for this purpose, also workedtogether with her. But in such a city it could not all end well. Princess Maria Scherbatova, together with Helen Stolypina, was shot by the Red Army in 1920.

2. Treatment and rehabilitation system

Treatment and rehabilitation system is a complex socially governed (partially self-governed) system with patient subjects as its central subsystem, and most ample satisfaction of their rehabilitation needs is its target function. According to V.S. Preobrazhenskiy (1988), there are basic model of treatment and rehabilitation system (fig. 1.)[5].

Let’s disclose the basic components of this system. Rehabilitation persons group is characterized by behavioral parameters described by the cycles of activities. Relations with other sub-systems are differentiated depending on the social, age, psychological, ethnic, professional, regional and individual selectivity of certain groups of people, surroundings and rehabilitation resources[6].

Technical systems provide usual activities of the patients and service employees as well as meet specific rehabilitation needs of the peoples. Service employees use technical systems for producing, collecting, preserving and providing a service package to the patients as well as for collection and disposal of wastes. Operating control compares information on satisfying the needs of the patients with information on condition of other sub-systems and on availability of materials and financial reserves, makes economic decisions. Interaction is a universal form of connection of

[5] Стафійчук В.І. Рекреалогія: навч. посібник для студ. вищ. навч. закл. 2-е вид., перероб. та доп. К.: Альтерпрес, 2008. 264 с.

[6] Кусков А.С., Голубева В.Л., Одинцова Т.Н. Рекреационная география: учеб.-метод. комплекс. М.: МПСИ; Флинта, 2005. 496 с.

subsystems in the treatment and rehabilitation system, objects, phenomena of objective reality, which manifests itself in the fact that a change in one object causes a change in the other[7].

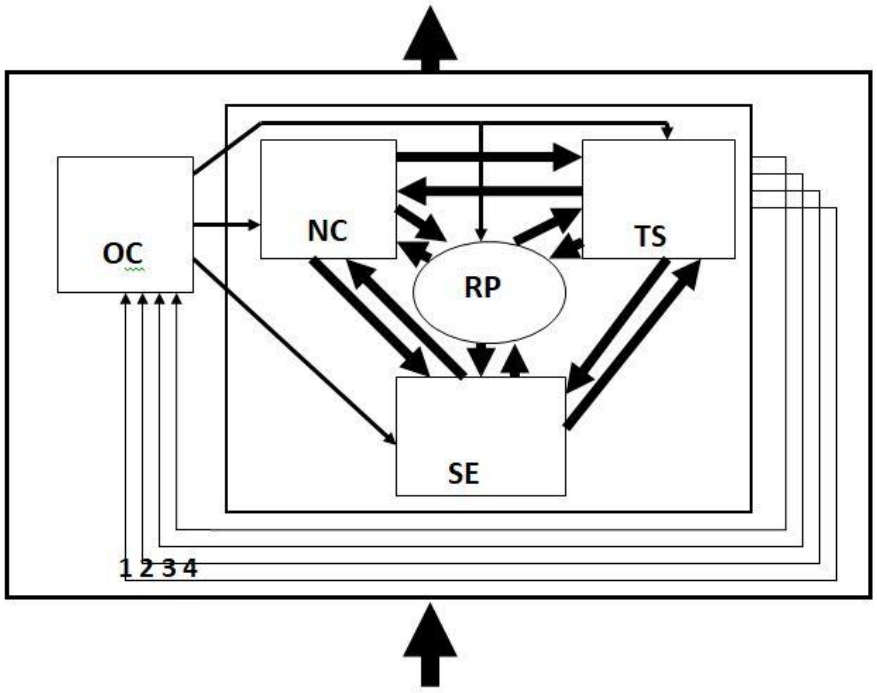


Fig. 1. Treatment and rehabilitation system by Prof. V.S. Preobrazhenskiy

RP – rehabilitation persons group; **NC** – natural complex; **TS** – technical systems (material resources for treatment and rehabilitation infrastructure); **OC** – operating control; **SE** – service employees.

– external connections of the system.

– connections between subsystems.

– control instructions.

– subsystem information: 1 – about rehabilitation persons' needs satisfaction; 2 – about the level of natural complexes accordance with rehabilitation persons' needs; 3 – about the level of useful properties and capabilities of technical systems safety; 4 – about service employees level.

[7] Николаенко Д.В. Рекреационная география: учеб. пособие для студ. высш. учеб. завед. М.: ВЛАДОС, 2001. 288 с.

Treatment and rehabilitation system structure includes: hierarchic and territorial structures. The first one is typical for a complex treatment and rehabilitation system where there is a division of sets of constituent elements into subsets of various levels – subsystems manifesting integrity, a certain degree of self-regulation, and connected by multi-stage relations of subordination of subsystems of lower levels to subsystems of higher levels and territorial structure is a set of regional links between elements of the system. Territorial structure, for example, includes: layout structure of the sanatorium; territorial structure of patient flows; territorial organization of rehabilitation activities; territorial organization of patients services; territorial structure of patients' demand; territorial structure of the rehabilitation services' market etc.[8]

We have different types of treatment and rehabilitation structures. One of them is medicinal type – is characterized by the primary function of treatment, which is based on natural factors: mineral waters, mud, climatic conditions. There are: climate therapy, balneotherapy, muds therapy, ozokerite therapy, hydropathic, phytocuring parts. Health-improving type of treatment and rehabilitation structure is characterized by the basic functions of the rehabilitation and development of physical and mental potentials, by prevention of different diseases, by working, everyday nervous and physical exhaustion relieving. This type is divided into bath-coastal and walking parts. Sport type of treatment and rehabilitation structure is characterized by the main function of physical development. It's composed of physical training group with coaching mode: sports games and competitions, mountaineering, hunting, fishing. Additional role is belonging to walking, swimming, sun and air baths, excursions. This type is divided into fishing-hunting, emulative and resort parts[9].

Clinical sanatorium "Avangard" is closely connected with first and second types of recreation structures. Its provide rehabilitation and treatment for circulatory system, respiratory system, neural system, loco-motor system, skin, digestive system, endocrine system, reproductive system as well as for organs of vision diseases.

[8] Про курорти: Закон України від 5 жовтня 2000 р. № 2026-III / Верховна Рада України. *Відомості Верховної Ради України*. 2000. № 50. Ст. 435.

[9] Про курорти: Закон України від 5 жовтня 2000 р. № 2026-III / Верховна Рада України. *Відомості Верховної Ради України*. 2000. № 50. Ст. 435.

3. Treatment and Rehabilitation in sanatorium "Avangard"

Taking into account the analysis of geographical, natural, historical, economic conditions, the sanatorium "Avangard" is a real participant in the progress of the social and economic life of the city of Nemyriv and the region. The result of recreational activities is the satisfaction of people's needs for rest, rehabilitation, development of their own physical, mental, spiritual and intellectual forces. Clinical sanatorium "Avangard" is provide rehabilitation and treatment for circulatory system, respiratory system, neural system, loco-motor system, skin, digestive system, endocrine system, reproductive system as well as for organs of vision diseases.

There are different rehabilitation types for circulatory system diseases in this sanatorium: 1) ischemic heart disease (stable exertional angina not higher than 3rd functional class); 2) cardiosclerosis; 3) hypertension not higher than stage IIB with benign course; 4) hypotonic disease and symptomatic hypotonia; 5) cardiomyopathy; 6) obliterate diseases of extremities vessels; 7) stages after the surgery for arteries obliterate diseases; 8) varicose veins, chronic thrombophlebitis[10].

Rehabilitation of patients with respiratory system diseases can solve different problems after acute inflammation of trachea, bronchi, lungs; chronic non-specific diseases of the lungs and also bronchial asthma in remission stage. Neural system diseases (Neuritis, polyneuritis, plexitis, radiculitis; affections of nervous roots, plexuses and nerves; neurological manifestations of spinal osteochondrosis; raynaud's disease; neurosis) can be rehabilitate also[11].

There are different types of loco-motor system diseases that can be cured in clinical sanatorium "Avangard" also: chronic arthritis in remission phase; spinal osteochondrosis; chronic bursitis, synovitis, tendovaginitis; myositis. Rehabilitation of patients with skin diseases includes solutions for neurodermatitis; psoriasis; trophic ulcers. Chronic cholecistitis, cholangitis, hepatitis, gall-stone diseases, biliary dyskinesia, stage after cholecystectomy as well as chronic gastritis with low secretory function in remission phase

[10] Clinical sanatorium "Avangard": official web-site. URL: http://sanatoriy-avangard.com/ua_en.

[11] Clinical sanatorium "Avangard": official web-site. URL: http://sanatoriy-avangard.com/ua_en.

are the main digestive system diseases that patients can rehabilitate in clinical sanatorium "Avangard"[12].

Endocrine system diseases (obesity of I–II stages, mild diabetes mellitus, urine acid diathesis) as well as reproductive system diseases (disturbances of ovary-menstrual cycle, chronic inflammation of uterus and appendages, male and female infertility, endometriosis, miscarriage, cystalgia, hysteromyoma if conservative treatment is prescribed) are one of the main directions of patient rehabilitation in above-mentioned sanatorium also[13].

Moreover, clinical sanatorium "Avangard" has different programs for organs of vision diseases rehabilitation. There are: 1) state after surgeries for inborn and acquired glaucoma, after cataract surgical treatment, after surgeries on cornea, after surgeries in retina pathologies, vitreous body (4–6 months after the surgery); 2) stable compensated glaucoma; 3) pigment degeneration of cornea; 4) age-related macular dystrophy, dry form; 5) consequences of inflammatory diseases of retinal and vascular membranes; 6) corneal dystrophy; 7) keratoleukoma (after viral or bacterial keratitis, trauma); 8) diabetic retinopathy (non-proliferative, pre-proliferative); 9) disturbances of refraction and accommodation: myopia, hyperopia, astigmatism, accommodation cramp; 10) amblyopy; 11) squint; 12) chronic blepharitis; 13) chronic blepharoconjunctivitis; 14) eye trauma consecuences; 15) improvement of health before surgery[14].

Clinical sanatorium "Avangard" has different medical and rehabilitation departments:

I. For burn patients:

1. Burn contractures and deformations of any localization which spread during 1,5 years after burning.

2. Afterburning scars maturing or healing, burn sites of III-A stage not in joint areas (during first year after burn wounds healing).

3. Maturing sites of free skin transplanting not in joint areas (during first year after skin transplanting).

4. Hypertrophic and keloid cicatrix after surgery or after self-healed burn wounds (during first 1,5 year after burn wounds healing).

---

[12] Clinical sanatorium "Avangard": official web-site. URL: http://sanatoriy-avangard.com/ua_en.

[13] Clinical sanatorium "Avangard": official web-site. URL: http://sanatoriy-avangard.com/ua_en.

[14] Clinical sanatorium "Avangard": official web-site. URL: http://sanatoriy-avangard.com/ua_en.

5. Thermal lesions of respiratory passages (inhalation lesions) – during first year after burn.

6. Burn pneumonias (during first year after burn wounds healing).

7. Toxic hepatitis, enteritis (bowel lesions).

8. After-burn pyelitis, pyelonephritis (during first year).

9. After-burn myocarditis (during first year)[15].

II. For women with pregnancy pathologies:

1. Normal pregnancy course in terms 12–35 weeks (including the term of staying in the sanatorium department).

2. Anemia of pregnancy (iron deficiency) without accompanying diseases.

3. Ordinary abortion.

4. Rheumatism, non-active phase.

5. Compensated rheumatic and inborn heart defects.

6. Chronic non-specific lung diseases in remission phase.

7. Hypertension of I stage.

8. Myocardiosclerosis and myocardiodystrophy without blood circulation deficiency and heart rhythm disturbances.

9. Obesity.

10. Diabetes mellitus (light form).

11. Thyrotoxicosis (light form).

III. For patients with chronic non-specific lung diseases:

1. Bronchial asthma of atonic, infective or mixed etiology in light or mild stage.

2. Chronic obstructive bronchitis with respiration failure signs of I–II stages.

3. Chronic non-obstructive bronchitis with respiration failure signs of I–II stages.

4. Acute pneumonia with prolonged course more than 8 weeks.

5. Acute pneumonia with obstructive syndrome and prolonged course more than 8 weeks.

6. Acute pneumonia with accompanying allergic diseases and prolonged course more than 8 weeks.

7. Persons who often have acute bronchitis, acute respiratory diseases working in conditions of high gas and dust pollution.

---

[15] Clinical sanatorium “Avangard”: official web-site. URL: http://sanatoriy-avangard.com/ua_en.

8. Persons with signs of bronchi hyperactivity appeared after influenza, acute respiratory diseases, acute bronchitis of viral and bacterial etiology.

IV. For patients with endocrinology pathologies:

1. Obesity, primary, alimentary-constitutional, of I–III stages, without signs of circulatory decompensation.

2. Diabetes mellitus, first found, latent, pronounced, of I–II types, mild stage in the phase of stable compensation.

3. Thyrotoxicosis, of primary and secondary genesis, of mild stage, without pronounced complications, with enough correction of hormonal disturbances.

4. Hypothyreosis, non-complicated forms accompanied with thyreogenic obesity, dystrophic changes in joints and spine.

5. Pathology of reproductive sphere of endocrinal genesis.

Also, clinical sanatorium "Avangard" provides innovative treatment with radon of mild action.

Sanatorium "Avangard" has a separate treatment and diagnostics with 47 rooms, a swimming pool, a shower and bath departments[16]. It also has several unique departments that are officially recognized as the best in the Ukraine – pulmonology, endocrinology, pathology pregnancy and burns. Very great pride and heritage "Avangard" is highly developed technology hydrotherapy. In addition, it is not the only unique services that are in the sanatorium, their number also includes: plasmapheresis, bioresonance stimulation, hirudotherapy, massage, cryotherapy, and others.

The list of treatment facilities that available at the sanatorium include: apparatuses for inhalation "Pari" (Germany); apparatus for salt inhalations "Haloneb"; apparatus "Stream" for electrophoresis and galvanization; apparatuses "Pole", "Mustang", "Mit", "Olympus" for magnetic and magnetic-laser therapy; apparatus "EOL" for vibration massage of lungs; apparatuses "UST", "Barvinok", "Gamma ENT" for ultrasound therapy; apparatuses "Zond" and "Rupor" for EHF therapy; Chizhevsky's lamp for aeroionotherapy; apparatus "Mit" for oxygen therapy; apparatus "UI" for aerosol therapy; apparatus "Mit" for UV and laser radiation of blood; apparatuses for washing of nasal sinuses and tonsils; apparatuses for massage of middle ear tympanic membrane; apparatus for electrosonotherapy; baths combined for curing swirling and massaging

[16] Clinical sanatorium "Avangard": official web-site. URL: http://sanatoriy-avangard.com/ua_en.

baths; showers for circular, descending, Charcot's, jet methods of shower; apparatus "Mit" for hydrolaser showers; endotracheal, endobronchial irrigation as a special method of treatment; swimming pool; massages; therapeutic ophthalmologic laser LTO-02R; electrostimulator ophthalmologic "Phosphen"; muscle trainer ophthalmologic perimeter MOP-1; accommodation trainer optical ATO-3; synoptophore Sinph-1; trainer for retina stimulation with panoramic fields "Ambliopanorama"; maculostimulator "MKS-C"; apparatus spectral ophthalmologic ACO-3; apparatus for vacuum massage ophthalmologic ABMO 2m; apparatus for visual field examination "Pericom"[17].

4. Natural resources management for treatment and rehabilitation in sanatorium "Avangard"

Natural resource management is theoretical and practical investigations connected with creation of optimal regime of natural resources usage for treatment and rehabilitation system development. Natural resource management include: management of technological impact from the patient material assets and treatment and rehabilitation infrastructure on the ecosystem; management of treatment and rehabilitation impact on the ecosystem; management of domestic anthropogenic impact on the ecosystem; management of production impact on the ecosystem and treatment and rehabilitation resources (fig. 2)[18].

[17] Clinical sanatorium "Avangard": official web-site. URL: http://sanatoriy-avangard.com/ua_en.

[18] Стафійчук В.І. Рекреалогія: навч. посібник для студ. вищ. навч. закл. 2-е вид., перероб. та доп. К.: Альтерпрес, 2008. 264 с.

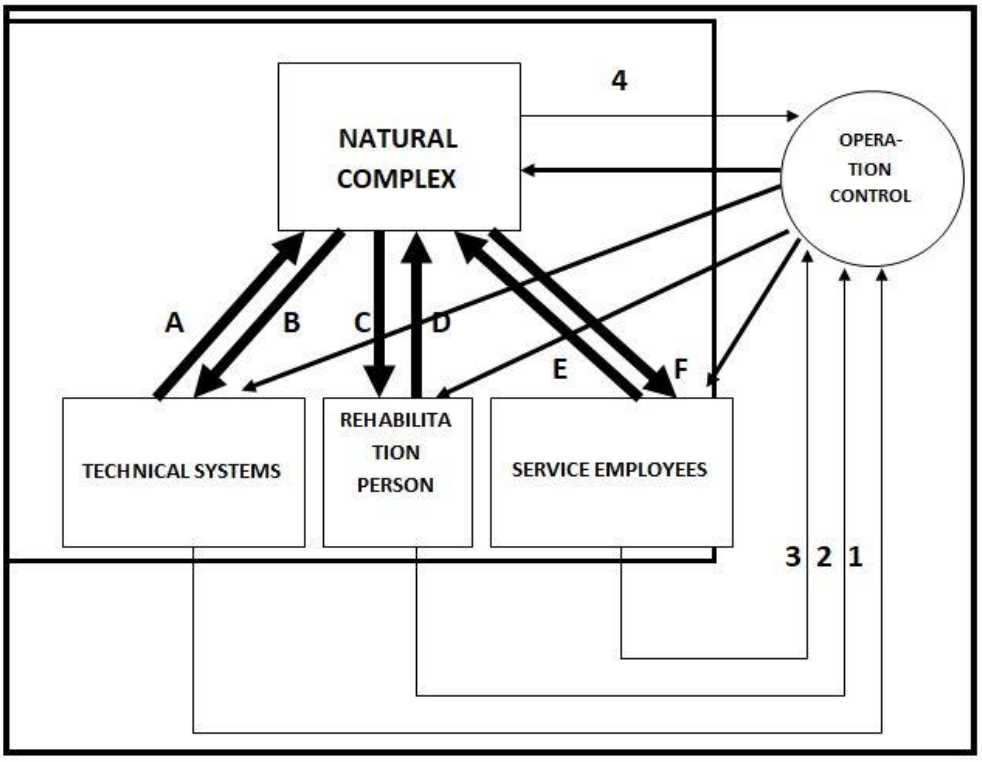


Fig 2. Functional model of treatment and rehabilitation natural resource management

⟶ – links off natural complex with another subsystems: A – technological influence on natural complexes; B – requests to technological resources; C – anthropogenic treatment and rehabilitation influences; D – selectivity of natural conditions; E – anthropogenic influence of life conditions; F – requests to environment of vital activity from resident population side.

⟶ – information about subsystems development: 1 – information about reserves of technological natural resources and environmental efficiency of technologies; 2 – information about correspondence between to natural conditions and patients' requirements and also about the level of treatment and rehabilitation needs satisfaction; 3 – information about level of natural conditions comfortable for different peoples; 4 – information about the sustainability of the natural complex.

⟶ – operating commands.

Natural resource management allows creating different treatment and rehabilitation zones: 1) protective sanitary zone is especially protected natural area with conditions for economic activities, residence, use of natural

resources established according to current legislation, which provides protection and conservation of natural healing systems, medicinal and recreational areas with adjacent territories from pollution and premature exhaustion; 2) recreation zone is a functioning zone intended for recreation; 3) personal territory zone is a zone of specific individual activities; 4) rehabilitation zone is specially organized area for receiving a large number of short-term visits (on the weekend), equipped with transport communication with a big city, water supply, sanitation, catering, leisure and entertainment facilities[19].

Sustainable recreational natural resource management consists in: replacing culture of intensive use with a culture of reasonable growth; balancing economic and ecological impact factors; finding common interests of the tourists and local population; distributing the generated profit among all members of the society and, in the first place among most needy and vulnerable populations[20].

The sanatorium "Avangard" occupies a leading position for treatment and rehabilitation in Vinnitsia region and is very popular. The territory of the health resort is buried in greenery, there is a dendrological park with a century-old history, exotic plants grow, beautiful picturesque lakes and water bodies are on the territory of the park.

The pride of the sanatorium "Avangard" is a deposit of its own radon mineral water. On the basis of the sanatorium, unique medical departments have been created, including a department for pregnant women. The powerful therapeutic and diagnostic base of the sanatorium and the latest equipment allow implementing innovative methods of healing, such as plasmapheresis, bioresonance stimulation, cryotherapy and others.

For climatotherapy in the sanatorium "Avangard" all natural factors are important: atmospheric or meteorological, space or radiation, telluric, or earthly. Meteorological factors have two components: chemical (gases and various impurities) and physical (air temperature, atmospheric pressure, humidity, cloudiness, precipitation, wind).

Heliotherapy is used in the form of general or local solar baths (on collar area, lumbar sacral region, upper or lower limbs, etc.). The procedure of heliotherapy is shown to all healthy rest as a preventive, hardening agent, especially those that arrived from areas in which there is a probable light

[19] Yukic T.S. Fundamentals of Recreation. 2nd ed. New York: Harpers & Row, 1970. 187 p.
[20] Gladkey A.V. Recreology. Lecture notes. Kyiv: KNTEU, 2016. 51 p.

hunger strike. In addition, heliotherapy is indicated with hypovitaminosis D; pyoderma, psoriasis; wounds, badly healing ulcers, bone fractures with slow consolidation; at chronic pathology of locomotor apparatus, radiculitis, diseases of the cardiovascular and respiratory systems. For the rest of the elderly (over 55 years) heliotherapy should be used with caution.

There are over 140 species of trees and bushes imported from many countries of the world in the dendrological park of the Avangard sanatorium. These are several varieties of oak, whose bark has tannin properties; Lonely Planet, whose homeland is North America; the mountain ash, in the fruits of which contains a lot of vitamin C, P, carotene, organic acids. Make the sight of the beauty of ordinary juniper, a hectare of plantations that allocates about 300 kg of phytoncides per day, enough to clear the microbes of the air of a large city. As a symbol of longevity and invulnerability, the beauty of the dark-green needles "red tree", which belongs to the endangered species, is amazing. The cork tree, or the Amur velvet, remains a vivid monument of subtropical forests that covered Europe, Siberia, East Asia in the distant times. The decoration of the park is a ginkgo biloba tree, the birthplace of which is considered China. In folk medicine, its seeds are used from cough, odor, tuberculosis – as carcinostats, and also from the extract of fresh leaves, preparations are prepared (ginkkor, tanakan, etc.). Often found chestnut horse, imported from Greece. The medicinal properties have leaves, fresh juice which is used for varicose veins, thrombophlebitis, atherosclerosis and hemorrhoids.

A magnificent magnolia vision is magnificent flower, the home of which is Japan. Its leaves are useful with high blood pressure and pain in the heart. Among many other trees and bushes of the park, you can note the laxative jester, whose fruits are used for atonics, spastic constipation and some skin diseases. Common viburnum, which is known as homeostatic and urethra, is indicated for nervous excitement, atherosclerosis, hypertension. Conventional maple has anaesthetizing, wound healing properties, enhances male sexual activity. Lime-tree Caucasian, used as tea, is a diaphoretic, antitussive and anti-inflammatory drug. Common lichen is used for anemia, atherosclerosis, diabetes, gallstone disease.

Aerotherapy is an integral part of sanatorium "Avangard" treatment. The effects of fresh air during walks, excursions and sports games are elements of aerotherapy. Sanatorium "Avangard" used air baths (dose effects), prolonged exposure of open air at verandas, balconies as well as at shores of

the lakes as special types of aerotherapy. Heliotherapy is used solar baths (on collar area, lumbar sacral region, upper or lower extremities, etc.) with general or local influence on human rehabilitation. Significant improvement and rehabilitation of disturbed functions are observed under the influence of climate treatments.

Atmospheric air, especially in Vinnytsya region, is saturated with ozone, oxygen, terpenes, phytoncides and other volatile phytogenesis substances. Clinical investigations of sanatorium "Avangard" had shown the positive effect of spa treatment and rehabilitation in more than 30 thousand patients with cardiovascular, respiratory and nervous system pathologies. Training effect of climatotherapy promotes hardening of the organism, increasing of its resistance to adverse effects of the environment as well as help with prevention of diseases. Long-term walking in dendrological park of sanatorium "Avangard" together with aromatherapy, hydrotherapy and also with usage of therapeutic factors of muds, stones, minerals, fauna and its representatives assist to optimal recovering and rehabilitation of different patients.

Using health-improving types of recreational activities, medical workers of sanatorium "Avangard" had established in their dendrological park some playgrounds for tennis, volleyball, basketball etc. Recreants and patients can use chinning bars, gymnastic apparatus as well as terencours for their health recovery. Besides the cultural-historical and pedagogical role, dendrological park of sanatorium "Avangard" has great recreational value due to healing radon water springs and unique healing microclimate.

It is advisable to recommend the following promotional activities for further development of treatment and rehabilitation in sanatorium "Avangard"[21]:

1. When working with consumers. It is necessary to change the attitude towards advertising, so that advertising not only demonstrated the range of services of the sanatorium, and where to go, but also gave other ideas about the brand "Avangard". When conducting an advertising campaign in the media in order to form a positive image of the company, the provision of advertising information should be based on the consumers' perception of the quality and capabilities of the Avangard brand, a wide range of services,

[21] Bauer H., Klein-Belting W. Model of the driving forces of brand equity. Value of premium brands and brand management issues. *Brand management*. 2011. № 1. P. 2–21.

Breckenridge D. PR 2.0: new media, new audiences, new tools. Moscow: EKSMO, 2010. 272 p.

provision of additional services, and differences between the Avangard brand and other sanatoriums[22]. Sanatorium "Avangard" should place in the advertising products information about the mission statement and strategic goals (reflecting the interests of consumers), as well as information on the diversity of the range of services and other benefits. One of the important tasks of advertising in creating an image is the creation of printing products (prestigious avenues, booklets, leaflets, postcards, etc.).

2. Work with the public. In order to strengthen the image of the Avangard brand in the public eye, one should take part in social events, such as: to sponsor an event that has a wide public resonance; to hold a charity event for veterans; to organize charity meals for the poor; to become an organizer of the holiday for disabled children, etc.[23] Then regularly give information material in the media about the work done. Publications will strengthen the business reputation of the enterprise and its leader.

3. Work with government agencies. The purpose of above mentioned actions aimed at enhancing treatment and rehabilitation functions of sanatorium "Avangard" when working with the authorities is to establish two-way communication to identify common interests or common ideas and to achieve mutual understanding[24]. Therefore, it is very important to have constant personal contacts between the managers and specialists of sanatorium "Avangard" with representatives of the regional administration and the city, ministries, public organizations, etc.

CONCLUSIONS

Thus, the sanatorium "Avangard", located in the city of Nemyriv play an important role in recreation and rehabilitation process of different types of patients and recreants. Its medical, health improving and cultural-historic potential, described above, are very significant for mental and physical rehabilitation of peoples.

Clinical sanatorium "Avangard" is a highly developed recreation system located in the center of Vinnytska region, Ukraine. It has long and fruitful experience in rehabilitation and treatment of circulatory, nervous, loco-motor systems, metabolism disturbances and endocrinology system, diseases

[22] Gladkey A.V. Recreology. Lecture notes. Kyiv: KNTEU, 2016. 51 p.

[23] Breckenridge D. PR 2.0: new media, new audiences, new tools. Moscow: EKSMO, 2010. 272 p.

[24] Bauer H., Klein-Belting W. Model of the driving forces of brand equity. Value of premium brands and brand management issues. *Brand management*. 2011. № 1. P. 2–21.

of skin, respiratory organs, female reproductive organs, etc. The implementation of above-mentioned methods of recreation system development will allow sanatorium “Avangard” to development innovative and high-impact system of rehabilitation and recreation of patients. Using this program, clinical sanatorium “Avangard” will have every reason to get the state status of regional value resort.

SUMMARY

The article deals with investigation of treatment and rehabilitation functions of sanatorium “Avangard” located in the Nemyriv, Vinnitska region, Ukraine. Historical background of sanatorium “Avangard” creation and development is defined. The different methods of treatment and rehabilitation in clinical sanatorium “Avangard” are explained. The different rehabilitation types for circulatory system diseases, respiratory system diseases, loco-motor system diseases, endocrine system diseases as well as for organs of vision diseases rehabilitation are explored. The treatment and rehabilitation procedures for burn patients, for women with pregnancy pathologies, for patients with chronic non-specific lung diseases as well as for patients with endocrinology pathologies are proposed.

REFERENCES

1. Clinical sanatorium “Avangard”: official web-site. URL: http://sanatoriy-avangard.com/ua_en.

2. Стафійчук В.І. Рекреалогія: навч. посібник для студ. вищ. навч. закл. 2-е вид., перероб. та доп. К.: Альтерпрес, 2008. 264 с.

3. Кусков А.С., Голубева В.Л., Одинцова Т.Н. Рекреационная география: учеб.-метод. комплекс. М.: МПСИ; Флинта, 2005. 496 с.

4. Николаенко Д.В. Рекреационная география: учеб. пособие для студ. высш. учеб. завед. М.: ВЛАДОС, 2001. 288 с.

5. Про курорти: Закон України від 5 жовтня 2000 р. № 2026-III / Верховна Рада України. *Відомості Верховної Ради України*. 2000. № 50. Ст. 435.

6. Yukic T.S. Fundamentals of Recreation. 2nd ed. New York: Harpers & Row, 1970. 187 p.

7. Gladkey A.V. Recreology. Lecture notes. Kyiv: KNTEU, 2016. 51 p.

8. Bauer H., Klein-Belting W. Model of the driving forces of brand equity. Value of premium brands and brand management issues. *Brand management*. 2011. № 1. P. 2–21.

9. Breckenridge D. PR 2.0: new media, new audiences, new tools. Moscow: EKSMO, 2010. 272 p.

Information about authors:
Hladkyi O. V.,
Doctor of Geographic Sciences, Professor,
Academician of Higher Education Academy of Sciences of Ukraine,
Professor at the Department of Tourism and Recreation,
Kiev National University of Trade and Economics
19, Kioto str., Kyiv, 02156, Ukraine
Kilivnik V. S.,
Candidate of Medical Sciences,
Senior Lecturer,
Academician of the International Academy of Applied Radio-Electronics,
Senior Lecturer at the Department of Medical Rehabilitation
and Medical Social Expertise,
National Pirogov Memorial Medical University
56 Pirogova str., Vinnitsa, 21018, Ukraine

PERSPECTIVES OF THE USE OF SPECIES OF ARTEMISIA IN MEDICINE[1]

Grycyk R. A., Struk O. A.

Nature treats…

INTRODUCTION

Current problem of modern pharmacy is the search for new species of medicinal plant material. The interest in medicinal plants and their significance in the production of medicines have grown in the drug market in recent years. Much attention of scientists is attracted by medicinal plants, which have experience of using in non-traditional medicine and a rich chemical composition. These plants include species of the *Artemisia* L. genus of the *Asteraceae* family. This genus includes more than 500 species in the world's flora, about 30 of which are found on the territory of Ukraine. Polymorphism of many *Artemisia* L. genus species leads to a divergence of views of various authors on the systematics of the genus. Taking into account the distribution of the *Artemisia* L. genus species on the territory of Ukraine, the possibility of cultivating them, it is promising to study their raw material base in the western regions of Ukraine.

1. Botanical characteristics and distribution of Artemisia genus plants

Genus *Artemisia* belongs to the family *Asteraceae*. It includes more than 500 species, about 30 species grow in Ukraine. A lot of species grow in Russia, the USA, Canada, China, Japan, India, Western Europe, the Middle East, Central Asia and North Africa.

According to the ordinary classification system, the genus *Artemisia* is divided into three subfamilies: *Artemisia* Less., *Dracunculus* (Bess.) Rydberg and *Seriphidium* (Bess.) Rouy. They differ mainly in the structure of inflorescences: in the species of the *Artemisia* subgenus there is one row of edge tubular petal flowers in the baskets, and the flowers of the disk are bisexual; in the species of *Dracunculus* subgenus, the flower of the disk is

[1] We express our sincere gratitude for the advisory assistance in completing the work to Kovaleva A.M. (D.Sc. in Pharmaceutical Sciences, Professor of the Pharmacognosy Department of National Pharmaceutical University) and Kireev I.V. (D.Sc. in Medicine, Professor, Head of the Pharmacotherahy Department of National Pharmaceutical University).

tinny (but with a reduced mast), in the species of the *Serphidium* subgenus all flowers in the baskets are bisexual, tubular, in the quantity up to 8–10[2].

The system of species of *Artemisia* L. genus of Ukrainian flora is represented in Table 1.

Table 1

The system of species of *Artemisia* L. genus of Ukrainian flora

<table>
<tr><th colspan="3">Genus Artemisia L.</th></tr>
<tr><th>Subgenus 1 – Artemisia Less.</th><th>Subgenus 2 – Dracunculus Bess.</th><th>Subgenus 3 – Seriphidium Rouy</th></tr>
<tr><td>Division 1 – Artemisia
A. vulgaris L.</td><td>Division 6 – Dracunculus Bess.
A. dracunculus L.</td><td rowspan="2">Division 9 – Seriphidium
A. dzevanovskyi Leonova
A. lerchiana Weber ex Stechm.
A. nutans Willd.
A. santonica L.
A. taurica Willd.</td></tr>
<tr><td>Division 2 – Stellerianum
Genuses do not grow in Ukraine</td><td>Division 7 – Salsoloides Leonova
A. salsoloides Willd.</td></tr>
<tr><td>Division 3 – Abrotanum
A. abrotanum L.
A. annua L.
A. armeniaca Lam.
A. pontica L.
A. tournefortiana Rchb.</td><td rowspan="3">Division 8 – Campestres Korobkov
A. arenaria DC.
A. campestris L.
A. marschalliana Spreng.
A. scoparia W.
A. trautvetteriana Besser
A. tschernieviana Besser</td><td rowspan="3">Division 10 – Junceum Poljak.
Genuses do not grow in Ukraine</td></tr>
<tr><td>Division 4 – Absinthium
A. absinthium L.
A. argentata Klok.
A. austriaca Jacq.
A. caucasica Willd.
A. hololeuca M.Bieb. ex Besser
A. sieversiana Willd.</td></tr>
<tr><td>Division 5 – Artanacetum
Genuses do not grow in Ukraine</td></tr>
</table>

[2] Доброчаева Д.Н., Котов М.И., Прокурин Ю.Н. и др. Определитель высших растений Украины. 2-е изд. К.: Фитосоциоцентр, 1999. 548 с.

Флора УРСР: в 12 т. / за ред. Д.К. Зерова. К.: Вид-во АН УРСР, 1962. Т. 11. С. 352–355.

Флора СССР: в 30 т. / под ред. В.Л. Комарова. М., Л.: Изд-во АН СССР, 1961. Т. 26. С. 642–645.

Polymorphism of many species of *Artemisia* L. leads to divergences in various authors views on the taxonomy of this genus. Thus, according to some data, *A. Chernyayev* is considered to be a type of *A. arenaria* DC., and *A. caucasica* Willd. is a type of *A. argentata* Klok., simultaneously *A. campestris* L. and *A. marschalliana* Spreng. are the same one. And only the chemical analysis solves the problem of systematic affiliation and species autonomy for such a polymorphous genus as *Artemisia* L.[3]

Artemisia genus plants are typical ruderous weeds that grow on the roadsides, in forest belts, in vacant lots, pastures, abandoned fields, slopes of quarries, logging and other various types of disturbed lands, but does not come in the crops[4]. Until the conditions of moisture and soil richness is unpretentious, although, like most weeds, it grows better on fresh soils rich in nitrates.

Areas and ecological conditions of growth of *Artemisia* L. genus plants are presented in Table 2.

Table 2

Distribution and places of growth of *Artemisia* L. genus plants in Ukraine

Genus	Distribution	Places of growth
Subgenus Artemisia Less.		
Division Artemisia		
A. vulgaris L.	Basin of Dniester river, Black Sea Coast, Crimea, Basin of Don river, Bessarabia	Deciduous forests, lawns, river valleys, ravines, meadows, wilderness, settlements
Division Abrotanum		
A. abrotanum L.	Black Sea Coast, Podniprovya, Basin of Don river,	Valleys of rivers, meadows, forest glades, settlements

[3] Флора УРСР: в 12 т. / за ред. Д.К. Зерова. К.: Вид-во АН УРСР, 1962. Т. 11. С. 352–355.

Мінарченко В.М., Тимченко І.А. Атлас лікарських рослин України. К., 2002. С. 15, 63, 97, 127, 142, 155.

Растительные ресурсы СССР: цветковые растения, их химический состав, использование. Семейство *Asteraceae (Compositae)* / под ред. П.Д. Соколова. СПб.: Наука, 1993. 352 с.

[4] Флора СССР: в 30 т. / под ред. В.Л. Комарова. М., Л.: Изд-во АН СССР, 1961. Т. 26. С. 642–645.

Лікарські рослини: енциклопедичний довідник / за ред. А.М. Гродзінського. К.: Гол. редакція УРЕ, 1990. С. 430–432.

Флора Европейской части СССР: в 11 т. / отв. ред. и ред. тома Н.Н. Цвелев. СПб.: Наука, 1994. Т. 7. 317 с.

Продовження таблиці 2

A. pontica L.	Black Sea Coast, Podniprovya, Basin of Don river, Bessarabia	Birch groves, river valleys, meadows, salt marshes, steppes
A. armenica Lam.	Basin of Samara river, Basin of Don river (uncommonly)	Alpine and feathery steppes, meadows
A. annua L.	Basin of Dniester river, Black Sea Coast	Settlements, gardens
A. tournefortiana Rchb.	Black Sea East Coast, Podniprovya	Settlements, gardens, near the roads
Division Absinthium		
A. caucasica Willd. (*A. lanulosa* Klok.)	Black Sea Coast, Steppe, Crimea	Steppe hills and lowlands
A. argentata Klok.	Donetsk region (uncommonly)	Cretaceous slopes
A. austriaca Jacq.	Basin of Dnipro river, Basin of Dniester river, Basin of Donets river, Black Sea Coast	Salted meadows, sands, steppes, settlements
A. hololeuca Bieb. ex Bess.	Basin of Dnipro river, Basin of Donets river,	Cretaceous slopes
A. absinthium L.	Basin of Dnipro river, Basin of Donets river, Basin of Dniester river, Bessarabia, Black Sea Coast, Crimea	Bows, pastures, forest lawns
A. sieversiana Willd.	Kyiv region	Introduced plant
Subgenus Dracunculus Bess.		
Division Dracunculus Bess.		
A. dracunculus L.	Basin of Dnipro river, Basin of Donets river, Black Sea Coast, Crimea	Salt meadows, meadow and steppe slopes
Division Salsoloides Leonova		
A. salsoloides Willd.	Black Sea Coast, Left Bank Steppe	Cretaceous slopes
Division Campestres Korobkov		
A. arenaria DC.	Black Sea Coast, Bessarabia, Steppe	Sands, seasides
A. tschernieviana Bess.	Basin of Dnipro river, Steppe	River sands

Закінчення таблиці 2

A. campestris L.	Basin of Dnipro river, Basin of Dniester river, Bessarabia, Steppe, Crimea, Black Sea Coast	Steppe bows, sandy steppes, pine forests, river valleys
A. marschalliana Spreng.	Basin of Dnipro river, Bessarabia, Steppe, Crimea, Black Sea Coast	Steppe bows, sandy steppes, pine forests, river valleys
A. scoparia W.	Basin of Dnipro river, Basin of Dniester river, Basin of Donets river, Black Sea Coast, Crimea	Steppe saline meadows, sands, river valleys
A. trautvetteriana Bess.	Black Sea Coast, (uncommonly)	Seaside sands
Subgenus Seriphidium Rouy		
Division Seriphidium		
A. nutans Willd.	Bessarabia Black Sea Coast, Crimea Basin of Don river	River terraces, ravines, salt marshes and saline soils, carbonate rocks
A. santonica L.	Steppe, Crimea	Salt marshes, salted meadows
A. taurica Willd.	Crimea	Clay salted soils
A. lerchiana Web. ex Stechm.	Crimea (uncommonly)	Steppe rocky slopes
A. dzevanovskyi Leonova	Crimea (uncommonly)	Cretaceous slopes

Differences in the morphological features of the most common species of *Artemisia* L. genus plants are presented in Table 3.

Herb and leaves of *A. absinthium* L. are used as officinal raw material. The herb is harvested at the beginning of flowering (July – August), cutting tips with the length of 20–25 cm, without rough bases of the stems. In case of delay with harvesting, the herb becomes dark gray when it is dry, and the baskets are drilled and scattered. Harvesting should be carried out at the beginning of the plant's flowering. Usually it lasts from 10 to 15 days[5].

Industrial harvesting is possible in Khmelnytsky, Vinnytsia, Kyiv, Cherkassy, Poltava, Kharkiv, Kirovograd, Dnipropetrovsk, Luhansk, Odessa, Kherson and Mykolaiv regions.

[5] Правила сбора и сушки лекарственных растений (сборник инструкций). М.: Медицина, 1985. 328 с.

Table 3

Basic distinctive morphological features of *Artemisia* L. genus species of Ukrainian flora

The main distinctive morphological features	*Artemisia* L. genus plants				
	***A. absinthium* L.**	***A. austriaca* Jacq.**	***A. vulgaris* L.**	***A. dracunculus* L.**	***A. campestris L.***
Taste	Very bitter	Very bitter	Slightly bitter	Slightly bitter	Slightly bitter
Smell	Strong, fragrant	Strong, peculiar	Weak	The smell of the fresh raw material is weak and pleasant, which intensifies after drying	Weak
Flower	Yellow, diameter up to 4 mm	Red-yellow, diameter up to 1,5 mm	Reddish, diameter 2–3 mm	Pale yellow, diameter up to 4 mm	Yellow or reddish
Leaf	Silver-gray, large (up to 20 cm in length)	Almost white, small (up to 3 cm in length)	Top green, bottom white (10 cm in length)	Pieceless, elongated-lanceolate	Young ones are covered with silky trichomes, old – are nake
Stem	Height 50–100 cm	Height 20–50 cm	Height up to 200 cm	Height 40–150 cm	Height 30–60 cm

According to the requirements of the SPU, plant raw material of *A. absinthium* L. are basal leafs, or pale-flowered flowering tops, or a mixture of these dried parts of *A. absinthium* L.[6]

Identification. A. Leaves are grayish or greenish, densely pubescent on both surfaces. The bottom leaves are long-limbed, with a triangular or oval double or thirty pinnate plate, with rounded or lanceolate segments. Stem leaves are less dismembered, the upper leaves are lanceolate. The stalk in the peduncle is greenish-gray, felted, about 2,5 mm in diameter, and usually

[6] Державна Фармакопея України: в 3 т. 2-е вид. Х.: ДП «Український науковий фармакопейний центр якості лікарських засобів», 2014. Т. 3. 328 с.

with 5 longitudinal flattened grooves. Baskets are collected in not tight panicles, developing in the axils of lanceolate or slightly pinnately dissected leaves. Baskets are spherical or flattened-semispherical, 2–4 mm in diameter. They consist of a gray felt wrap, the prickets of its outer row are linear, and the inner ones are oval, with a blunt apex and a thin filthy edge. The bases of baskets have the very long scales, about 1 mm long or sometimes longer, with numerous yellow, tubular, bipartite flowers about 2 mm in length and several yellow border unreal-tongue flowers.

B. Microscopic study (2.8.23). Powder is greenish-gray. The microscopy is provided with a help of a *chloral hydrate solution R*. The powder shows the following diagnostic characters (Fig. 1): numerous T-shaped covering trichomes [A] consisting of a short perpendicular single-row base with 1–5 small cells and very long, wavy apical cage with pointed ends; fragments of the epidermis (top view [D]) consist of the cells with the winding or wavy membranes, the anomocyte stomatas (2.8.3) [Da], dermal hairs [Db] and essential oil glands with oil [Ds] or without oil [Dd], each of them has a short two-row 2-cell base and a double-row head with 2–4 cells; isolated essential oil glands (side view [C]); fragments of corolla tubular and marginal flowers, some with small drosses of calcium oxalate [H]; numerous flower scales, each of which consists of small cells of the base and a very long cylindrical thin-walled end-cell of about 1–1,5 mm in length, more often their distal parts [B, E]; spherical pollen grains, about 30 microns in diameter, with 3 pores and finely-edible exine [G]; fragments of leaf vascular tissue [F] or stem [J] from vessels with spiral or cellular thickening [Fa] or vessels with fringed pores [Ja], fibers [Fb, Jb] and parenchyma cells with moderately thickened and porous membranes [Fs, Js][7].

Tests of *A. absinthium* L. herb were performed according to the following indicators:

Foreign matter (2.8.2). Maximum 5% of stems larger than 4 mm in diameter; not more than 2% of other foreign matter.

Indicator of bitterness (2.8.15). Minimum 10 000.

Loss on drying (2.2.32). Maximum 10.0%. 1,000 g powdered raw material (355) (2.9.12) by drying in an over at 105°C for 2 h.

Total ash (2.4.16). Maximum 12.0%.

Ash insoluble in hydrochloric acid (2.8.1). Maximum 1,0%.

[7] Державна Фармакопея України: в 3 т. 2-е вид. Х.: ДП «Український науковий фармакопейний центр якості лікарських засобів», 2014. Т. 3. 328 с.

A. absinthium L. herb must withstand the above requirements with such changes.

Identification. **B.** Essential oil glands are visible on fragments of a leaf plate as light spots with small increase of the microscope. With a large increase of the microscope, the essential oil glands occur sometimes along the edge of the fragments of the leaf plate or the ovary. They are deformed after the drying of the raw material and only the cuticle that surround the crumpled shells of the secretory cells is visible.

It is allowed to use raw materials with such standardization.

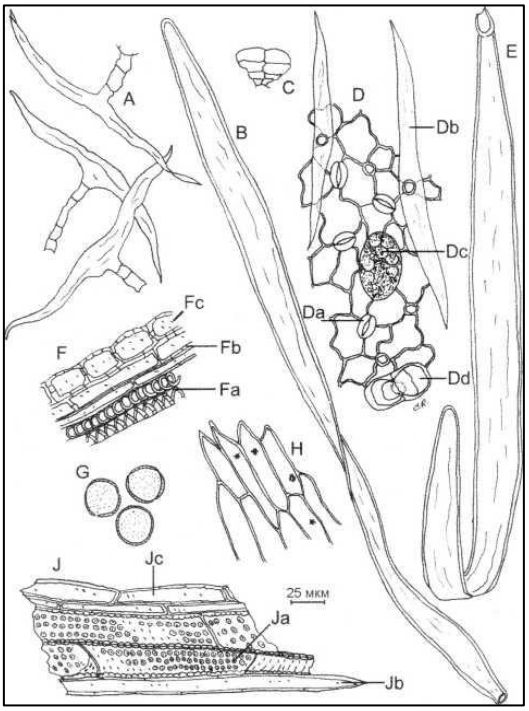


Figure 1. Diagnostic structures of *A. absinthium L.* (identification B)

Foreign matter (2.8.2). Maximum 3% of discoloured pieces; maximum 3% of stems greater than 3 mm in diameter; maximum 3,5% of foreign particles, including maximum 1,5% of impurities of mineral foreign matter.

Loss on drying (2.2.32). Maximum 13%. 1,000 g powdered raw material (355) (2.9.12) by drying in an over at 105°C for 2 h.

Raw materials of *A. absinthium* L. are stem apexes with leaves and flowers (*Herba Artemisiae vulgaris*) and roots (*Radix Artemisiae vulgaris*). The apexes of the stems, which are cutted at the beginning of the plant

flowering, bind to the beams, and suspended to dry in the shade or in the ventilated room. The yield of air-dry raw materials is 23–24%.

The roots should be dugged in the autumn, succulent lateral parts should be chiped, the ground should be carefully scraped with the flaps (do not wash them!) and quickly dried (possibly under the sun). The yield of air-dry raw materials is 20%.

Loss on drying. Maximum 13%; discoloured pieces maximum 4%; stems with a diameter of more than 5 mm maximum 10%; parts passing through a sieve with a diameter of holes 1 mm maximum 3%; organic foreign matter maximum 2%; mineral – maximum 1,5%.

The prepared raw material is stored in well-sealed cans or tins. Expiration date – 3 years. Pharmacies do not release raw materials[8].

2. Chemical composition of Artemisia genus plants

A. absinthium L. raw material contains essential oil (0,5–2%), flavonoids, tannins, lignans, organic acids, carotene and vitamin C. Essential oil includes bicyclic monoterpenoids: pinene, cadinene, ketone tuyon and tuyil alcohol; sesquiterpenoids: phellandrene, *β*-caryophyllene, *γ*-sepinene, sesquiterpene alcohols – absinthin, anabsinthin and artabsinthin, sesquiterpene and monocyclic lactones. The basic flavonoids of *A. absinthium* L. are isoquercitrin, artemisetin, artemisin, isorhamnetin, narcisin[9].

The State Pharmacopoeia of Ukraine, the 2nd edition standardizes *A. absinthium* L. herb according to the content of essential oils – minimum 2 mL/kg of essential oil (dried drug)[10].

---

[8] Державна Фармакопея України: в 3 т. 2-е вид. Х.: ДП «Український науковий фармакопейний центр якості лікарських засобів», 2014. Т. 3. 328 с.

[9] Растительные ресурсы СССР: цветковые растения, их химический состав, использование. Семейство *Asteraceae (Compositae)* / под ред. П.Д. Соколова. СПб.: Наука, 1993. 352 с.

Люй Годун. Фармакогностическое изучение отдельных представителей рода *Artemisia* L.: автореф. дисс. … канд. фармац. наук: 14.04.02; ГОУ ВПО «Московская медицинская академия им. И.М. Сеченова». М., 2011. 24 с.

Беленовская Л.М. Сесквитерпеновые лактоны некоторых видов рода *Artemisia* L. *Растительные ресурсы*. 2000. Т. 36. Вып. 1. С. 43–45.

Akhmedov I.S., Kasymov S.Z., Sidyakin G.P. A study of the composition of Artemisia absinthium. *Chemistry of natural compounds*. 1969. № 5(1). P. 50.

[10] Державна Фармакопея України: в 3 т. 2-е вид. Х.: ДП «Український науковий фармакопейний центр якості лікарських засобів», 2014. Т. 3. 328 с.

Identification. C. Thin-layer chromatography (2.2.27).

Test solution. Place 2 g of the powdered drug (355) (2.9.12) in 50 mL of boiling *water R* and allow to stand for 5 min, shaking the flask several times. After cooling, add 5 mL of a 100 g/L solution of *lead acetate R*. Mix and filter. Rinse the flask and the residue on the filter with 20 mL of *water R*. Shake the filter with 50 mL of *methylene chloride R*. Separate the organic layer, dry over anhydrous *sodium sulfate R*, filter and evaporate the filtrate to dryness on a water-bath. Dissolve the residue in 0,5 mL of *ethanol* (96%) *R*.

Reference solution. Dissolve 2 mg of *methyl red R* and 2 mg of *resorcinol R* in 10,0 mL of *methanol R*.

Plate: TLC *silica gel plate R*.

Mobile phase: *acetone R, glacial acetic acid R, toluene R, methylene chloride R* (10:10:30:50).

Application: 10 µL, as bands.

Development: over a path of 15 cm.

Drying: in air.

Detection A: spray with *acetic anhydride-sulfuric acid solution R* and examine in daylight.

Results A: the chromatogram obtained with the test solution shows a blue zone due to artabsin shortly above a red zone due to methyl red in the chromatogram obtained with the reference solution.

Detection B: examine in daylight while heating at 100–105°C for 5 min.

Results B: the chromatogram obtained with the reference solution shows in the middle third a red zone due to methyl red and below it a light pink zone due to resorcinol. The chromatogram obtained with the test solution shows an intense red or brownish-red zone due to absinthin with a similar RF value to that of the zone due to resorcinol in the chromatogram obtained with the reference solution. Other zones are visible, but less intense than that due to absinthin.

Quantitative determination

Essential oil (2.8.12). Use 50,0 g of the cut drug, a 1 000 mL round-bottomed flask and 500 mL of *water R* as the distillation liquid. Add 0,5 mL of *xylene R* in the graduated tube. Distil at a rate of 2–3 mL/min for not less than 3 h.

It is allowed to applied identification C of above methodology with such changes.

C. Thin-layer chromatography (2.2.27).

Mobile phase: acetone R – methylene chloride R (10:90).

Development: over a path of 10 cm.

Detection A: spray with *anisaldehyde solution R* and examine in daylight.

Results A: on the chromatogram of the test solution is the blue-violet zone, due to artabsins, almost at the level of the red zone due tothe methyl red on the chromatogram of the reference solution.

Detection B: viewed in daylight after heating at a temperature from 100°C to 105°C for 5 minutes.

Results B: on the chromatogram of the reference solution in the middle third there is a red zone due to methyl red, and below it is the yellow-orange zone due to resorcinol. The chromatogram of the test solution reveals an intense, brownish-red zone of absintine, which almost due to the resorcinol zone on the chromatogram of the reference solution by the value of R_f. Other visible zones are less intense than the area due to absinthin.

It is allowed to use raw materials with such standardization.

Content: Cut raw material: minimum 1,5 mL/kg of essential oil (dried drug).

Recommended Test.

Extractive substances: minimum 20%.

1,0 g (exact weighting) of powdered raw material (500) (2.9.12) is placed to a conical flask, 50 ml of *ethanol* (70%, v/v) *R* is added, the flask is sealed, weighed (accurately ± 0,01 g) stand for 1 hour, boil with reflux for 2 h and cool. The flask is covered with the same stopper, weighed, ethanol (70%, v/v) *R* is added to the initial mass, stirred and filtered. 25,0 ml of the resulting filtrate is evaporated in a water bath dry and dried at 100°C to 105°C to constant weight.

By the HPLC method in the *Artemisia absinthium* herb phenolic compounds were determined: hydroxycinnamic acids – chlorogenic, neochlorogenic, 4-*caffeoyl* quinic, 3,4-di*caffeoyl* quinic, 4,5-di*caffeoyl* quinic, 3,5-di*caffeoyl* quinic[11].

Artemisia absinthium contains proteinogenic amino acids; among the essential ones arginine, leucine and tyrosine are dominant; among the non-

[11] Очкур А.В., Чуксина А.Н., Ковалева А.М., Колесник Я.С. Органические кислоты экстрактов травы полыни песчаной (*Artemisia arenaria* DC.). *Gisap. Medical science, pharmacology*. 2014. № 3. P. 50–52.

Очкур О.В. Фармакогностичне дослідження видів роду Artemisia L. флори України: автореф. дис. … канд. фармац. наук: 15.00.02; НФаУ. Х., 2014. 25 с.

essential prolin, aspartic acid (with asparagine), glutamic acid (with glutamine) predominate[12].

Among the mineral elements of the *Artemisia absinthium* herb Potassium predominates (over 1 000 mg/100 g); a high concentration of Calcium, Silicon, Phosphorus and Magnesium – from 100 to 1 000 mg/100 g was determined; the content of Sodium, Aluminum and Manganese in the raw material is from 10 to 100 mg/100 g.

A. vulgaris L. herb contains essential oil (0,1–0,6%), alkaloids, carotene, ascorbic acid (in a leaf up to 175 mg per cent), and vitamins of group B, routine, tannins, bitter sesquiterpene lactones (tauremizin and others), mucous and resinous substances. The composition of the essential oil of the herb includes cineol, tuyon, borneol, camphor, paraffin and aldehydes. In the roots there are mucous membranes, resinous and tannic substances, inulin and essential oils, which include dihydroimagnetic ether and ketone[13].

---

[12] Люй Годун. Фармакогностическое изучение отдельных представителей рода *Artemisia* L.: автореф. дисс. … канд. фармац. наук: 14.04.02; ГОУ ВПО «Московская медицинская академия им. И.М. Сеченова». М., 2011. 24 с.

Derwich E., Benziane Z., Boukir A. Chemical compositions and insecticidal activity of essential oils of three plants *Artemisia sp: Artemisia herba-alba, Artemisia absinthiumand, Artemisia Pontica* (Moroccо). *Elect. J. Environ. Agric. Food Chem.* 2009. № 8. P. 1202–1211.

Очкур О.В., Ковальова А.М., Мороз А.О. та ін. Компонентний склад ефірних олій видів роду *Artemisia* L. підроду *Seriphidium* Rouy флори України. *Актуальні питання створення нових лікарських засобів: матеріали Всеукр. наук.-практ. конф. студентів та молодих вчених*. Х.: НФаУ, 2012. С. 99.

Sharopov F.S., Sulaimonova V.A., Setzer W.N. Composition of the Essential oil of the *Artemisia absinthium* from Tajikistan. *Rec. Nat. Prod*. 2011. № 6(2). P. 128–134.

Очкур О.В., Першко О.А., Шестак О.І. та ін. Компонентний склад ефірних олій *Artemisia absinthium* L. та *Artemisia vulgaris* L. *Актуальні питання створення нових лікарських засобів: зб. тез доп. Міжвуз. студентської наук.-практ. конф*. Х.: НФаУ, 2010. С. 87.

Гречана О.В., Мазулін О.В., Сур С.В. та ін. Фітохімічне вивчення ефірної олії полину гіркого. *Фармацевтичний журнал*. 2006. № 2. С. 82–86.

[13] Люй Годун. Фармакогностическое изучение отдельных представителей рода *Artemisia* L.: автореф. дисс. … канд. фармац. наук: 14.04.02; ГОУ ВПО «Московская медицинская академия им. И.М. Сеченова». М., 2011. 24 с.

Очкур О.В., Першко О.А., Шестак О.І. та ін. Компонентний склад ефірних олій *Artemisia absinthium* L. та *Artemisia vulgaris* L. *Актуальні питання створення нових лікарських засобів: зб. тез доп. Міжвуз. студентської наук.-практ. конф*. Х.: НФаУ, 2010. С. 87.

Халилова А.З., Шакуров Э.Р., Нуриев И.Ф. и др. Выделение и идентификация летучих органических соединений, выделяемых растениями рода *Artemisia* L. *Химия и технология растительных веществ: матер. II Всеросс. конф*. (24–27 июня 2002 г.). Казань, 2002. С. 55–56.

A. austriaca Jacq. herb contains essential oil (0,3–0,4%), alkaloids, organic acids, carotene, vitamin C and resins. The composition of essential oil includes cineol, keton tuyon, sesquiterpene alcohol absinthin and tuyol[14].

Ісакова Т.І., Ковальова А.М., Очкур О.В. та ін. Особливості складу ефірних олій деяких видів полину флори України. Повідомлення 1: монотерпеноїди ефірних олій полину гіркого та полину звичайного. *Український біофармацевтичний журнал*. 2010. № 1. С. 62–68.

Ісакова Т.І., Ковальова А.М., Очкур О.В. та ін. Особливості складу ефірних олій деяких видів полину флори України. Повідомлення 2: сесквітерпеноїди та ароматичні сполуки ефірних олій полину гіркого та полину звичайного. *Український біофармацевтичний журнал*. 2010. № 2. С. 54–60.

Очкур А.В., Ковалева А.М. Жирные кислоты травы *Artemisia vulgaris* L. и *Artemisia austriaca* Jacq. *Химия и технология растительных веществ: тез. докладов VIII Всеросс. науч. конф*. Сыктывкар; Калининград, 2013. С. 172.

Ханина М.А., Хан В.А., Березовская Т.П. Терпеноиды эфирного масла *Artemisia vulgaris*. *Химия природных соединений*. 1987. № 3. С. 457.

Ковальова А.М., Очкур О.В., Колісник Я.С. та ін. Хромато-мас-спектрометричне дослідження ліпофільних сполук екстрактів полину звичайного. *Український медичний альманах*. 2012. Т. 15. № 5. С. 126–128.

[14] Люй Годун. Фармакогностическое изучение отдельных представителей рода *Artemisia* L.: автореф. дисс. … канд. фармац. наук: 14.04.02; ГОУ ВПО «Московская медицинская академия им. И.М. Сеченова». М., 2011. 24 с.

Гречана О.В. Фармакогностичне дослідження азуленвміщуючих рослин роду *Artemisia* L. флори України з метою одержання лікарських засобів протизапальної дії: автореф. дис. … канд. фармац. наук: 15.00.02; НФаУ. Х., 2008. 17 с.

Коновалов Ю.Б. Фармакогностическое изучение полыни австрийской как источника фармакологически активных сесквитерпеновых лактонов: автореф. дисс. … канд. фармац. наук: 15.00.02; ГОУ ВПО «Пятигорская государственная фармацевтическая академия Федерального агентства по здравоохранению и социальному развитию». Пятигорск, 2007. 22 с.

Очкур О.В., Вальдовський А.О., Капкан В.П. та ін. Фітохімічне дослідження трави *Artemisia austriaca* Jacq. *Актуальні питання створення нових лікарських засобів: тези доп. Міжвуз. студент. наук.-практ. конф*. Х.: НФаУ, 2010. С. 88.

Великородов А.В., Морозова Л.В., Пилипенко В.П. и др. Химический состав эфирного масла четырех эндемичных видов полыни Астраханской области: *Artemisia lerchiana, Artemisia santonica, Artemisia arenaria* и *Artemisia austriaca*. *Химия растительного сырья*. 2011. № 4. С. 115–120.

Очкур О.В., Ковальова А.М., Ісакова Т.І. Компонентний склад ефірної олії полину однорічного. *Фармація України: погляд у майбутнє: матер. VII Національного з'їзду фармацевтів України*. Х.: НФаУ, 2010. Т. 1. С. 318.

Ходаков Г.В., Котиков И.В. Компонентный состав эфирного масла *Artemisia annua* и *A. scoparia*. *Химия природных соединений*. 2009. № 6. С. 759–761.

Verdian-rizi M. Variation in the essential oil composition of *Artemisia annua* L. of different growth stages cultivated in Iran. *Afr. J. of Plant Sci*. 2008. Vol. 2(2). P. 16–18.

Очкур О.В., Белоуши Ж., Півень Т.В. та ін. Фітохімічне дослідження трави *Artemisia dracunculus* L. *Актуальні питання створення нових лікарських засобів: тези доп. Всеукр. наук.-практ. конф. студентів та молодих вчених*. Х., 2011. С. 101.

A. annua L. herb contains essential oil (0,18–0,62%), which includes 1,8-cineol, artemisia ketone, myrcene, camphor, pinene, *β*-farnesene, terpinene-4-ol, *α*-terpineol, *δ*-cadinene, linalool, sabinene, caryophyllene oxide, thymol. Characteristic for this species are sesquiterpenenoids arteannuin (artemisinin), artemisinol, artemisinic acid[15].

A. dracunculus L. herb contains essential oil (0,1–0,72%), carotene (15 mg per cent), ascorbic acid (1 190 mg per cent) and bitter and tannic substances. In the essence of essential oil are sabinene, myrcene, methyl, estragole, etc.[16]

There are several basic chemotypes for the *A. dracunculus* L., which differ in their chemical composition and the yield of essential oils. German *A. dracunculus* L. contains about 35% of sabinene and more than 25% of methyl eugenol, French *A. dracunculus* L. contains 80–90% of methyl

---

Ходаков Г.В., Котиков И.В., Панковецкий В.Н. Компонентный состав эфирного масла *Artemisia abrotanum* и *A. dracunculus*. *Химия природных соединений*. 2009. № 6. С. 755–758.

Aglarova A.M., Zilfikarov I.N., Severtseva O.V. Biological characteristics and useful properties of tarragon (*Artemisia dracunculus*). *Pharmaceutical Chemistry J.* 2008. № 42. P. 81–86.

Новруз А.А., Нариман А.Н., Искендер А.М. и др. Компонентный состав и фумигантная активность эфирных масел видов рода *Artemisia* L. *Химия растительного сырья*. 2017. № 4. С. 235–240.

[15] Люй Годун. Фармакогностическое изучение отдельных представителей рода *Artemisia* L.: автореф. дисс. … канд. фармац. наук: 14.04.02; ГОУ ВПО «Московская медицинская академия им. И.М. Сеченова». М., 2011. 24 с.

Очкур О.В., Ковальова А.М., Мороз А.О. та ін. Компонентний склад ефірних олій видів роду *Artemisia* L. підроду *Seriphidium* Rouy флори України. *Актуальні питання створення нових лікарських засобів: матеріали Всеукр. наук.-практ. конф. студентів та молодих вчених*. Х.: НФаУ, 2012. С. 99.

Очкур О.В., Ковальова А.М., Ісакова Т.І. Компонентний склад ефірної олії полину однорічного. *Фармація України: погляд у майбутнє: матер. VII Національного з'їзду фармацевтів України*. Х.: НФаУ, 2010. Т. 1. С. 318.

Ходаков Г.В., Котиков И.В. Компонентный состав эфирного масла *Artemisia annua* и *A. scoparia*. *Химия природных соединений*. 2009. № 6. С. 759–761.

Verdian-rizi M. Variation in the essential oil composition of *Artemisia annua* L. of different growth stages cultivated in Iran. *Afr. J. of Plant Sci*. 2008. Vol. 2(2). P. 16–18.

[16] Очкур О.В., Белоуши Ж., Півень Т.В. та ін. Фітохімічне дослідження трави *Artemisia dracunculus* L. *Актуальні питання створення нових лікарських засобів: тези доп. Всеукр. наук.-практ. конф. студентів та молодих вчених*. Х., 2011. С. 101.

Ходаков Г.В., Котиков И.В., Панковецкий В.Н. Компонентный состав эфирного масла *Artemisia abrotanum* и *A. dracunculus*. *Химия природных соединений*. 2009. № 6. С. 755–758.

Aglarova A.M., Zilfikarov I.N., Severtseva O.V. Biological characteristics and useful properties of tarragon (*Artemisia dracunculus*). *Pharmaceutical Chemistry J.* 2008. № 42. P. 81–86.

chavicol, Russian (closer to wild-type forms) contains mainly methyl eugenol or elemycine, Japanese – up to 35% of anethole[17].

Separate baskets of *A. Maritima L. S. Str.* contain sesquiterpene lactone santonine (0,2–0,94%), glycosides absintin and anabsinthin, up to 1% essential oil, the main component of which are tuyil, tannins, hamasulene, carotene, vitamin C and B_6, and amber and malic acids[18].

A. santonica L. contains essential oil (about 1%, mainly in baskets), the main components of which are tuyol, 1,8-cyneol, pinene, borneol, camphene. The buds contain santonin, bitter glycosides, resins. The main active ingredient of *A. santonica* L. is santonin, the content of which in raw materials should be minimum 2,5%. There are also flavonoids – derivatives of quercetin, isoformine, apigenin, luteolin; tannins, derivatives of gallic acid, hydroxycinnamic acids, alkaloids, carotene, ascorbic acid[19].

Despite one of the synonyms – "odorless" – *A. campestris* L. herba contains up to 0,5% of essential oil. Its main components are α- and β-foam – about 25%, 1,8-cineole – about 8%, tuion – 4%, tuyil alcohol – 15%, geraniol – 13%[20]. Large amounts of hydroxycinnamic acids and their derivatives, in particular coumaric acid esters; higher aliphatic alcohols. The polyacetylene compounds (dehydrofalkarinone and artemisiacetone) are found in the roots. The above-ground part contain a number of flavonoids-5-hydroxy-3,7,4'-trimethoxiflavone, 5,3', 4'-trihydroxy-6,7-dimethoxyflavone, 5,8,4'-trihydroxy-7-methoxyflavone, 7-methyl amazonadedrine, penoprotein,

[17] Супильникова А.В. Качественный и количественный анализ лекарственного сырья и настойки полыни эстрагон. *Фармация*. 2002. № 3. С. 11–14.

Drobot K.O., Matvieieva N.A., Duplij V.P. et al. Study of Artemisinin and Sugar accumulation in *Artemisia dracunculus* "Hairi" root cultures. *Preparative biochemistry and biotechnology*. 2017. Т. 47. № 8. P. 776–781.

Obolskiy D., Pischel I., Feistel B. et al. *Artemisia dracunculus* L. (Tarragon): A Critical Review of Its Traditional Use, Chemical Composition, Pharmacology, and Safety. *J. of Agricult. and Food Chem*. 2011. № 9. P. 17–45.

[18] Новруз А.А., Нариман А.Н., Искендер А.М. и др. Компонентный состав и фумигантная активность эфирных масел видов рода *Artemisia* L. *Химия растительного сырья*. 2017. № 4. С. 235–240.

[19] Singh A., Sarin R. *Artemisia scoparia* – a new source of artemisinin. *Bangladesh J. Pharmacol.* 2010. № 5. P. 17–20.

Sharma S., Ali M. New compounds from Roots of *Artemisia scoparia. Journal of Herbs, Spices and Medicinal Plants*. 1998. Vol. 5. № 4. P. 77–86.

[20] Куатбеков Н.А., Кедельбаев Б.Ш., Сатаева Ж.И. Моносахариды *Artemisia cina* Berg. *Новые достижения в химии и химической технологии растительного сырья: матер. V Всеросс. конф. с международным участием* (24–26 апреля 2012 г.). Барнаул, 2012. С. 137–138.

pinocembrin, sakurantin, naringenin (5,7,4'-trihydroxyflavanone); sesquiterpene lactones[21].

Separate baskets of *A. cina Berg ex Poljak* contain sesquiterpene lactone santonin (up to 7%), tannins, bitter and color substances, malic and acetic acids, betaine and choline. Baskets of *A. cina Berg ex Poljak* are the source of lactone santonin. There is found also found essential oil (up to 3%), which consists of cineol (70–80%), camphor, corvacrol, terpineol, sesquiartemisol. The content of santonin in buds is up to 7%, in the apex of the stems – up to 5,4%. In inflorescences of *A. cina Berg ex Poljak* macro- and trace elements were found. The raw material concentrates Sr, Se, Ni[22].

The basic components of the essential oil of the *A. scoparia* W. are eugenol (about 20%), isoeugenol, methyl eugenol, α-pinene, 1,8-cineol, carvone, tuyon, capilene. Hydroxycinnamic acids (chlorogenic), hydroxycoumarins (6,7-dimethoxy-coumarin, esculental 7-methyl ether, scopoletin), flavonoids (7-methyl aromadendrine, rhamnocetrin, eupalitin, eupatolitin, cirsimaritin, routine, etc.) are also known[23].

---

[21] Супильникова А.В. Качественный и количественный анализ лекарственного сырья и настойки полыни эстрагон. *Фармация*. 2002. № 3. С. 11–14.

Очкур О.В., Ковальова А.М., Коваленко Я.Г. та ін. Фітохімічне дослідження трави *Artemisia campestris* L. *Актуальні питання створення нових лікарських засобів: матер. Всеукр. наук.-практ. конф. студентів та молодих вчених*. Х.: НФаУ, 2012. С. 98.

Новруз А.А., Нариман А.Н., Искендер А.М. и др. Компонентный состав и фумигантная активность эфирных масел видов рода *Artemisia* L. *Химия растительного сырья*. 2017. № 4. С. 235–240.

Люй Годун. Фармакогностическое изучение отдельных представителей рода *Artemisia* L.: автореф. дисс. … канд. фармац. наук: 14.04.02; ГОУ ВПО «Московская медицинская академия им. И.М. Сеченова». М., 2011. 24 с.

[22] Растительные ресурсы СССР: цветковые растения, их химический состав, использование. Семейство *Asteraceae (Compositae)* / под ред. П.Д. Соколова. СПб.: Наука, 1993. 352 с.

Куатбеков Н.А., Кедельбаев Б.Ш., Сатаева Ж.И. Моносахариды *Artemisia cina* Berg. *Новые достижения в химии и химической технологии растительного сырья: матер. V Всеросс. конф. с международным участием* (24–26 апреля 2012 г.). Барнаул, 2012. С. 137–138.

Очкур О.В., Вальдовський А.О., Ковальова А.М. та ін. Порівняльне дослідження елементного складу деяких представників роду полин. *Актуальні питання створення нових лікарських засобів: тез доп. Міжвуз. студент. наук.-практ. конф*. Х.: НФаУ, 2010. С. 85.

Ковальова А.М., Очкур О.В., Ісакова Т.І. та ін. Порівняльний аналіз складу ефірних олій деяких видів роду Полин флори України. *Фармаком*. 2010. № 2. С. 35–39.

[23] Singh A., Sarin R. *Artemisia scoparia* – a new source of artemisinin. *Bangladesh J. Pharmacol.* 2010. № 5. P. 17–20.

Sharma S., Ali M. New compounds from Roots of *Artemisia scoparia*. *Journal of Herbs, Spices and Medicinal Plants*. 1998. Vol. 5. № 4. P. 77–86.

The basic compounds of *A. marschalliana* Spreng. essential oil are chrysanthenol, chrysanthene acetate, 1,8-cineol, camphor. In smaller quantities, borneol, pinocarvone, spathulenol, terpinen-4-ol, β-eudesmol were found. In the herb flavonoids are identified – kaemperferol, luteolin, apigenin, luteolin-7-glycoside, routine[24].

The composition of essential oil of *A. arenaria* DC. is studied. However, in general, data on its chemical composition is extremely small[25].

A. taurica Willd. herb while the budding phase contains up to 1,5% of essential oil, the main component of which is sesquiterpenic lactone tauremisin. It exhibits cardiotonic activity and is able to stimulate the central nervous system, as well as sesquiterpenes tauricin, taurine, artemin; flavonoids: axillaroside, nuhenseine[26].

A. balchanorum Krasch. contains about 1,15% of essential oil, the dominant components of which are citral, geraniol and linalool. In the herb of this species sesquiterpenoids (kostunolide, balcanolide, isobalcanolide, hydroxybalcanolide, balchanin), flavonoids are founded[27].

Data on the chemical composition of *A. nutans* Willd. is very small. It is known that the plant contains essential oils, the basic component of which is tuyon, and a series of sesquiterpene lactones, in particular, santonin[28].

---

[24] Растительные ресурсы СССР: цветковые растения, их химический состав, использование. Семейство *Asteraceae (Compositae)* / под ред. П.Д. Соколова. СПб.: Наука, 1993. 352 с.

[25] Великородов А.В., Морозова Л.В., Пилипенко В.П. и др. Химический состав эфирного масла четырех эндемичных видов полыни Астраханской области: *Artemisia lerchiana, Artemisia santonica, Artemisia arenaria* и *Artemisia austriaca*. *Химия растительного сырья*. 2011. № 4. С. 115–120.

Великородов А.В., Морозова Л.В., Щепетова Е.В. Изучение химического состава эфирного масла некоторых дикорастущих видов рода *Artemisia* L. флоры Астраханской области. *Естественные науки*. 2010. № 4(33). С. 160–168.

[26] Ходаков Г.В., Котиков И.В. Компонентный состав эфирного масла *Artemisia taurica*. *Химия природных соединений.* 2008. № 2. С. 205–206.

Танагузова Б.М. Химическое исследование и стандартизация лекарственного сырья эфирномасличных растений Казахстана: автореф. дисс. … канд. фармац. наук: 15.00.02; АО «Науч.-произв. центр «Фитохимия» МОН РК. Караганда, 2007. 25 с.

[27] Клышев Л.К., Алюкина Л.С., Ряховская Т.В. Фенольные соединения полыней Казахстана. Алма-Ата: Наука, 1983. 159 с.

[28] Растительные ресурсы СССР: цветковые растения, их химический состав, использование. Семейство *Asteraceae (Compositae)* / под ред. П.Д. Соколова. СПб.: Наука, 1993. 352 с.

A.M. Kovaleva and co-authors (2009) investigated the terpene compounds of essential oils of *A. vulgaris* L. and A. *absinthium* L. by the chromatic-mass spectrometry method[29].

O.V. Grechana (2008) investigated *A. absinthium* L. of the southeast of Ukraine flora, in particular the *A. absinthium* L., *A. vulgaris* L. and *A. austriaca* Jacq. which contain azulene. O.V. Grechana and O.V. Mazulin (2008) studied the component composition of essential oils of these species by the method of gas-liquid chromatography. They identified: in the *A. absinthium* L. herb – up to 56 compounds, in *A. vulgaris* L. herb – up to 31, in *A. austriaca* Jacq. herb – to 43. The authors identified 14 compounds of flavonoid origin. For the first time, they have identified up to 17 amino acids by the HPLC method. Chlorogenic, neo-chlorogenic and caffeic acids have been isolated and identified in the herb of all these species. In *A. absinthium* L. herba rosmarinic acid is found, in the *A. vulgaris* L. herb – ursolic acid. In the raw materials of these species in the period of mass flowering the content of free catechins, sum of carotenoids, ascorbic acid and 21 inorganic elements was detected[30].

O.V. Grechana (2008) established the maximum concentrations of essential oils and azulenes during the flowering period of the plants. The accumulation of flavonoids in different phases of vegetation has been studied. The bacteriostatic, mycostatic effects of essential oils and anti-inflammatory – of drugs from the herb of *A. absinthium* L. and *A. austriaca* Jacq have been studied[31].

A.M. Kovaleva and O.V. Ochkur (2014) for the first time identified 292 substances on the basis of the results of physical and chemical analysis in 14 species of *Artemisia*: 10 aromatic and 16 aliphatic carboxylic acids,

---

[29] Ковальова А.М., Очкур О.В., Вальдовський А.О. Порівняльне хромато-мас-спектрометричне дослідження терпеноїдних сполук ефірних олій полину звичайного та полину гіркого. *Збірник наукових праць співробітників Національної медичної академії післядипломної освіти імені П.Л. Шупика*. 2009. Вип. 18. Кн. 3. С. 444–448.

[30] Гречана О.В. Фармакогностичне дослідження азуленвміщуючих рослин роду *Artemisia* L. флори України з метою одержання лікарських засобів протизапальної дії: автореф. дис. … канд. фармац. наук: 15.00.02; НФаУ. Х., 2008. 17 с.

Гречаная Е.В., Мазулин А.М., Денисенко О.Н., Зоря Б.П. Фармакогностическое изучение эфиромасличных видов рода *Artemisia* L. флоры юго-востока Украины. *Актуальні питання фармацевтичних та медичних наук та практики: збірник наукових праць*. Запоріжжя, 2003. Вип. Х. С. 27–28.

[31] Гречана О.В. Фармакогностичне дослідження азуленвміщуючих рослин роду *Artemisia* L. флори України з метою одержання лікарських засобів протизапальної дії: автореф. дис. … канд. фармац. наук: 15.00.02; НФаУ. Х., 2008. 17 с.

10 hydroxycholic acids, 5 coumarins, 6 flavonoids, 176 terpenoids and their derivatives, 17 hydrocarbons and their derivatives, 22 amino acids, 28 fatty acids, 2 chlorophylls[32].

A.M. Kovaleva and and co-authors (2013) determined the chemical profile of the studied continuum of the *Artemisia* L. species on the basis of chemotaxonomic study and selected perspective species for further study. The complex phytochemical study of the *A. vulgaris* L. *A. absinthium* L., *A. austriaca* Jacq., *A. dracunculus* L *and A. abrotanum* L. was conducted. The content of ascorbic acid, the sum of organic acids of the raw material and the content of tannins were determined by titrimetric methods; the content of hydroxycholic acids and flavonoids are determined by spectrophotometric method. The highest content of hydroxycinnamic acids was found in the herba of *A. vulgaris* L. (2,13%); The highest content of flavonoids was in the herb of *A. vulgaris* L. and *A. dracunculus* L. (by 1,60%)[33].

3. Pharmacological properties and applications in medicine

Wormwood herb exhibits various pharmacological properties, because of presence of various biologically active compounds, particularly terpenoids and volatile oils, in the medicinal raw material.

---

[32] Очкур А.В., Чуксина А.Н., Ковалева А.М., Колесник Я.С. Органические кислоты экстрактов травы полыни песчаной (*Artemisia arenaria* DC.). *Gisap. Medical science, pharmacology*. 2014. № 3. P. 50–52.

Очкур О.В. Фармакогностичне дослідження видів роду Artemisia L. флори України: автореф. дис. … канд. фармац. наук: 15.00.02; НФаУ. Х., 2014. 25 с.

Очкур А.В., Ковалева А.М. Компонентный состав эфирного масла *Artemisia nutans. Человек и лекарство: сб. матер. XX Росс. нац. конгресса* (15–19 апреля 2013 г.). М., 2013. С. 402.

Очкур А.В., Ковалева А.М. Получение и изучение биологически активных веществ экстрактов травы видов рода полынь. *Биологически активные вещества и материалы: фундаментальные и прикладные вопросы получения и применения: матер. науч.-практ. конф.* (27 мая – 1 июня 2013 г.). Новый Свет, 2013. С. 55–56.

Очкур О.В. Антистафілококова активність екстрактів з трави полину звичайного. *Матеріали XV міжнар. мед. конгресу студентів та молодих вчених*. Тернопіль, 2011. С. 357.

Очкур О.В., Кашпур Н.В., Ковальова А.М. та ін. Патент на корисну модель 104173 Україна, МПК A61K 36/282, A61P 31/00. Спосіб одержання засобу з антимікробною активністю з рослинної сировини. Заявник та патентоутримувач Національний фармацевтичний університет. № a 2011 12754. Заявл. 31.10.11; опубл. 10.01.14, Бюл. № 1.

[33] Онучак Л.А., Куркин В.А., Минахметов Р.А., Куркин А.В. Высокоэффективная жидкостная хроматография в анализе экстрактов *Artemisia dracunculus*. *Химия природных соединений*. 2000. № 2. С. 115–117.

For ages wormwood have been used by different nations for therapeutical and recreational purposes. Ancient Romans were convinced that wormwood juice remains and redoubles the strength and health of runners, so in races the winners were awarded with wormwood juice. It was believed that the traveler should put the wormwood in his shoes and keep the branch of this herb in his hand, would not feel tired, while walking.

Avicenna recommended the wormwood from dizziness, nausea, seasickness, to improve appetite and as an antidote.

In ancient Greece, working in the field, people drank the decoction of wormwood to reduce the harmful effects of direct sunlight. In Kievan Rus, wormwood was valued not only for medicinal, but also for magical properties. The wormwood herb was harvested on the Dormition of the Mother of God (August 28), consecrated in the church and dried. Then they strung up above the door and windows to protect themselves and their home from the evil spirits and various evil forces[34].

In the Middle Ages, it was believed that wormwood improves digestion, warms the body and expels poison and bile. In XVII century in Russia it was used as the remedy for septic wounds and tumors.

Artemisia absinthium is described in pharmacopoeias of most countries of the world[35]. Is used in homeopathy. Wormwood herb is included as a compound of appetite, choleretic and other species.

Galenic preparations of wormwood reflectory stimulate the function of gastrointestinal tract, enhance biliation and distinctly improve digestion. This effect is predicated on increasing of excitability and response of mucous membranes neuroreceptors of gastrointestinal tract to the intake of food products. Terpenoid compounds, containing in plant, exhibit anti-inflammatory effect, stimulate the function of the reticuloendothelial system and phagocytic activity. The wormwood volatile oil is similar to camphor by the pharmacological properties and has cardiotonic activity, innervates the central nervous system.

---

[34] Мінарченко В.М., Тимченко І.А. Атлас лікарських рослин України. К., 2002. С. 15, 63, 97, 127, 142, 155.

Лікарські рослини: енциклопедичний довідник / за ред. А.М. Гродзінського. К.: Гол. редакція УРЕ, 1990. С. 430–432.

[35] Державна Фармакопея України: в 3 т. 2-е вид. Х.: ДП «Український науковий фармакопейний центр якості лікарських засобів», 2014. Т. 3. 328 с.

British Pharmacopoeia. London: Stationery Office Books HMSO, 2001. Vol. 1. 1389 p.

There were reports of the bactericidal and fungicidal properties of unsaturated carbohydrates (capelin), isolated from wormwood[36].

The experiment proved that wormwood bitter principle stimulate the function of digestive glands, increase the secretion of bile, pancreatic and gastric juice. The effect of wormwood volatile oil is similar to camphor by the stimulating action on the central nervous system[37].

In folk medicine, flowering shoots are used for stomach diseases, dysentery, pulmonary tuberculosis, kidney and liver diseases, headaches, worms, coughs, obesity, inflammation of the cecum, yellow fever, edema, scurvy, malaria, gout, paralysis and scrofula. Wormwood is considered to be the remedy for treating anemia, hemorrhoids, intestinal diseases. It is externally used for maim, tumors, corns and wound irrigation[38].

The essence of fresh young leaves is used in homeopathy; in veterinary practice wormwood decoction is used for treating digestive disorders,

---

[36] Ковальова А.М., Ільїна Т.В., Кашпур Н.В. та ін. Біологічно активні речовини ліпофільних фракцій з трави видів родів *Artemisia* L., *Potentilla* L., *Melilotus* L. і дослідження їх протимікробної активності. *Биологически активные вещества: фундаментальные и прикладные вопросы получения и применения*: матер. науч.-практ. конф. (Новый Свет, 23–28 мая 2011 г.). Новый Свет, 2011. С. 263–264.

Кашпур Н.В., Волянський А.Ю., Осолодченко Т.П. та ін. Визначення антибактеріальної дії активних речовин роду *Artemisia*. *Теоретична і експериментальна медицина*. 2010. № 4. С. 41–44.

Очкур О.В., Кашпур Н.В., Ковальова А.М. та ін. Вплив ліпофільних фракцій видів роду *Artemisia* L. на адгезивні властивості мікроорганізмів. *Актуальні питання фармацевтичної і медичної науки та практики*. 2011. Вип. XXIV. № 2. С. 33–36.

Очкур О.В., Кашпур Н.В., Ковальова А.М. та ін. Вплив ліпофільних фракцій деяких видів роду *Artemisia* L. на протилізоцимні властивості мікроорганізмів. *Клінічна фармація*. 2011. Т. 15. № 3. С. 44–47.

Wani H., Shah S.A., Banday J.A. Chemical composition and antioxidant activity of the leaf essential oil of *Artemisia absinthium* growing wild in Kashmir. *The journal of phytopharmacology*. 2014. № 3(2). P. 90–94.

Taraghdari S.B., Mazidi M., Kamgar M. et al. The effect of hydro-alcoholic extract of *Artemisia absinthium* on appetite in male rats. *Avicenna Journal of Phytomedicine*. 2015. Vol. 5. № 2. P. 78–83.

[37] Wasim A., Azhar H., Ansani A., Tahera T. Medicinal importance of *Artemisia absinthium* Linn (Afsanteen) in Unani Medicine: A revive. *Hippocratic Journal of Unani Medicine*. 2010. Vol. 5. № 4. P. 117–125.

[38] Obolskiy D., Pischel I., Feistel B. et al. *Artemisia dracunculus* L. (Tarragon): A Critical Review of Its Traditional Use, Chemical Composition, Pharmacology, and Safety. *J. of Agricult. and Food Chem*. 2011. № 9. P. 17–45.

Wasim A., Azhar H., Ansani A., Tahera T. Medicinal importance of *Artemisia absinthium* Linn (Afsanteen) in Unani Medicine: A revive. *Hippocratic Journal of Unani Medicine*. 2010. Vol. 5. № 4. P. 117–125.

yellow fever, helminth infestation, washing animals from insect bites and to scare out external parasites[39].

The wormwood is one of the representative of bitters: it stimulates the appetite, reflexively stimulates the activity of digestive tract glands, increases secretion of bile, pancreatic and gastric juice. In moderate doses wormwood has sedative effect, in large doses it increases excitation effects with following inhibition. In addition, wormwood has anti-inflammatory, antiseptic, anti-ulcer and helminthagogic properties. Wormwood is prescribed in the form of soft extract, infusion or tincture as bitter, which improves appetite and stimulates the activity of digestive organs; in case of gastritis with hyper and hypoacidity; diseases of the liver and gall bladder; in all cases of physiological asthenia – general decline of nutrition; after prolonged consumptive diseases; anemia, scrofula and dyspepsia.

Wormwood is a part of the ingredients of bitter tincture *(Tinctura amara)*, gastric tablets *(Tabulettae Stomachicae cum extracto Belladonnae)*, appetizing and choleretic teas. Wormwood is very popular treatment in folk medicine. Except all the above mentioned cases, it is used internally at fever and malaria, tuberculosis and scrofula, diseases of the liver attended by yellow plague, spleen diseases, inflammatory processes in kidneys and bladder, edema, hemorrhoids and cholera (as disinfectant), insomnia, constant dizziness, spasms in the abdomen, asphyxia, at insufficient and irregular menstruation and nocturnal emission. Infusion from mixture of wormwood and wild thyme herb is used for the treatment of alcoholism[40].

Preparations of wormwood have analgetic, antiseptic, anti-inflammatory effect in external use. Dissolved in boiled water in the ratio of 1:10 tincture

---

[39] Мінарченко В.М., Тимченко І.А. Атлас лікарських рослин України. К., 2002. С. 15, 63, 97, 127, 142, 155.

Растительные ресурсы СССР: цветковые растения, их химический состав, использование. Семейство *Asteraceae (Compositae)* / под ред. П.Д. Соколова. СПб.: Наука, 1993. 352 с.

Derwich E., Benziane Z., Boukir A. Chemical compositions and insecticidal activity of essential oils of three plants *Artemisia sp: Artemisia herba-alba, Artemisia absinthiumand, Artemisia Pontica* (Morocco). *Elect. J. Environ. Agric. Food Chem*. 2009. № 8. P. 1202–1211.

[40] Растительные ресурсы СССР: цветковые растения, их химический состав, использование. Семейство *Asteraceae (Compositae)* / под ред. П.Д. Соколова. СПб.: Наука, 1993. 352 с.

Лікарські рослини: енциклопедичний довідник / за ред. А.М. Гродзінського. К.: Гол. редакція УРЕ, 1990. С. 430–432.

Derwich E., Benziane Z., Boukir A. Chemical compositions and insecticidal activity of essential oils of three plants *Artemisia sp: Artemisia herba-alba, Artemisia absinthiumand, Artemisia Pontica* (Morocco). *Elect. J. Environ. Agric. Food Chem*. 2009. № 8. P. 1202–1211.

is used in compresses and soaking therapy at injuries, scratch, wounds, eye diseases and insect bites. Fresh grinding leaves are successfully used in hemorrhages on the body as a result of injuries, dislocations and tendon strain. Wormwood herb infusion is used in the form of enema (after evacuation) with addition of garlic (middle garlic head is boiled in 1 cup infusion). And it is used for deriving pinworms. The enema is repeated several days until worms will disappear completely. Mouthwash with infusion is carried out because of bad breath. Galenic preparations of *Artemisia vulgaris* usually act on the nervous system as a calmative, suppress spasmodic stricture, exhibit lighysedative-hypnotic and sudatory activity, stimulate appetite and regulate the functional activity of the digestive tract, normalize the menstrual cycle, derive some types of worms. The decoction of wormwood herb is indicated in redused appetite, hyposthenic digestion, epilepsy, neurasthenia and other nervous diseases and in nervous insomnia[41].

In obstetrics and gynecology, decoction of wormwood herb is prescribed to amenorrhea and hypomenorrhea of central origin, to toxicosis of pregnancy and as a remedy, accelerating and anaesthetizing childbirth. In absence of menstruation, infusion of herb with root (2 tablespoons of herb and 1 tablespoon, or 15 g of roots on 200 ml of cold water, ingrain 1 day) is consumed for 1 table spoon every 3 hours before the onset of menstruation. If during the week menstruation does not start, the concentration of infusion is increased (take 30 g of herbs and roots). In case of helminth infestation, infusion of mixture of wormwood herb, chamomile flowers and yellow gentian roots is consumed[42].

In folk medicine *Artemisia abrotanum* is widely used in many diseases. In particular: dentalgia (rinsing), colds, rheumatic, gastrointestinal, infectious

---

[41] Растительные ресурсы СССР: цветковые растения, их химический состав, использование. Семейство *Asteraceae (Compositae)* / под ред. П.Д. Соколова. СПб.: Наука, 1993. 352 с.

Derwich E., Benziane Z., Boukir A. Chemical compositions and insecticidal activity of essential oils of three plants *Artemisia sp: Artemisia herba-alba, Artemisia absinthiumand, Artemisia Pontica* (Morocco). *Elect. J. Environ. Agric. Food Chem*. 2009. № 8. P. 1202–1211.

[42] Растительные ресурсы СССР: цветковые растения, их химический состав, использование. Семейство *Asteraceae (Compositae)* / под ред. П.Д. Соколова. СПб.: Наука, 1993. 352 с.

Derwich E., Benziane Z., Boukir A. Chemical compositions and insecticidal activity of essential oils of three plants *Artemisia sp: Artemisia herba-alba, Artemisia absinthiumand, Artemisia Pontica* (Morocco). *Elect. J. Environ. Agric. Food Chem*. 2009. № 8. P. 1202–1211.

and other diseases. Internally drink tea (1 tablespoon of grinded twig on 1–2 cups of boiling water) 2–3 cups per day. It is also used in mixtures with other plants in the case of kidney stones and other kidney diseases, as the remedy, that promotes the formation of bile, including the so-called "hepatic" herb mixes. It is considered as helminthic, analgesic, antispasmodic drug[43].

This plant is especially popular in Poland, used there in official medicine and homeopathy. It stimulates bile formation by the liver and increases the bile reflux to the duodenum. Bitter substances increase the secretion of digestive gastric juices. Herb acts anti-inflammatory, anthelmintic (especially in children).

It is used in liver disease, congestive phenomenon in biliary tract, hypoacidity, attended by abdominal pain, distention, loss of appetite, and belching. Adjunct in inflammation of the mucous membrane of the stomach, intestines[44].

Artemisia absinthium herb is officinal in many countries of Europe and America – Switzerland, Netherlands, France, Brazil, Poland, USA – and is used as appetizing, gastric, sudatory, anticonvulsant, sedative, antihelminthic, antialcoholic and stimulating childbirth drug[45].

Artemisia austriaca is used as the remedy for stimulating secretion of gastric juice and bile, increases diuresis and secretion of sweat, exhibits an antipyretic, hemostatic, anticonvulsant, light sedative-hypnotic and antiemetic effects and has antihelminthic properties.

Most often, infusion of Austrian wormwood is prescribed to improve appetite and digestion, to edema, malaria, helminth infestation, liver and spleen diseases, gout, as well as anticonvulsant and antiemetic remedy.

---

[43] Derwich E., Benziane Z., Boukir A. Chemical compositions and insecticidal activity of essential oils of three plants *Artemisia sp: Artemisia herba-alba, Artemisia absinthiumand, Artemisia Pontica* (Morocco). *Elect. J. Environ. Agric. Food Chem.* 2009. № 8. P. 1202–1211.

[44] Мінарченко В.М., Тимченко І.А. Атлас лікарських рослин України. К., 2002. С. 15, 63, 97, 127, 142, 155.

Растительные ресурсы СССР: цветковые растения, их химический состав, использование. Семейство *Asteraceae (Compositae)* / под ред. П.Д. Соколова. СПб.: Наука, 1993. 352 с.

[45] Растительные ресурсы СССР: цветковые растения, их химический состав, использование. Семейство *Asteraceae (Compositae)* / под ред. П.Д. Соколова. СПб.: Наука, 1993. 352 с.

Люй Годун. Фармакогностическое изучение отдельных представителей рода *Artemisia* L.: автореф. дисс. … канд. фармац. наук: 14.04.02; ГОУ ВПО «Московская медицинская академия им. И.М. Сеченова». М., 2011. 24 с.

Fresh plant herb is applied to the forehead and temples with insomnia. Preparations of Austrian wormwood are contraindicated to pregnancy and obesity in elderly people.

Artemisia dracunculus L. is used as the remedy for stimulating secretion of gastric juice and bile, increases diuresis, exhibits a general tonic effect. Herbal infusion is prescribed to improve appetite and digestion, catarrhal gastritis, specifically to chronic gastritis with reduced acidity of gastric juice, in the case of edema, flatulency and helminth infestation, and as an antiscorbutic agent. Taking medications of tarragon is contraindicated for pregnant women.

Artemisia cina Beerg ex Poljak. anthodium and suntonin, isolated from it, exhibits an anthelmintic effect (against round worms: ascarids and pinworms). In folk medicine flowers of this plant species have long been used against ascariasis and sandworm disease, used in the grinded form in a mixture with jam, honey, syrup, sugar in strictly established doses depending on age. Applies only to the prescription of doctor[46].

An infusion of *Artemisia annua* herb is used as the agent that stimulates appetite. Fresh juice is used to treat scabies[47].

In large doses suntonin causes poisoning; at the same time all the objects appear violet, then green and, finally, yellow colors; common vomiting, diarrhea, xanthopsia, respiratory and cardiac activity suppression, spasmodic stricture. Volatile oil (Darminol) has a strong bactericidal activity, exhibits anti-inflammatory and analgesic effect. It is used to balm as a revellent agent at muscle and joint rheumatic disease, neuralgia and lumbago. For today drugs from *Artemisia cina Beerg ex Poljak.* “Santonin” and “Sancaphen” are approved for use only in veterinary medicine. *A. nutans* Willd. was used as an antihelmintic agent along with *A. cina Beerg ex Poljak.* In XVIII – XIX centuries so-called “russian santonic seeds” were procured specifically from *Artemisia nutans* Willd. Medicinal raw material of *A. santonica* L. is unexpanded anthodium. Like other types of wormwood, this plant species is often used in folk medicine to bronchial asthma, amenorrhea, as detoxifying agent. Juice, infusion and tincture exhibit antihelminthic and antiacidic activity, volatile oil has antibacterial and fungicidal effect. Alcoholic

[46] Растительные ресурсы СССР: цветковые растения, их химический состав, использование. Семейство *Asteraceae (Compositae)* / под ред. П.Д. Соколова. СПб.: Наука, 1993. 352 с.

[47] Флора СССР: в 30 т. / под ред. В.Л. Комарова. М., Л.: Изд-во АН СССР, 1961. Т. 26. С. 642–645.

tincture of dry wormwood herb is used for compresses and balm at injury. In Bulgaria the antihelmintic drug "Santonin" was manufactured on the basis of *A. santonica* L. In veterinary medicine it is used similarly to *A. cina Beerg ex Poljak*.

Decoction of *Artemisia campestris* L. is drinking at uterine hemorrhage, cystitis, abdominal pain and bone ache. It is necessary to use it to wash mouth at a toothache. Ointment, based on fresh cut leaves and rendered pork fat, is used for the treatment of wounds and abscesses, for unction the feet at an excessive sweating.

More often *Artemisia maritima* is used as an agen with antihelminthic properties in the form of infusion or powders from anthodium mixed with honey. Along with this, infusion from anthodium is taken inside to stimulate the activity of the digestive organs and increase appetite, in the case of pulmonary tuberculosis, low and irregular menstruation. Stupes from anthodium of wormwood are applied to injury and areas of skin damage by the cancer, to the corns and warts[48].

Herb infusion of *Artemisia scoparia* exhibits diuretic effect. Wormwood volatile oil also has a laxative effect. It is low toxic. Wormwood volatile oil is included as a compound of complex preparation "Artemisole", which was used to treat patients with urinary stone disease.

In folk medicine *Artemisia scoparia* herb worm is used in the form of alcohol tincture for balm with radiculitis (2,5:10)[49].

Volatile oils, isolated from other species of the genus *Artemisia*, exhibit antibacterial, antifungal, tuberculostatic activity[50]. The chemical

---

[48] Растительные ресурсы СССР: цветковые растения, их химический состав, использование. Семейство *Asteraceae (Compositae)* / под ред. П.Д. Соколова. СПб.: Наука, 1993. 352 с.

Лікарські рослини: енциклопедичний довідник / за ред. А.М. Гродзінського. К.: Гол. редакція УРЕ, 1990. С. 430–432.

[49] Растительные ресурсы СССР: цветковые растения, их химический состав, использование. Семейство *Asteraceae (Compositae)* / под ред. П.Д. Соколова. СПб.: Наука, 1993. 352 с.

Лікарські рослини: енциклопедичний довідник / за ред. А.М. Гродзінського. К.: Гол. редакція УРЕ, 1990. С. 430–432.

[50] Очкур О.В., Мороз А.О., Осьмачко А.П. та ін. Дослідження хлороформного екстракту з трави *Artemisia austriaca* Jacq. *Здобутки та перспективи розвитку фармацевтичної та медичної галузі в сучасному світі: зб. тез доп. II Всеукр. наук.-практ. конф. молодих вчених та студентів*. Луганськ, 2012. С. 100.

Hofling J.F., Anibal P.C., Obando-Pereda G.A. et al. Antimicrobial potential of some plant extracts against Candida Species. *Braz. J. Biol.* 2010. Vol. 70. № 4. P. 1065–1068.

composition and physiological activity of wormwoods are analysed by phytochemicals of different countries.

A.M. Kovaliova and O.V. Ochkur (2011) determined antimicrobial and antifungal activity of the obtained substances of 5 species of the genus *Artemisia* against strains of *Staphylococcus aureus* 15 923, *Escherichia coli* 25 922, *Pseudomonas aeruginosa* 2 789, *Bacillus subtilis* 6 633, *Proteus vulgaris* 4 636, *Candida albicans* 885–563. The correlation relationship between the content of the main biologically active substances in the complexes and the level of their antimicrobial activity has been studied. The cytotoxic activity of the phenolic complex of *Artemisia absinthium* on the cells of Ehrlich's ascite carcinoma has been established. It has been determined that lipophilic and phenolic complexes of *A. absinthium, A. vulgaris, A. abrotanum, A. dracunculus and A. Austriaca* ones belong to the toxicity class V – practically non-toxic substances[51].

Literature data prove that genus *Artemisia* plants are widely spread, exhibit various pharmacological action due to the complex of biologically active substances and widely used in scientific and folk medicine.

Basa S.C. Natural biocoumarins. *Phytochemistry*. 1988. № 7. P. 1933–1941.

Ahameethunisa A.R., Hopper W. Antibacterial activity of *Artemisia nilagirica* leaf extracts against clinical and phytopathogenic bacteria. *Complementary and Alternative Medicine*. 2010. № 10. P. 6.

Baykan Erel S., Reznicek G., Senol S.G. et al. Antimicrobial and antioxidant properties of *Artemisia* L. species from western Anatolia. *Turk. J. Biol.* 2012. № 36. P. 75–84.

Jazani N.H., Zartoshti M., Babazadeh H. et al. Antibacterial Effects of *Artemisia dracunculus* Essential Oil on Multi-drug Resistant Isolates of Acinetobacter baumannii. *Bacteriology J*. 2011. № 1. P. 31–36.

[51] Очкур О.В., Кашпур Н.В., Ковальова А.М. та ін. Вплив ліпофільних фракцій видів роду *Artemisia* L. на адгезивні властивості мікроорганізмів. *Актуальні питання фармацевтичної і медичної науки та практики*. 2011. Вип. XXIV. № 2. С. 33–36.

Очкур О.В., Кашпур Н.В., Ковальова А.М. та ін. Вплив ліпофільних фракцій деяких видів роду *Artemisia* L. на протилізоцимні властивості мікроорганізмів. *Клінічна фармація*. 2011. Т. 15. № 3. С. 44–47.

Ковальова А.М., Очкур О.В., Колеснік Я.С. та ін. Антибактеріальна активність ліпофільних екстрактів трави *Artemisia abrotanum* L. *Проблемы и пути развития современного здравоохранения: сб. матер. XVI Междунар. науч.-практ. конф*. Одесса; Лондон, 2011. С. 137–139.

Очкур О.В., Кашпур Н.В., Волянський А.Ю. та ін. Антикандидозна активність ліпофільних витягів з трави видів роду полин. *Тези доповідей IV Національного з'їзду фармакологів України* (15–17 вересня 2010 р.). Х., 2010. С. 233–234.

Bonyadian M., Karim G. Study of the effects of some volatile oils of herbs against *Escherichia coli* and *Staphylococcus aureus* in broth media. *J. Fac. Vet. Med. Univ. Tehran*. 2002. № 57. P. 81–83.

4. Morphological and anatomic study of species of genus *Artemisia* L.

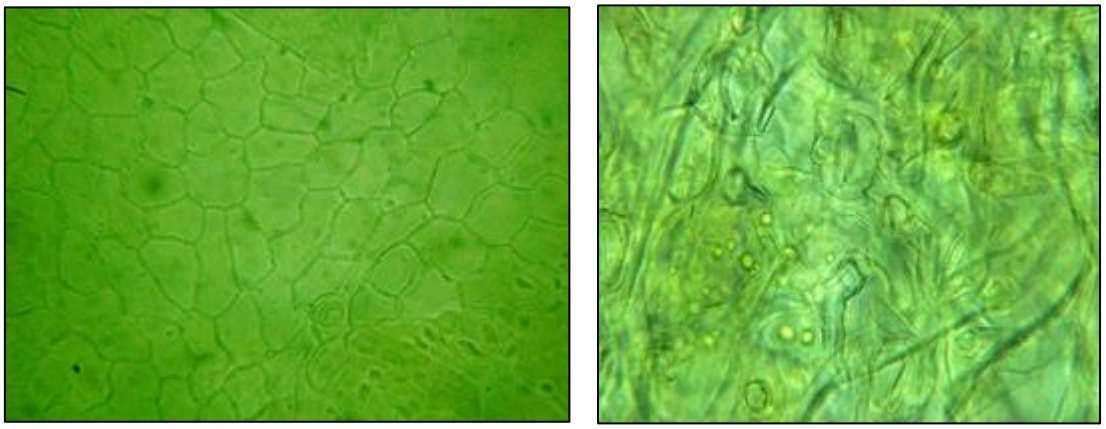
Aimed to identification of the raw material of species of genus *Artemisia* and differentiatiton between other species, that may be impurities, morphological and anatomical study of *A. abrotanum, A. austriaca, A. vulgaris, A. absinthium, A. dracunculus* was conducted in comparison with the pharmacopoeial species *Artemisia absinthium.*

The genus *Artemisia* L. is polymorphous and is characterized by the high level of interspecific hybridization; the significant number of species are identified against each other.

Therefore, appreciate the necessity of identification and standardization of plant material, it is necessary to establish diagnostic morphological and anatomical features of the studied species in comparison with the officinal species *Artemisia absinthium.*

Anatomical diagnostic features of stems, leaves and flowers of the studied species of genus are described in Figure 2–6.

Artemisia absinthium. Upper epidermis. Cells are parenchymal, wavy walled, uniformly thickened. Anomocytic stomata (Fig. 2).



A **B**

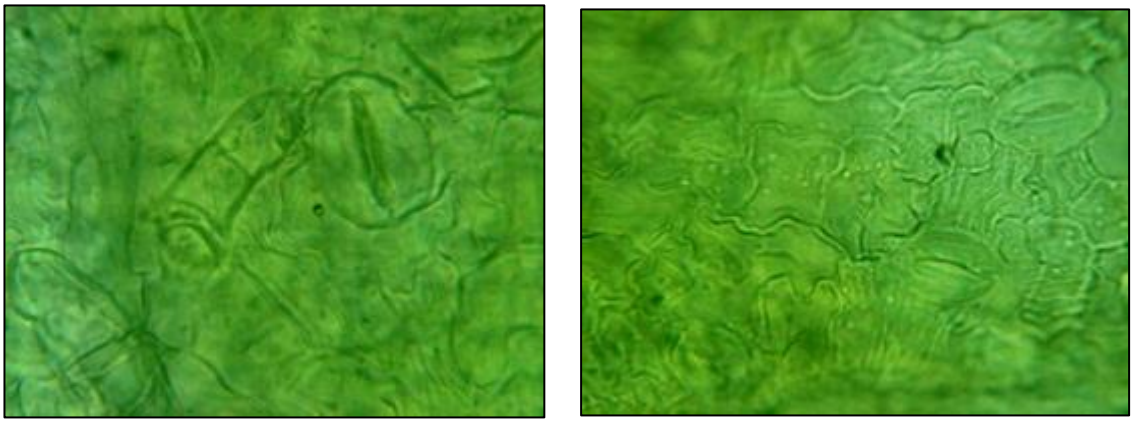
Fig. 2. Anatomical structure of the leaf:
A – upper epidermis; B – lower epidermis

Lower epidermis. Cells with wavy walls. Not much glands, they are small. T-shaped hairs on 2-5-cell stalk form downy pubescence. Two types of hairs: simple – very long, curly, with a narrow cavity, thin walled; T-shaped hairs on a 2-, 3-, 4-, 5-cell stalk and cross cell with long wavy ends.

Stem. Rounded ribbed, 3-5-layer collenchyma is in the edges. Vascular system of fasciculate structure, bundles of collateral type; sclerenchyma is a member of the bundles, well-defined, composed of 12 layers of cells. Phloem consists of the thin layer, located under the sclerenchyma. Xylem with libriform, parenchymal cells and vessels. The medulla cells are thin-walled, varying in size and shape, parenchymal, rounded.

Artemisia abrotanum

Upper epidermis. Cells are parenchymal, deep wavy walled, walls are uniformly thickened. Above the vein the cells are parenchymal prosenchymatous, with slightly wavy walls. Anomocytic stomata (Fig. 3).



A **B**

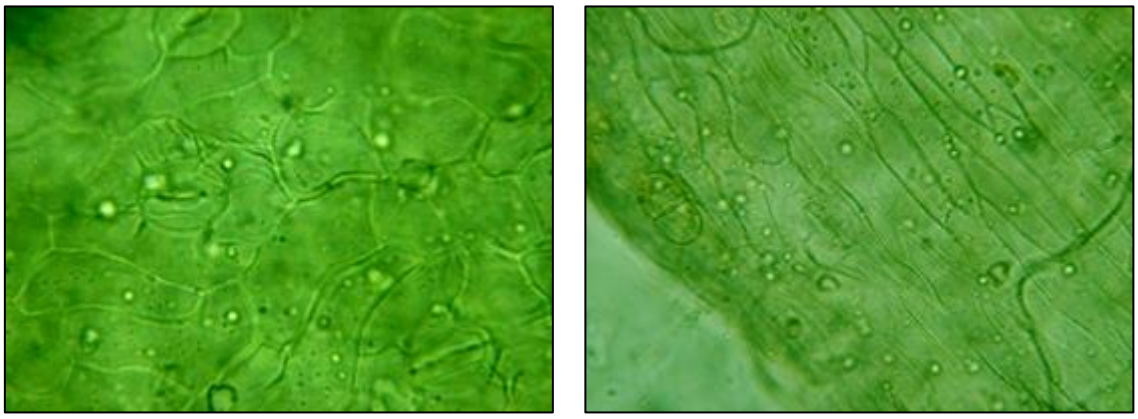
Fig. 3. Anatomical structure of *A. abrotanum* leaf: A – upper epidermis; B – lower epidermis

Lower epidermis. Cells with tightly wavy, almost laciniate walles. A lot of small glands are observed. The hairs are simple multicellular. Apical hair cells are falling away while storage. Rugosity of cuticle is longitudinal wrinkled, it is clearly visible along the edge and near the stomates.

Stem. Rounded ribbed, with 7–8 edges, collenchymas is in the edges (up to three layers), parenchyma – 3-layer, the endoderm is mild marked, represented by 1 layer of rectangular-rounded cells. Vascular system of fasciculate structure, bundles of collateral type; sclerenchyma is a member of the bundles, well-defined. Phloem consists of the thin layer, located under the sclerenchyma. Xylem vascular. The medulla cells are thin-walled, varying in size and shape, parenchymal, rounded. Libriform lying in the form of belt.

Artemisia austriaca

Upper epidermis. Cells are parenchymal, slightly wavy walled, uniformly thickened; sometimes straight walled. Anomocytic stomata. There are many glands that have a structure of *Asteraceae*-type. The glands are surrounded by the rosette of epidermal cells. Along the vein, cells are prosenchymatous, acuminated, with uniformly thickened walls.



A **B**

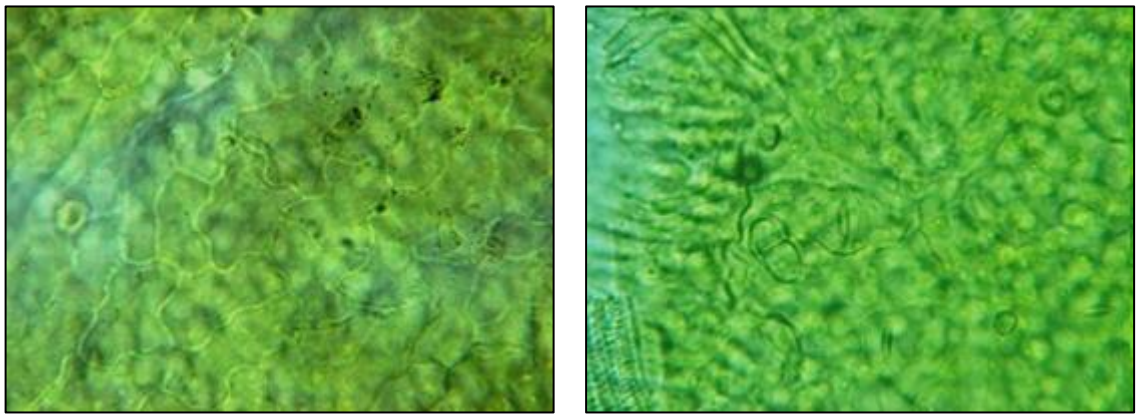
Fig. 4. Anatomical structure of *A. austriaca* leaf: A – upper epidermis; B – lower epidermis

Lower epidermis. Cells are parenchymal prosenchymatous, lightly wavy walled, walls are uniformly thickened. A lot of glands. Simple, thin walled hairs form a downy pubescence. Two types of hair: simple – very long, crisped, with a narrow cavity, thin walled; T-shaped hairs on 2-, 3-, 4-, 5-cell stalk and cross cell with long waving ends.

Stem. Rounded ribbed, 3-layer collenchyma is in the edges, parenchyma – 3-layer, the endoderm is represented by 1 layer of rectangular-rounded cells. Vascular system of fasciculate structure, bundles of collateral type; sclerenchyma is a member of the bundles, well-defined, composed of 12 layers of cells. Phloem consists of the thin layer, located under the sclerenchyma. Xylem with libriform, parenchymal cells and vessels. Presence of oxalate calcium crystals in the primary xylem cells. The medulla cells are thin-walled, varying in size and shape, parenchymal, rounded. Libriform lying in the form of belt.

Artemisia vulgaris

Upper epidermis. Cells are parenchymal, slightly wavy walled, uniformly thickened; small quantity of stomates. Anomocytic stomata. Small quantity of glands (Fig. 5).



A **B**

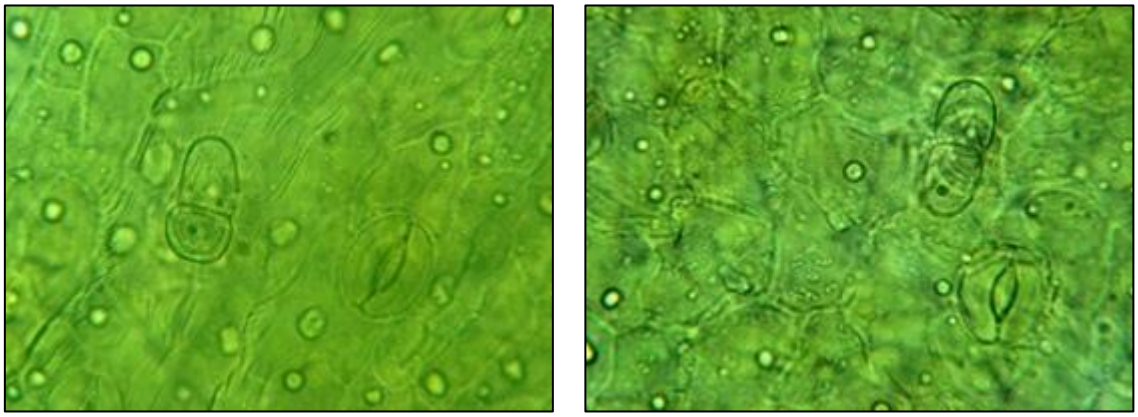
Fig. 5. Anatomical structure of *A. vulgaris* leaf: A – upper epidermis; B – lower epidermis

Lower epidermis. Cells are parenchymal prosenchymatous, lightly wavy walled, walls are uniformly thickened. Few glands, small, rounded. Three types of hair: 1) simple – very long, crisped, with a narrow cavity, thin walled; 2) simple – long, with a narrow cavity, warty surface; 3) T-shaped hairs on 2-, 3-, 4-, 5-cell stalk and cross cell with long waving ends.

Stem. Rounded ribbed, fasciculate structure, has 7–8 edges. Vascular system of fasciculate structure, bundles of collateral type; sclerenchyma is a member of the bundles, well-defined, composed of 8–10 layers of cells. The medulla cells are thin-walled, varying in size and shape, parenchymal, rounded. Libriform lying in the form of belt.

Artemisia dracunculus

Upper epidermis. Cells are parenchymal, deeply wavy walled, uniformly thickened. Above the vein cells are parenchymal prosenchymatous, elongated along the vein. Anomocytic stomata (Fig. 6).



A **B**

Fig. 6. Anatomical structure of *A. dracunculus* leaf: A – upper epidermis; B – lower epidermis

Lower epidermis. Cells of epidermis are polygonal, slightly wavy walled. A lot of small glands are observed. Hairs are simple, multicellular. Rugosity of cuticle is oblong.

Stem. Rounded ovated, visible schizogenic contents with brown content. 3-layer colenchyma places in edges, parenchyma – 3-layer, endoderm is strongly marked and placed between bark and central cylinder, consists of 1 cell column. Vascular system of fasciculate structure. Sclerenchymatous sections are strongly marked above the phloem sections. Phloem is presented with thin sections, xylem is vasculared. The medulla cells are thin-walled, varying in size and shape, parenchymal, rounded.

5. Cultivation conditions and methods of growing of wormwood *(Artemisia absinthium* L.*)* and common mugwort (*Artemisia vulgaris* L.)

The wormwood is reproducted by its seeds. Plants of the first year of the wormwood develop slowly, which greatly complicates the care of sowings. To keep the plantation clean from weeds, it is necessary to carry out two or three mechanical and manual weedings, which requires considerable expenses of fuel and labour. All this encourages us to study the seedling method of growing the wormwood that could ease the struggle against weeds at the expense of the terms of planting the seedlings in the soil[52].

[52] Лихочвор В.В., Борисюк В.С., Дубковецький С.В., Онищук Д.М. Лікарські рослини. Значення, ботанічні і біологічні особливості, технологія вирощування, заготівля. Львів: НВФ «Українські технології», 2003. 272 с.

Cuttings with buds of restoration and a part of the root were used for experiments and planted in boxes, filled with a wet mixture of peat and sand (1:1), and covered with a polyethylene film. Before sowing the soil was watered, and after sowing – it was mulched, to prevent soil from drying it was covered with agro fiber. Seedlings were planted in open ground according to the scheme of 45×25 cm in the third decade of June. Plants adapted more quickly after the transplantation, began earlier to grow up, reacted better to solar insolation. Due to this the plants of the seedlings, which were grown in boxes, grew more intensively. The productivity of plants of seedlings, which were grown on garden beds, was 58,8%.

During the vegetative reproduction of the wormwood the vegetative period is prolonged, which promotes the transition of plants to generative development. At the time of conducting the phenological observations for wormwood grown on garden beds it did not form shoots and inflorescences. At the same time, 47% of the seedlings grown in the boxes formed inflorescences and some individuals even bloomed.

The weight of plants leaves (table 4) planted in laboratory boxes is 69,2 g, stems – 28,1 g, inflorescence – 10,8 g.

Table 4

The structure of the above ground mass of the wormwood during the harvesting, depending on the methods of growing the seedlings

Method of cultivation	Leaves, g	Stems, g	Inflorescence, g
On the garden beds	58,8	24,1	9,4
In the laboratory boxes	69,2	28,1	10,8

Thus, for the first year of vegetation it is already possible to obtain the high-quality raw materials of the herb of wormwood.

Germination of the seeds of wormwood and common mugwort. Seeds are the main plant reproductive material both in the wild nature and in culture. In the process of evolution seeds have acquired a number of properties that give them the opportunity to maintain the viability until the

---

Лихочвор В.В. Рослинництво. Технології вирощування сільськогосподарських культур. Львів: НВФ «Українські технології», 2002. 800 с.

Бейдеман И.Н. Методика изучения фенологии растений и растительных сообществ. Новосибирск: Наука, 1974. 155 с.

Бабенко Л.М. Структурно-функціональні особливості насіння з різним типом спокою: дис. … канд. біол. наук: 03.00.01. К., 1995. 133 с.

time that is favourable for the germination of new seeds. To study the germination of the seeds we used the technique of Nikolaieva M. G. and co-authors (1985). Before the sowing stratification of seeds was carried out, which accelerates the biochemical processes in seeds[53].

For the experiment, seed material with a shelf life of 2 years was used, harvested from specimens growing wild in the territory of the Ivano-Frankivsk region.

The experiment included variants in which were used:

– mature seeds for sowing (control);

– immature seeds for sowing right after separation from initial root;

– heat (+20–25°C) stratification of mature seeds for sowing in spring;

– cold (+4–7°C) stratification of mature seeds for sowing in spring;

– germination of seeds in cups of Petri.

The laboratory germination was determined in cups of Petri on a wet filter paper at a constant temperature of air +20–25°C in a five-times repetition. It has been determined that seeds of wormwood and common mugwort in the laboratory conditions start to grow on the 17th day and its germination is 60–65%.

To determine the soil germination of seeds of wormwood and common mugwort, the experimental area was laid in field conditions. The seeds were sown in the spring period (the month of April, the temperature of the air – +12–17°C) in continuous rows to a depth of not more than 2 cm with a distance of 15 cm. Cold and warm stratifications of mature seeds were carried out during six months. Experiments were performed in three-times repetition. In each variant were studied 300 seeds (by 100 in each repetition).

The seeds of the control variant (ripen seeds without processing) in the first year after sowing germinated in spring with 65%. Unprocessed immature seeds sown in autumn, as well as any of the variants of mature stratified seeds did not germinate. In both variants of stratification

[53] Лихочвор В.В., Борисюк В.С., Дубковецький С.В., Онищук Д.М. Лікарські рослини. Значення, ботанічні і біологічні особливості, технологія вирощування, заготівля. Львів: НВФ «Українські технології», 2003. 272 с.

Лихочвор В.В. Рослинництво. Технології вирощування сільськогосподарських культур. Львів: НВФ «Українські технології», 2002. 800 с.

Бейдеман И.Н. Методика изучения фенологии растений и растительных сообществ. Новосибирск: Наука, 1974. 155 с.

Бабенко Л.М. Структурно-функціональні особливості насіння з різним типом спокою: дис. … канд. біол. наук: 03.00.01. К., 1995. 133 с.

(warm/cold) mature seeds in the year of processing germinated with 45%. The germination of such seeds is extremely low. The seeds of wormwood, like the majority of the *Asteraceae* species, are characterized by a state of organic calm. Such seeds, even in favorable conditions for germination, are either completely unable to germinate, or have a substantially reduced germination. This state of the wormwood and common mugwort is so deep that it takes a long and complex pre-sowing preparation to produce seedlings. In natural conditions the germination of the seeds of the *Artemisia* genus species begins only one or two years after sowing, and the appearance of the shoots extends for several year.

Plants of the second and next years of life in the spring grow at a temperature of 5–6°C. Optimal temperature for their growing and development is 18–25°C. High temperatures in the Summer months reduce the productivity of plants. In the first month after the appearance of the shoots the plants of wormwood and common mugwort grow slowly and suffer from shading by fast-growing weeds.

As a result of experiments, it was established that seeds of wormwood and common mugwort react positively to the influence of temperatures. The germination of non-stratified freshly harvested seeds at variable temperatures did not yield the desired results. In many seeds the process of germination slowed down immediately after the root was visible from the shall.

Phenological observation of the development of a wormwood. The annual cycle of development consists of two main periods – rest and vegetation, which are divided into 10 stages of organogenesis[54].

Period of rest – the first stage; it lasts in autumn, winter and spring months (about 160 days). During this period any external morphological changes are not observed on the underground part of the plant. The metabolism is slow.

The second stage – swelling of the buds – ends with the emergence and formation of shoots. The growth of the wormwood occurs in the soil without light, due to plastic substances, that were deposited last year in underground organs. The duration of the stage depends on the type of soil and temperature.

[54] Бейдеман И.Н. Методика изучения фенологии растений и растительных сообществ. Новосибирск: Наука, 1974. 155 с.

The third stage continues from the appearance of the shoots to the formation of the first pair of leaves on the shoots. At this time, the process of photosynthesis begins, but shoots still do not grow, mainly due to energy reserves and minerals of the underground part of the plant. Spring frosts delay the development of the wormwood.

The fourth stage lasts from the formation of the first pair of leaves to the beginning of the growth of branches. An intense growth of stems and leaves due to both the products of photosynthesis and the nutrients of the underground part is observed.

The fifth stage begins with the formation of branches and continues until the beginning of flowering. During this period, the main branches and leaves are growing intensively. On the lateral shoots in the axillaries of the leaves buds are formed, from which inflorescences are formed.

The sixth stage – the flowering – begins with the appearance of the first flowers and continues to the beginning of the formation of seeds. From 7 to 12 hours plants bloom most intensively and all the flowering lasts for about 24 days.

Seventh stage – the formation and growth of seeds – continues until their maturity. During the eighth stage (up to 15 days) the seeds become fully mature.

The ninth stage lasts until the beginning of the physiological dying of the above-ground part of the plant. At this stage the seeds ripen physiologically. The seeds lose water and density, some of them fall off. There is an intense outflow of assimilation products – plastic substances from the above-ground part of the plant into the underground organs, where they are deposited.

The tenth stage is characterized by the extinction of the above-ground part of plants, which begins with the upper, morphologically youngest parts of the stems. In the root system the accumulation of plastic substances continues.

CONCLUSIONS

The article is devoted to the prospects of study of the *Artemisia* L. genus species use in medicine. The botanical description, distinctive morphological features of the most common species, distribution, areas and ecological conditions of growth of the *Artemisia* L. genus plants are presented. The chemical composition, pharmacological properties and application in medicine of the *Artemisia* L. genus plants are studied. In order to identify raw materials of the *Artemisia* L. genus species and determine the

differences with other species that may be impurities, the authors conducted a comparative morphological and anatomical study of *Artemisia abrotanum* L., *Artemisia austriaca* Jacq., *Artemisia vulgaris* L., *Artemisia dracunculus* L. compared with the pharmacopoeial species *Artemisia absinthium* L.

Taking into account the distribution of the *Artemisia* L. genus species on the territory of Ukraine, the possibility of cultivating them, it is promising to study their raw material base in the regions of Ukraine.

SUMMARY

Analysis of primary literary data shows that plants of the *Artemisia* L. genus include more than 500 species in the world's flora, about 30 of which are found on the territory of Ukraine. Species of the Artemisia L. genus contain a great number of different groups of BAS, including essential oil, flavonoids, tannins, lignans, organic acids, carotene and vitamin C. Essential oil includes ketone tuyon, sesquiterpene lactones, tuyil alcohol, hydrocarbons (bisabolines, pinene, cadinene, phellandrene, caryophyllene and sepinene), sesquiterpene alcohols (absinthin, anabsinthin and artabsinthin, monocyclic lactones). Experience of application of these plants in non-traditional medicine supported by experimental studies confirms efficacy and safety of the *Artemisia* L. genus species and the possibility of their cultivation. It is a prerequisite for obtaining phyto-substances and development of new drugs based on them.

Pharmacopoeial raw materials are herb and leaves of *Artemisia absinthium* L. Other species of the Artemisia L. genus, which grow on the territory of Ukraine, are also available for resource-related, phytochemical and pharmacological research with the purpose of creating new drugs.

REFERENCES

1. Singh A., Sarin R. *Artemisia scoparia* – a new source of artemisinin. *Bangladesh J. Pharmacol*. 2010. № 5. P. 17–20.

2. Sharma S., Ali M. New compounds from Roots of *Artemisia scoparia. Journal of Herbs, Spices and Medicinal Plants*. 1998. Vol. 5. № 4. P. 77–86.

3. Aglarova A.M., Zilfikarov I.N., Severtseva O.V. Biological characteristics and useful properties of tarragon (*Artemisia dracunculus*). *Pharmaceutical Chemistry J.* 2008. № 42. P. 81–86.

4. Ahameethunisa A.R., Hopper W. Antibacterial activity of *Artemisia nilagirica* leaf extracts against clinical and phytopathogenic bacteria. *Complementary and Alternative Medicine*. 2010. № 10. P. 6.

5. Akhmedov I.S., Kasymov S.Z., Sidyakin G.P. A study of the composition of Artemisia absinthium. *Chemistry of natural compounds*. 1969. № 5(1). P. 50.

6. Jazani N.H., Zartoshti M., Babazadeh H. et al. Antibacterial Effects of *Artemisia dracunculus* Essential Oil on Multi-drug Resistant Isolates of Acinetobacter baumannii. *Bacteriology J*. 2011. № 1. P. 31–36.

7. Baykan Erel S., Reznicek G., Senol S.G. et al. Antimicrobial and antioxidant properties of *Artemisia* L. species from western Anatolia. *Turk. J. Biol*. 2012. № 36. P. 75–84.

8. Hofling J.F., Anibal P.C., Obando-Pereda G.A. et al. Antimicrobial potential of some plant extracts against Candida Species. *Braz. J. Biol*. 2010. Vol. 70. № 4. P. 1065–1068.

9. Obolskiy D., Pischel I., Feistel B. et al. *Artemisia dracunculus* L. (Tarragon): A Critical Review of Its Traditional Use, Chemical Composition, Pharmacology, and Safety. *J. of Agricult. and Food Chem*. 2011. № 9. P. 17–45.

10. Basa S.C. Natural biocoumarins. *Phytochemistry*. 1988. № 7. P. 1933–1941.

11. Bonyadian M., Karim G. Study of the effects of some volatile oils of herbs against *Escherichia coli* and *Staphylococcus aureus* in broth media. *J. Fac. Vet. Med. Univ. Tehran*. 2002. № 57. P. 81–83.

12. British Pharmacopoeia. London: Stationery Office Books HMSO, 2001. Vol. 1. 1389 p.

13. Derwich E., Benziane Z., Boukir A. Chemical compositions and insecticidal activity of essential oils of three plants *Artemisia sp: Artemisia herba-alba, Artemisia absinthiumand, Artemisia Pontica* (Morocco). *Elect. J. Environ. Agric. Food Chem.* 2009. № 8. P. 1202–1211.

14. Sharopov F.S., Sulaimonova V.A., Setzer W.N. Composition of the Essential oil of the *Artemisia absinthium* from Tajikistan. *Rec. Nat. Prod*. 2011. № 6(2). P. 128–134.

15. Wani H., Shah S.A., Banday J.A. Chemical composition and antioxidant activity of the leaf essential oil of *Artemisia absinthium* growing wild in Kashmir. *The journal of phytopharmacology*. 2014. № 3(2). P. 90–94.

16. Wasim A., Azhar H., Ansani A., Tahera T. Medicinal importance of *Artemisia absinthium* Linn (Afsanteen) in Unani Medicine: A revive. *Hippocratic Journal of Unani Medicine*. 2010. Vol. 5. № 4. P. 117–125.

17. Drobot K.O., Matvieieva N.A., Duplij V.P. et al. Study of Artemisinin and Sugar accumulation in *Artemisia dracunculus* "Hairi" root cultures. *Preparative biochemistry and biotechnology*. 2017. T. 47. № 8. P. 776–781.

18. Taraghdari S.B., Mazidi M., Kamgar M. et al. The effect of hydro-alcoholic extract of *Artemisia absinthium* on appetite in male rats. *Avicenna Journal of Phytomedicine*. 2015. Vol. 5. № 2. P. 78–83.

19. Verdian-rizi M. Variation in the essential oil composition of *Artemisia annua* L. of different growth stages cultivated in Iran. *Afr. J. of Plant Sci*. 2008. Vol. 2(2). P. 16–18.

20. Ковальова А.М., Очкур О.В., Колеснік Я.С. та ін. Антибактеріальна активність ліпофільних екстрактів трави *Artemisia abrotanum* L. *Проблемы и пути развития современного здравоохранения: сб. матер. XVI Междунар. науч.-практ. конф*. Одесса; Лондон, 2011. С. 137–139.

21. Очкур О.В., Кашпур Н.В., Волянський А.Ю. та ін. Антикандидозна активність ліпофільних витягів з трави видів роду полин. *Тези доповідей IV Національного з'їзду фармакологів України* (15–17 вересня 2010 р.). Х., 2010. С. 233–234.

22. Бабенко Л.М. Структурно-функціональні особливості насіння з різним типом спокою: дис. … канд. біол. наук: 03.00.01. К., 1995. 133 с.

23. Бейдеман И.Н. Методика изучения фенологии растений и растительных сообществ. Новосибирск: Наука, 1974. 155 с.

24. Беленовская Л.М. Сесквитерпеновые лактоны некоторых видов рода *Artemisia* L. *Растительные ресурсы*. 2000. Т. 36. Вып. 1. С. 43–45.

25. Ковальова А.М., Ільїна Т.В., Кашпур Н.В. та ін. Біологічно активні речовини ліпофільних фракцій з трави видів родів *Artemisia* L., *Potentilla* L., *Melilotus* L. і дослідження їх протимікробної активності. *Биологически активные вещества: фундаментальные и прикладные вопросы получения и применения*: матер. науч.-практ. конф. (Новый Свет, 23–28 мая 2011 г.). Новый Свет, 2011. С. 263–264.

26. Великородов А.В., Морозова Л.В., Щепетова Е.В. Изучение химического состава эфирного масла некоторых дикорастущих видов

рода *Artemisia* L. флоры Астраханской области. *Естественные науки*. 2010. № 4(33). С. 160–168.

27. Кашпур Н.В., Волянський А.Ю., Осолодченко Т.П. та ін. Визначення антибактеріальної дії активних речовин роду *Artemisia*. *Теоретична і експериментальна медицина*. 2010. № 4. С. 41–44.

28. Очкур О.В., Кашпур Н.В., Ковальова А.М. та ін. Вплив ліпофільних фракцій видів роду *Artemisia* L. на адгезивні властивості мікроорганізмів. *Актуальні питання фармацевтичної і медичної науки та практики*. 2011. Вип. XXIV. № 2. С. 33–36.

29. Очкур О.В., Кашпур Н.В., Ковальова А.М. та ін. Вплив ліпофільних фракцій деяких видів роду *Artemisia* L. на протилізоцимні властивості мікроорганізмів. *Клінічна фармація*. 2011. Т. 15. № 3. С. 44–47.

30. Халилова А.З., Шакуров Э.Р., Нуриев И.Ф. и др. Выделение и идентификация летучих органических соединений, выделяемых растениями рода *Artemisia* L. *Химия и технология растительных веществ: матер. II Всеросс. конф*. (24–27 июня 2002 г.). Казань, 2002. С. 55–56.

31. Онучак Л.А., Куркин В.А., Минахметов Р.А., Куркин А.В. Высокоэффективная жидкостная хроматография в анализе экстрактов *Artemisia dracunculus*. *Химия природных соединений*. 2000. № 2. С. 115–117.

32. Гречана О.В. Фармакогностичне дослідження азуленвміщуючих рослин роду *Artemisia* L. флори України з метою одержання лікарських засобів протизапальної дії: автореф. дис. … канд. фармац. наук: 15.00.02; НФаУ. Х., 2008. 17 с.

33. Державна Фармакопея України: в 3 т. 2-е вид. Х.: ДП «Український науковий фармакопейний центр якості лікарських засобів», 2014. Т. 3. 328 с.

34. Очкур О.В., Мороз А.О., Осьмачко А.П. та ін. Дослідження хлороформного екстракту з трави *Artemisia austriaca* Jacq. *Здобутки та перспективи розвитку фармацевтичної та медичної галузі в сучасному світі: зб. тез доп. II Всеукр. наук.-практ. конф. молодих вчених та студентів*. Луганськ, 2012. С. 100.

35. Клышев Л.К., Алюкина Л.С., Ряховская Т.В. Фенольные соединения полыней Казахстана. Алма-Ата: Наука, 1983. 159 с.

36. Ковальова А.М., Очкур О.В., Вальдовський А.О. Порівняльне хромато-мас-спектрометричне дослідження терпеноїдних сполук ефірних олій полину звичайного та полину гіркого. *Збірник наукових праць співробітників Національної медичної академії післядипломної освіти імені П.Л. Шупика*. 2009. Вип. 18. Кн. 3. С. 444–448.

37. Очкур О.В., Першко О.А., Шестак О.І. та ін. Компонентний склад ефірних олій *Artemisia absinthium* L. та *Artemisia vulgaris* L. *Актуальні питання створення нових лікарських засобів: зб. тез доп. Міжвуз. студентської наук.-практ. конф*. Х.: НФаУ, 2010. С. 87.

38. Очкур О.В., Ковальова А.М., Мороз А.О. та ін. Компонентний склад ефірних олій видів роду *Artemisia* L. підроду *Seriphidium* Rouy флори України. *Актуальні питання створення нових лікарських засобів: матеріали Всеукр. наук.-практ. конф. студентів та молодих вчених*. Х.: НФаУ, 2012. С. 99.

39. Новруз А.А., Нариман А.Н., Искендер А.М. и др. Компонентный состав и фумигантная активность эфирных масел видов рода *Artemisia* L. *Химия растительного сырья*. 2017. № 4. С. 235–240.

40. Коновалов Ю.Б. Фармакогностическое изучение полыни австрийской как источника фармакологически активных сесквитерпеновых лактонов: автореф. дисс. … канд. фармац. наук: 15.00.02; ГОУ ВПО «Пятигорская государственная фармацевтическая академия Федерального агентства по здравоохранению и социальному развитию». Пятигорск, 2007. 22 с.

41. Куатбеков Н.А., Кедельбаев Б.Ш., Сатаева Ж.И. Моносахариды *Artemisia cina* Berg. *Новые достижения в химии и химической технологии растительного сырья: матер. V Всеросс. конф. с международным участием* (24–26 апреля 2012 г.). Барнаул, 2012. С. 137–138.

42. Лихочвор В.В. Рослинництво. Технології вирощування сільськогосподарських культур. Львів: НВФ «Українські технології», 2002. 800 с.

43. Лікарські рослини: енциклопедичний довідник / за ред. А.М. Гродзінського. К.: Гол. редакція УРЕ, 1990. 544 с.

44. Лихочвор В.В., Борисюк В.С., Дубковецький С.В., Онищук Д.М. Лікарські рослини. Значення, ботанічні і біологічні особливості, технологія вирощування, заготівля. Львів: НВФ «Українські технології», 2003. 272 с.

45. Люй Годун. Фармакогностическое изучение отдельных представителей рода *Artemisia* L.: автореф. дисс. … канд. фармац. наук: 14.04.02; ГОУ ВПО «Московская медицинская академия им. И.М. Сеченова». М., 2011. 24 с.

46. Мінарченко В.М., Тимченко І.А. Атлас лікарських рослин України. К., 2002. 172 с.

47. Доброчаева Д.Н., Котов М.И., Прокурин Ю.Н. и др. Определитель высших растений Украины. 2-е изд. К.: Фитосоциоцентр, 1999. 548 с.

48. Очкур А.В., Чуксина А.Н., Ковалева А.М., Колесник Я.С. Органические кислоты экстрактов травы полыни песчаной (*Artemisia arenaria* DC.). *Gisap. Medical science, pharmacology*. 2014. № 3. P. 50–52.

49. Ісакова Т.І., Ковальова А.М., Очкур О.В. та ін. Особливості складу ефірних олій деяких видів полину флори України. Повідомлення 1: монотерпеноїди ефірних олій полину гіркого та полину звичайного. *Український біофармацевтичний журнал*. 2010. № 1. С. 62–68.

50. Ісакова Т.І., Ковальова А.М., Очкур О.В. та ін. Особливості складу ефірних олій деяких видів полину флори України. Повідомлення 2: сесквітерпеноїди та ароматичні сполуки ефірних олій полину гіркого та полину звичайного. *Український біофармацевтичний журнал*. 2010. № 2. С. 54–60.

51. Очкур А.В., Ковалева А.М. Жирные кислоты травы *Artemisia vulgaris* L. и *Artemisia austriaca* Jacq. *Химия и технология растительных веществ: тез. докладов VIII Всеросс. науч. конф*. Сыктывкар; Калининград, 2013. С. 172.

52. Очкур А.В., Ковалева А.М. Получение и изучение биологически активных веществ экстрактов травы видов рода полынь. *Биологически активные вещества и материалы: фундаментальные и прикладные вопросы получения и применения: матер. науч.-практ. конф*. (27 мая – 1 июня 2013 г.). Новый Свет, 2013. С. 55–56.

53. Очкур О.В. Антистафілококова активність екстрактів з трави полину звичайного. *Матеріали XV міжнар. мед. конгресу студентів та молодих вчених*. Тернопіль, 2011. С. 357.

54. Очкур О.В., Ковальова А.М., Ісакова Т.І. Компонентний склад ефірної олії полину однорічного. *Фармація України: погляд у*

майбутнє: матер. VII Національного з'їзду фармацевтів України. Х.: НФаУ, 2010. Т. 1. С. 318.

55. Очкур О.В. Фармакогностичне дослідження видів роду Artemisia L. флори України: автореф. дис. … канд. фармац. наук: 15.00.02; НФаУ. Х., 2014. 25 с.

56. Очкур О.В., Кашпур Н.В., Ковальова А.М. та ін. Патент на корисну модель 104173 Україна, МПК A61K 36/282, A61P 31/00. Спосіб одержання засобу з антимікробною активністю з рослинної сировини. Заявник та патентоутримувач Національний фармацевтичний університет. № a 2011 12754. Заявл. 31.10.11; опубл. 10.01.14, Бюл. № 1.

57. Очкур О.В., Вальдовський А.О., Ковальова А.М. та ін. Порівняльне дослідження елементного складу деяких представників роду полин. *Актуальні питання створення нових лікарських засобів: тез доп. Міжвуз. студент. наук.-практ. конф*. Х.: НФаУ, 2010. С. 85.

58. Ковальова А.М., Очкур О.В., Ісакова Т.І. та ін. Порівняльний аналіз складу ефірних олій деяких видів роду Полин флори України. *Фармаком*. 2010. № 2. С. 35–39.

59. Правила сбора и сушки лекарственных растений (сборник инструкций). М.: Медицина, 1985. 328 с.

60. Растительные ресурсы СССР: цветковые растения, их химический состав, использование. Семейство *Asteraceae (Compositae)* / под ред. П.Д. Соколова. СПб.: Наука, 1993. 352 с.

61. Супильникова А.В. Качественный и количественный анализ лекарственного сырья и настойки полыни эстрагон. *Фармация*. 2002. № 3. С. 11–14.

62. Танагузова Б.М. Химическое исследование и стандартизация лекарственного сырья эфирномасличных растений Казахстана: автореф. дисс. … канд. фармац. наук: 15.00.02; АО «Науч.-произв. центр «Фитохимия» МОН РК. Караганда, 2007. 25 с.

63. Гречана О.В., Мазулін О.В., Сур С.В. та ін. Фітохімічне вивчення ефірної олії полину гіркого. *Фармацевтичний журнал*. 2006. № 2. С. 82–86.

64. Гречаная Е.В., Мазулин А.М., Денисенко О.Н., Зоря Б.П. Фармакогностическое изучение эфиромасличных видов рода *Artemisia* L. флоры юго-востока Украины. *Актуальні питання фармацевтичних*

та медичних наук та практики: збірник наукових праць. Запоріжжя, 2003. Вип. X. С. 27–28.

65. Очкур О.В., Белоуши Ж., Півень Т.В. та ін. Фітохімічне дослідження трави *Artemisia dracunculus* L. *Актуальні питання створення нових лікарських засобів: тези доп. Всеукр. наук.-практ. конф. студентів та молодих вчених*. Х., 2011. С. 101.

66. Очкур О.В., Вальдовський А.О., Капкан В.П. та ін. Фітохімічне дослідження трави *Artemisia austriaca* Jacq. *Актуальні питання створення нових лікарських засобів: тези доп. Міжвуз. студент. наук.-практ. конф*. Х.: НФаУ, 2010. С. 88.

67. Очкур О.В., Ковальова А.М., Коваленко Я.Г. та ін. Фітохімічне дослідження трави *Artemisia campestris* L. *Актуальні питання створення нових лікарських засобів: матер. Всеукр. наук.-практ. конф. студентів та молодих вчених*. Х.: НФаУ, 2012. С. 98.

68. Флора Европейской части СССР: в 11 т. / отв. ред. и ред. тома Н.Н. Цвелев. СПб.: Наука, 1994. Т. 7. 317 с.

69. Флора СССР: в 30 т. / под ред. В.Л. Комарова. М., Л.: Изд-во АН СССР, 1961. Т. 26. 720 с.

70. Флора УРСР: в 12 т. / за ред. Д.К. Зерова. К.: Вид-во АН УРСР, 1962. Т. 11. 591 с.

71. Ханина М.А., Хан В.А., Березовская Т.П. Терпеноиды эфирного масла *Artemisia vulgaris*. *Химия природных соединений*. 1987. № 3. С. 457.

72. Ковальова А.М., Очкур О.В., Колісник Я.С. та ін. Хромато-мас-спектрометричне дослідження ліпофільних сполук екстрактів полину звичайного. *Український медичний альманах*. 2012. Т. 15. № 5. С. 126–128.

73. Великородов А.В., Морозова Л.В., Пилипенко В.П. и др. Химический состав эфирного масла четырех эндемичных видов полыни Астраханской области: *Artemisia lerchiana*, *Artemisia santonica*, *Artemisia arenaria* и *Artemisia austriaca*. *Химия растительного сырья*. 2011. № 4. С. 115–120.

74. Ходаков Г.В., Котиков И.В., Панковецкий В.Н. Компонентный состав эфирного масла *Artemisia abrotanum* и *A. dracunculus*. *Химия природных соединений*. 2009. № 6. С. 755–758.

75. Ходаков Г.В., Котиков И.В. Компонентный состав эфирного масла *Artemisia taurica*. *Химия природных соединений.* 2008. № 2. С. 205–206.

76. Ходаков Г.В., Котиков И.В. Компонентный состав эфирного масла *Artemisia annua* и *A. scoparia*. *Химия природных соединений.* 2009. № 6. С. 759–761.

77. Очкур А.В., Ковалева А.М. Компонентный состав эфирного масла *Artemisia nutans. Человек и лекарство: сб. матер. XX Росс. нац. конгресса* (15–19 апреля 2013 г.). М., 2013. С. 402.

Information about authors:

Grytsyk R. A.,

Physician, Postgraduate Student of the Department of Pharmacotherahy
National Pharmaceutical University
4, Valentynska st., Kharkiv, 61002, Ukraine

Struk O. A.,

Candidate of Pharmaceutical Sciences,
Associate Professor of the Department of Pharmacy
SHEE "Ivano-Frankivsk State Medical University"
2, Halytska Str., Ivano-Frankivsk, 76018, Ukraine

РІВЕНЬ АНТИМІКРОБНОГО ПЕПТИДУ КАТЕЛІЦИДИНУ У ДІТЕЙ, ХВОРИХ НА МУКОВІСЦИДОЗ

Дудник В. М., Демянишина В. В.

ВСТУП

Муковісцидоз (МВ) є одним із найпоширеніших аутосомно-рецисивних захворювань, що зумовлене мутацією гену CFTR (трансмембранний регуляторний білок муковісцидозу) і характеризується клінічним поліморфізмом.

МВ поширений серед усього населення Землі, але найчастіше зустрічається у осіб європеоїдної раси. Найбільша захворюваність спостерігається в таких країнах, як Ірландія (1:1800), Шотландія (1:1984), Швейцарія (1:2000), Франція (1:2350). Серед населення країн Азії частота МВ складає 1:40000-100000 (Індія), 1:100000-350000 (Японія). За даними щорічного звіту The European Cystic Fibrosis Society Patient Registry (ECFSPR) за 2014 рік, у 26 європейських країнах, у тому числі і в Україні, зареєстровано 35 582 пацієнтів, хворих на МВ, з яких 48,2% дітей[1]. В США на 2015 рік згідно з щорічним звітом Cystic Fibrosis Foundation Patient Registry проживає 28 983 хворих на МВ, серед яких 48,4% дитячого населення[2]12.

Станом на 2016 рік за офіційною статистикою МОЗ України на обліку перебуває 674 дитини. За результатами неонатального скринінгу на МВ 2013–2014 рр., середня частота в Україні становила 1:8400. Точну частоту МВ в Україні складно визначити через припинення програми масового неонатального скринінгу в 2015 році[3].

Згідно з даними ДУ «Інститут спадкової патології НАМН України» в Україні гетерозиготним носієм мутацій гену CFTR є кожний 29 житель. За підрахунками, в Україні мало б проживати від 1 700 до 4 000 хворих на муковісцидоз, отже, переважна більшість пацієнтів не виявлена[4].

---

[1] Cystic Fibrosis in Europe – Facts and Figures. The European Cystic Fibrosis Society. 2015.

[2] Patient registry annual data report. Cystic Fibrosis Foundation. 2016.

[3] Makukh H., Křenková P., Tyrkus M. A high frequency of the Cystic Fibrosis 2184insA mutation in Western Ukraine: Genotype–phenotype correlations, relevance for newborn screening and genetic testing. *Journal of Cystic Fibrosis*. 2010. № 9. p. 371–375.

[4] Makukh H., Křenková P., Tyrkus M. A high frequency of the Cystic Fibrosis 2184insA mutation in Western Ukraine: Genotype–phenotype correlations, relevance for newborn screening and genetic testing. *Journal of Cystic Fibrosis*. 2010. № 9. p. 371–375.

Клінічні прояви та важкість хвороби варіюють залежно від виду мутації, що спричинила хворобу, віку пацієнтів та багатьох інших умов. Дієвих методів виліковування МВ немає, але сучасні дослідження ведуть до покращення медикаментозного супроводу таких хворих, який допомагає покращити якість та подовжити тривалість життя. Сьогодні у розвинених країнах медіана тривалості життя хворих на МВ збільшилась. Згідно з даними Національного Інституту Здоров'я США (NIH) за 2016 рік середня тривалість життя пацієнтів, хворих на МВ, становила більше 37 років[5]. Щорічний звіт UK Cystic Fibrosis Registry 2015 року показав, що середній очікуваний вік хворих на МВ в Британії становить 45,1 рік, це означає, що половина зареєстрованих хворих житимуть більше за вказаний вік, але інша половина може померти, не досягнувши цього показника[6].

Це захворювання зберігає свою високу медико-соціальну значущість, що пов'язано із низькою тривалістю життя хворих, раннім формуванням ускладнень, ранньою інвалідизацією, проблемами своєчасної діагностики, необхідністю постійного диспансерного спостереження, складнощами лікування та високим рівнем смертності.

З віком у хворих виникають такі ускладнення, як дихальна недостатність, легенева гіпертензія, серцева недостатність, зниження нутритивного статусу, цироз печінки, цукровий діабет, печінкова недостатність, кишкова непрохідність. Для МВ характерний прогресуючий перебіг, часті загострення, зумовлені активністю інфекційних агентів (Haemophilus influenzae, Pseudomonas aeruginosa, Staphylococcus aureus, Burkholderia cepacia). Прогресування легеневої та серцевої недостатності є найчастішою причиною смерті хворих (95%). Серед інших причин в економічно розвинутих країнах виділяють: ускладнення після трансплантації органів (12%), захворювання печінки та печінкова недостатність (2,3%), травми (2,1%), суїцид (0,8%), інші (1,3%).

Прогноз даного захворювання більшою мірою залежить від наявності хронічної інфекції і персистуючого запалення дихальних шляхів. За останнє десятиріччя розроблено багато терапевтичних програм, в тому числі і антибіотикотерапії, що покращують прогноз

[5] Cystic Fibrosis Life Expectancy. Cystic fibrosis news today. 2016. URL: https://cysticfibrosisnewstoday.com/cystic-fibrosis-research/.

[6] UK Cystic Fibrosis Registry 2015 Annual Data Report. 2016.

для хворих. Але проблема антибіотикорезистентності більшості штамів, висіяних з респіраторного тракту хворих на МВ, залишається. За окремими даними, 85,2% штамів мають резистентність до 1 і більше бактерицидних засобів.

Хоча проблемі патогенезу захворювання приділено багато уваги, залишаються невизначеними механізми генетичних, імунних та імунорегуляторних порушень, що сприяють прогресуванню захворювання у дітей.

Враховуючи імунний генез поліорганного ураження при МВ, подальші перспективи вивчення пов'язані з переводом досліджень на молекулярно-генетичний рівень, пошуком і детальним дослідженням механізмів специфічного та неспецифічного захисту організму, вплив на які покращить прогноз та якість життя хворих.

Роль антимікробних пептидів у захисті респіраторного тракту у здорових людей та у хворих на муковісцидоз

За останнє десятиліття інтерес до системи захисту дихальних шляхів значно зріс. Важлива роль у цій системі відводиться епітелію дихальних шляхів, на поверхні якого відбувається взаємодія між зовнішніми агентами та системою захисту організму. Епітеліальний шар дихальних шляхів є першою точкою контакту між внутрішнім середовищем організму та речовинами, що вдихаються з повітрям. Це перший бар'єр, що стоїть на захисті організму[7], будучи механічним бар'єром між зовнішнім та внутрішнім середовищем, здійснює циліарний кліренс, забезпечує відповідну гідратацію поверхневого шару, регулює транспорт іонів та рідини, секрецію слизу залозами[8]. Крім функції неспецифічного імунітету, епітеліальний шар забезпечує захист проти мікроорганізмів та стимулює відповідь адаптативного імунітету.

Епітеліальний шар дихальних шляхів є першою точкою контакту між внутрішнім середовищем організму та речовин, що вдихаються з повітрям. Він сприймає бактеріальний вплив і відповідає, активуючи захисні механізми організму. Ця реакція складається із вивільнення так

[7] Bals R., Hiemstra P.S. Innate immunity in the lung: how epithelial cells fight against respiratory pathogens. *European respiratory journal*. 2004. №23. p. 327–333.

[8] Hartl D., Gaggar A., Bruscia E. Innate immunity in cysticfibrosis lung disease. *Journal of Cystic Fibrosis*. 2012. №11. p. 363–382.

званих антимікробних пептидів в просвіт дихальних шляхів і вивільнення хемокінів і цитокінів в підслизову, що ініціює запальну реакцію. Ця запальна реакція включає рекрутинг фагоцитів, які елімінують мікроорганізми, що не були очищені віями епітелію безпосередньо, і дендритні клітини та лімфоцити, які допомагають підвищити адаптивну імунну відповідь[9],[10].

Епітеліальні клітини дихальних шляхів секретують велику кількість молекул, що залучаються до запальних та імунних процесів. Деякі з секретованих речовин мають пряму антимікробну дію та діють як ендогенні антибіотики[11]. Ці молекули включають катіонні антимікробні пептиди, як, наприклад, b-дефензини і кателіцидини (LL-37), а також і більші антимікробні протеїни, як, наприклад, лізозим, лактоферин і інгібітор секреторної лейкоцитарної протеінази. Ці молекули здійснюють мікробоцидну активність або інгібують ріст мікроорганізмів, що вдихаються, доки їх не елімінує мукоциліарний апарат, завербовані фагоцити та/або не розвинеться відповідь адаптивного імунітету.

Антимікробні пептиди (АМП) – ефекторні молекули вродженої імунної системи. Нещодавно було визнано, що вони мають множинні ефекти та, окрім їх антимікробної функції, здійснюють протигрибковий, протипаразитарний та противірусний захист. АМП залучаються в запальний процес не лише як ендогенні антибіотики, але і як медіатори запалення. Дефензини і кателіцидини є головними сім'ями АМП, які експресуються в дихальному тракті. Антимікробні пептиди в людській легенях здебільшого продукуються і секретуються епітеліальними і фагоцитарними клітинами[12].

Класичний механізм дії АМП включає їх здатність спричиняти ушкодження клітинної мембрани. АМП можуть взаємодіяти з мікроорганізмами за допомогою електростатичних сил між їх

[9] Cystic Fibrosis in Europe – Facts and Figures. The European Cystic Fibrosis Society. 2015.

[10] Patient registry annual data report. Cystic Fibrosis Foundation. 2016.

[11] Bals R. Epithelial antimicrobial peptides in host defense against infection. *Respiratory Research*.2000. №1. p. 141–150.

[12] Bals R., Hiemstra P.S. Innate immunity in the lung: how epithelial cells fight against respiratory pathogens. *European respiratory journal*. 2004. №23. p. 327–333.

позитивно зарядженими амінокислотними залишками і негативно зарядженими часточками, які виставлені на клітинних поверхнях[13].

Були запропоновані кілька моделей, які пояснюють механізм руйнування мембран антимікробними пептидами, як, наприклад, модель «бочок», тороїдальна і «килимова» моделі[14].

АМП-контрольована мікробна смерть може викликатися іншими механізмами, на додаток до мембранного руйнування, що завершується клітинним лізисом. Багато фактів вказують на те, що деякі АМП можуть взаємодіяти із внутрішньоклітинними цілями та спричиняти клітинні ушкодження, як, наприклад, інгібування ДНК, РНК і синтезу білків. Окрім того, один пептид може діяти на кілька клітинних цілей[15,16].

Сьогодні виконано багато робіт, де досліджувалися антимікробні пептиди (АМП). Визначено, що ці речовини є ланкою вродженого імунітету і рівень АМП можна розцінити як маркер системної активації при інфекційних, аутоімунних та запальних захворюваннях. АМП залучаються в запальний процес не лише як ендогенні антибіотики, але і як медіатори запалення та в дихальних шляхах в основному продукуються і секретуються епітеліальними і фагоцитарними клітинами.

Експресія і секреція генів антимікробних пептидів жорстко регулюється. Експресія збільшується внаслідок контакту клітин із мікробними продуктами або прозапальними медіаторами. Вроджена імунна система сприймає молекулярні образи, користуючись рецепторами розпізнавання образів (PRR). Клітини системи вродженого імунітету, у тому числі фагоцити, дендритні клітини і епітеліальні клітини, користуються PRR, щоб зв'язати молекулярні образи, які присутні на мікроорганізмах[17,18]. Образ-розпізнаючі

---

[13] Zhang L., Parente J., Harris S. M. Antimicrobial Peptide Therapeutics for Cystic Fibrosis. *Antimicrobial Agents and Chemotherapy*. 2005. № 49.

[14] Guilhelmelli F., Vilela N., Albuquerque P. Antibiotic development challenges: the various mechanisms of action of antimicrobial peptides and of bacterial resistance. *Frontiers in Microbiology*. 2013. № 4. P. 1–12.

[15] Cystic Fibrosis in Europe – Facts and Figures. The European Cystic Fibrosis Society. 2015.

[16] Makukh H., Křenková P., Tyrkus M. A high frequency of the Cystic Fibrosis 2184insA mutation in Western Ukraine: Genotype–phenotype correlations, relevance for newborn screening and genetic testing. *Journal of Cystic Fibrosis*. 2010. № 9. P. 371–375

[17] Hartl D., Gaggar A., Bruscia E. Innate immunity in cysticfibrosis lung disease. *Journal of Cystic Fibrosis*. 2012. № 11. P. 363–382.

[18] Bals R., Hiemstra P.S. Innate immunity in the lung: how epithelial cells fight against respiratory pathogens. *European respiratory journal*. 2004. № 23. P. 327–333.

молекули можуть бути присутніми у виділеннях і циркулювати в розчинній формі, як, наприклад, манан-зв'язучий лектин, або вони можуть бути трансмембранними молекулами, що опосередковують пряму клітинну відповідь на мікробний вплив 2.

Toll-like рецептори (TLR) – це найбільш вивчена група PRR[19]. Більшість TLR розпізнають бактерійні патерни, так звані патерн-зв'язуючі молекулярні образи (PAMP), (TLR1, TLR2, TLR4, TLR5, TLR6 і TLR9, TLR10, TLR11). Наприклад, TLR4 чутливий до ліпополісахаридів (ЛПС), TLR5 – до флагеліну, TLR9 – до CpG олігонуклеотидів і TLR11 уропатогенні бактерії та/або профілін протозойних. Вірусні PAMP розпізнаються через TLR3 (дволанцюгова РНК, dsРНК), TLR7 і TLR8 (одноланцюгова РНК, ssРНК). TLR експресуються різноманітними імунними і структурними клітинами, у тому числі моноцитами/макрофагами, дендритними клітинами, лімфоцитами, ендотеліальними клітинами, міоцитами, епітеліальними клітинами, нейтрофілами тощо[20].

Клітини епітелію дихальних шляхів також експресують велику кількість TLR [21,22], які допомагають їм підвищити адекватну відповідь на мікробний вплив. Активація TLR на епітеліальних клітинах залучена до регулювання експресії різноманітних генів, у тому числі тих, що кодують цитокіни, хемокіни і антимікробні пептиди.

Велика кількість бактеріальних, грибкових і вірусних продуктів була ідентифікована як ліганди для різних TLR та інших рецепторів розпізнавання образів, що експресуються на клітинах епітелію дихальних шляхів [23,24].

---

[19] Guilhelmelli F., Vilela N., Albuquerque P. Antibiotic development challenges: the various mechanisms of action of antimicrobial peptides and of bacterial resistance. *Frontiers in Microbiology*. 2013. №4.p. 1–12.

[20] Cystic Fibrosis in Europe – Facts and Figures. The European Cystic Fibrosis Society. 2015.

[21] Hartl D., Gaggar A., Bruscia E. Innate immunity in cysticfibrosis lung disease. *Journal of Cystic Fibrosis*. 2012. №11. p. 363–382.

[22] Bals R., Hiemstra P.S. Innate immunity in the lung: how epithelial cells fight against respiratory pathogens. *European respiratory journal*. 2004. №23. p. 327–333.

[23] Greene C.M., Carroll T.P., Smith S. G. TLR-Induced Inflammation in Cystic Fibrosis and Non-Cystic Fibrosis Airway Epithelial Cells. *The Journal of Immunology*. 2005. №174. p. 1638–1646.

[24] Gerritn J., Yildirim A. O., Rubin B.K. TLR-4–Mediated Innate Immunity Is Reduced in Cystic Fibrosis Airway Cells. *American journal respiratory cell molecular biology*. 2010. № 42. p. 424–431.

Відомо, що TLR також регулюють експресію антимікробних пептидів. З'ясовано, що CD14, частина рецепторного комплексу TLR4 важлива в ЛПС-індукованій продукції hBD-2 в трахеобронхіальних епітеліальних клітинах. Згодом було з'ясовано, що TLR2 регулює експресію hBD-2 у відповідь на бактерійний ліпопротеїн в епітеліальних клітинах легені, і hBD-2 і IL-8, у відповідь на пептидоглікани і частки стіни клітини дріжджових грибів в людських кератиноцитах[25].

Було показано, що експресія hBD-2, hBD-3, hBD-4, LL-37 і декількох інших антимікробних пептидів стимулюється in vitro бактеріальними продуктами і запальними медіаторами. Ці дослідження клітинних культур підтверджуються декількома дослідженнями на пацієнтах, показуючи, що концентрація антимікробних пептидів, таких як b-дефензини, підвищується в різних рідинах біологічного походження під час запального або інфекційного процесу, як, наприклад, при пневмонії чи муковісцидозі[26].

Миші з видаленим cathelin-подібним антимікробним протеїном-18, зокрема щурячий гомолог LL-37, мають більш виражене інфікування після шкірного введення бактерій. З іншого боку, надмірна продукція LL-37 за допомогою вірусного переносу гена призводить до збільшення вродженого захисту організму у бронхіальній ксенотрансплантатній моделі муковісцидозу і в щурячих тваринних моделях пневмонії і септичного шоку[27].

Використовуючи системи культури епітеліальних клітин дихальних шляхів від хворих на муковісцидоз, було продемонстровано, що отримані з епітеліальних клітин антимікробні пептиди можуть бути інактивовані високою концентрацією солі в поверхневому шарі рідини епітеліального шару.

Мутації у гені CFTR спричиняють критичне зниження вродженої захисної системи організму легень, що призводить до ранніх та тяжких форм хронічної хвороби дихальних шляхів, яка характеризується обструкцією, запальним процесом з нейтрофільним переважанням та бактеріальною інфекцією, в результаті яких прогресує ураження легень

[25] Patient registry annual data report. Cystic Fibrosis Foundation. 2016.

[26] Bals R. Epithelial antimicrobial peptides in host defense against infection. *Respiratory Research*.2000. № 1. p. 141–150.

[27] Balsa R., J. Wilson. Cathelicidins – a family of multifunctional antimicrobial peptides. *Cellular and Molecular Life Sciences*. 2003. № 60.

з розвитком бронхоектазів, пневмофіброзу[28]. У пацієнтів антибактеріальна активність поверхні дихальних шляхів знижується внаслідок високої міцності іонних зв'язків та прямої взаємодії АМП із ДНК та філаментним F-актином.

Зростаюча резистентність мікрофлори до антибіотиків, особливо в дихальних шляхах у хворих на МВ, вимагає нових підходів до антибіотикотерапії. Оскільки бактерії не розвивають резистентність до АМП, як, наприклад, до кателіцидину, АМП можуть бути ефективним лікувальним засобом.

Саме тому *метою* нашого дослідження було визначення вмісту антимікробного пептиду кателіцидину в сироватці крові дітей, хворих на муковісцидоз, залежно від важкості та періоду захворювання.

Матеріали та методи. Проведено комплексне клінічне та лабораторно-інструментальне обстеження 74 дітей віком від 2 місяців до 18 років, хворих на муковісцидоз.

Критерії включення:

– діти, хворі на муковісцидоз, віком до 18 років;

– діти, хворі на муковісцидоз, в періоді загострення та в період ремісії;

– діти, у яких діагноз муковісцидоз підтверджений двома позитивними потовими пробами, та/або виявлена мутація гена CFTR;

Критерії виключення: діти з підозрою на муковісцидоз, який не підтверджений двома позитивними потовими пробами та/або виявленою мутацією гена CFTR.

Клініко-лабораторне обстеження дітей, хворих на муковісцидоз, проводили відповідно до Наказу МОЗ України №723 від 15.07.2016 р. «Уніфікований клінічний протокол первинної, вторинної (спеціалізованої) та третинної (високоспеціалізованої) медичної допомоги хворим на муковісцидоз».

Діти були опитані на наявність скарг, був вивчений анамнез життя та захворювання, зібраний спадковий анамнез. Під час об'єктивного обстеження, яке проводили за загальновизнаними методиками, враховували наявність та відсутність таких синдромів, як дихальна недостатність, задишка, тривалий вологий кашель, утруднення носового дихання, наявність в'язкого густого мокротиння, ціаноз,

[28] Cohen T., Prince A. Cystic fibrosis: a mucosal immunodeficiency syndrome / T. Cohen, // *Nat Med*.2013.№18. p. 509–519.

затримка фізичного розвитку, втрата маси тіла, деформація пальців у вигляді «барабанних паличок», «годинникових скелець», частий зловонний стул, стеаторея, поліфекалія.

Вміст антимікробного пептиду LL-37 у сироватці крові визначали імуноферментним методом за набором "Human LL-37 ELISA kit" (Hycult Biotech, Нідерланди) у відповідності до інструкції фірми-виробника. LL-37 є антимікробним С-кінцевим пептидом катіонного антимікробного протеїну людини hCAP-18 із родини кателіцидинів. У лунки планшетів, на стінках яких адсорбовані антитіла до LL-37, додавали по 100 мкл стандартних розчинів (з концентраціями LL-37 – 0; 0,1; 0,4; 1,2; 3,7; 11; 33 та 100 нг/мл), 100 мкл проб (1:10), герметизували адгезивною плівкою, інкубували 60 хвилин при 25°С. Далі лунки 4 рази промивали буферним розчином, вносили 100 мкл біотинолових антитіл, інкубували 60 хвилин при 25°С. Далі лунки 4 рази промивали буферним розчином, вносили 100 мкл ензимного кон'югату (стрептавідин-пероксидази), перемішували, закривали лунки адгезивною плівкою. Інкубували 60 хв при 25°С для утворення на твердій фазі комплексу АТ-АГ-АТ-ензим. Лунки відмивали від надлишку незв'язаних реагентів, вносили в них по 100 мкл хромогенного субстрату. Перемішували, інкубували 15 хв при 25°С, реакцію зупиняли 100 мкл стоп-розчину і фотометрували при 450 нм (диференційний фільтр 630 нм) на автоматичному аналізаторі STAT FAX 303/PLUS. Чутливість набору < 0,1 нг/мл, коефіцієнт варіації – < 10%.

Проведений аналіз дітей основної групи за статтю та віком. За статтю обстежені діти розподілені, відповідно, на 45 хлопчиків (51,7 ± 5,35 %) та 42 дівчинки (48,3 ± 5,35 %).

Всі обстежені діти були розділені на наступні вікові групи: від 0 до 3 років – 19 (21,8 ± 4,43 %), від 4 до 6 років – 16 (18,4 ± 4,15 %), від 7 до 14 років 40 – (46,0 ± 5,34 %), від 15 до 18 років – 12 (13,8 ± 3,70 %). Середній вік обстежених становив 9,39 ± 0,535 років.

В якості контрольної групи обстежено 40 практично здорових дітей віком від 2 до 18 років (середній вік 9,20 ± 1,06 років), серед яких було 23 хлопчики (57,7 ± 10,3 %) та 17 дівчаток (42,5 ± 11,98 %). В контрольну групу включали практично здорових дітей за умов відсутності скарг та об'єктивних ознак спадкових і хронічних захворювань, без відхилень показників при клініко-лабораторних, інструментальних дослідженнях, із відсутністю гострих інфекційних

захворювань. У всіх дітей відзначались нормальні результати клініко-лабораторних та інструментальних досліджень.

Нашими дослідженнями було встановлено, що серед обстежених дітей переважала форма з панкреатичною недостатністю 84 дитини (96,6 ± 1,94 %), і лише 3 дитини (3,4%) не мали панкреатичної недостатності.

Проаналізувавши залежність перебігу захворювання від віку дітей, ми встановили, що важка форма муковісцидозу достовірно частіше зустрічається серед дітей 7–14 років (51,11 ± 5,36%), а діти молодшого віку хворіють здебільшого середньоважкою формою.

У результаті проведеного дослідження встановлено, що у період загострення хвороби обстежено 67 дітей (77,0 ± 4,51 %), в ремісії – 20 (23,0 ± 4,51%).

Всім дітям для верифікації діагнозу було проведено потову пробу та ДНК аналіз мутації гену CFTR. Серед усіх мутацій 46,3% займає класична F508del/ F508del, решта являються компаунд-гетерозиготами або гомозиготні за іншою мутацією. Аналіз мутацій за статтю показав, що хлопчики та дівчатка мають практично однаковий розподіл мутантних генів.

Визначення хлоридів поту у хворих на МВ є золотим стандартом діагностики захворювання. Так, за результатами нашого дослідження, потова проба у 51 пацієнта (87,9 ± 4,28 %) була позитивною, у 5 дітей (8,6 ± 2,86 %) – сумнівна та у 2 (3,4 ± 2,39 %) – негативна. Середнє значення проби становить 92,85 ± 3,17 мкмоль/л. Проаналізувавши результати потової проби залежно від важкості перебігу МВ, ми встановили, що 78,6 ± 5,39 % дітей із важкими перебігом мали позитивну потову пробу.

Результати дослідження. Встановлено, що вміст катіонного протимікробного пептиду кателіцидину LL-37 в сироватці крові дітей, хворих на МВ, достовірно відрізняється від значень у здорових дітей ($p \leq 0,001$) та характеризується підвищенням його рівня. Так, середній вміст кателіцидину LL-37 в сироватці групи дітей із МВ становив 24,73 ± 0,33 нг/мл, тоді як у групі контролю відповідний пептид у середньому склав 7,74 ± 0,27 нг/мл.

Результати визначення антимікробного пептиду кателіцидину у сироватці крові дітей, хворих на МВ, нами було розподілено на 4 квартилі. У І квартиль потрапили значення LL-37 нижче 18,90 нг/мл,

що склало 28,4% від усіх спостережень, II квартиль – 18,91 – 25,60 нг/мл (23% спостережень), до II квартилю ввійшли показники 25,61 – 31,50 нг/мл (25,7%) та до IV квартилю віднесено значення вище 31,51 нг/мл (23,0 %) (рис. 1).

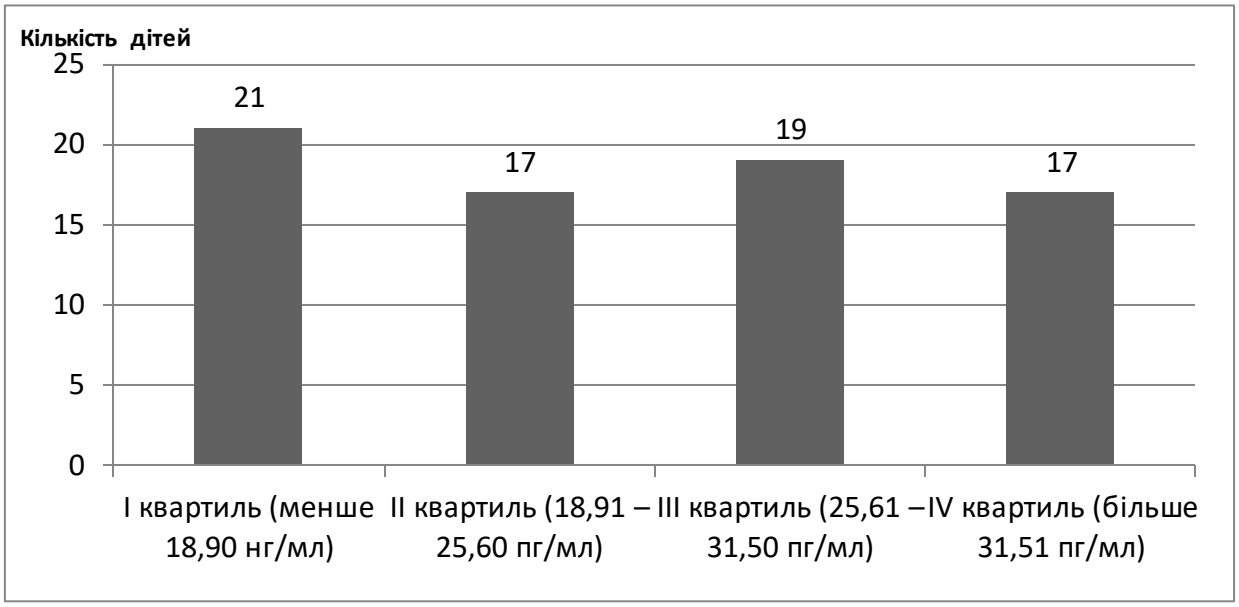


Рис. 1. Розподіл дітей, хворих на МВ, залежно від вмісту антимікробного пептиду кателіцидину

Оцінивши дані залежно від статі, достовірної різниці не виявлено. Так, у 38 хлопчиків, яким визначали рівень LL-37, середній показник становив 25,004 ± 1,37 нг/мл, у дівчаток дещо менший – 24,86 ± 1,42 нг/мл відповідно.

Проаналізувавши отримані дані, залежно від періоду захворювання, з'ясовано, що середнє значення LL-37 в періоді загострення та ремісії практично не відрізнялось та становило відповідно 24,9 ± 0,98 та 25,06 ± 2,76, однак достовірно відрізнялось від групи здорових дітей у сторону підвищення (табл. 1).

Таблиця 1

Вміст антимікробного пептиду кателіцидину LL-37 в сироватці крові дітей, хворих на МВ, залежно від періоду захворювання

	Загострення		Ремісія	
	n	**M±m**	**n**	**M±m**
25(OH)Д, нг/мл	57	24,9±0,98*	20	25,06±2,7*
Здорові діти	7,74 ± 0,27			

*Примітка: * – p ≤ 0,001 – різниця вірогідна відносно показників здорових дітей*

Залежність між рівнем кателіцидину у дітей, хворих на МВ, та ступенем важкості нами не виявлена. Так, серед хворих із середньоважким перебігом в середньому рівень кателіцидину складав 24,46 ± 1,69 нг/мл, із важким – 25,27 ± 1,21 нг/мл. Але хворі на МВ діти із важким і середньоважким перебігом мали достовірно вищі значення кателіцидину LL-37 порівняно з групою здорових дітей (p ≤ 0,001) (табл. 2).

Таблиця 2

Вміст антимікробного пептиду кателіцидину LL-37 у сироватці крові дітей, хворих на МВ, залежно від важкості захворювання

	Середньоважкий перебіг		Важкий перебіг	
	n	**M ± m**	**n**	**M ± m**
LL-37, нг/мл	31	24,498 ± 1,69*	43	25,28 ± 1,22*
Здорові діти	7,74 ± 0,27			

*Примітка: * – p ≤ 0,001 – різниця вірогідна відносно показників здорових дітей.*

ВИСНОВКИ

У дітей, хворих на муковісцидоз, відмічається значне підвищення антимікробного пептиду кателіцидину в сироватці крові, порівняно із групою здорових дітей. Так, середнє значення кателіцидину у пацієнтів з муковісцидозом у 3,16 рази перевищувало значення здорових пацієнтів. Було показано, що вміст кателіцидину не залежить від статі хворих дітей. Проведений аналіз вмісту LL 37 залежно від періоду та важкості захворювання показав, що даний пептид однаково підвищений у всіх хворих та достовірно відрізняється від групи контролю. Діагностична чутливість тесту складає 71,62%, діагностична специфічність тесту складає 77,5%.

АНОТАЦІЯ

Стаття присвячена вивченню ролі антимікробних пептидів у захисті респіраторного тракту у дітей, хворих на муковісцидоз. Визначені основні механізми неспецифічного захисту поверхні дихальних шляхів від бактеріальних та вірусних агентів у здорових та хворих на муковісцидоз дітей, та роль у цьому антимікробних пептидів. Обговорюються механізми антимікробної дії антимікробного пептиду кателіцидину та можливі шляхи розробки та застосування пептиду як протимікробного засобу у пацієнтів із муковісцидозом. У роботі наведені результати визначення антимікробного пептиду кателіцидину LL 37 у сироватці крові дітей, хворих на муковісцидоз, та проаналізовано його вміст залежно від періоду та важкості хвороби.

ЛІТЕРАТУРА

1. Bals R. Epithelial antimicrobial peptides in host defense against infection. *Respiratory Research*.2000. № 1. p. 141–150.

2. Bals R., Hiemstra P.S. Innate immunity in the lung: how epithelial cells fight against respiratory pathogens. *European respiratory journal*. 2004. № 23. p. 327–333.

3. Balsa R., J. Wilson. Cathelicidins – a family of multifunctional antimicrobial peptides. *Cellular and Molecular Life Sciences*. 2003. № 60.

4. Cohen T., Prince A. Cystic fibrosis: a mucosal immunodeficiency syndrome. *Nat Med*. 2013. № 18. p. 509–519.

5. Cystic Fibrosis in Europe – Facts and Figures. The European Cystic Fibrosis Society. 2015.

6. Cystic Fibrosis Life Expectancy. Cystic fibrosis news today. 2016. URL: https://cysticfibrosisnewstoday.com/cystic-fibrosis-research/.

7. Gerritn J., Yildirim A.O., Rubin B.K. TLR-4–Mediated Innate Immunity Is Reduced in Cystic Fibrosis Airway Cells. *American journal respiratory cell molecular biology*. 2010. № 42. p. 424–431.

8. Greene C.M., Carroll T.P., Smith S. G. TLR-Induced Inflammation in Cystic Fibrosis and Non-Cystic Fibrosis Airway Epithelial Cells. *The Journal of Immunology*. 2005. № 174. p. 1638–1646.

9. Guilhelmelli F., Vilela N., Albuquerque P. Antibiotic development challenges: the various mechanisms of action of antimicrobial peptides and of bacterial resistance. *Frontiers in Microbiology*. 2013. № 4. p. 1–12.

10. Hartl D., Gaggar A., Bruscia E. Innate immunity in cysticfibrosis lung disease. *Journal of Cystic Fibrosis*. 2012. № 11. p. 363–382.

11. Makukh H., Křenková P., Tyrkus M. A high frequency of the Cystic Fibrosis 2184insA mutation in Western Ukraine: Genotype–phenotype correlations, relevance for newborn screening and genetic testing. *Journal of Cystic Fibrosis*. 2010. № 9. p. 371–375.

12. Patient registry annual data report. Cystic Fibrosis Foundation. 2016.

13. UK Cystic Fibrosis Registry 2015 Annual Data Report. 2016.

14. Zhang L., Parente J., Harris S. M. Antimicrobial Peptide Therapeutics for Cystic Fibrosis. *Antimicrobial Agents and Chemotherapy*. 2005. № 49.

Information about authors:
Dudnyk V. M.,
Doctor of Medical Science, Professor
Head of Department of Pediatrics № 2
Vinnytsya National Pirogov Memorial Medical University
56, Pirogov str., Vinnytsya, 21018, Ukraine
Demianyshyna V. V.,
Postgraduate student
Department of pediatrics № 2
Vinnytsya National Pirogov Memorial Medical University
56, Pirogov str., Vinnytsya, 21018, Ukraine

EFFECTS OF AQUEOUS EXTRACT FROM *LEMNA MINOR* FROND ON HORMONAL, METABOLIC AND ELEMENTAL STATUS IN HYPOTHYROID RATS

Kononenko A. G., Kravchenko V. M.

INTRODUCTION

The literature data indicate that diseases of the thyroid gland are not the last among the endocrine pathology, and remain one of the most difficult problems in the clinical endocrinology. The ever-increasing level of morbidity, as well as high rates of disability in this pathology, due to the deterioration of the environmental situation, the lack of iodine intake, and the increase in the frequency of autoimmune diseases confirm this. Almost every second or third person on Earth has a particular pathology of the thyroid gland, in the structure of which hypothyroidism is one of the leading places in its frequency and social significance[1], [2], [3].

The basic physiological effects of thyroid hormones are quite clearly verified and due not only to their direct effect on the expression of genes that control the synthesis of structural and functional proteins in cells of different systems of the body, but also by the indirect mechanism – through interaction with catecholamines. The main effects of thyroid hormones include: 1) ensuring normal growth, development and differentiation of tissues and organs; 2) increase of efficiency of mitochondria and myocardial contractility; 3) activation of sympathetic effects; 4) increased excitability of the central nervous system and the activation of mental processes; 5) increase of renal blood flow, glomerular filtration and diuresis; 6) participation in increasing the heat formation and body temperature; 7) maintaining a normal sexual and reproductive function[4]. Undoubtedly, the change in thyroid status directly affects the functioning of almost all systems of the body, and, consequently, mechanisms of non-specific adaptation can be

[1] Кравченко В.І., Постол С.В. Динаміка захворюваності на патологію щитоподібної залози в Україні. *Междунар. эндокрин. журн*. 2011. №3 (35). С. 26–31.

[2] Лебединець Н.В., Парубоча О.М. Сучасні аспекти динаміки ендокринної патології дитячого населення. *Environment Health*. 2012. №3. С. 21–25.

[3] Bajaj J.K., Salwan P., Salwan S. Various possible toxicants involved in thyroid dysfunction: A Review. *Journal of Clinical and Diagnostic Research*. 2016. Vol. 10. № 1. P. FE01-FE03. doi: 10.7860/JCDR/2016/15195.7092.

[4] Фролов Б.А. Физиология и патология нейроэндокринной регуляции. *М.: Медицина*, 2006. С. 86–177.

used. Thyroid dysfunction is one of the endocrine pathologies, which in most cases is associated with metabolic disorders. The thyroid hormonal status is also important for homeostasis of glucose. Hypothyroidism reduces the ability to glycogenesis, partly due to a decrease in hepatic glycogen synthesis. In studies of a number of authors found that the decrease in thyroid function affects the adaptive capacity of the body and its elemental status[5, 6, 7, 8, 9].

It should be noted that for the treatment of hypothyroid conditions, mostly used substitution therapy – thyroid hormones. Substitution hormonal therapy used in hypothyroidism, corrects various symptoms of the disease, including the disruption of metabolic processes, due to artificial restoration of blood levels of thyroid hormones. However, traditional oral methods of hormone therapy do not always provide a stable compensated flow of hypothyroidism, and in some cases cause side effects, not always prevent the onset and progression of complications, often lead to hormone resistance[10, 11]. Correction of biochemical parameters in thyroid dysfunction may be related to both the incidence of thyroid hormones and biologically active substances in the bloodstream from the means used to treat disorders of metabolic processes[12, 13]. The most promising source of biologically active substances is medicinal plants. The expediency of studying medicinal plants

---

[5] Андрусишина И.Н. Информативная значимость определения микроэлементов в биологических средах пациентов с эндокринной патологией. *Scientific Journal «ScienceRise»*. 2015. №7/4(12). С. 5–10.

[6] Барышева Е.С. Роль микроэлементов в функциональном и структурном гомеостазе щитовидной железы: клинико-экспериментальное исследование. *Международный эндокрилогический журнал*. 2010. №7(31).

[7] Мирошников С.В., Нотова С.В., Тимашева А.Б., Кван О.В. Влияние экспериментального тиреотоксикоза и гипотиреоза на элементный статус лабораторных животных. *Научное обозрение. Медицинские науки*. 2014. № 2. С. 67–77.

[8] Рустембекова С.А., Аметов А.С., Тлиашинова А.М. Элементный дисбаланс при патологии щитовидной железы. *РМЖ*. 2008. № 16. С. 1078.

[9] Терещенко И.В. Дефицит магния в практике эндокринолога. *Клиническая медицина*. 2008. № 7. С.47–51.

[10] Третьяк С.И., Хрыщанович В.Я. Современные методы лечения гипотиреоза. *Минск: БГМУ*. 2011. 150 с.

[11] Javed Z., Sathyapalan Th. Levothyroxine treatment of mild subclinical hypothyroidism: a review of potential risks and benefits. *Ther. Adv. Endocrinol. Metab*. 2016. Vol. 7. № 1. P.12–23. doi: 10.1177/2042018815616543.

[12] Никифоров Л.А., Белоусов М.В., Фурса Н.С. Изучение аминокислотного состава ряски малой (*Lemna minor* L.). *Бюл. сибирской медицины*. 2011. № 5. С. 74–77.

[13] Каджарян В.Г., Мельник А.И., Бидзиля П.П., Соловьюк А.О. Оценка состояния липидного обмена при дисфункции щитовидной железы. *Запорожский медицинский журнал*. 2014. №1 (82). С. 20–22

in this direction is determined by a wide spectrum of their pharmacological activity and relative safety with long-term application[14, 15].

One of the promising plants containing a complex of biologically active substances is *Lemna minor*. Phytochemical studies of the *Lemna minor* frond are revealed iodine and 14 other elements (calcium, potassium, copper, sodium, etc.). Thirty-two biological active substances of various chemical groups (phytosterols, saturated hydrocarbons, aldehydes and ketones, fatty acids, etc.) were identified[16]. The presence of amino acids, including asparagine and glutamic acid, arginine, leucine, alanine, valine and lysine, is also established. The results of our previous screening studies in healthy rats showed the presence of a powerful thyroid stimulating action of *Lemna minor* frond in the form of aqueous extract[17].

Therefore, the purpose of our study was to study the effect of aqueous extract from *Lemna minor* frond on the hormonal, metabolic and elemental status of blood serum in rats with experimental hypothyroidism.

1. Materials and methods

Experiments were conductcarried out on white non-linear male rats weighing 120-150 g in compliance with the requirements of the NUPh Bioethics Commission and the “General Ethical Principles of Animal Experiments” (Kyiv, 2001), which are in accordance with the provisions of the European Convention on the Protection of Vertebrate Animals used for experiments or for other scientific purposes "(Strasbourg, 1986). Rats were kept under standard vivarium conditions in natural light and in the diet recommended for this species of animals.

The objects of the study were Iodomarin (Berlin-Schiemie, Germany) and aqueous extract from *Lemna minor* frond, obtained at the Department of Quality, Standardization and Certification of drugs of National University of Pharmacy and standardized in accordance with the requirements of the State

[14] Фадеев В.В. Проблемы заместительной терапии гипотиреоза: современность и перспективы. *Клин. и эксперимен. тиреоидология*. 2012. № 8(3). С. 12–17.

[15] Constantinou C., Margarity M., Valcana T. Region-specific effects of hypothyroidism on the relative expression of thyroid hormone receptors in adult rat brain. *Molecular and Cellular Biochemistry*. 2005. Vol. 278. P. 93–100.

[16] Владимирова И.Н., Георгиянц В.А. Биологически активные соединения Lemna minor S. F. gray. *Химико-фармацевтический журн*. 2013. № 47(11). С. 29–31.

[17] Кравченко В.Н., Георгиянц В.А., Владимирова И.Н., Кононенко А.Г., Орлова В.А., Щербак Е.А. Влияние лекарственных растений на уровень йодсодержащих гормонов щитовидной железы в крови крыс. *Биолог. журн. Армении*. 2014. № 4(66). С. 17–21.

Pharmacopoeia of Ukraine and the European Pharmacopoeia. Experimental hypothyroidism was induced by daily administration of aqueous solution of Mercazolil (500 mg in 1 liter) instead of drinking water for 30 days[18]. Experimental animals were divided into 4 groups of 10 rats in each: 1st – intact control (IC); 2nd – rats receiving thyreostatic Mercazolil (hypothyroid rats (control pathology (CP); 3rd – hypothyroid rats receiving aqueous extract from *Lemna minor* frond at a dose of 0.5 ml/100 g body weight; 4th – hypothyroid rats, which received a reference drug Iodomarin 12 μg iodine/kg body weight. The dose of Iodomarin was calculated according to Yu.R. Rybolovlev's method, proceeding with an average daily human iodine dose of 200 μg/day. The test animals in the 3rd and 4th experimental groups receved investigated extract and Iodomarine intragastrically during 21 days, starting with the 13th day of administration of Mercazolil.

At day 13, blood from the caudal vein of some experimental animals was taken to determine the content of thyroid hormones in the serum to confirm the development of the hypothyroid state. At the end of the experimental period, animals were devitalized by instantaneous decapitation under thiopental anesthesia (20 mg/kg), blood was collected. In blood serum, the concentration of iodine-containing thyroid hormones – triiodothyronine total (T3) and free (fT3) and total thyroxine (T4) and free (fT4) by immunoassay analysis using test systems was determined ("Chema", Russia).

The metabolic status was evaluated by the alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), lactate dehydrogenase (LDH), total protein (TP), albumin, urea, creatinine, cholesterol, triglycerides (TG), using a set of tests "Biosystems" (Spain). The glucose content was determined using the Glucometer "One Touch Ultra".

To determine the activity of ALT, the unified dinitrophenylhydrazine method (the Rietman-Frenkel method) was used. Due to the reaction of reamination, which occurs with the participation of ALT, pyruvic acid is formed, which in the presence of 2,4-dinitrophenylhydrazine in alkaline medium gives a colored hydrazone (λ=500-560 nm). To determine the

[18] Пат. № 109608 UA, МПК G09B 23/28 A61K 9/08 A61K 31/00 A61P 5/14. Спосіб моделювання експериментального гіпотиреозу у лабораторних тварин / Кравченко В. М., Кононенко А. Г., Щербак О. А., Орлова В. О., Карпенко Н. О., Коренєва Є. М. Опубл. 25.08.2016, Бюл. № 16.

activity of the AST used the unified method of Reitman-Frenkel. Because of the amination of 2-oxo-glutaric acid with L-aspartic acid, which occurs under the action of AST, the latter is decarboxylated unwittingly with the formation of pyruvic acid. The definition is based on the measurement of the optical density of 2,4-dinitrophenyl hydrazones in alkaline medium.

The content of TP was determined by the biuret method. The method is based on the ability of peptide groups of proteins and polypeptides to form a complex compound colored in violet with Cu^{2+} ions in alkaline medium and characterized by a maximum absorption at 540-580 nm.

The level of creatinine was determined by colorimetric method. The picric acid in an alkaline medium forms a yellow-red product with creatinine (a derivative of 2,4,6-tri-nitrocyclohexadenium). The urea content was evaluated by color reaction with diacetylmiooxime. Urea in the presence of thiosemicarbazide and iron salts in a strongly acidic medium forms a red colored diacetyl monooxime compound with a maximum absorption at 500-560 nm.

In the method of determination of cholesterols the indicator is the Tringer reaction – an oxidative azo combination of 4-aminophenazone with phenol with the participation of hydrogen peroxide, which is formed in the previous reaction of oxidation of cholesterol with cholesterol oxidase. Colorimetric analysis is performed at λ=498 (460-540) nm.

The determination of TG was carried out by an enzymatic colorimetric method, in which the hydrolysis of triglycerides with the formation of glycerol, which, under the action of metaperiodota sodium, is oxidized to formaldehyde, which, in turn, forms with the acetylacetone and ammonium ions a colored compound (3,5-diacetyl-1,4- dihydroluthene) with a maximum absorption at 500 nm.

Elemental status in rats was evaluated by the content of potassium, sodium, calcium, phosphorus, chlorine, iron, zinc, magnesium and copper in blood serum. Determination of the elemental composition of the serum was carried out by colorimetric method using the test kits “Medbioalance” (Ukraine).

The statistical analysis of the results was carried out using the standard statistical program package “Statistica 6.0”. The obtained experimental data are presented as arithmetic mean ($\bar{x}$), its error ($\pm$ S). Non-parametric methods (Mann-Whitney’s criterion) were used to obtain statistical

conclusions. Differences between groups were considered probable at the accepted level of statistical significance $p<0,05$[19,20].

2. Results

The results of the study showed that the level of iodine-containing thyroid hormones in serum on the 12th day of administration of Mercazolil was reduced. Concentrations of both thyroxine and triiodothyronine were statistically significantly lower than in intact animals by 36.8% and 43.9%, respectively. At the end of the trial, the level of thyroid hormones was even lower, which confirmed the correctness of the experiment to create a model of hypothyroidism induced Mercazolil. Concentration of fT3 and fT4 in the blood serum of the rats of the CP group in comparison with the IC group also decreased in 1.78 and 2.63 times ($p <0.05$), respectively (Table 1, Fig. 1, 2).

The course administration of aqueous extract from *Lemna minor* frond contributed to the normalization of the functional state of the thyroid gland and increased its synthetic function, which was manifested in a probable increase in the level of T4, fT4, T3 and fT3 at 2.33, 2.67, 1.50 and 1.82 times ($p <0.05$), respectively, in comparison with the CP group. The influence of investigated extract on thyroid hormones content exceeded the effect of the Iodomarin, which resulted in a probable increase in the T4 and T3 content in comparison with the CP group in 1.7 and 1.5 times, respectively (Table 1, Fig. 1, 2).

[19] Реброва О.Ю. Статистический анализ медицинских данных. Применение пакета прикладных программ STATISTICA. *М.: Медиа Сфера*, 2006. 312 с.

[20] Халафян А.А. STATISTICA 6. Статистический анализ данных. 3-е изд. Учебник. *М.: ООО «Бином-Пресс»*, 2007. 512 с.

Table 1

Concentration of thyroid hormones (T3, fT3, T4, fT4) in blood serum of experimental rats (n=10)

Indicator	IC	CP		AELMF	Iodomarine
		12 days	33 days		
T_3, nmol/l	1,90±0,11	1,20±0,08*	1,13±0,07*	1,70±0,13$^{\#}$	1,36±0,04*/$^{\#}$
fT_3, pmol/l	6,96±0,20	–	3,90±0,12*	7,10±0,26$^{\#}$	5,86±0,31*/$^{\#}$
T_4, nmol/l	57,50±2,33	32,24±0,95*	25,25±1,20*	58,76±1,03$^{\#}$	39,98±2,76*/$^{\#}$
fT_4, pmol/l	11,09±0,60	–	4,21±0,11*	11,24±0,49$^{\#}$	6,60±0,65*/$^{\#}$

*Notes: Values are mean ± SEM; * p <0,05 versus intact control group; # p <0,05 versus control pathology group; n – number of animals in each group; AELMF – aqueous extract from Lemna minor frond.*

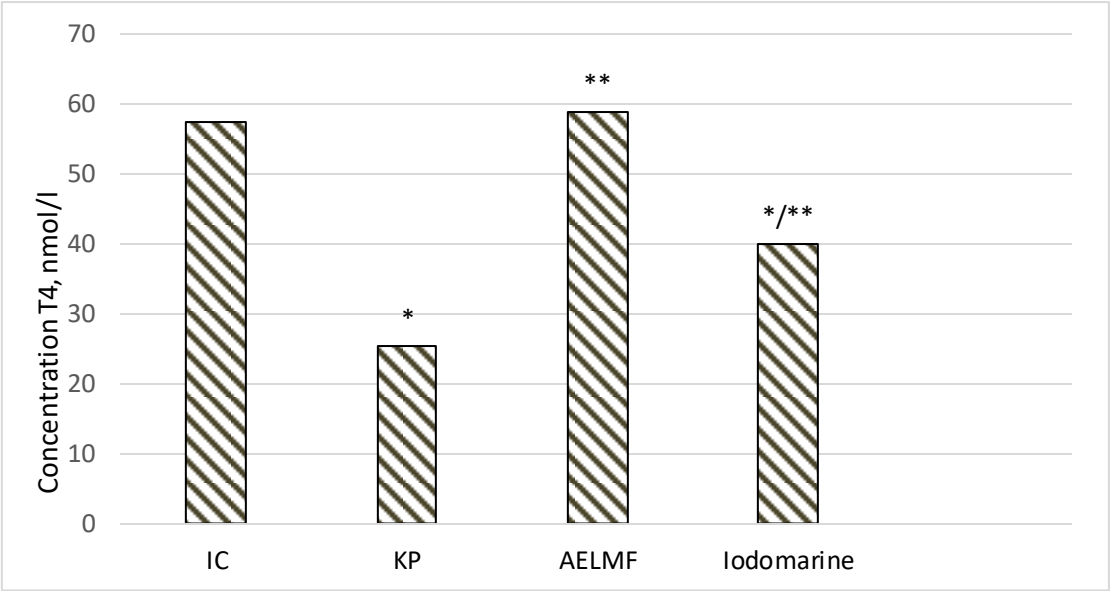


Fig. 1. The content of thyroxine in blood serum of rats of different experimental groups

*Notes: * p <0,05 versus intact control group; # p <0,05 versus control pathology group; AELMF – aqueous extract from Lemna minor frond.*

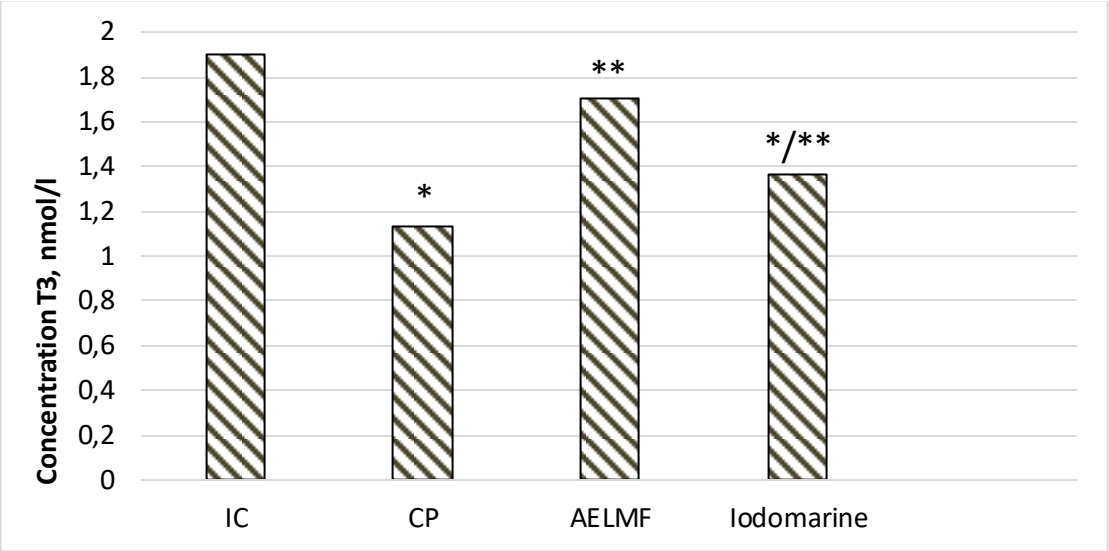


Fig. 2. The content of triiodothyronine in blood serum of rats of various experimental groups

*Notes: * p <0,05 versus intact control group; # p <0,05 versus control pathology group; AELMF – aqueous extract from Lemna minor frond.*

In the group of animals with hypothyroidism, there were no significant changes in the level of total protein and albumin; the serum creatinine and urea levels in serum of rats relative to the parameters of the IR group increased by 22.6% and 67.1%, respectively. The introduction of aqueous extract from *Lemna minor* frond to hypothyroid rats contributed to a 1.4-fold decrease in urea and a reduction in creatinine levels compared to animals in the CP group. The introduction of the Iodomarine did not lead to a decrease in the content of creatinine and urea (Table 2).

The development of experimental hypothyroidism was accompanied by a change in the content of ALT in serum. The activity of ALT in the CP group increased by 1.4 times compared with the group of IC. The introduction of aqueous extract from *Lemna minor* frond to hypothyroid rats led to a decrease in this indicator to the level of intact control. At the same time, it was found that aqueous extract from *Lemna minor* frond showed greater efficacy in relation to ALT than the reference drug Iodomarin, which reduced its activity by 1.2 times compared with the CP group. Experimental hypothyroidism was not accompanied by a change in the activity of the enzyme AST, its level remained at the level of values of the group of animals IC (Table 2). Experimental hypothyroidism led to an increase in the activity of CK in the blood serum of experimental animals in 1,3 times compared with the group of

animals IC. LDH activity remained at the level of intact animals. The use of aqueous extract from *Lemna minor* frond resulted in a decrease in the content of CK in serum blood rats to the level of animals IC. The use of the Iodomarin also had a positive effect on the activity of the CK, but to a lesser extent than the investigated extract (Table 2).

Table 2

Biochemical indices in blood serum of rats with experimental hypothyroidism and in the application of aqueous extract from *Lemna minor* frond (n=10)

Indicator	Group			
	IC	CP	AELMF	Iodomarine
ALT, μkat/l	0,383±0,021	0,523±0,022*	0,379±0,042**	0,452±0,019*/**
AST, μkat/l	0,579±0,032	0,626±0,039	0,559±0,031	0,617±0,022
CK, units/L	264,40±9,73	351,90±26,53*	273,89±17,48**	332,77±30,45
LDH, μkat/l	4,09±0,10	4,11±0,11	3,89±0,22	3,87±0,26
Total protein, g/l	60,11±1,01	63,44±2,84	71,05±3,17*	62,21±2,79
Albumin, g/l	34,14±0,48	33,61±1,34	34,82±1,01	32,86±0,56
Urea, mmol/l	5,56±0,23	9,29±0,35*	6,82±0,30*/**	7,81±0,344*/**
Creatinine, μmol/l	97,69±2,94	119,72±5,14*	100,96±3,31**	119,37±5,48*
Cholesterol, mmol/L	2,96±0,12	4,11±0,11*	3,28±0,16**	3,38±0,12*/**
TG, mmol/l	0,58±0,02	0,90±0,04*	0,64±0,02**	0,73±0,02*/**
Glucose, mmol/l	5,63±0,14	4,63±0,06*	6,04±0,24**	5,11±0,12**

*Notes: Values are mean ± SEM; * p <0,05 versus intact control group; # p <0,05 versus control pathology group; n – number of animals in each group; AELMF – aqueous extract from Lemna minor frond.*

The introduction of Mercazolil was also accompanied by changes in lipid metabolism indices (Table 2). The content of cholesterol increased by 38.9% and TG – by 55.2%. After 21 days of use of aqueous extract from *Lemna minor* frond in experimental hypothyroid rats, the serum cholesterol and TG in serum from experimental animals decreased by 20.2% and 28.9%, respectively ($p \leq 0.05$), respectively, compared to animals in the CP group (Table 2)

Concentrations of glucose in serum decreased by 17.8% in hypothyroid rats. Against the background of the introduction of aqueous extract from

Lemna minor frond, its concentration increased and slightly exceeded this level in the group of animals IC. The action of the referent drug Iodomarin was also manifested in increased glucose levels, which reached the values of the group of IC (Table 2).

Table 3

The content of macro- and microelements in blood serum of rat of experimental groups (n=10)

Indicator	IC	CP	AELMF	Iodomarine
Sodium, mg/dl	363,71±6,65	289,13±7,23*	345,32±4,45#	291,18±8,76*
Potassium, mg/dl	21,58±0,52	18,29±0,85*	19,95±0,86	19,19±1,06
Chlorides, mg/dl	388,23±8,61	321,72±16,24*	346,68±14,94*	341,13±7,19*
Calcium, mg/dl	10,79±0,32	13,58±0,43*	10,72±0,43#/o	12,02±0,30*/#
Phosphorus, mg/dl	6,37±0,09	5,27±0,15*	5,91±0,14*/#	5,20±0,11*
Magnesium, mg/dl	5,75±0,12	4,42±0,18*	5,54±0,14#	4,62±0,19*
Iron, μg/dl	100,67±3,31	76,87±3,02*	97,73±5,25#	89,71±4,73
Copper, μg/	94,12±3,31	146,57±3,14*	107,84±5,64#	134,80±5,13*
Zinc, μg / dl	63,87±1,93	53,58±1,80*	62,00±2,29#	59,23±1,52#

*Notes: Values are mean ± SEM; * p <0,05 versus intact control group; # p <0,05 versus control pathology group; n – number of animals in each group; AELMF – aqueous extract from Lemna minor frond.*

The introduction of thyreostatics Mercazolil resulted in a decrease in sodium concentration (by 20.5%), potassium (by 18.0%), chlorine (by 17.1%), phosphorus (by 17.3%), magnesium (by 30.1%), iron (30.9%) and zinc (by 16.1%) and increase the content of copper (by 55.7%) and calcium (by 25.9%) compared with the intact control group (Table 3).

The use of aqueous extract from *Lemna minor* frond in hypothyroid rats led to an increase in the concentration of sodium, chlorine, zinc, iron, phosphorus, magnesium, and a decrease in the content of calcium and copper in serum of rats compared with the CP group to the level of these elements in the IC group (Table 3). The use of Iodomarin in hypothyroid animals, unlike aqueous extract from *Lemna minor* frond, did not lead to significant changes in the elemental status compared with the control group (Table 3).

3. Discussion

Merkazolil is a well-known thyreostatic agent whose mechanism of action is to block the synthesis of thyroid hormones at the level of interaction between mono and diiodotyrosine, as well as in the inhibitory effect on the iodization process of tyrosine fragments of thyroglobulin. In our study, the use of Mercazolil resulted in a decrease in the synthetic thyroid function in experimental animals, which was manifested in reducing the level of thyroid hormones. The development of experimental hypothyroidism in experimental animals was also confirmed by a reduced level of fT4 and fT3. The evaluation of their content is the most adequate and direct marker of thyroid hormone function, since it is known that the biological action of thyroid hormones corresponds to free T4 and T3 and their content does not depend on the concentration of binding proteins.

The use of aqueous extract from *Lemna minor* frond facilitated the restoration of the functional state of thyroid in rats with experimental hypothyroidism to the level of these indicators in animals of the intact control group. It is possible to assume that the efficacy of aqueous extract from *Lemna minor* frond is related to the direct effect of the investigated extract on the synthetic thyroid function, which is likely to be realized due to its effect on the enzyme systems involved in the processes of synthesis, deiodination and binding to the proteins of thyroid hormones. The mechanism of action of aqueous extract from *Lemna minor* frond on the functional activity of the thyroid gland needs further detailed study.

Result of the analysis of dynamics of biochemical parameters in blood serum of rats, it was found that in the group of animals with hypothyroidism there were no significant changes in the level of total protein and albumin. Introduction of Mercazolil resulted in an increase in creatinine levels. It is known from the literature that accumulation of creatinine in serum may indicate renal insufficiency due to decreased glomerular filtration in the kidneys. The introduction of aqueous extract from *Lemna minor* frond led to the restoration of this indicator.

The development of the hypothyroid state was accompanied by an increase in the level of urea in the blood serum of experimental animals, which may indicate a glomerular filtration disorder on the one hand, and a violation of the urea-forming function of the liver, on the other. In addition, an increase in this indicator may occur due to increased catabolism of proteins. The introduction of aqueous extract from *Lemna minor* frond

against the background of hypothyroidism contributed to a decrease in the urea content.

The analysis of experimental data on the activity of aminotransferases showed that the development of experimental hypothyroidism was accompanied by a change in the content of ALT in serum. The introduction of aqueous extract from *Lemna minor* frond in hypothyroid rats led to a decrease in this indicator to the level of intact control. Increasing the content of ALT may indicate a violation of the liver function, which may be due to the lack of thyroid hormones, and the hepatotoxic action of thyreostatic mercazolil. On the other hand, as a result of the study, it was shown that experimental hypothyroidism was not accompanied by a change in the activity of the enzyme AST. Its level remained at the level of values of the group of animals IC.

In the study of the activity of enzymes CK and LDH, it was found that the development of experimental hypothyroidism is accompanied by an increase in the activity of CK in the blood serum of experimental animals in 1,3 times compared with the group of animals intact control. LDH activity remained at the level of intact animals. Creatine kinase and LDH – enzymes involved in the formation of energy and its cleavage, and their release into the bloodstream testifies to damage to the integrity of the muscles. In the case of hypothyroidism, the increase in CK activity may be due to increased catabolic processes in the skeletal muscle. In addition, one should take into account the fact that thyroxine suppresses the activity of the CK. The use of aqueous extract from Lemna minor frond resulted in a decrease in the serum CK content in rat serum to the level of infant animals.

Analysis of indicators of lipid metabolism showed that the hypothyroid state caused by the introduction of Mercazolilum was also accompanied by changes. The content of cholesterol and TG in the animal group CP was increased. Hypercholesterolemia associated with hypothyroidism in humans and animals to a significant extent is usually due to the increase in the content of cholesterol with low density lipoprotein and very low density lipoprotein, although there is evidence of an increase in the concentration of high density lipoprotein. After 21 days of application of aqueous extract from Lemna minor frond in experimental rats with experimental hypothyroidism, positive changes were observed. The content of cholesterol and TG in blood serum of experimental animals decreased by 20.2% compared to animals of the CP group.

The development of the hypothyroid state was also accompanied by a decrease in glucose concentration in serum. One of the reasons for a fixed decrease in glucose levels may be the reduction of its absorption in the intestine because of a decrease in the activity of hexokinase and a violation of its transport through the blood. An analysis of the experimental data on serum glucose in rats showed that in the group of animals, which received aqueous extract from Lemna minor frond on the background of hypothyroidism, its concentration increased and slightly exceeded the level in the group of infants.

Features of the elemental status of the organism and its influence on the thyroid status were studied from the middle of the last century. The influence of Cu, Fe, Mg, Mn, P, Zn, Cd, Co, Mo and their correlation bonds on thyroid function is shown. Other researchers also found the inverse relationship between the influences of thyroid status on mineral metabolism.

Data from the study of the elemental status of blood serum of hyporhyroid rats showed that the introduction of thyreostatics of Mercazolil resulted in a decrease in the concentration of sodium, potassium, chlorine, phosphorus, magnesium, iron and zinc, and an increase in the content of copper and calcium.

Reducing the concentration of sodium, potassium and chlorine can be due to increased elimination of these elements by the kidneys. It is known from the literature that thyroid hormones are involved in the mechanism of regulation of excretion of sodium, potassium and chlorine, namely, they affect the velocity of glomerular filtration. It is also known that thyroxine stimulates the activity of Na^+-K^+ -ATPase. In the development of the hypothyroid state, these processes are violated, which leads to an elevated excretion of sodium, potassium and chlorine with urine. In addition, literature data suggests that lowering the level of magnesium leads to a decrease in potassium content.

The mechanism of reducing the magnesium content in hypothyroidism may be the reduction of its flow through the mucous membrane of the stomach and intestines due to ion diffusion and slowdown of the active absorption process observed in the hypothyroidism of the thyroid gland.

Reductions in the content of phosphorus, zinc and iron in blood serum of experimental animals with experimental hypothyroidism can be explained by the interrelation of these elements with thyroid status, since zinc is a

component of the nuclear receptor of triiodothyronine, and iron is involved in the conversion of L-phenylalanine to L-tyrosine.

The increase in blood serum in rats in the control group of copper concentration may be due to a decrease in zinc content, since it is a functional copper antagonist. On the other hand, there are literature data that in rats, unlike humans, thyroxine reduces the level of copper in the blood.

The obtained data concerning the increase of the level of calcium in the blood serum of rats with hypofunction of the thyroid gland can be explained, probably, by the fact that hypothyroidism leads to inhibition of thyroid extraction not only thyroid hormones, but also thyrocalcitonin, which participates in the regulation of calcium content. Reducing the level of thyocalcitonin, in turn, leads to an increase in the allocation of parathormone by the pterygoid glands, which is its antagonist and contributes to an increase in the concentration of calcium in the blood.

The use of aqueous extract from *Lemna minor* frond in hypothyroid rats led to the restoration of elemental status in experimental animals, which was manifested in increasing the concentration of sodium, chlorine, zinc, iron, phosphorus, magnesium, and lowering the content of calcium and copper in blood serum of rats compared with the control group to the level of these elements in a group of intact animals. The resulting effect of aqueous extract from *Lemna minor* frond on the elemental status of blood serum of experimental animals may be explained, probably not only, by its positive effect on the functional activity of the thyroid gland in the experimental hypothyroidism. It is also possible to assume that the investigated extract also affects other physiological and biochemical processes due to its content of macro- and trace elements and other biologically active substances, which requires further research.

CONCLUSIONS

Experimental hypothyroidism, induced by the administration of 0.05% of Mercazolil solution, is characterized by a decrease in thyroid function, which is manifested in the reduction of the thyroid hormones content in the blood serum of experimental animals. Hypofunction of the thyroid gland contributed to the development of negative manifestations of the biochemical reactions of the body in the form of an increase in serum cholesterol, triglycerides, urea, creatinine, activity of ALT and CK enzymes and a decrease in glucose levels, indicating a violation of lipid, protein and carbohydrate metabolism in the background of hypothyroidism. As a result,

experimental hypothyroidism is characterized by changes in the content of macro- and microelements, which manifests itself in decreasing the concentration of sodium, potassium, chlorine, phosphorus, magnesium, iron, zinc and increasing the content of copper and calcium in serum from experimental animals.

The use of aqueous extract from *Lemna minor* frond at a dose of 0.5 ml/100 g of the body weight of the animal had a corrective effect on the hypothyroid state of the thyroid, as evidenced by the probable increase in the level of T4, fT4, T3 and fT3 in the blood serum of experimental animals to the level of the intact control group. Introduction aqueous extract from *Lemna minor* frond contributed to the restoration of biochemical parameters in serum. In the experimental group receiving the test extract in the background of hypothyroidism, there were positive changes in biochemical parameters that could be regarded as a sign of recovery of metabolic processes. Aqueous extract from *Lemna minor* frond contributed to the restoration of elemental status in animals with experimental hypothyroidism.

The resulting effect of aqueous extract from *Lemna minor* frond on the hormonal, metabolic and elemental status of blood serum of experimental animals may be explained not only by its positive effect on the functional activity of the thyroid gland in the experimental hypothyroidism. It is also possible to assume that the investigated extract also affects other physiological and biochemical processes due to the content of macro- and microelements and other biologically active substances in it.

In conclusion, aqueous extract from *Lemna minor* frond is promising for further study of its effectiveness and mechanisms of action on other models of experimental hypothyroidism as an independent agent and in the composition of pharmacological combinations for the prevention and treatment of hypothyroid conditions and for the correction of the consequences of hypothyroidism.

SUMMARY

The article presents the study of the effect of aqueous extract from *Lemna minor* frond on the hormonal, metabolic and elemental status of rats with experimental hypothyroidism. For the treatment of hypothyroid conditions use substitution hormonal therapy. However, there was a need to find alternative methods for compensating thyroid insufficiency, one of which is herbal preparations. Experimental hypothyroidism was induced by daily

administration of aqueous Mercasolil solution instead of drinking water for 30 days. It was established that administration of aqueous extract from *Lemna minor* frond contributed to a probable increase in T4, fT4, T3 and fT3 levels in 2.33, 2.67, 1.50 and 1.82 times ($p < 0.05$), respectively, compared with the CP group. As a result of the experiment, it was found that the use of aqueous extract from *Lemna minor* frond to hypothyroid rats leads to a decrease in the maintenance of cholesterol, triglycerides, urea, creatinine, ALT and creatine kinase, and an increase in blood glucose levels in blood serum of rats. The introduction of aqueous extract from *Lemna minor* frond led to the restoration of elemental balance in rats, which was manifested in increasing the sodium, chlorine, phosphorus, magnesium, zinc, iron content and lowering the content of copper and calcium in serum. Thus, aqueous extract from *Lemna minor* frond had a corrective effect on the hypothyroid state of the thyroid gland.

REFERENCES

1. Кравченко В.І., Постол С.В. Динаміка захворюваності на патологію щитоподібної залози в Україні. *Междунар. эндокрин. журн*. 2011. № 3 (35). С. 26–31.
2. Лебединець Н.В., Парубоча О.М. Сучасні аспекти динаміки ендокринної патології дитячого населення. *Environment Health*. 2012. № 3. С. 21–25.
3. Bajaj J.K., Salwan P., Salwan S. Various possible toxicants involved in thyroid dysfunction: A Review. *Journal of Clinical and Diagnostic Research*. 2016. Vol. 10. № 1. P. FE01-FE03. doi: 10.7860/JCDR/2016/15195.7092.
4. Фролов Б.А. Физиология и патология нейроэндокринной регуляции. *М.: Медицина*, 2006. С. 86–177.
5. Андрусишина И.Н. Информативная значимость определения микроэлементов в биологических средах пациентов с эндокринной патологией. *Scientific Journal «ScienceRise»*. 2015. №7/4(12). С. 5–10.
6. Барышева Е.С. Роль микроэлементов в функциональном и структурном гомеостазе щитовидной железы: клинико-экспериментальное исследование. *Международный эндокрилогический журнал*. 2010. №7(31).
7. Мирошников С.В., Нотова С.В., Тимашева А.Б., Кван О.В. Влияние экспериментального тиреотоксикоза и гипотиреоза на

элементный статус лабораторных животных. *Научное обозрение. Медицинские науки*. 2014. № 2. С. 67-77.

8. Рустембекова С.А., Аметов А.С., Тлиашинова А.М. Элементный дисбаланс при патологии щитовидной железы. *РМЖ*. 2008. №16. С. 1078.

9. Терещенко И.В. Дефицит магния в практике эндокринолога. *Клиническая медицина*. 2008. № 7. С.47–51.

10. Третьяк С.И., Хрыщанович В.Я. Современные методы лечения гипотиреоза. *Минск: БГМУ*. 2011. 150 с.

11. Javed Z., Sathyapalan Th. Levothyroxine treatment of mild subclinical hypothyroidism: a review of potential risks and benefits. *Ther. Adv. Endocrinol. Metab*. 2016. Vol. 7. № 1. P. 12–23. doi: 10.1177/2042018815616543.

12. Никифоров Л.А., Белоусов М.В., Фурса Н.С. Изучение аминокислотного состава ряски малой (*Lemna minor* L.). *Бюл. сибирской медицины*. 2011. № 5. С. 74–77.

13. Каджарян В.Г., Мельник А.И., Бидзиля П.П., Соловьюк А.О. Оценка состояния липидного обмена при дисфункции щитовидной железы. *Запорожский медицинский журнал*. 2014. №1 (82). С. 20–22.

14. Фадеев В.В. Проблемы заместительной терапии гипотиреоза: современность и перспективы. *Клин. и эксперимен. тиреоидология*. 2012. № 8(3). С. 12–17.

15. Constantinou C., Margarity M., Valcana T. Region-specific effects of hypothyroidism on the relative expression of thyroid hormone receptors in adult rat brain. *Molecular and Cellular Biochemistry*. 2005. Vol. 278. P. 93–100.

16. Владимирова И.Н., Георгиянц В.А. Биологически активные соединения Lemna minor S. F. gray. *Химико-фармацевтический журн*. 2013. № 47(11). С. 29–31.

17. Кравченко В.Н., Георгиянц В.А., Владимирова И.Н., Кононенко А.Г., Орлова В.А., Щербак Е.А. Влияние лекарственных растений на уровень йодсодержащих гормонов щитовидной железы в крови крыс. *Биолог. журн. Армении*. 2014. № 4(66). С. 17–21.

18. Пат. № 109608 UA, МПК G09B 23/28 A61K 9/08 A61K 31/00 A61P 5/14. Спосіб моделювання експериментального гіпотиреозу у лабораторних тварин / Кравченко В. М., Кононенко А. Г.,

Щербак О. А., Орлова В. О., Карпенко Н. О., Коренєва Є. М. Опубл. 25.08.2016, Бюл. № 16.

19. Реброва О.Ю. Статистический анализ медицинских данных. Применение пакета прикладных программ STATISTICA. *М.: Медиа Сфера*, 2006. 312 с.

20. Халафян А.А. STATISTICA 6. Статистический анализ данных. 3-е изд. Учебник. *М.: ООО «Бином-Пресс»*, 2007. 512 с.

21. Побігун Н.Г. Кальцій-фосфорний баланс та мінеральна щільність кісткової тканини при експериментальній гіпотиреоїдній дисфункції. *Галицький лікарський вісник*. 2013. Т. 20, № 4. С. 137–139.

22. Liamis G., Filippatos T.D., Liontos A., Elisaf M.S. Management of endocrine disease: Hypothyroidism-associated hyponatremia: Mechanisms, implications and treatment. *Eur J Endocrinol*. 2016. P. 16-0493

Information about authors:
Kononenko A. G.,
Candidate of Pharmaceutical Sciences,
Associate Professor at the Department of Physiology
and Human Anatomy
National University of Pharmacy
53, Pushkinskay Str., Kharkiv, 61000, Ukraine
Kravchenko V. M.,
Doctor of Biological Sciences,
Professor, the Head of the Department of
Biological Chemistry
National University of Pharmacy
53, Pushkinskay Str., Kharkiv, 61000, Ukraine

CLINICAL AND EXPERIMENTAL RATIONALE FOR THE TREATMENT OF PERIODONTAL DISEASES IN INDIVIDUALS WITH PSYCHO-EMOTIONAL STRESS

Kononova O. V.

INTRODUCTION

Influence of psycho-emotional stress on the state of periodontal tissues

According to WHO, periodontal diseases revealed in 80% of children and 95% of the adult population of the planet. Periodontal disease is one of the most widespread human diseases. The most frequent inflammatory lesions are gingivitis and chronic periodontitis – lesions involving the destruction of periodontal tissues (alveolar bone) and subsequent tooth loss. To date, there is no country or region of the world where periodontal diseases were absent[1].

Periodontal disease, especially chronic periodontitis, is the main cause of loss of teeth in people over 30 years of age. In Ukraine, according to epidemiological studies the prevalence of periodontal disease among the population aged 35–44 ranges from 92% to 98%. In recent years, there is a steady tendency to increase the diseases incidence among young people, and increase in the number of patients with chronic periodontitis. The prevalence of periodontal diseases among people aged 19-24 reaches 30%, and 25–30 years – more than 60%[2].

The degree, severity and course of periodontal diseases depend on a number of factors, such as individual oral hygiene, diet, heredity, etc. Consideration should also be given to the level of dental care provided by health authorities. Periodontal diseases occur in every age group, but more often in adults, regardless of sex, race, education, place of residence, or socio-economic status.

The development of periodontal disease (chronic periodontitis) depends on many local or systemic risk factors. Important place is given to

[1] Pistorius A., Krahwinkel T., Willerhausen B., Bockstegen C. Relationship between stress factors and periodontal disease. Eur. J. Med. Res. 2002. Vol. 7(9). P. 393–398

[2] Косенко К.М. Епідеміологія основних стоматологічних захворювань у населення України і шляхи їх профілактики [автореферат]. Київ: Національний медичний університет. 1994. 45

parodontopathogenic microorganisms that induce an increase in the level of proinflammatory cytokines with subsequent destruction of periodontal tissues. Most often it is Porphyromonas gingivalis (P. gingivalis), Prevotella intermedia (P. intermedia), Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans)[3]. In addition to the action of bacteria and host reaction factors, viruses also play a role in the pathogenesis of chronic periodontitis.

But the effect of periodontal pathogenic bacteria is not sufficient for the development and progression of periodontal disease. Possible risk factors are age, tobacco use. systemic diseases, psychological stress, etc.

Stress is a proven and important factor in the etiology and development of many inflammatory diseases, including periodontal diseases[4]. Several studies have shown the relationship between psychological stress and inflammatory diseases, such as rheumatoid arthritis and chronic periodontitis. In the event of chronic stress, chronic systemic inflammatory processes, such as rheumatoid arthritis, diabetes mellitus, cardiovascular disease or periodontal disease occur.

A number of clinical studies have examined the possible relationship between psychological stress and periodontal disease. The authors suggested that stress can play a role in the development of periodontal diseases[5]. It has been shown that individuals who are under psychological stress are more prone to development of periodontal diseases, loss of alveolar bone and increase of lesions by chronic periodontitis.

Stress inhibits healing of connective and bone tissue, apical migration of the conjunctive epithelium and the formation of a periodontal pocket[6]. It occurs under the influence of changes in the organism's protective forces that acquire immunosuppressive action, increasing the propensity to cause the disease.

---

[3] Castillo DM., Sanchez-Beltran MC, Castellanos JE, Sanz I, Mayorda-Fayad I, Sanz M, Lafaurie GI. Detection of specific periodontal microorganisms from bacteriemia samples after periodontal therapy using molecular-based diagnostics. J. Clin. Periodontol. 2011. Vol. 38. P. 418–427

[4] Keshava P.K., Sangeeta N.U. Stressing the stress in periodontal disease. J. Pharm. Biomed. Sci. 2013. V. 26. P. 345–348

[5] Aksali A., Huck O, Tenenbaum H, Davideau JL, Buduneli N. Periodontal diseases and stress: a brief review. J. Oral. Rehabil. 2013. Vol. 40(1). P. 60–68

[6] Chandna S., Bathla M. Stress and periodontium: A review of concepts. J. Oral Health Comm. Dent. 2010. Vol. 4(1). P. 17–22

In the process of destruction of periodontium from the point of view of the inflammatory and immune response of the host organism, cytokines or matrix metalloproteinases are involved.

Interesting research results were obtained in the case of studying the impact of psychological stress on students. The conducted studies showed a high level of anxiety among students of medical and dental departments[7]. They also performed a determination of the state of periodontal tissues. Students taking part in the large exam showed significantly more dental plaque and gingival disease than did not participate in any exam. The researchers concluded that the academic stress test seemed to negatively affect the state of the periodontal tissue of students. Such psychological stress resulted in an increase in blood serum and gingival fluid in students of inflammatory cytokines, IL-1β, IL-6 and IL 10. There is also a stimulation of the hypothalamic-pituitary adrenal system by cytokines.

Croucher et al.[8] have shown that negative life events lead to the risk of periodontal disease development. They believe that in order to overcome psychological stress, there are many opportunities for changing the behavior of patients who can be stressed.

It was found that psychological stress can affect lifestyle and the hygienic state of the oral cavity. Stress caused by psychosocial factors can affect the microbial ecology of periodontium. Conducted by Roberts et al.[9], in 2005, in vitro studies showed that changes in the growth of 43 microorganisms were detected in human gingival fluid in stress response.

The development of gingivitis under the influence of stress occurs as follows. Stress reduces salivation and increases the formation of dental plaque (biofilms). Neurotransmitters and neuropeptides, neuroendocrine

[7] Omigbodun O.O., Odukogbe A.T., Omigbodun A.O., Yusuf O.B., Bella T.T., Olayemi O. Stressors and physiological symptoms in students of medicine and allied health professions in Nigeria. Soc. Psychiatry Psychiatr. Epidemiol. 2006. Vol. 41(5). P. 415–421; Smith C.K., Peterson D.F., Degenhardt B.F., Johnson J.C. Depression, anxiety, and perceived hassels among entering medical students. Psychol. Health. Med. 2007. Vol. 12(1).

[8] Croucher R., Marcenes W.S, Torres M.C., Hughes F., Sheiham A. The relationship between life-events and periodontitis. A case-control study. J. Clin. Periodontol. 1997. Vol. 24(1). P. 39–43

[9] Roberts A., Matthews B. J, Socransky S.S., Freestone, Williams H. P, Chapple I.L. Stress and periodontal diseases: growth responses of periodontal bacteria to Escherichia coli stress-associated autoinducer and exogenous. *Fe. Molecular. Oral Microbiology*. 2005. Vol. 20(3). P. 147–153.

(hormones) substances that can modulate the immune response to bacteria[10] are allocated. Emotional stress changes the pH of saliva and secretion of salivary IgA. Increased levels of IL-1 in gingival fluid and decreased oral hygiene have been noted.

Psychoemotional factors are beneficial factors for the development of ulcerative-necrotizing diseases of the periodontal disease. In 1964, Giddon et al. discovered more ulcerative-necrotizing periodontal disease in college students during the exam period[11]. Compared with other periods of the year, college students report an increase in the incidence of acute ulcerative-necrotizing gingivitis during exams.

A number of clinical studies have examined the possible relationship between psychological stress and generalized periodontitis. It was assumed that stress can play an important role in the development and increase in the severity of periodontal diseases[12]. It has been shown that individuals who were stressed were more prone to developing periodontal diseases than subjects without stress[13].

On the other hand, Castro et al.[14], in 2006, could not show any connection between life events, anxiety and depression and generalized periodontitis.

Thus, a number of conducted studies show the relationship between psycho-emotional stress and diseases of periodontal tissues, in particular, generalized periodontitis. This relationship should be taken into account in the case of complex treatment of periodontal diseases. The analysis of literature data shows that such treatment cannot be considered fully worked out. Thus, the study of the influence of psycho-emotional stress on the course of periodontal diseases and the development of appropriate regimens of medical treatment of periodontal diseases (generalized periodontitis) in people with psycho-emotional stress is an urgent problem of the periodontology.

---

[10] Breivik T, Thrane PS. Breivik T, Thrane PS. Psychoneuroimmune interaction in periodontal disease. In: Psychoneuroimmunology. In: Ader R., Fetten D.L., Cohen N. 3nd ed. San Diego: Academic Press, 2001. P. 627–644

[11] Giddon D.B., Zaskin, S.J., Goldhaber P. Acute necrotizing ulcerative gingivitis in college students. J. Am. Dent. Assoc. 1964. Vol. 68. P. 381–386

[12] Sateesh C.P., Santosh K.R., Pushpalatha G. Relationship between stress and periodontal disease. J. Dent. Sci. Res. 2010. Vol. 1. P. 54–61

[13] Akhter R., Hannan M, Okhuba R, Morita M. Relationship between stress factor and periodontal disease in a rural area population in Japan. J. Med. Res. 2005. Vol. 10(8). P. 352–357

[14] Castro GDC., Oppermann RV, Haas AN, Winter R, Alchieri JC. Association between Psychosocial factors and periodontitis. J. Clin. Periodontol. 2006. Vol. 33(2). P. 109–114

2. The prevalence of periodontal disease in individuals with different levels of anxiety (psycho-emotional stress)

A number of clinical studies have examined the possible relationship between psychological stress and periodontal disease. It was suggested, that stress could play a certain provocative role in the development of periodontal diseases. In particular, persons who are in a state of psychological stress are more prone to the development of generalized periodontitis.

Measuring anxiety as a personal quality is especially important, because this quality largely determines the behavior of the subject. A certain level of anxiety is a natural and obligatory feature of an active person[15].

Given these circumstances, it was of interest to determine the possible relationship between living conditions (illumination), the psychosomatic state of the inhabitants and the state of their periodontal tissues.

The aim of this investigation was to determine the effect of housing lighting conditions on the psychosomatic state and periodontal tissue of the inhabitants.

Material and methods of research. To study the subjective reactions of a person to the action of various environmental factors it is advisable to use specially designed questionnaires. The advantage of a questionnaire survey over other methods is the possibility of obtaining significant amounts of empirical information in short terms.

A total of 350 residents of different districts of Kyiv, living in different housing conditions, were questioned and examined. The contingent of respondents consisted mainly of young people (average age – 31, at the maximum 68 years and minimum 18 years). Among the respondents, women dominated – 63.14%, men accounted for only 36.86%.

The information gathered through questionnaires. The interviewer filled out questionnaires during the conversation. At present, 350 respondents were interviewed for the preliminary adaptation of the questionnaire to the research and adaptation of the methods of statistical data processing. According to the received data, electronic databases are created in the format Excel. A qualitative analysis of respondents' answers to the questions was also carried out.

[15] Астапов В.М., редактор. Функциональный подход к изучению состояния тревоги. СПб.: Питер, 2001. Раздел Тревога и тревожность; с. 156–165

Diagnosis of anxiety self-esteem was performed by determining the Spielberger-Khanin test of reactive and personality anxiety[16]. The test allows you to assess the emotional state and, in particular, the level of emotional stress. Evaluated reactive and personality aspects of anxiety. The subjects were filled with questionnaire Spielberger-Khanin, with which help determine personal and situational anxiety. Subsequently, the responses were evaluated according to the keys and counted the total number of points by all the judgments separately for each of the scales (situational anxiety and personal anxiety).

At examination of the oral cavity, the color and consistency of the mucous membrane of vestibule, its depth, the condition and height of attachment of frenulums were estimated. The condition of the mucous membrane of the cheeks, soft palate, hard palate, tongue, the floor of the oral cavity was determined. Gingiva examined from the vestibular and oral sides. Evaluated its color, presence or absence of edema, consistency, relief of the clear margin, etc. The presence, localization and intensity of the inflammatory process of the gingiva were determined using the Pisarev-Schiller test. Particular attention was paid to dental deposits: their type, consistency, quantity and localization. Diagnostic dyes were used to detect dental plaque (biofilm).

All patients with generalized periodontitis had a thorough clinical examination of the oral cavity: they determined the condition of the hard tissues of the teeth, dental arches, anatomical features of the structure of the vestibule, the level of attachment of the frenulums of the mucosa, the condition of the mucous membrane of the gingiva, periodontal pockets, the width of the attached gums, the state of periodontium and bone tissue alveolar processes of the jaws. The examination included anamnesis, actual clinical examination and X-ray examination. When making a diagnosis, the classification of periodontal diseases was used for MF. Danilevsky.

The assessment of the hygienic state of the oral cavity was carried out using the hygienic index of Fedorov-Volodkin and the Green-Vermillion index. To determine the degree of inflammation of the gingiva, the index of PMA was used.[17]

[16] Spielberger C.D. Test Anxiety Inventory. Sampler Set. Manual, Test, Scoring. Redwood City: Mind Garden, 1980. 240 p

[17] Заболевания пародонта / под ред. Борисенко А.В. Киев: Медицина; 2011. 616 с.

Statistical processing of the obtained results was carried out in the package "STATISTICA 6.1" using parametric and nonparametric methods. Evaluated the correct distribution of signs for each of the variation series, average values for each sign and their standard errors and deviations[18].

RESEARCH RESULTS

The analysis of questionnaires of respondents showed that 308 (88.0%) people favorably assessed the direct penetration of direct sunlight in their home. Only 42 (12.0%) of the respondents indicated that penetration of direct sunlight into their homes causes unpleasant sensations. 221 (63.14%) of respondents believe that direct sunlight leads to better illumination of housing. 53 (15.14%) of the respondents believe that it improves the conditions of the microclimate of housing, 42 (12.0%) people - it is better to feel well, 34 (9.71%) of respondents believe that direct sunlight promotes health improvement. Thus, the solar insolation of the room has a significant positive effect on the psychological state of the human organism, located in the home.

Certain insufficiency of natural light and solar insolation of residential premises has a corresponding negative impact on the health of inhabitants. 154 (44.0%) of the respondents are ill 2 times a year, 119 (34.0%) – once a year, 59 (16.86%) more than 2 times a year and only 18 (5.14%) – do not get sick during the year. In 308 (88.0%) of respondents in their premises live patients with bronchial asthma, only 42 (12.0%) – no. Perhaps this is due to the fact that there are few inhabitants in the fresh air, which is very important for health, especially children. 221 (63.14%) of respondents are in the open air for more than 1 hour and 129 (36.86%) – less than 1 hour. This is very small to maintain the optimal health of apartment dwellers.

Thus, the results of the survey showed that 204 (58.29%) of the respondents have insufficient levels of natural lighting of the living space, and 146 (41.71%) of the respondents are satisfactory.

More precise data on the incidence rate of the respondents showed that deterioration of health by one or more indicators (morbidity, adaptation, physical condition, psycho-emotional status) was detected in 301 (86.0%) of the polled. 126 (36.0%) had different chronic systemic diseases (digestive tract, cardiovascular system, diabetes, etc.) in the stage of compensation and

[18] Мінцер О.П., Вороненко Ю.В, Власов В.В. Оброблення клінічних і експериментальних даних у медицині. Київ: Вища школа; 2003. 350 с.

subcompensation, 221 (63.14%) had unsatisfactory levels of adaptation, 94 (26.86%) of the polled considered their condition unsatisfactory.

The tested Spielberger test showed in the respondents a moderate level of reactive anxiety – 34.72±2.45 and a high level of personal anxiety – 50.64 ±3.58.

The following patterns of distribution of reactive anxiety were found among respondents who lived in conditions unsatisfactory in conditions of natural light conditions (Table 1).

Table 1

The level of reactive anxiety depending on the diseases among respondents who lived in unsatisfactory conditions of natural illumination of housing

Presence of the disease	Quantity	%	Reactive anxiety
Deterioration of health by one or more indicators	149	73,39	55,93±3,95
The presence of chronic diseases	57	28,08	51,27±3,62
They considered their condition unsatisfactory	24	11,82	58,22±4,11

Analysis of personal anxiety data also revealed their close connection with the presence of diseases in the respondents. In particular, the respondents living in satisfactory conditions regarding natural light conditions revealed the following patterns of distribution of personal anxiety (Table 2).

Table 2

The level of personal anxiety depending on the diseases among respondents who lived in unsatisfactory conditions of natural illumination of housing

Presence of the disease	Quantity	%	Personal anxiety
Deterioration of health by one or more indicators	139	94,56	49,13±3,47
The presence of chronic diseases	61	41,49	49,67±3,51
They considered their condition unsatisfactory	56	38,09	56,22±3,97

The table 2 data shows that the presence of diseases increases the level of personal anxiety of the respondents. In 32 (22.76%) of respondents who did not notice the clinical manifestations of the disease, the level of personal anxiety was in the low range – 28.33.

Comparison of personal anxiety data depending on residence in conditions of satisfactory or unsatisfactory natural illumination of housing showed the presence of certain differences between them (Table 3). It is evident from the data in the table that revealed significant (<0.05) differences in the indicators of personal anxiety in the presence of diseases depending on the residence in conditions of satisfactory or unsatisfactory natural illumination of housing.

Table 3

Comparison of the level of personal anxiety depending on the conditions of natural illumination of housing

Presence of the disease	Personal anxiety		
	Satisfactory living conditions	Unsatisfactory living conditions	p
Deterioration of health by one or more indicators	49,13±3,47	65,83±4,65	<0.05
The presence of chronic diseases	48,67±3,43	61,33±4,33	<0.05
They considered their condition unsatisfactory	56,22±3,97	68,14±4,81	<0.05

There is a certain relationship between the level of natural insolation of housing and the level of morbidity of the inhabitants. The level of reactive anxiety under different conditions of insolation and the presence of diseases is almost the same. It was noted, that the presence of diseases increases the level of personal anxiety of the respondents. Detected reliable (<0.05) differences in the indicators of personal anxiety in the presence of diseases depending on the residence in conditions of satisfactory or unsatisfactory natural illumination of housing.

To assess the condition of the tissues of the periodontal examination were divided into two groups: I group – 204 (58.29%) of respondents with insufficient levels of natural lighting of the living space; and the second group – 146 (41.71%) of the respondents who have satisfactory levels of natural lighting of the living space.

The conducted epidemiological survey showed that in general, the prevalence of periodontal disease in the examined group I was 95.09±6.7% (Table 4). Approximately the same prevalence of periodontal diseases was detected and in the examined group II – 81.51±7.6%. The difference between these indicators is statistically significant (p > 0.05).

Table 4

The prevalence and structure of periodontal diseases in the surveyed inhabitants (%)

Group	Number of surveyed	Chronic catarrhal gingivitis		Generalized periodontitis		Total periodontal diseases	
		abs.	%	abs.	%	abs.	%
Under unsatisfactory conditions (Group I)	204	13	6,38±1,7	181	88,72±6,5 *	194	95,09±6,7*
With satisfactory conditions (group II)	146	11	7,53±2,3	108	73,97±6,9 *	119	81,51±7,6*

** – reliability (p<0, 05) between the data of I and II groups of surveyed inhabitants*

The analysis of the structure of periodontal diseases showed that in the examined group I the most common periodontal disease was generalized periodontitis, which was detected in 181 subjects (88.72±6.5%). In 13 (6.38±1.7%) chronic catarrhal gingivitis was diagnosed, in 7 (3.43±1.3%) – periodontal disease. Clinically healthy periodontal tissues were found only in 3 (1.47±0.7%) surveyed inhabitants with insufficient levels of natural lighting of the living space.

Approximately the same structure of the morbidity of periodontal tissues was found in the examined group II: generalized periodontitis was found in 108 examined subjects – 73.97±6.9%, chronic catarrhal gingivitis – in 11 patients (7.53±2.3%), periodontal disease – in 7 examined (4.79±0.9%) and clinically healthy periodontal tissues were found in 20 surveyed patients – 13.69±3.9%. It can be stated that despite the approximate equality of the prevalence of periodontal tissue diseases, the surveyed residents of this group have a statistically significant (p <0.05) lower level of generalized

periodontitis in the presence of practically the same level of inflammatory periodontal disease

The study of the prevalence and structure of periodontal diseases in the subjects was carried out taking into account the presence or absence of somatic diseases of one or another origin (according to the data of the questionnaire). A higher level of systemic somatic illness was detected among inhabitants with insufficient living conditions. It also affected a greater level of personal anxiety of the inhabitants.

The results of the survey may indicate the presence of inadequate conditions of illumination of housing on the psychosomatic state of the inhabitants and on the periodontal tissue. In case of a better psychosomatic state, the level of morbidity for generalized periodontitis was less

Thus, the study of the influence of psycho-emotional stress on the course of periodontal diseases and the further development of appropriate schemes of rational medical treatment of periodontal diseases (generalized periodontitis) in people with psycho-emotional stress is a promising urgent task of therapeutic dentistry.

The conducted investigations have shown the relationship between the prevalence of systemic diseases, the level of anxiety (reactive and personality) and the prevalence and structure of periodontal diseases. The higher level of personal anxiety surveyed positively correlates with the widespread occurrence of generalized periodontitis in them.

3. Experimental substantiation the treatment of periodontal diseases in patients with psycho-emotional stress

Among the main general etiological factors of periodontal diseases, psychological stress also plays an important role. Clinical investigations have shown that people who are in a state of psychological stress revealed a greater prevalence of generalized periodontitis. The researchers concluded that a possible negative impact of psychological stress on the state of periodontal tissues of young people.

Considering the etiological role of the microflora, an experimental substantiation of the antidysbiotic effect of the proposed drug composition was important.

Aim: to determine the psychosomatic state of the inhabitants, the influence of psychological stress on the periodontal tissue and the

experimental substantiation of the antidysbiotic effect of the drug composition for the neutralization of the stress state.

The purpose of the experimental study was a comparative determination of the action on the biochemical parameters of the blood serum of the proposed complex of adrenoblockers (zoxon + nitsergoline + sibazon)[19][20] and antidisbiotic quertulin (quercetin + inulin + calcium citrate).

MATERIAL AND METHODS OF RESEARCH

In the presence of anxiety state (psychoemotional stress) in patients, for its neutralization, a medicinal composition was proposed that included zoxone (0.002 g 1 time per day), nicergoline (0.005 g 3 times a day), sibazon (0.005 g 1 time a day). It was recommended to take 1–2 hours before each session of the treatment of periodontal diseases.

To substantiate the periodontoprotective (membrane-stabilizing) effect of this drug composition, experimental studies were performed on rats in which adrenalin stress was modeled. It was induced by the introduction of adrenaline and lincomycin in rats. This causes the development in experimental animals of the inflammatory process in the periodontium against the background of violations of lipid peroxidation processes.

For experimental studies, white rats of the Vistar line were selected: 21 animals, females, 12–13 months old and live weight 290–330 g. Adrenalin stress was modeled with daily additions of gel with adrenaline (0.36 mg / kg of animal weight) and the introduction of lincomycin (it was added to drinking water) for 10 days. Experimental animals were divided into three groups of 7 rats. The first group consisted of animals that modeled adrenalin stress, but did not carry out drug treatment (control group). The second (main) group consisted of animals that modeled adrenalin stress and, on the background of this stress, they received adrenaline stress daily gel application of the proposed drug composition with adrenergic blockers (zoxone, nicergoline, sibazon). The third group (comparisons) were animals that received daily quertulin gel application in the background of adrenaline stress. The experiment lasted 10 days. Euthanasia of rats was performed on

[19] Кононова О.В. Влияние линкомицина на состояние пародонта у крыс с адреналиновым стрессом. *Вісник стоматології.* 2016; 3(96): с. 26-28

[20] Кононова О.В., Борисенко А.В, Левицкий А.П. Влияние оральных гелей квертулина и адреноблокаторов на состояние пародонта у крыс с адреналиновым стрессом. *Вісник стоматології.* 2016; 4(97): с. 8–11

day 11 under thiopental anesthesia (20 mg / kg) by total blood flow from the heart.

To determine the presence of inflammatory process in the periodontium of experimental animals in serum, the activity of catalase was determined. The activity of urease, lysozyme, elastase and catalase was determined to determine the effect of the proposed drug composition on the processes of dysbiosis in serum. The blood serum was determined by the content of malondialdehyde (MDA) and the ratio of the activity of catalase and the content of MDA - antioxidant-prooxidant index API. According to the ratio of relative activity of urease and lysozyme, the degree of dysbiosis according to Levitsky was calculated Levitsky[21].

For histological research, the animals were carved gingival tissue, placed in 10% neutral formalin, prepared cuts that stained with hematoxylin and eosin.

In the second series of experimental pathogistological studies in animals, the defect of the mandible bone was prepared. This experimental study was conducted on 36 white rats in accordance with the Law of Ukraine "On the Protection of Animals from Cruel Treatment" (No. 1759-VI of 15.12.2009). Experimental pathogistological studies were carried out during modeling in animals (rats) of adrenalin stress.

Animals were divided into three groups of 12 animals in each. The first group consisted of 12 rats, which had a bone marrow defect that had not been treated (control group). The rats of the second (primary) and the third group (comparative) defect of bone tissue were loosely filled with a suspension based on Bio-Oss. Animals of the second (main) group on the background of adrenaline stress received daily gel treatment with proposed blockers (zoxon + nitrogolin + sibazon). Duration of administration of these drugs was 10 days. Animals of the third group (comparisons) did not conduct drug therapy for adrenalin stress.

In each group, on the 10 th and 30 th day of the experiment, euthanasia of 6 animals was performed by total bloodletting from the heart under

[21] Левицкий А.П., Макаренко А.П, Деньга О.В. Экспериментальные методы исследования стимуляторов остеогенеза: методические указания. Киев: ГФЦ; 2005. 50 с., Левицкий А.П, Макаренко А.П, Селиванская И.А. Ферментативный метод определения дисбиоза полости рта для скрининга про- и пребиотиков: метод. рекомендации. Киев: ГФЦ; 2005. 22 с., Левицкий А.П, Деньга О.В, Макаренко А.П. Биохимические маркеры воспаления тканей ротовой полости. Методические рекомендации. Одесса; 2010. 16 с.

thiopental anesthesia, 20 mg / kg. Allocated bone tissue in the zone of bone defect.

The resulting bone tissue of the jaws was subjected to decalcification, poured into paraffin and stained with hematoxylin and eosin according to the standard procedure[2223].

The results were processed using standard statistical methods[24].

RESULTS

3.1. Biochemical investigations

To reduce the negative influence of psycho-emotional stress on the patient's organism, a range of medicines was proposed: zoxone (0.002 g 1 time per day), nicergoline (0.005 g 3 times a day), sibazon (0.005 g 1 time per day). This complex of medication preparations was recommended for patients to receive before each session of dental treatment.

For the experimental research, the model of adrenalin stress was chosen. When evaluated it was shown that the introduction of adrenaline and lincomycin causes in the serum of blood reduces the activity of catalase and the content of total cholesterol, which increases with adrenalin stress. Introduction of lincomycin against the background of adrenalin stress causes a decrease in the activity of lysozyme, an increase in the degree of dysbiosis and the activity of the marker of inflammation of the elastase.

The analysis of the blood of experimental animals (Table 5) showed that in rats with adrenaline stress in the background of dysbiosis in the blood, the content of leukocytes is significantly increased, but the proportion of monocytes is reduced by almost 2 times. The content of neutrophils and lymphocytes does not change significantly.

In rats with adrenaline stress, an increase in glucose levels (to 7.46 ± 0.3 mmol / l), threeglycerides (to 1.42 ± 0.1 mmol / l) and cholesterol (to 1.56 ± 0.08 mmol / l) was revealed. Administration of gel with adrenergic blockers in the rats of the main group causes a certain decrease in these parameters: glucose up to 7.63 ± 0.41 mmol / l, threeglycerides up to 1.25 ± 0.37 mmol / l and cholesterol up to 1.85 ±0, 11 mmol / l. In animals of the control group there was a similar decrease in these parameters: glucose to

[22] Меркулов Г.А. Курс патогистологической техники. Ленинград: Медицина; 1969. 424 с.

[23] Саркисов Д.С. Микроскопическая техника. Москва: Медицина; 1996. 544 с.

[24] Мінцер О.П., Вороненко Ю.В, Власов В.В. Оброблення клінічних і експериментальних даних у медицині. Київ: Вища школа; 2003. 350 с.

7.15 ± 0.22 mmol / l, threeglycerides - to 1.03 ± 0.09 mmol / l and cholesterol - to 1.90 ± 0.05 mmol / l .

Table 5

Indicators of the leukocyte formula of experimental animals (M ± m, n = 7)

Animal groups	Leucocytes (x10^9/l)	Neutrophil (%)	Lymphocytes (%)	Monocytes (%)
Control	12,1±0,3	28,0±0,7	65,0±1,5	4,6±0,2
The main	12,3±0,7 p>0,5 p_1>0.05	27,6±2,0 p>0,5 p_1>0,5	67,6±4,6 p>0,3 p_1>0,5	3,0±0,7 p<0.05 p_1>0,3
Comparison	14,4±1,4 p<0.05	27,2±2,0 p>0,3	68,6±3,7 p>0,2	2,6±0,6 p<0,01

In rats with adrenaline stress, an increase in the marker of microbial contamination of urease (to 1.40 ± 0.14 nkat / l), a decrease in the level of protection – lysozyme (up to 63 ± 3 units / l) and an increase in the level of dysbiosis to 1.81 ± 0.22 units (Table 6). Gel applications with blockers reduce the urease activity to 0.66 ± 0.21 nk / l and increase lysozyme levels to 73 ± 6 units / l. This leads to a significant reduction in the degree of dysbiosis – to 0.74 ± 0.20 units. A similar improvement in these parameters was observed in the control group: decrease of urease activity to 1.47 ± 0.43 ncat / l and increase of lysozyme level to 73 ± 5 units / l. The degree of dysbiosis decreased to 1.63 ± 0.18 units. Thus, application of gel with adrenoblockers is significantly better (p <0.05) improving the biochemical parameters of dysbiosis.

Table 6

The activity of urease, lysozyme and degree of dysbiosis in the gums of rats with experimental periodontitis (M ± m, n = 7)

Animal groups	Urease (μ-cat / kg)	Lysozyme, (unit / kg)	Degree of dysbiosis
Control	1,40±0,14	63±3	1,81±0,22
The main	0,66±0,21 p>0,5 p_1>0.05	73±6 p>0,5 p_1>0,5	0,74±0,20 p>0,3 p_1>0,5
Comparison	1,47±0,43 p<0.05	73±5 p>0,3	1,63±0,18 p>0,2

The development of adrenaline stress causes in animal's changes in inflammation (elastase) and peroxidation (malonic dialdehyde – MDA) in animals. In particular, the level of elastase increases to 138 ± 10,4 micron-cat / l, and the MDA content rises to 1,06 ± 0,06 mmol / l. (Table 7). Gel applications with adrenoblockers result in animals of the 2nd group to a significant decrease in these parameters: elastase to 112.4 ± 12.0 micron-cat / l and the MDA content to 0.90 ± 0.02 mmol / l. Applications of quertulin give a more pronounced anti-inflammatory effect, reducing the activity of elastase to 99.7 ± 8.9 μ-kat / l and slightly affect the processes of peroxidation, reducing the content of MDA to 1.01 ± 0.04 mmol / l. The obtained results indicate that gels with adrenoblockers have anti-inflammatory effects at the level of quertulin and significantly normalize the processes of lipid peroxidation.

Table 7

The level of biochemical markers of inflammation in the serum of blood of experimental animals (M ± m, n = 7)

Animal groups	MDA (mmol / l)	Elastase (μ-cat / l)
Control	1,06±0,06	138±10,4
The main	0,90±0,02 p>0.05 p_1<0.05	112,4±12,0 p<0.05 p_1<0.05
Comparison	1,01±0,04 p<0.05	99,7±8,9 p<0,01

In the case of the development of adrenaline stress in animals of the 1st group significantly lower other indicators; the activity of catalase decreases to 0.22 ± 0.02 mA /l and the API index decreases to 2.07 ± 0.17 units. (Table 8). Application of gel with blockers in animals in group 2 increases the activity of catalase to 0.30 ± 0.01 mA / l and the API to 3.33 ± 0.21 units. Applications of quertulin gel in animals of the 3rd group of comparison increase the activity of catalase to 0.37 ± 0.03 mA / l and the API to 3.66 ± 0.25 units. This indicates the ability of both investigating drugs to positively affect the antioxidant status of the organism.

Table 8

The level of biochemical parameters of lipid peroxidation processes in serum of experimental animals (M ± m, n = 7)

Animal groups	Catalase (μ-cat / l)	API
Control	0,22±0,02	2,07±0,17
The main	0,30±0,01 p>0,3 p_1>0,2	3,33±0,21 p>0,1 p_1>0.05
Comparison	0,37±0,03 p<0.05	3,66±0,25 p<0.05

The determination of gyaluronic acid content in the gingiva and the degree of resorption of the alveolar bone (Table 9) showed that in rats with adrenaline stress and experimental periodontitis, the content of hyaluronic acid only tends to decrease (p> 0.05). At the same time, the degree of the alveolar bone resorption is significantly increasing. Applications to the mucous membrane of the mouth of the proposed drug composition do not affect the processes of resorption of the alveolar bone.

The obtained results indicate that gels with adrenoblockers have anti-inflammatory effects at the level of quertulin and greatly improve the biochemical parameters of dysbiosis.

This testifies to the ability of the investigational drugs proposed to positively effect the disturbed psychosomatic state of the organism, which is promising in the case of their clinical application in the complex treatment of patients with generalized periodontitis in the presence of psychosomatic stress in them.

Table 9

The content of hyaluronic acid in the gingiva and the degree of resorption of the alveolar process of rats with experimental periodontitis (M ± m, n = 8)

Animal groups	Hyaluronic acid (mg / kg)	Degree of resorption (%)
Control	679,8±50,9	32,1±1,2
The main	633,3±50,6 p>0,3 p_1>0,5	27,2±1,2 p<0.05 p_1<0.05
Comparison	617,3±52,1 p>0,2	34,7±2,1 p<0.05

Experimental studies have shown that the drug composition of adrenergic blockers produces anti-inflammatory and antidysbiotic effects (more pronounced than applications for the preparation of the comparison of quertulin). This indicates the positive effect of the proposed drug combination on the dystrophic-inflammatory process in periodontal tissues. Thus, application of a gel with adrenoblockers produces an anti-inflammatory effect, but more definitely normalizes the processes of peroxidation of lipids.

The performed experimental studies indicate that the proposed combination of adrenoblockers produces antidisbiotic and antioxidant effects (more pronounced than applications of quertulin) and anti-inflammatory effects at the level of the drug comparison of quercetin. This indicates the positive effect of the proposed drug combination on the processes of lipid peroxidation and, accordingly, on the state of cell membranes.

In the complex treatment of patients with generalized periodontitis should take into account the existing violations of certain parts of his pathogenesis. In particular, for today, the presence of psychosomatic stresses in patients is important. These manifestations should be suppressed during complex treatment. The proposed drug composition allows to reduce the negative manifestations of stress.

3.2. Histopathological investigation

In the gums of rats of the 1st group (control) pathological changes in the gums were not revealed. In the gingival epithelium only the phenomena of apoptosis were detected, and in the gingival proper connective tissue a slight diffuse infiltration with lymphocytes and plasmacytes (Fig. 1, 2).

In rats of the 3rd group, which modeled adrenalin stress in the epithelium, acanthosis was observed with the phenomena of vacuolic degeneration of epithelial cells. In gingival proper connective tissue there are expressed lymphocytic-plazmocytic infiltration and edema of the basic substance. Locally infiltration of the subepithelial layer by eosinophils is detected (Fig. 3).

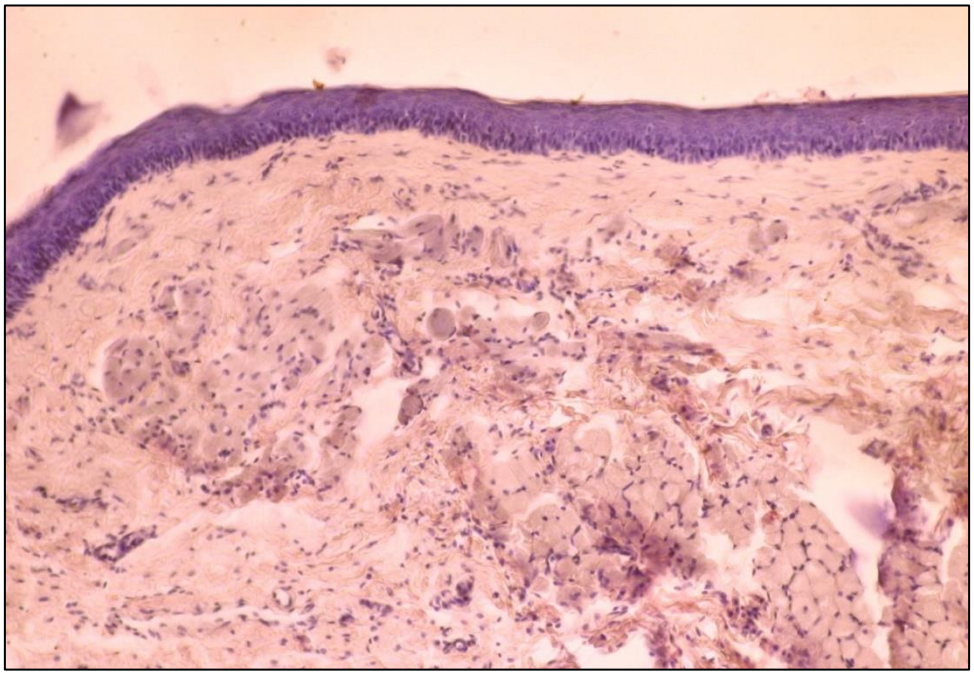


Fig. 1. Photomicrograph of the gingiva of the 1st group (control) rats. Hyperkeratosis of the epithelium, insignificant lymphocytic-plazmocytic infiltrate

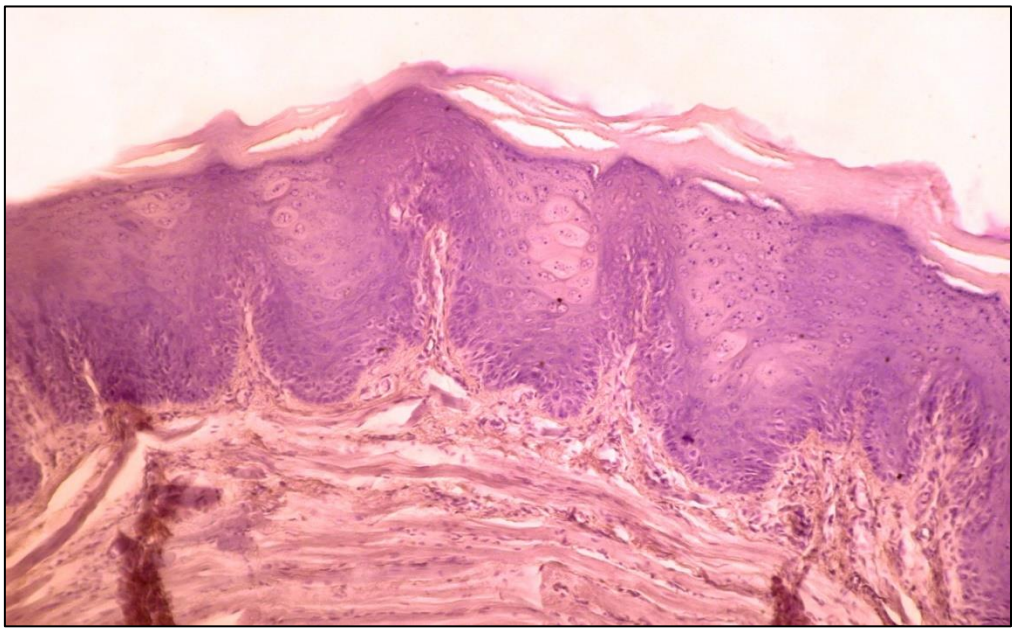


Fig. 2. Photomicrograph of the gingiva of the 1st group (control) rats. Hyperkeratosis of the epithelium, insignificant lymphocytic-plazmocytic infiltrate

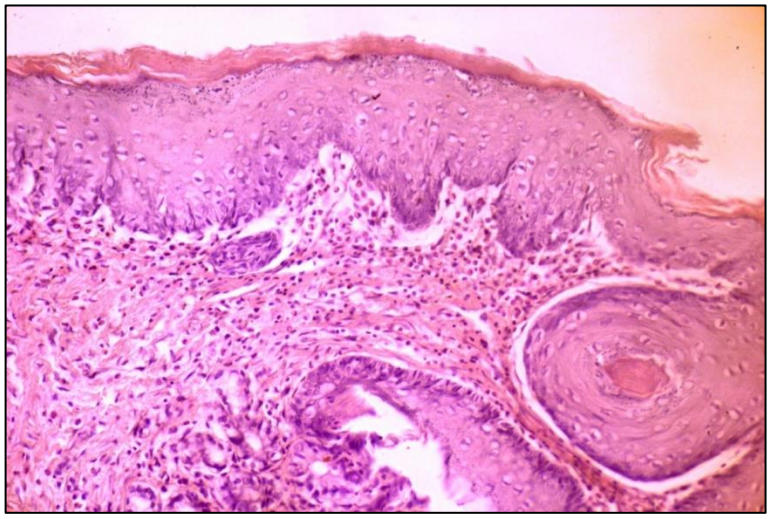


Fig. 3. Photomicrograph of the gingiva of the 2 nd group (dysbiosis) rats. Hyperkeratosis, acantosis of the epithelium. Diffuse lymphocytic-plazmocytic infiltrate, edema of connective tissue

Applications to the gingival mucosa gel with adrenoblockers in animals of the 2nd group also significantly reduce the epithelial damage and reduce the number of lymphocytes and plasmacytes in the gingival proper connective tissue (Fig. 4). Gingiva enough quickly restored practically to the level of animals in the control group without stress.

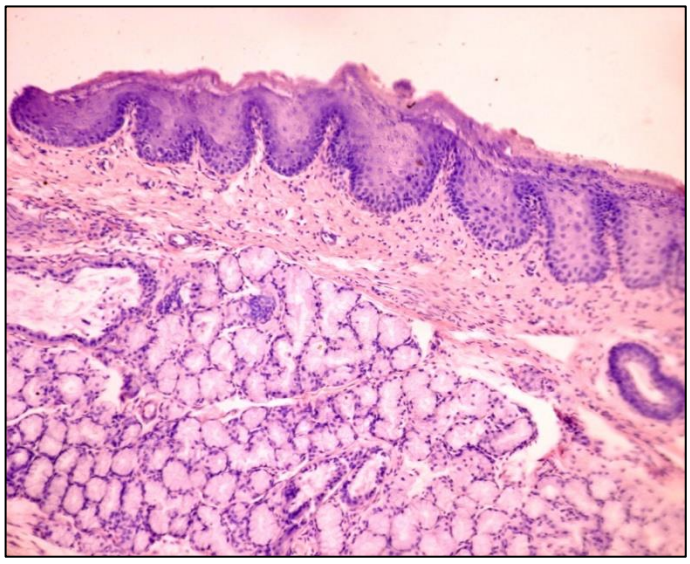


Fig. 4. Photomicrograph of the gingiva of the 3rd group group (dysbiosis + adrenoblockers) rats. Hyperkeratosis, acantosis of the epithelium. Insignificant lymphocytic-plazmocytic infiltrate

The presented results of the histological study to a certain extent confirm the results of biochemical studies of gums in rats receiving adrenaline and adrenoblockers.

In the complex treatment of patients with generalized periodontitis should take into account the existing violations of certain parts of his pathogenesis. In particular, for today, the presence of psychosomatic stresses in patients is important. These manifestations should be suppressed during complex treatment. The proposed drug composition allows to reduce the negative manifestations of stress. Experimental biochemical and pathologist studies have shown that this composition also produces anti-dysbiotic, anti-inflammatory and antioxidant effects, which indirectly indicates its membrane-stabilizing periodontioprotective properties. A similar effect of the drug composition will increase the effectiveness of its clinical application in the complex treatment of patients with generalized periodontitis.

In the second series of histopathological investigation of animals, the defect of the mandible bone was prepared.

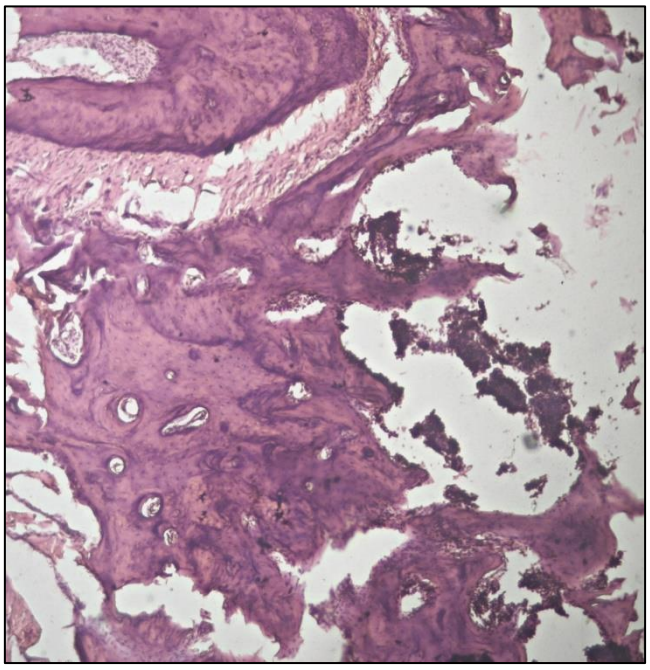


Fig. 5. Photomicrograph of the alveolar bone of the first group rats, the 10th day. Marked areas of destruction of basophilic zone and homogenization of bone plates

In rats of the first group (with a bone effect, without general treatment, 10 days) in the preparation small fragments of bone tissue that have undergone necrotic changes are determined. Bone tissue undergoes partial resorption with the formation of voids. After 30 days, marked bone fragments are surrounded by newly formed connective tissue. This indicates the beginning of regeneration of bone tissue by replacing the defect with fibrous tissue (Fig. 5).

In rats of the third group (comparison), 10 days later marked small fragments of bone tissue that were exposed to necrotic changes. Part of the bone plates undergoes resorption. Marked morphological signs of degeneration of osteocytes (Fig. 6). After 30 days, accumulation of finely divided basophilic material, hemolysis blood and fibrin clots is noted (Fig. 7). Thus, 30 days after the formation of an experimental defect, signs of repair and bone formation are practically absent.

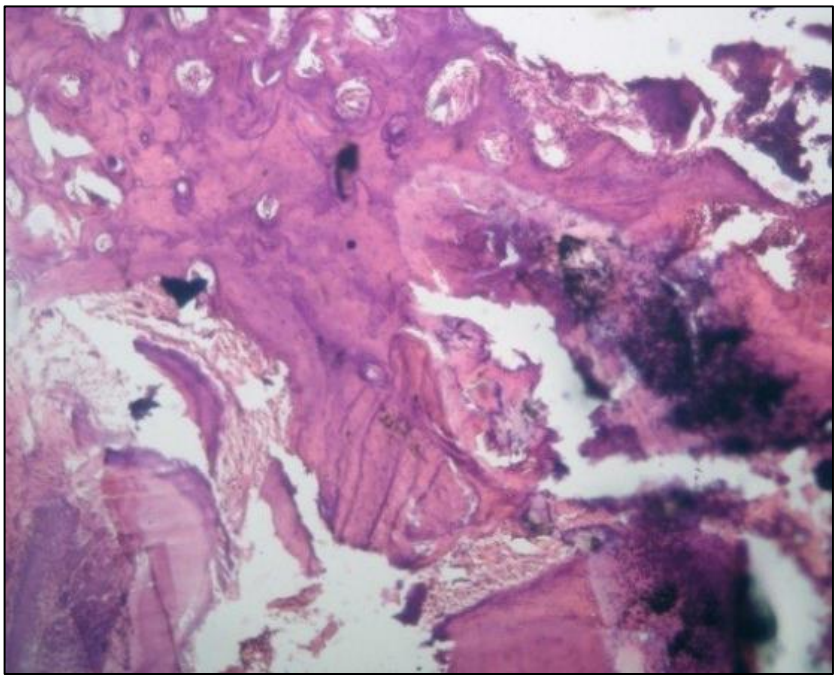


Fig. 6. Photomicrograph of the alveolar bone of the first group rats, the 10th day. The individual fragments of bone tissue, areas of basophilia of bone plates were noted

In the animals of the second group, which was treated with drug therapy for adrenalin stress 10 days after the defect was also marked destruction and resorption of bone plates. At the same time were revealed the less

pronounced resorption of fragments of bone tissue, signs of insignificant formation of connective tissue formations (Fig. 8).

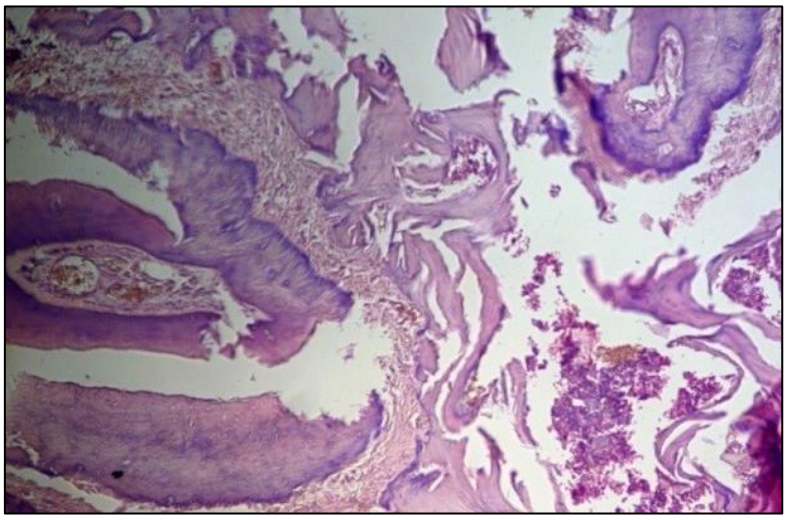


Fig. 7. Photomicrograph of the alveolar bone of the third group animals with adrenaline stress, 30 days. Marked lysis of bone plates surrounded by a fibrous tissue that is infused with blood

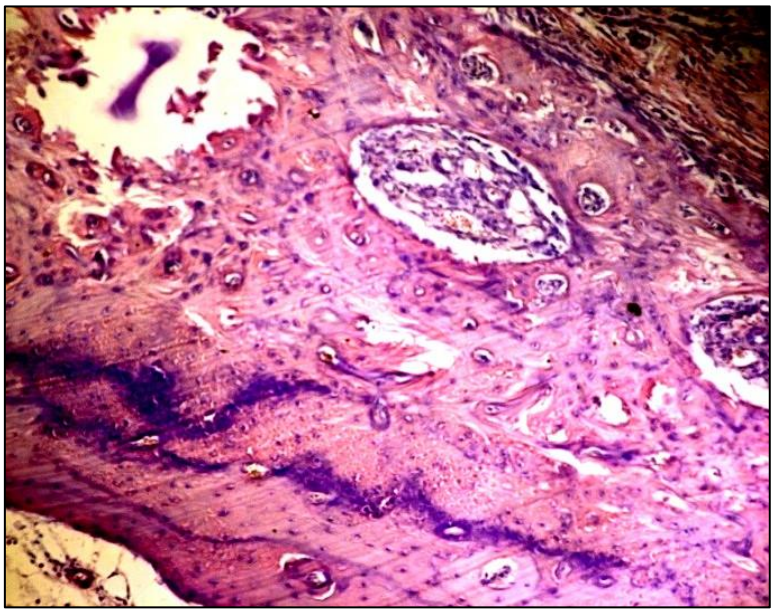


Fig. 8. Photomicrograph of the alveolar bone of the second group of animals with adrenaline stress, 10 days. Marked areas of focal destruction of bone plates, small concentrations of basophilic matter

30 days after the formation of an experimental defect in the second group of animals there are signs of repair. The formation of dense unformed connective tissue around bone fragments with active osteoblasts (Fig. 9) was noted. The formation of sections of well-vascularized cartilaginous bone tissue was marked on separate areas (Fig. 10)

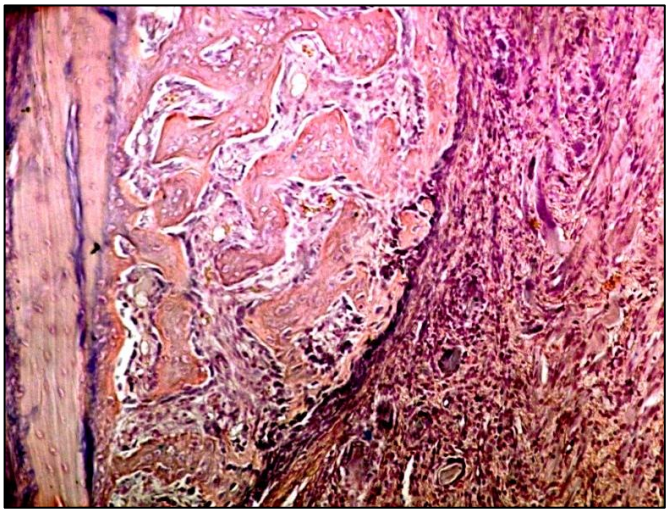


Fig. 9. Photomicrograph of the alveolar bone of the second group of animals with adrenaline stress, 30 days. Marked signs of formation of unformed bone tissue with osteocytes, insignificant bisophilia

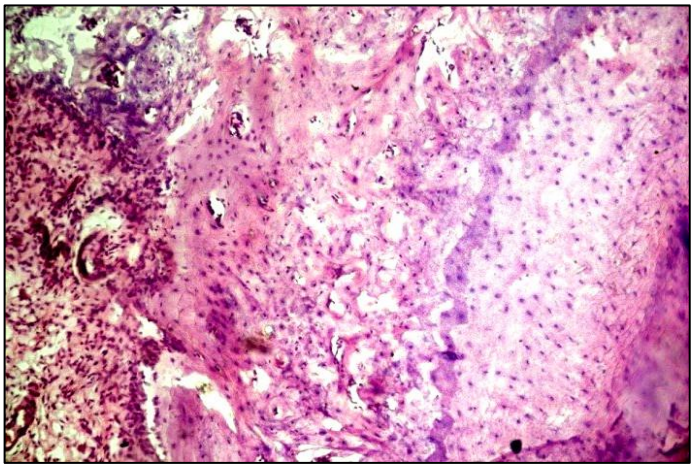


Fig. 10. Photomicrograph of the alveolar bone of the second group of animals with adrenaline stress, 30 days. Marked areas of well-vascularized bone tissue

The conducted histopathological investigation showed that 10 days after the formation of an experimental defect in the mandible bones in all groups of animals marked varying degrees of destruction of bone plates, accumulation of basophilic substance, infiltration of connective tissue structures. After 30 days in animals of the first and third groups marked weakly expressed phenomena of restoration of bone elements with dense fibrous tissue. Animals of the third group with medicinal treatment of adrenalin stress also noted signs of bone formation. The obtained data give some grounds for further application of the proposed composition of medicinal preparations in the complex treatment of patients with generalized periodontitis.

SUMMARY

The article is devoted a relationship between psychosocial stress and periodontal diseases, including generalized periodontitis. This relationship must be considered in the case of complex treatment of periodontal diseases. Analysis of the literature shows that such treatment cannot be fully spent.

More than half of respondents – 204 (58.29%) have insufficient levels of natural insolation. Poor conditions of insolation premises increase the level of personal anxiety of inhabitants. Poor conditions of natural light also affect the level of population morbidity. This leads to the high prevalence of periodontal disease, especially generalized periodontitis among these residents. A high level of personal anxiety – 50.64±3.58 leads to a significant increase in the prevalence of periodontal diseases – 95.09±6.7%, especially generalized periodontitis – 88.72±6.5%.

Experimental studies have shown that gel adrenoblockers decreases the urease activity, increase lysozyme levels and this leads to a significant reduction in the degree of dysbiosis. Oral application of the gel with adrenoblocators made an antidysbiotic effect after the modeling of adrenaline stress by administering animals to adrenalin and lincomycin.

To substantiate the effectiveness of the proposed complex of adrenoblockers for the treatment of periodontal diseases, an experimental animal study was conducted. Adrenalin stress was modeled in rats by oral administration of gel with adrenaline at a dose of 0.36 mg / kg for 10 days. Lincomycin was taken with drinking water at a dose of 60 mg / kg. The animals formed a defect in the mandible bone, which was filled with the drug Bio-Oss. The gel of adrenoblockers (zoxon + nicergolin and sibazon)

was administered by application at a dose of 0.6 mg / kg. Histological bone studies were performed in 10 and 30 days using hematoxylin and eosin.

The conducted experimental studies have shown that the development of adrenaline stress inhibits regeneration of the mandible bone. The applications of adrenoblockers stimulate the regeneration of the experimental defect of the mandible bone.

So the gel of adrenoblockers (zoxon + nicergolin and sibazon) may be recommended for the treatment of patients with chronic periodontitis and psychosocial stress.

REFERENCES

1. Функциональный подход к изучению состояния тревоги. / под ред. Астапова В.М. СПб.: Питер, 2001. Раздел: Тревога и тревожность; С. 156–165.
2. Заболевания пародонта. / под ред. Борисенко А.В. Киев: Медицина; 2011. 616 с.
3. Кононова О.В. Влияние линкомицина на состояние пародонта у крыс с адреналиновым стрессом. *Вісник стоматології*. 2016; 3(96): С. 26–28.
4. Кононова О.В, Борисенко А.В, Левицкий А.П. Влияние оральных гелей квертулина и адреноблокаторов на состояние пародонта у крыс с адреналиновым стрессом. *Вісник стоматології*. 2016; 4(97): с. 8–11.
5. Косенко К.М. Епідеміологія основних стоматологічних захворювань у населення України і шляхи їх профілактики [автореферат]. Київ: Національний медичний університет. 1994. 45 с.
6. Левицкий А.П, Макаренко А.П, Деньга О.В. Экспериментальные методы исследования стимуляторов остеогенеза: методические указания. Киев: ГФЦ; 2005. 50 с.
7. Левицкий А.П, Макаренко А.П, Селиванская ИА. Ферментативный метод определения дисбиоза полости рта для скрининга про- и пребиотиков: метод. рекомендации. Киев: ГФЦ; 2005. 22 с.
8. Левицкий А.П, Деньга О.В, Макаренко А.П. Биохимические маркеры воспаления тканей ротовой полости. Методические рекомендации. Одесса; 2010. 16 с.

9. Меркулов ГА. Курс патогистологической техники. Ленинград: Медицина; 1969. 424 с.

10. Мінцер О.П, Вороненко Ю.В, Власов В.В. Оброблення клінічних і експериментальних даних у медицині. Київ: Вища школа; 2003. 350 с.

11. Саркисов Д.С. Микроскопическая техника. Москва: Медицина; 1996. 544 с.

12. Aksali A, Huck O, Tenenbaum H, Davideau JL, Buduneli N. Periodontal diseases and stress: a brief review. J. Oral. Rehabil. 2013. Vol. 40(1). P. 60-68.

13. Akhter R, Hannan M, Okhuba R, Morita M. Relationship between stress factor and periodontal disease in a rural area population in Japan. J. Med. Res. 2005. Vol. 10(8). P. 352–357.

14. Breivik T, Thrane PS. Psychoneuroimmune interaction in periodontal disease. In: Psychoneuroimmunology. In: Ader R., Fetten D.L., Cohen N. 3nd ed. San Diego: Academic Press, 2001. P. 627–644.

15. Castillo DM, Sanchez-Beltran MC, Castellanos JE, Sanz I, Mayorda-Fayad I, Sanz M, Lafaurie GI. Detection of specific periodontal microorganisms from bacteriemia samples after periodontal therapy using molecular-based diagnostics. J. Clin. Periodontol. 2011. Vol. 38. P. 418–427.

16. Castro GDC, Oppermann RV, Haas AN, Winter R, Alchieri JC. Association between Psychosocial factors and periodontitis. J. Clin. Periodontol. 2006. Vol. 33(2). P. 109–114.

17. Chandna S., Bathla M. Stress and periodontium: A review of concepts. J. Oral Health Comm. Dent. 2010. Vol. 4(1). P. 17–22.

18. Croucher R., Marcenes W.S, Torres M.C., Hughes F., Sheiham A. The relationship between life-events and periodontitis. A case-control study. J. Clin. Periodontol. 1997. Vol. 24(1). P. 39–43.

19. Giddon D.B., Zaskin, S.J., Goldhaber P. Acute necrotizing ulcerative gingivitis in college students. J. Am. Dent. Assoc. 1964. Vol. 68. P. 381–386.

20. Keshava P.K., Sangeeta N.U. Stressing the stress in periodontal disease. J. Pharm. Biomed. Sci. 2013. V. 26. P. 345–348.

21. Omigbodun O.O., Odukogbe A.T., Omigbodun A.O., Yusuf O.B., Bella T.T., Olayemi O. Stressors and physiological symptoms in students of medicine and allied health professions in Nigeria. Soc. Psychiatry Psychiatr. Epidemiol. 2006. Vol. 41(5). P. 415–421.

22. Pistorius A., Krahwinkel T., Willerhausen B., Bockstegen C. Relationship between stress factors and periodontal disease. Eur. J. Med. Res. 2002. Vol. 7(9). P. 393–398.

23. Roberts A., Matthews B. J, Socransky S.S., Freestone, Williams H. P, Chapple I.L. Stress and periodontal diseases: growth responcses of periodontal bacteria to Escherichia coli stress-associated autoinducer and exogenous. *Fe. Molecular. Oral Microbiology*. 2005. Vol. 20(3). P. 147–153.

24. Sateesh C.P., Santosh K.R., Pushpalatha G. Relationship between stress and periodontal disease. J. Dent. Sci. Res. 2010. Vol. 1. P. 54–61.

25. Smith C.K., Peterson D.F., Degenhardt B.F., Johnson J.C. Depression, anxiety, and perceived hassels among entering medical students. Psychol. Health. Med. 2007. Vol. 12(1). P. 31–39.

26. Spielberger C.D. Test Anxiety Inventory. Sampler Set. Manual, Test, Scoring. Redwood City: Mind Garden, 1980. 240 p.

Information about author:
Kononova O. V.,
Candidate of Medical Sciences,
Senior Research Fellow,
„O. M. Marseev" Institute of Public Health of the NAMS of Ukraine
Bogomolets National Medical University
13, Taras Shevchenko Boulevard, Kyiv, 01601, Ukraine

ЭКСПЕРИМЕНТАЛЬНАЯ ТОКСИЧЕСКАЯ КУПРИЗОНОВАЯ МОДЕЛЬ ДЕМИЕЛИНИЗАЦИИ *IN VIVO* И *IN VITRO* КАК ИНСТРУМЕНТ ДЛЯ ИЗУЧЕНИЯ ПАТОГЕНЕЗА ЭТОЙ ПАТОЛОГИИ И ПОИСКА СРЕДСТВ С РЕМИЕЛИНИЗИРУЮЩИМ ДЕЙСТВИЕМ (ОБЗОР ДАННЫХ ЛИТЕРАТУРЫ И СОБСТВЕННЫХ ИССЛЕДОВАНИЙ)

Лабунец И. Ф.

ВВЕДЕНИЕ

Известно, что рассеянный склероз – одно из наиболее распространенных прогрессирующих демиелинизирующих заболеваний центральной нервной системы (ЦНС), которое сопровождается развитием двигательных, эмоциональных, сенсорных, вегетативных и когнитивных нарушений[1]. Это заболевание развивается преимущественно у людей трудоспособного возраста (в основном молодых), приводя к их инвалидизации, что имеет большое социально-экономическое значение.

Основным патоморфологическим проявлением при демиелинизирующих заболеваниях является разрушение миелиновой оболочки нервных волокон. Поэтому исследование механизмов развития демиелинизации и поиск медикаментозных средств с ремиелинизирующим эффектом представляется актуальной задачей современной неврологии. Одними из патогенетических факторов повреждений нервных клеток при демиелинизирующей патологии считают оксидативный стресс и нейровоспаление[2].Так, показано, что клетки микроглии и макрофаги головного мозга продуцируют свободные радикалы, активные формы кислорода и провоспалительные

[1] Міщенко Е.С., Шульга О.Д., Бобрик Н.В. та ін. Розсіяний склероз: глобальні перспективи. *Укр. мед. часопис*. 2014. Т. 101. №3. С. 84–87; Негрич Т.І., Сорокін Б.В., Євтушенко С.К. Від вірогідної діагностики до ефективної терапії розсіяного склероз. *Міжнар. Неврол. журн*. 2012. Т. 3 (49). С.152–158; Соколова Л.И., Сепиханова М.М. Сравнительный анализ эффективности дифференцированного лечения рассеянного склероза у пациентов разного возраста. Збірн. наук. праць спів роб. НМАПО ім. П.Л. Шупика. 2014. Т. 23. №2. С.289–298; Чуприна Г.М. Розсіяний склероз: Клініко-патогенетичні характеристики та терапевтичні підходи з урахуванням коморбідності. Дис. д-ра мед. наук. Київ, 2017. 409 с

[2] Пивнева Т.А. Механизмы демиелинизации при рассеянном склерозе. *Нейрофизиология*. 2009. Т.41. №5. С. 429–437.

цитокины (фактор некроза опухолей-α (TNF-α), интерферон-γ (IFN-γ), интерлейкин (IL)-1β)). Кроме указанных клеток, источником провоспалительных цитокинов при нейровоспалении могут быть Т-лимфоциты, которые инфильтрируют головной мозг[3].

Изучение роли указанных выше клеточных факторов при развитии демиелинизирующей патологии, а также их изменений под влиянием нейропротекторных средств возможно при использовании адекватных экспериментальных моделей этой патологии.

1. Подходы к индукции демиелинизации *in vivo*

Из данных литературы известны несколько экспериментальных подходов к индукции демиелинизации *in vivo*, в частности, это модель экспериментального аллергического энцефаломиелита (ЭАЭ), вирус- и токсин-индуцированные модели[4]. Так, модель ЭАЭ в основном воспроизводится путем введения животным энцефалитогенной смеси (эмульсия головного или спинного мозга взрослых крыс и полный адъювант Фрейнда) и применяется для изучения аутоиммунных механизмов развития демиелинизации[5]. С помощью вирусиндуцированных и токсин-индуцированных моделей демиелинизации можно изучать ее инфекционную и токсическую природу[6]. Так, модель мышиного энцефаломиелита Тейлора предусматривает инъекции одноцепочной рибонуклеиновой кислоты в ЦНС, в результате чего происходит имуноопосредованная гибель олигодендроцитов. Моделирование гибели олигодендроцитов с помощью локального введения токсинов бромистый этидий или лизолецитин позволяет воспроизвести демиелинизацию в определенных участках ЦНС.

---

[3] Praet J., Guglielmetti C., Berneman Z. et al. Cellular and molecular neuropathology of the cuprizone mouse model: Clinical relevance for multiple sclerosis. *J. Neubiorev*. 2014. Vol. 47. P. 485–505. doi: 10.1016/j.neubiorev.2014.10.004.

[4] Denic A., Johnsonb A. J., Bieber A. J. et al. The relevance of animal models in multiple sclerosis research. *Pathophysiology*. 2011. Vol.18. P. 21–29. doi: 10.1016/j.pathophys.2010.04.004

[5] Півнева Т.А. Морфофункціональний стан гліальних клітин при експериментальних впливах, що індукують нейродегенерацію: Дис. д-ра. біол. наук. Київ, 2010. 328 с; Цимбалюк В.І., Касяненко Ю.А. Особливості моделювання та перебігу експериментального алергічного енцефаломієліту. *Укр. нейрохір. журн*. 2005. №1. С.45–50.

[6] Denic A., Johnsonb A. J., Bieber A. J. et al. The relevance of animal models in multiple sclerosis research. *Pathophysiology*. 2011. Vol.18. P.21–29. doi: 10.1016/j.pathophys.2010.04.004

Среди указанных выше экспериментальных моделей демиелинизации *in vivo* привлекает внимание токсическая купризоновая модель этой патологии. Известно, что нейротоксин купризон [бис (циклогексанон)-оксалдигидразон] – это медный хелатор, который при пероральном использовании первично избирательно повреждает медьсодержащие ферменты митохондрий зрелых олигодендроцитов (в частности, цитохромоксидазу, моноаминооксидазу) и вызывает их апоптоз; в головном мозге наблюдается ранняя и выраженная супрессия экспрессии миелинассоциированного гликопротеина (MAG) mRNA, с его ранней потерей[7]. При этом повреждение миелина не носит аутоиммунного характера. Как результат, наблюдается демиелинизация аксонов нейронов и двигательные нарушения. Кроме того, у животных после употребления купризона в головном мозге происходит активация микроглии с усилением продукции провоспалительных цитокинов (TNF-α и IFN-γ) и развивается оксидативный стресс[8]. Повреждения в головном мозге не являются перивенозными, а инфильтрат в зоне повреждения преимущественно включает клетки микроглии, макрофаги и, в меньшей степени, Т-лимфоциты[9].

Путем изменения дозы купризона *in vivo* и использования мышей определенной линии, пола и возраста можно получить

[7] Acs P., Kalman B. Pathogenesis of multiple sclerosis: what can we learn from the cuprizone model. *Methods Mol Biol*. 2012. Vol. 900. P. 403–31. doi: 10.1007/978-1-60761-720-4-20; Gudi V., Gingele S., Skripuletz Th. Et. al. Glial response during cuprizon-induced de- and remyelination in the CNS: lessons learned. *Front Cell Neurosci*. 2014. 8 (Article 73). 24 p. DOI: 10.3389/fncel.2014.00073, Praet J., Guglielmetti C., Berneman Z. et. al. Cellular and molecular neuropathology of the cuprizone mouse model: Clinical relevance for multiple sclerosis. *J. Neubiorev*. 2014. Vol. 47. P. 485–505. DOI: 10.1016/j.neubiorev.2014.10.004.

[8] Acs P., Kalman B. Pathogenesis of multiple sclerosis: what can we learn from the cuprizone model. *Methods Mol Biol*. 2012. Vol. 900. P. 403-31. doi: 10.1007/978-1-60761-720-4-20; Praet J., Guglielmetti C., Berneman Z. et. al. Cellular and molecular neuropathology of the cuprizone mouse model: Clinical relevance for multiple sclerosis. *J. Neubiorev*. 2014. Vol. 47. P. 485–505. DOI: 10.1016/j.neubiorev.2014.10.004.

[9] Acs P., Kalman B. Pathogenesis of multiple sclerosis: what can we learn from the cuprizone model. *Methods Mol Biol*. 2012. Vol. 900. P. 403-31. DOI: 10.1007/978-1-60761-720-4-20; Praet J., Guglielmetti C., Berneman Z. et. al. Cellular and molecular neuropathology of the cuprizone mouse model: Clinical relevance for multiple sclerosis. *J. Neubiorev*. 2014. Vol. 47. P. 485–505. DOI: 10.1016/j.neubiorev.2014.10.004., Serra-de-Oliveira N., Boilesen S.N., Prado de França Carvalho C. et al. Behavioural changes observed in demyelination model shares similarities with white matter abnormalities in humans. *Behav Brain Res*. 2015. Vol. 287. P.265–275. DOI: 10.1016/j.bbr.2015.03.038.

демиелинизирующие повреждения соответствующих участков головного мозга (мозолистое тело, мозжечок, гиппокамп, кора)[10]. Кроме того, изменяя длительность приема купризона (от 3–6 недель до 12 недель) можно воспроизвести острую или хроническую демиелинизацию головного мозга, а также ремиелинизацию, которая происходит после завершения приема этого нейротоксина[11]. Усиление демиелинизации и удлинение длительности периода ремиелинизации можна достичь, используя купризон вместе с рапамицином[12]. Именно поэтому купризоновая модель является важной для исследования факторов, которые влияют как на демиелинизацию, так и ремиелинизацию в структурах ЦНС. Эта модель также дает возможность исследовать механизмы демиелинизации в спинном мозге, которая может возникать независимо от повреждений головного мозга[13].

По данным ряда исследователей патологические изменения в нервной системе у животных с купризоновой моделью демиелинизации напоминают некоторые проявления демиелинизирующей патологии у человека (в частности, рассеянный склероз)[14]. По мнению авторов, купризоновая модель может быть еще одним вариантом экспериментальных моделей для изучения механизмов демиелинизации.

В лаборатории экспериментального моделирования Института генетической и регенеративной медицины (ИГРМ) впервые в Украине

---

[10] Doucette, Jiao R., Nazarali A.J. Age-related and cuprizone-induced changes in myelin and transcription factor gene expression and in oligodendrocyte cell densities in the rostral corpus callosum of mice. *Cell Mol Neurobiol*. 2010. Vol. 30. P. 607–629. DOI: 10.1007/s10571-009-9486-z; Kipp M., Clarner T. Dang J. et al. The cuprizone animal model; new insights into an old story. *Acta neuropathol*. 2009. Vol. 118. P. 723–736. DOI: 10.1007/s00401-009-0591-3; Zhen W., Liu A., Lu J. et al. Wang J. An alternative cuprizone-induced demyelination and remyelination mouse model. *ASN Neuro* July-August. 2017. P. 1–9. DOI:10.1177/1759091417725174.

[11] Kipp M., Clarner T. Dang J. et al. The cuprizone animal model; new insights into an old story. *Acta neuropathol*. 2009. Vol. 118. P. 723–736. DOI: 10.1007/s00401-009-0591-3.

[12] Sachs H. Y., Bercury K. K., Popescu D. C. et al. A new model of cuprizone-mediated demyelination/remyelinatio. *ASN Neuro*. 2014. P.1–16. DOI:10.1177/1759091414551955.

[13] Herder V., Hansmann F., Stangel M. Et al. Lack of cuprizone-induced demyelination in the murine spinal cord despite oligodendroglial alterations substantiates the concept of site-specific susceptibilities of the central neurons system. *Neuropathol Appl Neurobiol.* 2011. Vol.37. P. 676–684. DOI: 10.1111/j.1365-2990.2011.01168. x.

[14] Lucchinetto C., Bruck W., Parisi J. Et al. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann Neurol*. 2000. Vol.55. P. 458–468; Serra-de-Oliveira N., Boilesen S.N., Prado de França Carvalho C. et al. Behavioural changes observed in demyelination model shares similarities with white matter abnormalities in humans. *Behav Brain Res*. 2015. Vol. 287. P.265–275. DOI: 10.1016/j.bbr.2015.03.038.

была воспроизведена купризоновая модель демиелинизации *in vivo*. Отработку этой модели проводили на мышах разных линий (129/Sv, FVB/N, C57Bl/6), пола и возраста (3–17 месяцев). При введении нейротоксина купризона и тестируемых биологически активных веществ применяли разные схемы (доза веществ, длительность введения). Использование таких подходов в экспериментальных исследованиях в течение 2014–2018 годов дало нам возможность получить следующие новые результаты:

1. При воспроизведении купризоновой модели демиелинизации на молодых мышах разных линий и пола были определены наиболее и наименее чувствительные линии мышей к токсическому влиянию купризона на нервные клетки[15], что позволило нам в дальнейшем исследовать значение генетических и гендерных факторов для развития этой патологии.

2. Установлен не только демиелинизирующий эффект, но и токсическое действие купризона на структуру нейронов ЦНС (кора головного мозга, клетки Пуркинье мозжечка, поясничный отдел спинного мозга) молодых мишей[16]. Данные раскрывают новые звенья патогенеза демиелинизирующей патологии и могут быть основанием для поиска нейропротекторных средств.

3. Показана согласованность степени повреждений миелина, структуры нейронов ЦНС и выраженности нарушений двигательной,

---

[15]Лабунець І.Ф., Мельник Н.О., Кузьмінова І.А. та ін. Вплив нейротоксину «купризон» на поведінкові реакції та морфофункціональні зміни нейронів головного та спинного мозку у мишей. Журн. НАМН України. 2014. Т. 20. №4. С. 402–408; Патент України на корисну модель № 94458 u UA. Реєстраційний номер заявки u 2014 06622; МПК G09B 23/28 (2006.01).Спосіб моделювання структурних змін нейронів центральної нервової системи при демієлінізуючих захворюваннях/ Лабунець І.Ф., Мельник Н.О., Кузьмінова І.А., Бутенко Г.М. Опубл. 10.11.2014. Бюл. № 21. 3 с.

[16]Лабунець І.Ф., Мельник Н.О., Кузьмінова І.А. та ін. Вплив нейротоксину «купризон» на поведінкові реакції та морфофункціональні зміни нейронів головного та спинного мозку у мишей. *Журн. НАМН України.* 2014. Т. 20. №4. С.402–408; Патент України на корисну модель № 94458 u UA. Реєстраційний номер заявки u 2014 06622; МПК G09B 23/28 (2006.01).Спосіб моделювання структурних змін нейронів центральної нервової системи при демієлінізуючих захворюваннях/ Лабунець І.Ф., Мельник Н.О., Кузьмінова І.А., Бутенко Г.М. Опубл. 10.11.2014. Бюл. № 21. 3 с.; Лабунец И.Ф., Мельник Н.А., Кузьминова И.А. Изменения поведенческих реакций и структуры нейронов центральной нервной системы у мышей при купризоновой модели демиелинизации. *Интер-медикал*. 2014. №5. С. 25–31. ISSN 0370-1069D

эмоциональной и исследовательской активности молодых мышей, употреблявших купризон[17].

4. Определены возможные патогенетические факторы токсического влияния купризона на развитие структурных изменений ЦНС у молодых мышей[18]. В частности, это такие иммунные факторы, как макрофаги и Т-лимфоциты, число которых в головном мозге повышается после приема купризона, тогда как на фоне ремиелинизации (через 2 месяца после отмены нейротоксина) снижается. Для повреждений нервных клеток купризоном имеет также значение повышение в головном мозге содержания малонового диальдегида и снижение активности антиоксидантных ферментов.

5. Установлено, что повышение количества активных макрофагов в головном мозге и снижение двигательной активности животных наблюдается уже на ранних сроках приема купризона (через 7 дней)[19].

---

[17]Лабунець І.Ф., Мельник Н.О., Кузьмінова І.А. та ін. Вплив нейротоксину «купризон» на поведінкові реакції та морфофункціональні зміни нейронів головного та спинного мозку у мишей. Журн. НАМН України. 2014. Т. 20. №4. С.402–408., Патент України на корисну модель № 94458 u UA. Реєстраційний номер заявки u 2014 06622; МПК G09B 23/28 (2006.01).Спосіб моделювання структурних змін нейронів центральної нервової системи при демієлінізуючих захворюваннях/ Лабунець І.Ф., Мельник Н.О., Кузьмінова І.А., Бутенко Г.М. Опубл. 10.11.2014. Бюл. № 21. 3 с.

[18]Лабунец И.Ф., Родниченко А.Е., Утко Н.А. и др. Нейропротекторное влияние мелатонина при экспериментальных моделях патологии нервной системы. Тези доповідей Науково-практичної конференції з міжнародною участю «Іноваційні напрями в генетичній та регенеративній медицині» (9–10 листопада 2017 р., Київ, Україна). *Клітинна та органна трансплантологія*. Додаток. 2017. Т. 5. №2. С. 41–42., Лабунец И.Ф., Таланов С.А., Родниченко А.Е. и др. Изучение в эксперименте влияния цитокинов и гормонов на отдельные звенья патогенеза нейродегенеративных заболеваний как возможный путь к повышению эффективности клеточной терапии Збірник тез науково-практичної конференції з міжнародною участю «Трансплантація – сьогодення, минуле та майбутнє» (м. Київ, 7 листопада 2014 р.). Київ. 2014. С. 28., Labunets I.F., Melnyk N.O., Rodnichenko A.E. et al. Cuprizone-induced disorders of central nervous system neurons, behavioral reactions, brain activity of macrophages and antioxidant enzymes in the mice of different ages: Role of Leukemia Inhibitory Factor in their improvement. *J Aging Geriatr Med*. 2017. Vol. 1. N2. 8 p. DOI: 10.4172/AGM.1000104.

[19]Лабунец И.Ф., Родниченко А.Е., Утко Н.А. и др. Нейропротекторное влияние мелатонина при экспериментальных моделях патологии нервной системы. *Тези доповідей Науково-практичної конференції з міжнародною участю «Іноваційні напрями в генетичній та регенеративній медицині»* (9–10 листопада 2017 р., Київ, Україна) / *Клітинна та органна трансплантологія*. Додаток. 2017. Т. 5. №2. С. 41–42., Labunets I.F., Melnyk N.O., Rodnichenko A.E. et al. Cuprizone-induced disorders of central nervous system neurons, behavioral reactions, brain activity of macrophages and antioxidant enzymes in the mice of different ages: Role of Leukemia Inhibitory Factor in their improvement. *J Aging Geriatr Med*. 2017. Vol. 1. N2. 8 p.

Эти результаты были основанием для разработки эффективных схем применения ряда нейропротекторных средств, вводя их именно с указанного выше срока и до конца приема нейротоксина, а не после завершения его приема.

6. Показано значение нарушений эндокринной функции центрального органа иммунной системы тимуса для развития изменений иммунных факторов в головном мозге мышей, получавших купризон, а также важность повышения в крови таких мышей уровня тимического гормона тимулина для реализации эффекта нейропротекторных средств[20].Тимулин влияет на дифференцировку Т-лимфоцитов в тимусе, активность макрофагов, проявляет антивоспалительный эффект в головном мозге, угнетая синтез провоспалительных цитокинов[21].

7. Установлен нейропротекторный эффект цитокинов ((лейкемия-ингибиторный фактор (LIF), интерлейкин-10 (IL-10)) и гормонов (мелатонин) у мышей с купризоновой моделью демиелинизации, который в значительной степени связан с изменением активности тимуса, факторов иммунных и антиоксидантной защиты (таблицы 1–3)[22].

DOI: 10.4172/AGM.1000104., Патент України на корисну модель № 104976 (UA). Номер заявки u 2015 09252. Спосіб моделювання регенерації ушкоджених нейронів головного мозку при нейродегенеративних захворюваннях. / Лабунець І.Ф., Мельник Н.О., Римар С.Ю. Опубл. 25.02.2016. Бюл.№4. 4 с.

[20] Лабунец И.Ф. Изменения эндокринной функции тимуса, макрофагов и Т-лимфоцитов головного мозга у мышей разного возраста после введения нейротоксина купризона и цитокина. *Междунар неврол журн.* 2018. №4 (98). С. 155–161. DOI:10.22141/2224-0713.4.98.2018.139434

[21] Лабунец И.Ф. Изменения эндокринной функции тимуса, макрофагов и Т-лимфоцитов головного мозга у мышей разного возраста после введения нейротоксина купризона и цитокина. *Междунар неврол журн.* 2018. №4 (98). С. 155–161. DOI:10.22141/2224-0713.4.98.2018.139434

[22] Лабунец И.Ф. Изменения эндокринной функции тимуса, макрофагов и Т-лимфоцитов головного мозга у мышей разного возраста после введения нейротоксина купризона и цитокина. *Междунар неврол журн.* 2018. №4 (98). С. 155–161. DOI:10.22141/2224-0713.4.98.2018. 139434, Лабунец И.Ф., Родниченко А.Е., Утко Н.А. и др. Нейропротекторное влияние мелатонина при экспериментальных моделях патологии нервной системы. *Тези доповідей Науково-практичної конференції з міжнародною участю «Інноваційні напрями в генетичній та регенеративній медицині»* (9–10 листопада 2017 р., Київ, Україна). *Клітинна та органна трансплантологія*. Додаток. 2017. Т. 5. №2. С. 41–42; Лабунець І.Ф., Мельник Н.О., Родніченко А.Є. та ін. Вплив рекомбінантного інтерлейкіна-10 людини на структуру нейронів центральної нервової системи та поведінкові реакції у мишей із купризоновою моделлю розсіяного склерозу. *Тези доповідей Науково-проктичної конференції з міжнародною участю «Інноваційні напрями в генетичній та регенеративній медицині»*

Полученные данные являются основанием для изучения клеточных и эндокринных механизмов восстановления нарушенной структуры ЦНС, а также поиска новых лекарственных средств и биотехнологических подходов к клеточной терапии демиелинизирующей патологии. В настоящее время для патогенетической терапии последней (рассеянный склероз) используют разные препараты, в частности, интерфероны, цитостатики, моноклональные антитела, препараты, которые улучшают кровоток, и так далее[23].

Таблица 1

Число активных макрофагов, CD3+- CD4+- клеток в головном мозге мышей экспериментальных групп, M±m

Группа Мышей	Фагоцитарный индекс, %	Фагоцитарное число, усл.ед.	$CD3^+$, %	$CD4^+$, %
Интактные	89,12±1,72	3,57±0,16	0,70±0,10	2,80±0,59
Купризон, 7 дней	95,20±0,86*	4,75±0,21*	-	-
Купризон, 3 недели	94,66±1,67*	3,44±0,71	1,90±0,18*	32,40±4,77*
Купризон+ rhLIF	94,31±1,60*	3,68±0,32**	1,24±0,20*$^{\#}$	17,43±2,47*$^{\#}$
Интактные	84,51±0,90$^{\&}$	4,32±0,59$^{\&}$	1,20±0,20$^{\&}$	2,20±0,19
Купризон, 7 дней	86,20±1,20$^{\&}$	4,50±0,29	-	-
Купризон, 3 недели	97,51±0,89* **	5,65±0,10* **$^{\&}$	2,30±0,39*	19,34±3,50*$^{\&}$
Купризон+ rhLIF	91,66±2,11*$^{\#}$ **	3,93±0,08$^{\#}$	1,91±0,20*$^{\&}$	18,40±3,91*

*Примечание: * – p<0,05 по сравнению с интактной группой; ** – p<0,05 по сравнению с купризоном (7 дней); $^{\#}$ – p<0,05 по сравнению с купризоном (3 недели); $^{\&}$ – p<0,05 по сравнению с возрастной группой 3–5 месяцев; rhLIF – рекомбинантный лейкемия ингибиторный фактор человека*

(9–10 листопада 2017 р., Київ, Україна). *Клітинна та органна трансплантологія.* Додаток 2017. Т. 5. №2. С. 240–241; Labunets I.F., Melnyk N.O., Rodnichenko A.E. et al. Cuprizone-induced disorders of central nervous system neurons, behavioral reactions, brain activity of macrophages and antioxidant enzymes in the mice of different ages: Role of Leukemia Inhibitory Factor in their improvement. *J Aging Geriatr Med*. 2017. Vol. 1. N2. 8 p. DOI: 10.4172/AGM.1000104.

[23] Черненко М.Є., Вовк В.І. Розсіяний склероз:сучасні підходи до патогенетичної терапії. *Междунар. мед. журнал*. 2015. №1. С. 58–62.

Таблица 2

Число $CD3^+$-клеток и активных макрофагов в головном мозге, а также содержание тимулина в крови мышей экспериментальных групп, M±m

Показатель	Экспериментальная группа		
	Интактная	Купризон+ 0,9% NaCl	Купризон+ мелатонин
$CD3^+$, %	0,9±0,1	1,6±0,2*	0,9±0,1#
Фагоцитарный индекс, %	47,1±2,1	65,0±3,5*	55,2±2,5*#
Фагоцитарная активность, у.ед.	1,5±0,1	2,3±0,2*	1,6±0,1#
Тимулин, $\log_2$ титра	5,5±0,2	6,1±0,5	8,3±0,6 *#

*Примечание: * – p<0,05 по сравнению с интактной группой; # – p<0,05 по сравнению с купризоном (3 недели).*

Таблица 3

Показатели оксидативного стресса и антиоксидантной защиты в головном мозге мышей экспериментальных групп, M±m

Показатель	Экспериментальная группа		
	Интактная	Купризон+ 0,9% NaCl	Купризон+ мелатонин
Малоновый диальдегид (нмоль/мг)	3,8±0,2	5,0±0,3*	3,7±0,2#
Супероксиддисмутаза (ед/мг·мин)	8,5±0,5	8,2±0,3	8,9±0,4
Каталаза (мкмоль/мг·мин)	1,9±0,1	2,3±0,2	2,2±0,3
Глутатионпероксидаза (нмоль/мг·мин)	12,5±0,5	11,1±0,3*	13,6±0,4#
Глутатионредуктаза (нмоль/мг·мин)	22,5±0,6	18,8±0,5*	28,5±0,8*#

*Примечание: * – p<0,05 по сравнению с интактной группой; # – p<0,05 по сравнению с купризоном (3 недели).*

8. Хотя такая демиелинизирующая патология, как рассеянный склероз встречается преимущественно у лиц молодого возраста, в настоящее время ее все чаще регистрируют в возрасте старше 45 лет. Поэтому исследование возрастного аспекта моделирования

демиелинизирующей патологии важно как с точки зрения патогенеза этого заболевания, так и его терапии.

При этом показано, что изменения с возрастом количества клеток микроглии, макрофагов и Т-лимфоцитов в головном мозге, уровня тимулина в крови могут отразиться не только на эффективности восстановления нейрогенеза после повреждений, но и на реакции нервных клеток на регуляторное влияние клеточных и эндокринных факторов[24]. Нами установлены возрастные особенности изменений патогенетических факторов демиелинизации и нейродегенерации в условиях применения купризона (в частности, изменение числа Т-лимфоцитов, макрофагов, активности антиоксидантних ферментов), а также инъекций цитокинов (LIF) и гормонов (мелатонин) (см. табл. 1–3)[25]. Результаты могут быть полезными при разработке подходов к терапии демиелинизирующей патологии в организмах разного возраста.

Таким образом, результаты проведенных нами экспериментальных исследований на купризоновой модели демиелинизации *in vivo* дали возможность не только подтвердить имеющиеся данные литературы об

---

[24] Лабунец И.Ф. Изменения эндокринной функции тимуса, макрофагов и Т-лимфоцитов головного мозга у мышей разного возраста после введения нейротоксина купризона и цитокина. *Междунар неврол журн*. 2018. №4 (98). С.155–161. DOI:10.22141/2224-0713.4.98.2018.139434; Doucette, Jiao R., Nazarali A.J. Age-related and cuprizone-induced changes in myelin and transcription factor gene expression and in oligodendrocyte cell densities in the rostral corpus callosum of mice. *Cell Mol Neurobiol.* 2010. Vol. 30. P. 607–629. DOI: 10.1007/s10571-009-9486-z., Labunets I.F., Melnyk N.O., Rodnichenko A.E. et al. Cuprizone-induced disorders of central nervous system neurons, behavioral reactions, brain activity of macrophages and antioxidant enzymes in the mice of different ages: Role of Leukemia Inhibitory Factor in their improvement. *J Aging Geriatr Med*. 2017. Vol. 1. N2. 8 p. DOI: 10.4172/AGM.1000104.

[25] Лабунец И.Ф. Изменения эндокринной функции тимуса, макрофагов и Т-лимфоцитов головного мозга у мышей разного возраста после введения нейротоксина купризона и цитокина. *Междунар неврол журн.* 2018. №4 (98). С. 155–161. DOI:10.22141/2224-0713.4.98.2018.139434, Лабунец И.Ф., Родниченко А.Е., Утко Н.А. и др. Нейропротекторное влияние мелатонина при экспериментальных моделях патологии нервной системы // *Тези доповідей Науково-практичної конференції з міжнародною участю «Інноваційні напрями в генетичній та регенеративній медицині»* (9–10 листопада 2017 р., Київ, Україна). *Клітинна та органна трансплантологія*. Додаток. 2017. Т. 5. №2. С. 41–42., Labunets I.F., Melnyk N.O., Rodnichenko A.E. et al. Cuprizone-induced disorders of central nervous system neurons, behavioral reactions, brain activity of macrophages and antioxidant enzymes in the mice of different ages: Role of Leukemia Inhibitory Factor in their improvement. *J Aging Geriatr Med*. 2017. Vol. 1. N2. 8 p. DOI: 10.4172/AGM.1000104.

эффективности использования этой модели при изучении механизмов развития демиелинизации в ЦНС, но и получить новые данные о патогенетических факторах развития структурных изменений нервных клеток, а также механизмов нейропротекторного эффекта некоторых цитокинов и гормонов.

2. Подходы к индукции демиелинизации *in vitro*

В последнее время большое внимание привлекают модели патологий нервной системы *in vitro,* которые дают возможность не только изучать закономерности нейрогенеза, взаимодействия нервных и глиальных клеток, механизмы изменений миелинизации, но и оценивать динамику морфологических и функциональных изменений культуры нервных клеток в процессе ее развития под непосредственным действием биологически активных веществ, медикаментозных средств, повреждающих факторов, а также веществ, которые уменьшают действие последних[26]. Кроме того, использование моделей патологии нервной системы *in vitro* позволяет уменьшить количество животных для экспериментальных исследований. Вместе с тем эти модели не дают возможности оценивать внутрисистемные и межсистемные регуляторные влияния, которые можно изучать в системе *in vivo*[27].

Известны несколько способов моделирования повреждений миелогенеза *in vitro,* а именно путем добавления: а) к диссоциированной культуре клеток мозжечка новорожденных крыс сыворотки крови пациентов с рассеянным склерозом в стадии обострения или сыворотки крови кроликов с ЭАЭ в присутствии комплемента; б) к культуре нервных клеток спинного мозга вируса Тейлора; в) к культуре срезов мозжечка лизолецитина или антител к MAG; г) к культуре нервных клеток нейротоксина купризона[28]. Во всех

[26] Пивнева Т.А. Механизмы демиелинизации при рассеянном склерозе. *Нейрофизиология.* 2009. Т.41. №5. С. 429-437., Baraterio A., Fernandes A. Temporal oligodendrocyte lineage progression: in vitro models of proliferation, differentiation and myelination. *Biochym Biophys Acta*. 2014. Vol. 1843. P. 1917–1929. DOI: 10.1016/j.bbamcr.2014.04.018., Jarjour A. A, Zhang H, Bauer N. et al. In vitro modeling of central nervous system myelination and remyelinationю *Glia*. 2012. Vol.60. P.1–12. DOI: 10.1002/glia.21231.

[27] Merrill J.E. In vivo and in vitro pharmacological models to asses demyelination and remyelination. *Neuropsychopharmacology*. 2009. V.34. P.55–73. DOI: 10.1038/npp.2008.145.

[28] Півнева Т.А. Морфофункціональний стан гліальних клітин при експериментальних впливах, що індукують нейродегенерацію: дис. д-ра. біол. наук. Київ, 2010. 328 с.; Cammer W. The neurotoxicant, cuprizone, retards the differentiation of oligodendrocytes in vitro. *J Neuorol*

указанных выше способах оценивается содержание олигодендроцитов или их предшественников в культуре клеток головного мозга с помощью методов иммуноцитохимии, иммуногистохимии и, кроме того, анализируется ультраструктура этих клеток методами электронной и конфокальной микроскопии. Одними из преимуществ купризоновой модели демиелинизации *in vitro* является возможность не только учитывать дозу токсического вещества, и, как результат, оценивать дозозависимые изменения количества миелинпродуцирующих клеток в культуре, но и избежать этических проблем при использовании повреждающего фактора.

Подходы к усилению/ускорению ремиелинизации демиелинизированных аксонов нейронов в культуре нервных клеток включают применение *in vitro* разных препаратов и биологически активных веществ[29]. В частности, это препараты для лечения рассеянного склероза (Fingolimod), факторы с ремиелинизирующим эффектом ((17-бета эстрадиол, Forskolin, фактор роста фибробластов-бета (FGF), LIF, ретиноевая кислота, трийодтиронин, экстракт гриба *Hericium erinaceus* и так далее)). Известен способ повышения *in vitro* числа олигодендроцитов и нейронов путем нанесения нейральных стволовых клеток, которые продуцируют ростовые факторы и цитокины, на органотипическую культуру гиппокампа после гипоксии[30]. Для оценки ремиелинизирующего эффекта упомянутых способов, как правило, используют дорогостоящие методы иммуногистохимии, иммуноцитохимии и электронной микроскопии.

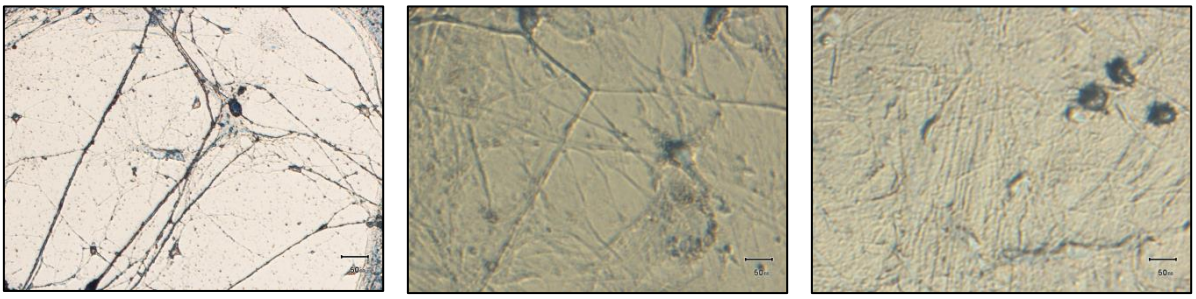
В лаборатории экспериментального моделирования ИГРМ впервые в Украине была отработана токсическая купризоновая модель

Sci. 1999. Vol.168. P. 116–120; Jarjour A. A, Zhang H, Bauer N. et al. In vitro modeling of central nervous system myelination and remyelination *Glia*. 2012. Vol.60. P.1–12. DOI: 10.1002/glia.21231.

[29] Півнева Т.А. Морфофункціональний стан гліальних клітин при експериментальних впливах, що індукують нейродегенерацію: дис. д-ра. біол. наук. Київ, 2010. 328 с., Jarjour A. A, Zhang H, Bauer N. et al. In vitro modeling of central nervous system myelination and remyelinationю *Glia*. 2012. Vol.60. P.1–12. DOI: 10.1002/glia.21231., Merrill J.E. In vivo and in vitro pharmacological models to asses demyelination and remyelination. *Neuropsychopharmacology*. 2009. V. 34. P. 55–73. DOI: 10.1038/npp.2008.145.

[30] Kopach O., Rybachuk O., Krotov V.et al. Maturation of neural stem cells and integration into hippocampal circuits: functional study in post-ischemia in situ. *J Cell Sci*. 2018. Vol. 131. N4. 35 p. DOI: 10.1242/jcs.210989.

демиелинизации *in vitro*[31]. При воспроизведении этой модели использовали простой, доступный, малозатратный способ оценки состояния миелинизации аксонов нейронов. Две повреждающие дозы нейротоксина купризона (25 μM і 500 μM) добавляли к диссоциированной культуре клеток мозжечка новорожденных мышей линии FVB/N с дальнейшей оценкой состояния миелинизации гистохимическим методом (окрашивание Суданом черным В). В результате внесения в культуру клеток мозжечка купризона в дозе 25 μM или 500 μM наблюдается соответственно частичная или полная демиелинизация аксонов нейронов по сравнению с контрольной культурой (без токсина) (рисунок 1).



А Б В

Рис. 1. Диссоциированная культура клеток мозжечка мышей: контрольная (А), с добавлением купризона в дозе 25 μM (Б-частичная демиелинизация) и 500 μM (В-полная демиелинизация) (26 сутки культивирования, окрашивание нервных волокон суданом черным В), х200[32].

[31] Патент України на корисну модель № 125486 (UA). Номер заявки u 2017 12275 від 12.12.2017 р. G01N 33/50 (2006.01). Спосіб оцінки демієлінізації аксонів нейронів при моделюванні *in vitro* ушкоджень клітин головного мозку, зокрема мозочка / Лабунець ІФ, Родніченко АЄ. Опубл.10.05.2018, Бюл. № 9.3 с., Родніченко А.Є. Відтворення токсичної купризонової моделі демієлінізації в системі in vitro. *Клітинна та органна трансплантологія.* 2018. Т. 6. № 1. С. 86–92. DOI:10.22494/cot.v6i1.84.

[32] Патент України на корисну модель № 125486 (UA). Номер заявки u 2017 12275 від 12.12.2017 р. G01N 33/50 (2006.01). Спосіб оцінки демієлінізації аксонів нейронів при моделюванні *in vitro* ушкоджень клітин головного мозку, зокрема мозочка / Лабунець ІФ, Родніченко АЄ. Опубл.10.05.2018, Бюл. № 9.3 с., Родніченко А.Є. Відтворення токсичної купризонової моделі демієлінізації в системі in vitro. *Клітинна та органна трансплантологія.* 2018. Т. 6. № 1. С. 86–92. DOI:10.22494/cot.v6i1.84.

Итак, использованный нами экспериментальный подход дает возможность доступно оценивать дозозависимый демиелинизирующий эффект нейротоксина купризона.

На отработанной купризоновой модели демиелинизации *in vitro* нами также была показана возможность оценки гистохимическим методом состояния ремиелинизации нервных волокон, поврежденных купризоном, под влиянием биологически активных веществ с ремиелинизирующим действием[33]. На примере цитокинов (LIF) и гормонов (мелатонин), которые выступают в качестве соответственно факторов микроокружения и макроокружения для нервных клеток, было показано, что после внесения их в культуру клеток мозжечка, предварительно обработанную купризоном, происходит полная ремиелинизация аксонов нейронов. Ремиелинизирующий эффект мелатонина и LIF согласуется с увеличением в культуре нервных клеток, обработанных купризоном, количества зрелых олигодендроцитов ($Olig2^+$-клетки). Под влиянием нейротоксина число последних в культуре существенно снижалось.

Следовательно, отработанная в лаборатории экспериментального моделирования купризоновая модель демиелинизации *in vitro* дает возможность информативно и доступно изучать факторы, которые усиливают/ускоряют демиелинизацию, а также осуществлять поиск медикаментозных средств с ремиелинизирующим эффектом.

Важно подчеркнуть, что одновременное использование нами купризоновой модели демиелинизации *in vivo* и *in vitro* при проведении экспериментальных исследований позволило получить наиболее полную информацию о механизмах и уровнях развития патологических изменений в ЦНС. О необходимости применения экспериментальных моделей патологии нервной системы *in vivo* как финального этапа доклинических исследований медикаментозних средств после их

[33] Лабунец И.Ф., Родниченко А.Е., Утко Н.А. и др. Нейропротекторное влияние мелатонина при экспериментальных моделях патологии нервной системы. *Тези доповідей Науково-практичної конференції з міжнародною участю «Іноваційні напрями в генетичній та регенеративній медицині»* (9–10 листопада 2017 р., Київ, Україна). *Клітинна та органна трансплантологія*. Додаток. 2017. Т. 5. №2. С. 41–42; Rodnichenko A, Utko N, Labunets I. In vitro cuprizone model as a tool to study remyelination factors». 11th FENS Forum of neuroscience (7-11 July 2018, Berlin, Germany). Abstract number F18-0774

изучения на моделях *in vitro* свидетельствуют результаты исследований и других авторов[34]

ВЫВОДЫ

1. Токсическая купризоновая модель демиелинизации *in vivo* может быть инструментом для изучения: патогенетических факторов (иммунных, эндокринных, оксидативного стресса) демиелинизации и нейродегенерации в ЦНС; механизмов ремиелинизации; значения генотипа, пола и возраста для развития демиелинизирующей патологии; механизмов нейропротекторного эффекта некоторых цитокинов (LIF, IL-10), факторов роста (FGF) и гормонов (мелатонин, половые и тиреоидные гормоны, глюкокортикоиды), а также поиска средств с ремиелинизирующим/нейропротекторным эффектом.

2. Токсическая купризоновая модель демиелинизации *in vitro* в нашей модификации может быть использована для: исследования факторов, которые усиливают/ускоряют демиелинизацию; поиска медикаментозных средств с ремиелинизирующим эффектом; получения наиболее полной информации о механизмах и уровнях развития патологических изменений в ЦНС (совместно с купризоновой моделью демиелинизации *in vivo*).

3. Купризоновая модель вместе с другими моделями демиелинизации может быть полезной при изучении патогенетических звеньев этой патологии, оценке механизмов действия терапевтических средств и их эффективности.

4. Токсическая купризоновая модель демиелинизации может быть использована при изучении других патологий нервной системы[35]. В частности, показано, что применение купризона в адаптированных схемах дает возможность получать структурные повреждения участков головного мозга и развитие в них нейрохимических изменений, которые в значительной степени напоминают патологические изменения при шизофрении и эпилепсии.

---

[34] Baraterio A., Fernandes A. Temporal oligodendrocyte lineage progression: in vitro models of proliferation, differentiation and myelination. *Biochym Biophys Acta*. 2014. Vol.1843. P.1917-1929. DOI: 10.1016/j.bbamcr.2014.04.018.

[35] Praet J., Guglielmetti C., Berneman Z. et al. Cellular and molecular neuropathology of the cuprizone mouse model: Clinical relevance for multiple sclerosis. *J. Neubiorev*. 2014. Vol. 47. P. 485–505. DOI: 10.1016/j.neubiorev.2014.10.004.

5. Таким образом, купризоновая модель демиелинизации – важный инструмент для фундаментальных и прикладных исследований в экспериментальной неврологии. Кроме того, полученные экспериментальные результаты могут быть полезными при разработке усовершенствованных терапевтических подходов в клинической неврологии.

АННОТАЦИЯ

Статья посвящена сравнительной оценке научных данных и результатов исследований лаборатории экспериментального моделирования Института генетической и регенеративной медицины НАМН Украины о возможности использования токсической купризоновой модели демиелинизации *in vivo* и *in vitro* в экспериментальной неврологии. Приводятся данные, свидетельствующие об эффективности применения токсической купризоновой модели демиелинизации *in vivo* для изучения роли факторов иммунных, эндокринных и оксидативного стресса в патогенезе этой патологии, а также значения возраста, пола и генотипа животных для ее развития. Обосновывается возможность использования купризоновой модели демиелинизации *in vivo* для поиска средств с ремиелинизирующим/нейропротекторным эффектом и изучения механизмов их действия. Описываются преимущества купризоновой модели демиелинизации перед другими экспериментальными моделями этой патологии. На собственном примере оценки ремиелинизирующего эффекта цитокинов (лейкемия ингибиторный фактор) и гормонов (мелатонин) показана перспективность применения купризоновой модели демиелинизации *in vitro* для скрининга ремиелинизирующих факторов. Обосновывается возможность использования экспериментальной купризоновой модели при изучении других патологий нервной системы (шизофрения, эпилепсия). Кроме того, аргументируется целесообразность применения экспериментальных результатов при разработке усовершенствованных подходов для терапии демиелинизирующей патологии в клинической практике.

ЛИТЕРАТУРА

1. Лабунец И.Ф. Изменения эндокринной функции тимуса, макрофагов и Т-лимфоцитов головного мозга у мышей разного возраста после введения нейротоксина купризона и цитокина. *Междунар неврол журн.* 2018. №4 (98). С. 155–161. DOI:10.22141/2224-0713.4.98.2018.139434

2. Лабунец И.Ф., Мельник Н.А., Кузьминова И.А. Изменения поведенческих реакций и структуры нейронов центральной нервной системы у мышей при купризоновой модели демиелинизации. *Интермедикал*. 2014. №5. С. 25–31. ISSN 0370-1069D

3. Лабунец И.Ф., Родниченко А.Е., Утко Н.А. и др. Нейропротекторное влияние мелатонина при экспериментальных моделях патологии нервной системы. *Тези доповідей Науково-практичної конференції з міжнародною участю «Інноваційні напрями в генетичній та регенеративній медицині»* (9–10 листопада 2017 р., Київ, Україна). *Клітинна та органна трансплантологія*. Додаток. 2017. Т. 5. №2. С. 41–42.

4. Лабунец И.Ф., Таланов С.А., Родниченко А.Е. и др. Изучение в эксперименте влияния цитокинов и гормонов на отдельные звенья патогенеза нейродегенеративных заболеваний как возможный путь к повышению эффективности клеточной терапии Збірник тез науково-практичної конференції з міжнародною участю «Трансплантація – сьогодення, минуле та майбутнє» (м. Київ, 7 листопада 2014 р.). Київ. 2014. С. 28.

5. Лабунець І.Ф., Мельник Н.О., Кузьмінова І.А. та ін. Вплив нейротоксину «купризон» на поведінкові реакції та морфофункціональні зміни нейронів головного та спинного мозку у мишей. *Журн. НАМН України.* 2014. Т. 20. №4. С. 402–408.

6. Лабунець І.Ф., Мельник Н.О., Родніченко А.Є. та ін. Вплив рекомбінантного інтерлейкіна-10 людини на структуру нейронів центральної нервової системи та поведінкові реакції у мишей із купризоновою моделлю розсіяного склерозу. *Тези доповідей Науково-практичної конференції з міжнародною участю «Інноваційні напрями в генетичній та регенеративній медицині»* (9–10 листопада 2017 р., Київ, Україна). *Клітинна та органна трансплантологія.* Додаток 2017. Т. 5. №2. С. 240–241.

7. Міщенко Е.С., Шульга О.Д., Бобрик Н.В. та ін. Розсіяний склероз: глобальні перспективи. *Укр. мед. часопис*. 2014. Т. 101. №3. С. 84–87.

8. Негрич Т.І., Сорокін Б.В., Євтушенко С.К. Від вірогідної діагностики до ефективної терапії розсіяного склероз. *Міжнар. Неврол. журн*. 2012. Т.3 (49). С. 152–158.

9. Патент України на корисну модель № 104976 (UA). Номер заявки u 2015 09252. Спосіб моделювання регенерації ушкоджених нейронів головного мозку при нейродегенеративних захворюваннях. / Лабунець І.Ф., Мельник Н.О., Римар С.Ю. Опубл. 25.02.2016. Бюл.№4. 4 с.

10. Патент України на корисну модель № 125486 (UA). Номер заявки u 2017 12275 від 12.12.2017 р. G01N 33/50 (2006.01). Спосіб оцінки демієлінізації аксонів нейронів при моделюванні *in vitro* ушкоджень клітин головного мозку, зокрема мозочка / Лабунець І.Ф., Родніченко А.Є. Опубл.10.05.2018, Бюл. №9. 3 с.

11. Патент України на корисну модель № 94458 u UA. Реєстраційний номер заявки u 2014 06622; МПК G09B 23/28 (2006.01).Спосіб моделювання структурних змін нейронів центральної нервової системи при демієлінізуючих захворюваннях/ Лабунець І.Ф., Мельник Н.О., Кузьмінова І.А., Бутенко Г.М. Опубл. 10.11.2014. Бюл. № 21. 3 с.

12. Пивнева Т.А. Механизмы демиелинизации при рассеянном склерозе. *Нейрофизиология*. 2009. Т.41. №5. С. 429–437.

13. Півнева Т.А. Морфофункціональний стан гліальних клітин при експериментальних впливах, що індукують нейродегенерацію: Дис. д-ра. біол. наук – Київ, 2010. – 328 с.

14. Родніченко А.Є. Відтворення токсичної купризонової моделі демієлінізації в системі in vitro. *Клітинна та органна трансплантологія.* 2018. Т. 6. №1. С. 86–92. doi:10.22494/cot.v6i1.84.

15. Соколова Л.И., Сепиханова М.М. Сравнительный анализ эффективности дифференцированного лечения рассеянного склероза у пациентов разного возраста. Збірн. наук. праць спів роб. НМАПО ім. П.Л. Шупика. 2014. Т. 23. №2. С. 289–298.

16. Цимбалюк В.І., Касяненко Ю.А. Особливості моделювання та перебігу експериментального алергічного енцефаломієліту. *Укр. нейрохір. журн*. 2005. №1. С. 45–50.

17. Черненко М.Є., Вовк В.І. Розсіяний склероз: сучасні підходи до патогенетичної терапії. *Междунар. мед. журнал*. 2015. №1. С. 58–62.

18. Чуприна Г.М. Розсіяний склероз: Клініко-патогенетичні характеристики та терапевтичні підходи з урахуванням коморбідності: дис. д-ра мед. наук. Київ, 2017. 409 с.

19. Acs P., Kalman B. Pathogenesis of multiple sclerosis: what can we learn from the cuprizone model. *Methods Mol Biol*. 2012. Vol. 900. P. 403–31. DOI: 10.1007/978-1-60761-720-4-20.

20. Baraterio A., Fernandes A. Temporal oligodendrocyte lineage progression: in vitro models of proliferation, differentiation and myelination. *Biochym Biophys Acta*. 2014. Vol.1843. P.1917-1929. doi: 10.1016/j.bbamcr.2014.04.018.

21. Cammer W. The neurotoxicant, cuprizone, retards the differentiation of oligodendrocytes in vitro. *J Neuorol Sci*. 1999. Vol. 168. P. 116–120.

22. Denic A., Johnsonb A. J., Bieber A. J. et al. The relevance of animal models in multiple sclerosis research. *Pathophysiology*. 2011. Vol. 18. P. 21–29. doi:10.1016/j. pathophys. 2010.04.004.

23. Doucette, Jiao R., Nazarali A.J. Age-related and cuprizone-induced changes in myelin and transcription factor gene expression and in oligodendrocyte cell densities in the rostral corpus callosum of mice. *Cell Mol Neurobiol*. 2010. Vol. 30. P. 607-629. DOI: 10.1007/s10571-009-9486-z.

24. Gudi V., Gingele S., Skripuletz Th. Et al. Glial response during cuprizon-induced de- and remyelination in the CNS: lessons learned. *Front Cell Neurosci*. 2014. 8 (Article 73). 24 p. DOI: 10.3389/fncel.2014.00073.

25. Herder V., Hansmann F., Stangel M. Et al. Lack of cuprizone-induced demyelination in the murine spinal cord despite oligodendroglial alterations substantiates the concept of site-specific susceptibilities of the central neurons system. *Neuropathol Appl Neurobiol*. 2011. Vol.37. P. 676–684. DOI: 10.1111/j.1365-2990.2011.01168. x.

26. Jarjour A. A, Zhang H., Bauer N. et al. In vitro modeling of central nervous system myelination and remyelinationю *Glia*. 2012. Vol.60. P. 1–12. DOI: 10.1002/glia.21231.

27. Kipp M., Clarner T. Dang J. et al. The cuprizone animal model; new insights into an old story. *Acta neuropathol*. 2009. Vol. 118. P. 723–736. DOI: 10.1007/s00401-009-0591-3.

28. Kopach O., Rybachuk O., Krotov V.et al. Maturation of neural stem cells and integration into hippocampal circuits: functional study in post-ischemia in situ. *J Cell Sci*. 2018. Vol. 131. N4. 35 p. DOI: 10.1242/jcs.210989.

29. Labunets I.F., Melnyk N.O., Rodnichenko A.E. et al. Cuprizone-induced disorders of central nervous system neurons, behavioral reactions, brain activity of macrophages and antioxidant enzymes in the mice of different ages: Role of Leukemia Inhibitory Factor in their improvement. *J Aging Geriatr Med*. 2017. Vol. 1. N2. 8 p. DOI: 10.4172/AGM.1000104.

30. Lucchinetto C., Bruck W., Parisi J. et al. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann Neurol*. 2000. Vol.55. P. 458–468.

31. Merrill J.E. In vivo and in vitro pharmacological models to asses demyelination and remyelination. *Neuropsychopharmacology*. 2009. V. 34. P. 55–73. DOI: 10.1038/npp.2008.145.

32. Praet J., Guglielmetti C., Berneman Z. et al. Cellular and molecular neuropathology of the cuprizone mouse model: Clinical relevance for multiple sclerosis. *J. Neubiorev*. 2014. Vol. 47. P. 485–505. DOI: 10.1016/j.neubiorev.2014.10.004.

33. Rodnichenko A., Utko N., Labunets I. In vitro cuprizone model as a tool to study remyelination factors». 11th FENS Forum of neuroscience (7-11 July 2018, Berlin, Germany). Abstract number F18-0774.

34. Sachs H.Y., Bercury K.K., Popescu D.C. et al. A new model of cuprizone-mediated demyelination/remyelinatio. *ASN Neuro*. 2014. P. 1–16. DOI:10.1177/1759091414551955.

35. Serra-de-Oliveira N., Boilesen S.N., Prado de França Carvalho C. et al. Behavioural changes observed in demyelination model shares similarities with white matter abnormalities in humans. *Behav Brain Res*. 2015. Vol. 287. P.265–275. doi: 10.1016/j.bbr.2015.03.038.

36. Zhen W., Liu A., Lu J. et al. Wang J. An alternative cuprizone-induced demyelination and remyelination mouse model. *ASN Neuro* July-August. 2017. P. 1–9. doi:10.1177/1759091417725174.

Information about author:
Labunets I. F.,
Doctor of Medical Sciences, Senior Research Officer,
Head of the Experimental Modeling Laboratory
Cell and Tissue Technologies Department
State Institute of Genetic and Regenerative Medicine
National Academy of Medical Sciences of Ukraine (IGRM)
67, Vyshgorodskaya Street, Kyiv, 04114, Ukraine

ФІТОХІМІЧНЕ ВИВЧЕННЯ ОСНОВНИХ ГРУП БІОЛОГІЧНО АКТИВНИХ РЕЧОВИН РОБІНІЇ ЗВИЧАЙНОЇ

Мандзій Т. П., Ерстенюк Г. М.

Актуальною проблемою сьогодення є раціональне використання рослинних ресурсів Землі, збагачення народного господарства України новими високопродуктивними видами, формами і сортами рослин.

Серед великого різноманіття рослин, які населяють нашу планету, провідне місце посідають деревні рослини, які ростуть у різних ґрунтово-кліматичних умовах і в процесі еволюції набули низки цінних господарських, фармакологічних, декоративних особливостей тощо. Велике народногосподарське значення має збереження генофонду рослин, як ендемів так і інтродуцентів. Досить цікавими у цьому плані є види роду *Robinia* L. (родина *Fabaceae* Lindl.), природні ареали яких розташовані у Північній і Центральній Америці. У світовій флорі відомо близько 20 видів, 6 з яких інтродуковано в Україні[1]. Найбільшого поширення набув вид *Robinia pseudoacacia* L. (робінія звичайна).

Перспективним є дослідження рослин, які широко використовують у народній медицині та мають достатні сировинні запаси[2]. До таких рослин належить, зокрема, робінія звичайна (*Robinia pseudoacacia* L.) – багаторічна рослина родини Бобових, також відома під назвами «робінія псевдоакація», «колюча акація» та «біла акація». Це цінна і дуже поширена медоносна, лікарська, фарбувальна, ефіроолійна, танідоносна, деревинна, декоративна й фітомеліоративна культура.

Робінія звичайна – це велике листопадне дерево з розлогою, негустою кроною, яке може сягати у висоту 20–35 м. Кора сіра, темно-сіра або ж коричнювата, вздовж стовбурів і старих гілок потріскана; молоді гілки зеленуваті або червонуваті[3].

---

[1] Деревья и кустарники СССР: определитель в 6 т. / сост. Соколов С.Я., Шепчинский Н.В.; ред. Соколов С.Я. М.-Л.: АН СССР, 1958. Т.4. 974 с., Дендрофлора України. Дикорослі й культивовані дерева і кущі. Покритонасінні: довідник в 2 т. / опис. Галкін С.І.; ред. Кохно М.А. К.: Фітосоціоцентр, Ч. ІІ. 2005. 715 с.

[2] Деревья и кустарники СССР: определитель в 6 т. / сост. Соколов С.Я., Шепчинский Н.В.; ред. Соколов С.Я. М.-Л.: АН СССР, 1958. Т.4. 974 с.

[3] Корнієвський Ю.І., Корнієвська В. Г. Фітотоксиклогія. Запоріжжя, 2013. 177 с.

Листки 18–20 см завдовжки, чергові, непарнопірчасті з 4–10 парами довгастих, довгасто-еліптичних або еліптичних листочків від 2 до 4 (6) см завдовжки[4].

Листочки цілокраї з округлою або трохи звуженою основою і тупою верхівкою, яка закінчується вістрям. Зверху листочки зелені, знизу блідо-зелені або сірувато-зелені, по жилках трохи опушені. Прилистки (до 3 см завдовжки) мають вигляд прямих або трохи зігнутих колючок.

Суцвіття – негусті пониклі китиці, завдовжки 10–20 см, розташовані в пазухах листків. Квітки до 2 см завдовжки, двостатеві, зигоморфні, дуже запашні. Оцвітина подвійна. Чашечка 6–8 мм завдовжки, 4–5 мм завширшки, зрослолиста, п'ятизубчаста, короткоопушена. Віночок метеликового типу з п'яти вільних пелюсток, білий або блідо-рожевий. Тичинок десять, з них дев'ять зрослися нитками в трубочку. Маточка одна, зав'язь верхня, стовпчик зігнутий, з головчастою приймочкою.

Плід – довгасто-лінійний біб 4–8 см завдовжки. Насінини вузько-ниркоподібні, коричневі або темно-бурі, матові.

Дерево світлолюбиве, жаро- та посухостійке, водночас доволі морозостійке і невибагливе до якості ґрунтів. У себе на батьківщині – у Східній частині Сполучених Штатів Америки – росте невеликими групами або поодиноко у листяних лісах. За межами природного ареалу цей вид найчастіше зростає у лісозахисних смугах та лісопарках, де можуть траплятися змішані і чисті насадження цієї культури.

Навесні біла акація розпочинає вегетацію найпізніше серед усіх дерев: її листя розпускається лише на початку травня. Цвітіння відбувається у травні-червні.

Хімічний склад. Квітки робінії містять цукри, органічні кислоти, флавоноїд робінін, ефірну олію, у складі якої переважає піперонал. У корі є дубильні речовини, ефірна олія, стерини, фітостерин і стігмастерин.

Застосування. Квітки робінії використовують як сировину для виготовлення флароніну – препарату з гіпоазотемічною дією.

Настій квіток робінії використовують як відхаркувальний, жарознижуючий, протизапальний, спазмолітичний, кровоспинний, діуретичний, гіпоазотемічний і легкий послаблюючий засіб.

[4] Корнієвський Ю.І., Корнієвська В. Г. Фітотоксиклогія. Запоріжжя, 2013. 177 с.

Назначають від кашлю і грипу, при болях у шлунку й кишківнику, при шлункових кровотечах і запальних процесах сечовивідних шляхів (пієлонефрит, нирковокам'яна хвороба, цистит).

Настій кори вживають при підвищеній кислотності шлункового соку, виразках шлунка й кишківника та при запорі. Застосовують ефірну олію робінії в парфумерії, косметиці, аромотерапії. Настій кори приймають при шлунково-кишкових виразках, підвищеній кислотності шлункового соку. Кора повинна застосовуватись чітко дозовано[5].

Білу акацію широко культивують по всій Україні. Найбільші площі її насаджень зосереджені в Лісостепу і Степу. Трапляється в парках, садах, полезахисних і протиерозійних смугах, у захисних насадженнях уздовж доріг, поблизу пасік тощо. Заготовляють сировину в районах її вирощування. Запаси сировини великі[6].

Метою роботи було фармакогностичне дослідження робінії звичайної та встановлення можливості створення лікарських засобів на основі біологічно активних речовин досліджуваної сировини.

Для досягнення поставленої мети необхідно було виконати наступні завдання:

– провести аналіз наукових першоджерел з питань ботанічної характеристики, поширення, хімічного складу робінії звичайної і застосування її у медицині;

– встановити якісний склад та кількісний вміст основних груп біологічно активних речовин (БАР) робінії звичайної;

– виділити індивідуальні сполуки та ідентифікувати їх.

Нами проведено кількісне визначення аскорбінової кислоти в листках та квітках робінії звичайної за фармакопейною методикою.

Вміст суми флавоноїдів визначали в перерахунку на рутин за модифікованою методикою.

Визначення кількісного визначення окиснюваних поліфенолів в перерахунку на танін проводили за фармакопейною методикою[7].

Матеріали та методи дослідження. Об'єктами дослідження були листки та квітки робінії звичайної (*Robinia pseudoacacia* L.) родини

[5] Корнієвський Ю.І., Корнієвська В. Г. Фітотоксиклогія. Запоріжжя, 2013. 177 с.

[6] Робінія звичайна, псевдоакація, колюча (біла) акація. *Вишнева пасіка.* URL: https://beegarden.com.ua/ukr/honeyplants/21/ (дата звернення: 25.10.2018).

[7] Державна фармакопея України. Державне підприємництво «Науково-експертний фармакопейний центр», 1-е вид. Х.: РІРЕГ, 2001. 556 с.

Бобових (*Fabaceae*), яку заготовляли під час цвітіння на території Івано-Франківської та Тернопільської областей у 2017 р.

Кількісне визначення вмісту аскорбінової кислоти. Нами проведено кількісне визначення аскорбінової кислоти в листках та квітках робінії звичайної. З грубо подрібнених листків аналітичної проби брали наважку масою 20 г, поміщали у фарфорову ступку, де ретельно розтирали зі скляним порошком (близько 5 г), поступово додаючи 300 мл води, і настоювали 10 хв. Потім суміш розмішували і витяг фільтрували, далі за методикою[8].

Вміст аскорбінової кислоти в перерахунку на абсолютно суху сировину у відсотках обчислювали за формулою:

$$X = \frac{V \times 0{,}000088 \times 300 \times 100 \times 100}{m \times 1 \times (100 - W)},$$

де 0,000088 – кількість аскорбінової кислоти, що відповідає 1 мл розчину 2,6-дихлорфеноліндофеноляту натрію (0,001 моль / л), в г;

V – об'єм розчину 2,6-дихлорфеноліндофеноляту натрію (0,001 моль/л), витрачений на титрування, в мл;

m – маса сировини, в г;

W – втрата в масі при висушуванні сировини, у відсотках.

Точну наважку подрібненої сировини або лікарського рослинного препарату просіювали крізь сито з отвором діаметром 3 мм, поміщали в конічну колбу ємністю 500 мл, заливали 250 мл нагрітої до кипіння води і кип'ятили зі зворотнім холодильником на електричній плитці із закритою спіраллю протягом 30 хв при періодичному перемішуванні. Отриману рідину охолоджували до кімнатної температури і фільтрували крізь вату в мірну колбу об'ємом 250 мл так, щоб частини сировини не потрапили у колбу, і доводили водою до мітки.

25 мл водної витяжки поміщали в колбу ємністю 750 мл, додавали 500 мл води, 25 мл розчину індигосульфокислоти і титрували при перемішуванні 0,02 моль/л розчином калію перманганату до золотисто-жовтого забарвлення. Паралельно проводили контрольний дослід: в конічну колбу ємністю 750 мл додавали 525 мл води, 25 мл розчину

[8] Государственная фармакопея СССР, вып. 2. Общие методы анализа. Лекарственное растительное сырье. МЗ СССР, XI-е изд. М.: Медицина, 1989. 400 с., Бензель І.Л. Дармограй Р.Є., Бензель Л.В. Дослідження вмісту аскорбінової кислоти та вільних органічних кислот у фітосубстанціях бадану товстолистого. *Фармацевт. журн.* 2010. № 2. С. 98–101.

індигосульфокислоти і титрували при перемішуванні 0,02 моль/л розчином калію перманганату до золотисто-жовтого забарвлення[9].

Вміст окиснюваних поліфенолів в перерахунку на танін і абсолютно суху сировину обчислювали за формулою:

$$X = \frac{(V_1 - V_0) \times 0{,}004157 \times 250 \times 100 \times 100}{m \times 25 \times (100 - W)},$$

де V_1 – об'єм розчину калію перманганату (0,02 моль/л), витраченого на титрування витяжки, в мл;

V_0 – об'єм розчину калію перманганату (0,02 моль/л), витраченого на титрування витяжки в контрольному досліді, в мл;

0,004157 – кількість окиснюваних фенолів, що відповідає 1 мл розчину калію перманганату (0,02 моль/л), в г;

m – маса сировини, в г;

W – втрата в масі при висушуванні сировини, у відсотках;

250 – загальний об'єм витяжки, в мл;

25 – об'єм витяжки, який взятий на титрування, в мл.

Точну наважку 1,0 г подрібненої сировини вміщували в патрон з фільтрувального паперу і обробляли хлороформом в апараті Сосклета до повного знебарвлення розчинника. Патрон з наважкою висушували на повітрі позбавленому запаху хлороформу. Після цього патрон поміщали в колбу ємністю 150 мл, заливали 30 мл 70% етанолу і нагрівали на киплячому водяному нагрівнику зі зворотнім холодильником протягом 30 хв.

Ідентифікацію флавоноїдів у сировині робінії звичайної проводили методом паперової хроматографії у системі розчинників етилацетат – кислота мурашина безводна – вода очищена (10:2:3). На хроматограмах речовини виявляли до і після обробки хромогенними реактивами (парами розчину аміаку концентрованого, 10% розчином натрію

[9] Державна фармакопея України. Державне підприємство «Науково- експертний фармакопейний центр», 1-е вид. Х.: ТОВ «РІРЕГ», доповнення 2. 2008. 608 с.; Державна Фармакопея України / Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів». 2-е вид. Доповнення 2. Харків: Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів», 2018. 336 с.; Визначення вмісту окислювальних фенолів у траві рути садової: матер. 83 наук.-практ. конф. студентів і молодих учених з міжнар. участю (27–28 березня 2014 р., Івано-Франківськ). Івано-Франківськ 2014. С. 198.

гідроксиду у 96% етанолі та 2% розчином алюмінію хлориду у 95% етанолі) за забарвленням у денному світлі та за флуоресценцією в УФ-світлі.

Приготування 2% розчину алюмінію хлориду в 95% етанолі: 2 г алюмінію хлориду х. ч. або ч. д. а. за ГОСТ 3759-85 розчиняли у 50 мл 95% етанолі в мірній колбі ємністю 100 мл, доводили об'єм розчину тим же розчинником до мітки і перемішували.

Оцінювали результати шляхом порівняння величини R_f зон на хроматограмі розчину порівняння та випробовуваного розчину. Для приготування розчину порівняння використовували стандартні зразки (*Fluka*) речовин-свідків: кверцетину, рутину, апігенін-7-глюкозиду, гіперозиду 14–19.

Приготування розчину стандартного зразку рутину: близько 0,05 г (точна наважка) рутину-стандарту (ФС 42-2508-87), висушеного до постійної маси при температурі 130–135ºС, розчинили в мірній колбі на 100 мл у невеликій кількості 96% етанолу при нагріванні на киплячому водяному нагрівнику, охолоджували і доводили об'єм розчину 96% етанолом до мітки (1 мл розчину стандартного зразку містить 0,0005 г рутину).

Вміст суми флавоноїдів визначали в перерахунку на рутин за модифікованою методикою. Метод оснований на реакції комплексоутворення флавоноїдів з алюмінію хлоридом безпосередньо у витяжках з сировини. Максимум поглинання комплексу флавоноїду з алюмінію хлоридом відмічено при 415 нм[10].

Витяжку охолоджували і фільтрували в мірну колбу ємністю 100 мл, а сировину екстрагували в аналогічних умовах ще двічі. Фільтрати

[10] Глущенко А.В. Методика визначення кількісного вмісту флавоноїдів в екстрактах *Salsola collina* L. *Український біофармац. журн.* 2014. № 2. С. 46-49.; Вишневська Л.І., Євтіфєєва О.А., Гарна С.В. Валідаційні характеристики методики кількісного визначення флавоноїдів методом УФ-спектрофотометрії «Бронхофіт». *Український медичний альманах*. 2010. Т. 13. №1. С. 33–35; Смалюх О.Г., Нестер М.І., Сур С.В. Стандартизація цмину піскового квітів за складом і вмістом флавоноїдів. *Актуальні питання фармацевтичної і медичної науки та практики.* 2013. №3 (13). С. 95–98; Евдокимова О.В. Валидация методики количественного определения суммы флавоноидов в столбиках с рыльцами кукурузы. *Фармация.* 2008. № 7. С. 14–17; Khokhlova K., Vishnevska L., Yakovenko V. Development and validation of the quantitative determination method of flavonoids in the preparations. *42nd IUPAC, Quality Assurance of Medicines and Detection of Counterfeits.* Glasgow. 2009. P. 107–108; Сливанчекова И.Б., Лякина М.Н., Костенникова З.П. Количественное определение флавоноидов в гомеопатических настойках туи методом спектрофотометрии.*Фармация*. 2001. №6. С. 14–17.

об'єднували в мірній колбі, об'єм розчину доводили до мітки 70% етанолом і перемішували. 5 мл виготовленого екстракту переносили в мірну колбу ємністю 25 мл, додавали алюмінію хлориду 2% спиртового розчину і доводили об'єм розчину до мітки 0,5% розчином натрію ацетату. Через 15 хв визначали оптичну густину розчину на спектрофотометрії СФ Cary-50 при довжині хвилі 415 нм в кюветі з товщиною шару 10 мм, як розчин порівняння використовували розчин, виготовлений з 5 мл екстракту, доведеного до мітки 70% етанолом в мірній колбі ємністю 25 мл. Концентрацію флавоноїдів у розчині обчислювали за калібрувальним графіком, побудованим з стандартним зразком рутину[11].

В мірну колбу ємкістю 25 мл вносили по 0,2; 0,4; 0,6; 0,8; 1,0 і 1,2 мл розчину рутину-стандартного (0,5 мг/мл), після чого додавали 5 мл 2% розчину алюмінію хлориду і доводили його 0,5% розчином натрію ацетату до мітки. Вимірювали оптичну густину розчину рутину.

Вміст суми флавоноїдів в перерахунку на рутин і абсолютно суху сировину у відсотках обчислювали за формулою:

$$X = \frac{A_1 \times m_0 \times V_0 \times 100 \times 25 \times 100 \times 100\ \%}{A_0 \times m_1 \times 100 \times V_1 \times 25 \times (100 - W),}$$

де A_0 – оптична густина розчину стандартного зразка рутину при довжині хвилі 415 нм;

[11] Глущенко А.В. Методика визначення кількісного вмісту флавоноїдів в екстрактах *Salsola collina* L. *Український біофармац. журн.* 2014. № 2. С. 46–49; Вишневська Л.І., Євтіфєєва О.А., Гарна С.В. Валідаційні характеристики методики кількісного визначення флавоноїдів методом УФ-спектрофотометрії «Бронхофіт». *Український медичний альманах*. 2010. Т. 13. №1. С. 33–35; Смалюх О.Г., Нестер М.І., Сур С.В. Стандартизація цмину піскового квітів за складом і вмістом флавоноїдів. *Актуальні питання фармацевтичної і медичної науки та практики.* 2013. №3 (13). С. 95–98; Евдокимова О.В. Валидация методики количественного определения суммы флавоноидов в столбиках с рыльцами кукурузы. *Фармация.* 2008. № 7. С. 14–17; Khokhlova K., Vishnevska L., Yakovenko V. Development and validation of the quantitative determination method of flavonoids in the preparations. *42nd IUPAC, Quality Assurance of Medicines and Detection of Counterfeits.* Glasgow. 2009. P. 107–108; Бурда Н. С., Журавель І. О., Кисличенко В.С. Кількісне визначення флавоноїдів у траві гадючника в'язолистого. *Фітотерапія.* 2010. № 3. С.58–60; Сливанчекова И.Б., Лякина М.Н., Костенникова З.П. Количественное определение флавоноидов в гомеопатических настойках туи методом спектрофотометрии.*Фармация*. 2001. №6. С. 14–17.

A_1 – оптична густина досліджуваного розчину при довжині хвилі 415 нм;

V_1 – об'єм витяжки стандартного зразка рутину, в мл;

m_0 – маса стандартного зразка рутину, в г;

m_1 – маса сировини, в г;

V0 – об'єм витяжки для аналізу, в мл;

W – втрата в масі при висушуванні сировини, у відсотках.

Результати дослідження та їх обговорення.

Визначення оптичної густини отриманих випробовуваних розчинів і розчину стандартного зразка рутину визначали на спектрофотометрі SPECORD M40 при довжині хвилі 415 нм в кюветі з товщиною шару 10 мм в «Центрі біоелементології» Івано-Франківського національного медичного університету (атестат акредитації № 037/14, видано 29 квітня 2014 р.).

Результати проведених досліджень кількісного вмісту аскорбінової кислоти наведено в табл. 1.

Таблиця 1

Кількісний вміст аскорбінової кислоти у листках та квітках робінії звичайної з різних місць зростання

Місце і рік заготівлі сировини	**Вміст аскорбінової кислоти, %, x±Δx, n=9**
Околиці м. Яремче Івано-Франківської обл., 2017 р.	0,276 ± 0,0135
	0,272 ± 0,0131
	0,276 ± 0,0134
	0,279 ± 0,0136
Околиці м. Монастирська Тернопільської обл., 2017 р.	0,282 ± 0,0074
	0,284 ± 0,0077
	0,277 ± 0,0059
	0,284 ± 0,0077
	0,279 ± 0,0068

Для визначення відносної похибки методу кількісного визначення вмісту аскорбінової кислоти нами проведено 9 паралельних визначень в зразках сировини[12], дані яких наведені в табл. 2.

[12]Vera A., Ly-Chatain M.H., Rigobello V., Demarigny Y. Description of a French natural wheat sourdough over 10 consecutive days focussing on the lactobacilli present in the microbiota.

Таблиця 2

Метрологічна характеристика результатів кількісного визначення вмісту аскорбінової кислоти в листках та квітках робінії звичайної

f	S^2	S	P	t (P, f)	Кількісний вміст	E, %
Івано-Франківська обл.						
9	0,000214000	0,0049	0,95	2,79	0,0136	4,8588
Тернопільська обл.						
9	0,000069000	0,0028	0,95	0,284	0,0077	2,7104

Результати проведених досліджень кількісного вмісту окиснюваних поліфенолів наведено в табл. 3.

Таблиця 3

Кількісний вміст суми окиснюваних поліфенолів у листках та квітках робінії звичайної з різних місць зростання

Місце і рік заготівлі сировини	Вміст суми окиснюваних фенолів, %, $x \pm \Delta x$, n=9
Околиці м. Яремче Івано-Франківської обл., 2017 р.	5,463 ± 0,117
	5,463 ± 0,117
	5,463 ± 0,117
	5,597 ± 0,099
Околиці м. Монастириська Тернопільської обл., 2017 р.	5,729 ± 0,0615
	5,730 ± 0,0628
	5,730 ± 0,0628
	5,729 ± 0,0615
	5,730 ± 0,0628

Для визначення відносної похибки методу кількісного вмісту суми окиснюваних поліфенолів нами проведено 9 паралельних визначень в зразках сировини, дані яких наведені в табл. 4.

International Journal of General and Molecular Microbiology. 2012. Vol. 101. Iss. 2. P. 369–377; Lücke F.-K., Zangerl P. Food safety challenges associated with traditional foods in German-speaking. *Food Control*. 2014. Vol. 43. P. 217–230.

Таблиця 4

Метрологічна характеристика методу кількісного визначення суми окиснюваних поліфенолів в листках та квітках робінії звичайної

f	S^2	S	P	t (P, f)	Кількісний вміст	E, %
Івано-Франківська обл.						
9	0,011431000	0,0356	0,95	5,597	0,0991	1,7702
Тернопільська обл.						
9	0,004600000	0,0226	0,95	5,730	0,0628	1,0969

Результати проведених досліджень на вміст флавоноїдів наведено в табл. 5.

Таблиця 5

Кількісний вміст суми флавоноїдів з різних місць зростання

Місце і рік заготівлі сировини	Вміст флавоноїдів, %, x±Δx, n=6
Околиці м. Яремче Івано-Франківської обл., 2017 р.	0,1361 ± 0,00027
	0,1368 ± 0,00033
	0,1367 ± 0,00031
	0,1298 ± 0,00022
Околиці м. Монастирська Тернопільської обл., 2017 р.	0,1296 ± 0,0054
	0,1300 ± 0,0059
	0,1311 ± 0,0038
	0,1284 ± 0,0049
	0,1286 ± 0,0043

Для визначення відносної похибки методу визначення кількісного вмісту суми флавоноїдів нами проведено 6 паралельних визначень в зразках сировини, дані яких наведені в табл. 6.

Таблиця 6

Метрологічна характеристика методу кількісного визначення суми флавоноїдів в листках та квітках робінії звичайної

f	S^2	S	P	t (P, f)	Кількісний вміст	E, %
Івано-Франківська обл.						
6	0,000008567	0,0012	0,95	0,1368	0,00033	2,4276
Тернопільська обл.						
6	0,000011200	0,0014	0,95	0,1311	0,0038	2,8994

На рис. 1 наведено експериментальні спектри поглинання спиртових витяжок робінії звичайної.

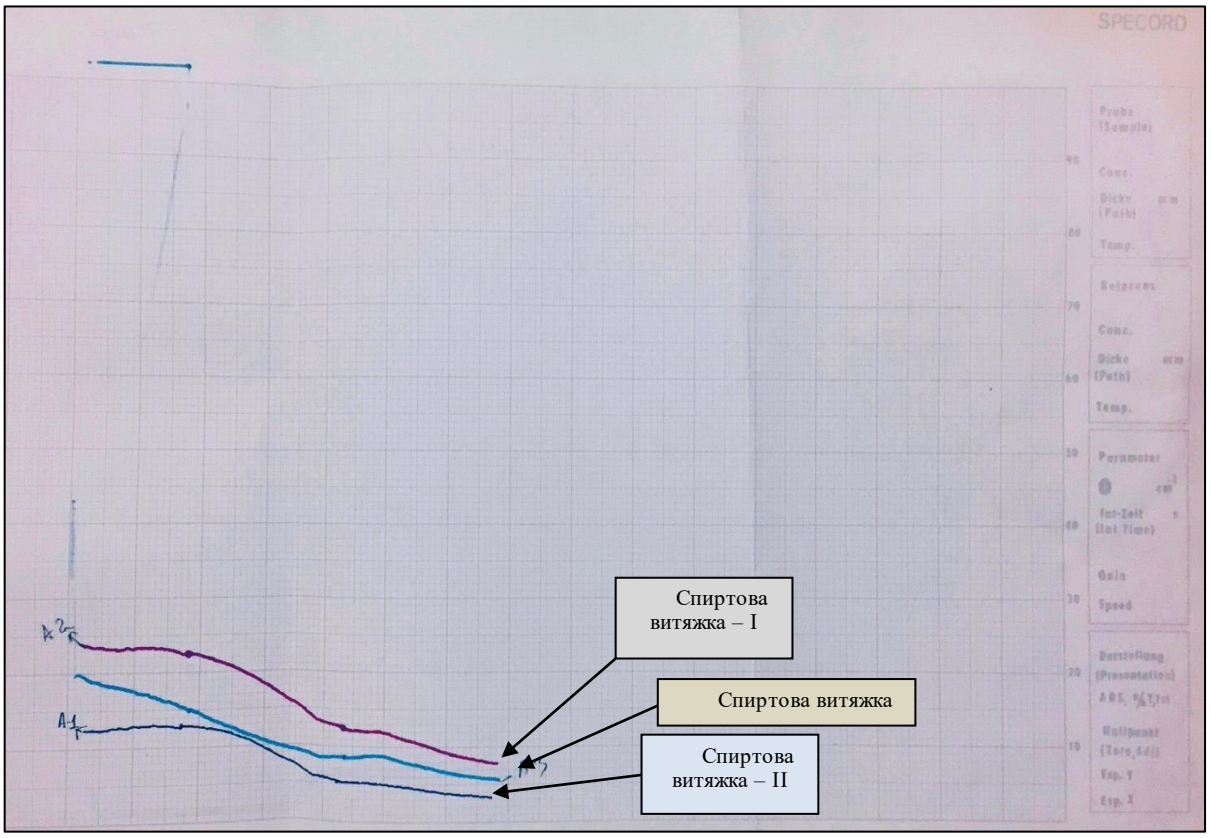


Рис. 1. Спектри поглинання спиртових витяжок робінії звичайної:
A_1 – спектр поглинання першої спиртової витяжки,
A_2 – спектр поглинання другої спиртової витяжки,
A_3 – спектр поглинання третьої спиртової витяжки.

Встановлено, що абсорбційні спектри досліджуваних витяжок з сировини робінії звичайної характеризуються наявністю максимуму поглинання при довжині хвилі 411 нм, що відповідає максимуму поглинання розчину рутину після реакції взаємодії з розчином алюмінію хлориду у кислому середовищі.

Нами встановлено, що у сировині робінії звичайної максимальний вміст аскорбінової кислоти становить від 0,284% до 0,272%.

Результати кількісного визначення суми окиснюваних поліфенолів вказують, що у листках та квітах робінії звичайної максимальний вміст суми окиснюваних фенолів знаходиться в межах від 5,730% до 5,463%.

На основі отриманих результатів можна зробити висновок, що кількісний вміст флавоноїдів у перерахунку на рутин у сировині робінії звичайної знаходиться в межах від 0,136% до 0,128%.

ВИСНОВКИ

1. Наявність у сировині БАР, що відповідають за її основні фармакологічні властивості, таких як флавоноїди, окиснювані поліфеноли та аскорбінова кислота, підтверджує можливість застосування та використання у фармацевтичній промисловості робінії звичайної.
2. У листках та квітках робінії звичайної встановлено кількісний вміст аскорбінової кислоти (0,272% – 0,284%), окиснюваних поліфенолів (5,463% – 5,730%) та флавоноїдів (0,128% – 0,136%).
3. Виявлені флавоноїди, окиснювані поліфеноли та аскорбінова кислота розширюють відомості про хімічний склад досліджуваної рослини і створюють передумови для подальшого її фітохімічного дослідження.

АНОТАЦІЯ

Розроблена методика кількісного визначення флаовноїдів у листках та квітках робінії звичайної. Вивчено накопичення біологічно активних речовин в залежності від місця зростання.

У результаті проведеного фітохімічного аналізу сировини робінії звичайної встановлено наявність різних гру БАР та визначено кількісний вміст аскорбінової кислоти, суму окиснюваних фенолів та флавоноїдів. Вміст аскорбінової кислоти знаходиться в межах до 0,28%, суми окиснюваних фенолів до 5,63%, флавоноїдів до 0,13%.

Результати досліджень показали, що максимальна кількість аскорбінової кислоти та суми окиснюваних фенолів накопичувалась на околицях Тернопільскої обл., а максимальна кількість флавоноїдів знаходилась на околицях Івано-Франківської обл.

ЛІТЕРАТУРА

1. Деревья и кустарники СССР: определитель в 6 т. / [сост. Соколов С.Я., Шепчинский Н.В.; ред. Соколов С.Я.]. М.-Л.: АН СССР, 1958. Т.4. 974 с.
2. Дендрофлора України. Дикорослі й культивовані дерева і кущі. Покритонасінні: довідник в 2 т. / [опис. Галкін С.І.; ред. Кохно М.А.]. К.: Фітосоціоцентр, Ч. ІІ. 2005. 715 с.

3. Корнієвський Ю.І., Корнієвська В.Г. Фітотоксикологія. Запоріжжя, 2013. 177 с.

4. Робінія звичайна, псевдоакація, колюча (біла) акація. *Вишнева пасіка.* URL: https://beegarden.com.ua/ukr/honeyplants/21/ (дата звернення: 25.10.2018).

5. Державна фармакопея України. Державне підприємництво «Науково – експертний фармакопейний центр», 1-е вид. Х.: РІРЕГ, 2001. 556 с.

6. Государственная фармакопея СССР, вып. 2. Общие методы анализа. Лекарственное растительное сырье. МЗ СССР, XI-е изд. М.: Медицина, 1989. 400 с.

7. Бензель І.Л. Дармограй Р.Є., Бензель Л.В. Дослідження вмісту аскорбінової кислоти та вільних органічних кислот у фітосубстанціях бадану товстолистого. *Фармацевт. журн.* 2010. № 2. С. 98–101.

8. Бензель І.Л. Дармограй Р.Є., Бензель Л.В. Дослідження вмісту аскорбінової кислоти та вільних органічних кислот у фітосубстанціях бадану товстолистого. *Фармац журн*. 2010. № 2. С. 98–101.

9. Неробеев В.Д., Неробеев Д.В. Системные стандарты и доказательная база математической статистики в научных медицинских исследованиях. *Новости медицины и фармации в мире*. 2013. № 6 (450). С. 24–26.

10. Лапач С.М., Чубенко А.В., Лапач С.М., Бабіч П.М. Статистичні методи в медико – біологічних дослідженнях із застосуванням *Exel*. Київ, МОРІОН, 2000. 320 с.

11. Державна фармакопея України. Державне підприємство «Науково-

експертний фармакопейний центр», 1-е вид. Х.: ТОВ «РІРЕГ», доповнення 2. 2008. 608 с.

12. Державна Фармакопея України / Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів». 2-е вид. Доповнення 2. Харків: Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів», 2018. 336 с.

13. Визначення вмісту окислювальних фенолів у траві рути садової: матер. 83 наук.-практ. конф. студентів і молодих учених з міжнар. участю (27–28 березня 2014 р., Івано-Франківськ). Івано-Франківськ 2014. С. 198.

14. Глущенко А.В. Методика визначення кількісного вмісту флавоноїдів в екстрактах *Salsola collina* L. *Український біофармац. журн.* 2014. № 2. С. 46–49.

15. Вишневська Л.І., Євтіфєєва О.А., Гарна С.В. Валідаційні характеристики методики кількісного визначення флавоноїдів методом УФ-спектрофотометрії «Бронхофіт». *Український медичний альманах*. 2010. Т. 13. №1. С. 33–35.

16. Смалюх О.Г., Нестер М.І., Сур С.В. Стандартизація цмину піскового квітів за складом і вмістом флавоноїдів. *Актуальні питання фармацевтичної і медичної науки та практики.* 2013. №3 (13). С. 95–98.

17. Евдокимова О.В. Валидация методики количественного определения суммы флавоноидов в столбиках с рыльцами кукурузы. *Фармация.* 2008. № 7. С. 14–17.

18. Khokhlova K., Vishnevska L., Yakovenko V. Development and validation of the quantitative determination method of flavonoids in the preparations. *42nd IUPAC, Quality Assurance of Medicines and Detection of Counterfeits*. Glasgow. 2009. P. 107–108.

19. Бурда Н.С., Журавель І.О., Кисличенко В.С. Кількісне визначення флавоноїдів у траві гадючника в'язолистого. *Фітотерапія.* 2010. № 3. С. 58–60.

20. Сливанчекова И.Б., Лякина М.Н., Костенникова З.П. Количественное определение флавоноидов в гомеопатических настойках туи методом спектрофотометрии. *Фармация*. 2001. №6. С. 14-17.

Information about authors:

Mandzii T. P.,
Candidate of Pharmaceutical Sciences,
Assistant of the department of pharmacy
Ivano-Frankivsk National Medical University
2, Galytska str., Ivano-Frankivsk, 76018, Ukraine

Ersteniuk A. M.,
Doctor of Biological Sciences, Professor,
Head of the Department of Biological and Medical Chemistry with the course of Physical and Bioorganic Chemistry
Ivano-Frankivsk National Medical University
2, Galytska str., Ivano-Frankivsk, 76018, Ukraine

RESEARCH ABOUT DEVELOPMENT OF DRUGS FROM WHITE WILLOW BARK AND SAGE

Postoy V. V., Mykhailyk D. O.

INTRODUCTION

Nature gives us a lot of useful things and it is important to be able to use these gifts properly. Medical plant is one of such miracles and gifts of nature. From ancient times, people, thanks to observation and experience, used the natural benefits of the plant world and as a source of their own consumption and to alleviate their afflictions.

Today, with the development of science and research opportunities, it is possible to explore widely the herbs and to understand why and how they can be used, which components could help and which could harm.

There are about 12 thousand plants that have healing properties and are used in both traditional and folk medicine[1]. In this case, medicinal plants are often combined with other treatment methods.

Usage of plants as drugs came from ancient times and still plays a significant role in the arsenal of drugs in modern medicine. This is due to some of the benefits of phytotherapy compared with synthetic drugs. The interest in phytotherapy is a cause of changes in the age structure of the population: an increase of persons of elderly and senile age who usually suffer from these or that diseases that require long-term use of drugs and the risk of development of side effects should be small. Phytotherapy plays a special role in pediatric practice, because plants act softer and rarely give negative complications. Uncommon side effects can be explained by the fact that "Herbal medicine is a valuable biogenetic complex, which includes active substances – proteins, essential oils, trace elements, vitamins and more"[2].

It is believed that such a complex was formed in a living cell, therefore, it has a greater resemblance to the human body than an isolated, chemically pure active substance, and therefore it is easier to assimilate and gives fewer side effects. Herbal drugs play big sense in the prevention of diseases. No wonder, that absolutely healthy people are not present in our time, most of

[1] Чухно Т. Большая энциклопедия лекарственных растений. М.: Эксмо, 2007. 1024 с.

[2] Bisset N.G. Herbal Drugs and Phytopharmaceuticals. Stuttgart, Germany: Medpharm Scientific Publishers, 2004. P. 534–536.

us are in so-called the third condition – between health and disease, on the verge of failure of adaptive mechanisms, that is, when the body needs an easily corrective action of the plant, normalizes slightly modified functions of the organism[34]. It should be noted that the plant raw material is a relatively cheaper and affordable source of medicinal products.

Detailed studying of the chemical composition, pharmacological properties, as well as clinical trials of plants allows the introduction of new effective drugs based on medicinal plant raw material (MPRM) annually. At the moment, the range of various dosage forms based on medicinal plants shows the tendency to increase. In addition to traditionally known tablets, drops, syrups, there are produced such ancient medicinal forms as therapeutic baths, teas, lozenges, which makes the convenient use of MPRM in pediatric and geriatric practice. The most commonly found now are drugs, like dietary soups, which are excellent nutrition for patients with various diseases. Medical wines, which are extracts of different plants in natural wines, are widespread. Herbal medicines are used to prevent diseases; they support the adaptive forces of the organism in the level necessary for normal existence. And this ability makes herbal medicines and medical nutrition essential and indispensable in the treatment of the elderly people, as well as those affected by chronic ailments, and those who do not tolerate chemo-drugs that cause them allergies[5]. Chemical compounds that have therapeutic properties (and the effect of their constituent parts, sometimes synergistic, and sometimes antagonistic, complex); perform particularly important tasks in the human body. Such compounds include flavonoids (routine, quercetin, hesperidin, campferol, apigenin and more than 400 others), which, with slight toxicity, have different properties. So, some of them, such as routine, in combination with ascorbic acid reduce the fragility and permeability of capillaries, acting as vitamin-like (like vitamin P); the others, in particular, quercetin, produce bacteriostatic effects, inactivate some antibiotics and sulfanilamide drugs, so they cannot be used at the same time[6]. Other

[3] Bisset N.G. Herbal Drugs and Phytopharmaceuticals. Stuttgart, Germany: Medpharm Scientific Publishers, 2004. P. 534–536.

[4] Мазнев Н. И. Золотая книга лекарственных растений. 15-е изд., доп. М.: ООО «ИД РИПОЛ Классик», ООО Издательство «ДОМ. XXI век», 2008. 621 с.

[5] Blumenthal M. The Complete German Commission E Monographs. Austin, TX: American Botanical Council. Boston: Integrative Medicine Communications; 1998. 684 p.

[6] Мазнев Н.И. Золотая книга лекарственных растений. 15-е изд., доп. М.: ООО «ИД РИПОЛ Классик», ООО Издательство «ДОМ. XXI век», 2008. 621 с.

substances activate oxidation-reduction processes. By acting antihistamically, anti-blastic, they inhibit the effects of hyaluronidase and have antioxidant properties and prevent the oxidation of adrenaline and ascorbic acid. Flavonoids are used in hemorrhagic diathesis, atherosclerosis, hypertension, myocardial infarction, rheumatism, diabetes mellitus, with allergic diseases such as urticaria, hay fever, with anaphylactic shock, frostbite, blistering. Glycosides, such as anthracene derivatives – antraglycosides, which, by increasing peristalsis of the large intestine, contribute to the emptying of the intestine and thus improve blood circulation in the pelvic organs, are equally important. Saponins promote the assimilation of other therapeutic agents, have anti-inflammatory action, inhibit the release of free histamine. These include antiallergic terpenoids of azulenes, antiviral polyphenols (tannins), acetylcholine mediator (enhances bowel peristalsis, urinary and biliary tract), glycokinins (replacing insulin, but not digested with digestive juices), organic acids and mineral salts (provide acid-base balance in the body), metabolites, vitamins and trace elements and enzymes associated with them (fissile and synthesizing proteins), antibiotics and phytoncides[78].

1. White willow as a source drug development

Taking into account the above, the object of our study was the willow white (Salix alba, Fig. 1). It is a tree, 12–15 meters high, and at a young age it is a shrub, in small polar regions and hilly areas there are small dwarf willows. Crown of willow is semi-spherical, flabby, smooth, the bark has a grayish color, the branches are very flexible and thin, grow up to 2 m. There are about 170 species of willow. Willow is the most widespread in Europe, Iran, Asia Minor, on the territory of Ukraine, except for the highlands of the Carpathians.

The willow blooms from April to May until the young leaves begin to spread. This tree can be found on wet meadows, on floodplains of rivers, in damp forests. It is grown as a decorative crop and phytomelioration plant. In Ukraine, about 30 species are distributed, among which 27 are wild and 3 are cultivated.

[7] Болотов Б.В. Травник академика Болотова. Спб.: Питер, 2012. 128 с.

[8] Bisset N.G. Herbal Drugs and Phytopharmaceuticals. Stuttgart, Germany: Medpharm Scientific Publishers, 2004. P. 534–536.

For medicinal purposes, bark is used more commonly than leaves[9]. Hippocrates, Celsius, Pliny the Elder, Dioscoride, Galen recommended to use the broths of willow in fever and to reduce the severity of pain.

Fig. 1. ***White willow***

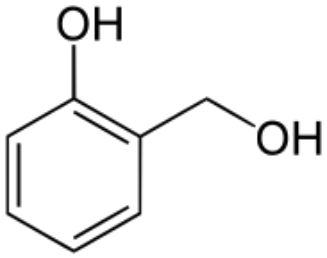
Biologically active substances in the complex have hemostatic, antiseptic, diuretic action. They expand coronary vessels and reduce the rhythm of the heart, prevent the formation of blood clots, increase bronchial secretion, promote blood coagulation, have the ability to increase the allocation of pancreatic juice, have an antiviral effect. Willow white revitalizes blood circulation and reduces the risk of developing heart attacks, strokes and varicose veins. Willow extract is effective in reducing pain and inflammation. The main active ingredient is salicin (0.5%) – a compound similar in quality to aspirin[10].

[9] Мазнев Н.И. Золотая книга лекарственных растений. 15-е изд., доп. М.: ООО «ИД РИПОЛ Классик», ООО Издательство «ДОМ. XXI век», 2008. 621 с.

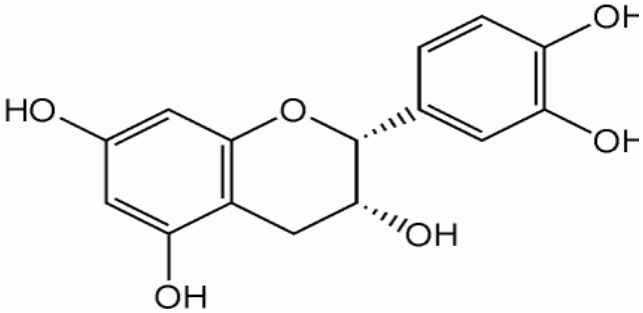
[10] Akao, T., Yoshino, T., Kobashi, K., and Hattori, M. Evaluation of salicin as an antipyretic prodrug that does not cause gastric injury. Planta Med 2002. Vol. 68(8). P. 714–718.

White willow bark contains a large number of biologically active substances: glucose responsible for stress relief, metabolic processes, brain nutrition, flavonoids (about 2% of catechins, flavones, flavonols, flavanones) produce antioxidant and anti-inflammatory effects, glycosides (3%) decrease the level of cholesterol affects the immune processes, tannins (about 12%) have anesthetic, anti-inflammatory effect, tannin (11% – 12%) produces astringent effect, so the bark helps with diseases of the gastrointestinal tract[11].

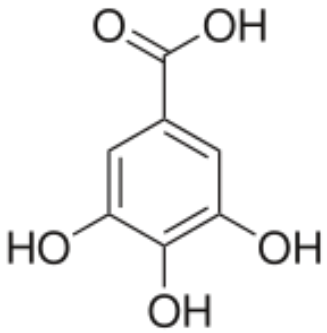
Salicin



Catechinin



Tannin



Once in the body, salicin splits in the liver to salicylic acid, which leads to a violation of the synthesis of inflammatory mediators by blocking the enzyme cyclooxygenase. As a result, these active substances do not cause inflammatory reactions, including pain. On the one hand, to achieve the maximum concentration of tannins, the willow bark is better to boil on low

[11] Blumenthal M. The Complete German Commission E Monographs. Austin, TX: American Botanical Council. Boston: Integrative Medicine Communications; 1998. 684 p.

heat for 10–20 minutes. But, when extracting tannins by boiling, the bark largely loses salicin. Therefore, although broths are used internally, it is better to use them externally (lotions, rinses), and for internal consumption, to prepare infusions[12].

To feel the effect when the infusion is used as a prophylactic, general-purpose drug, it should be taken regularly for 2 weeks, then a weekly break is done and the course is repeated. When administered internally willow contributes to improvement in diarrhea and gastritis, dysentery, colitis, arthritis, rheumatism, neuralgia, fever, bronchitis, pleurisy, gout, dispersion, neurosis. Also willow is used for urogenital system diseases, liver and spleen, bleeding. When applied externally helps with sweating, sore throat, has a bactericidal effect on the oral cavity during rinsing[13].

Drugs of white willow bark (Fig. 2) are presented in the form of crushed powder (extract), tablets, capsules, tea. Extract, in the composition of capsules and tablets, is obtained by vacuum extraction. An adult should take powder half a teaspoon three times a day, drinking with water. The capsule is taken once a day during meals. Tablets should be taken one or two times a day. Pour a pack of tea into a glass of boiling water, infuse for 30 minutes in a water bath, strain and use a tablespoon four times a day half an hour before meal.

Fig. 2. ***White willow bark***

[12] Болотов Б.В. Травник академика Болотова. Спб.: Питер, 2012. 128 с.

[13] Мазнев Н. И. Золотая книга лекарственных растений. 15-е изд., доп. М.: ООО «ИД РИПОЛ Классик», ООО Издательство «ДОМ. XXI век», 2008. 621 с.

Nowadays, there are drugs with willow white bark in the pharmaceutical markets of different countries (Table 1).

Table 1

White willow bark drugs in the pharmaceutical markets of different countries

Drug	Producing country	Composition		Pharmaco-therapeutic action
1	2	3		4
Tea "Bronchikum"	France	Willow bark, anise fruit, fennel, aniseed oil, grass honey, licorice root		Expectorant effect
Lotion "Alleviate"	USA	Extract of willow bark, arnica, ginseng, peppermint oil, menthol, lysine aspartate, methyl salicylate		Anesthetics for stretching athletes
Granules "Insti"	Pakistan	Extract of Willow White, Eucalyptus, Fennel, Valerian, Licorice, Menthol		Flu therapy
Capsules "Solaray"	USA	Extract of Willow White	Anti-inflammatory action, rheumatoid diseases	Capsules "Solaray"
Tabletes "Megaprin"	USA	White Willow Bark, Cardamom, Cumin, Mineral Calcium Extract	Anti-inflammatory action, rheumatoid diseases	Tabletes "Megaprin"
Capsules "Pharma-med"	Canada	Extract of willow, cranberries, raspberry leaves, ascorbic acid	Treatment of genitourinary system	Capsules "Pharma-med"

Продовження таблиці 1

1	2	3	4
Water-alcohol solution “Digestodoron”	France	Extract of willow bark of white, purple, corients of ferns	Inflammation of the gastrointestinal tract
Tea “Uroflux”	Ukraine	Extract of white willow bark, leaves of mint, birch, licorice roots, steelmaker	Anti-inflammatory and diuretic action
Supplements “Revmavit”	Netherlands	Extract of willow whitewash, ash, roots of gripogophyte, glucosamine, routine, taurine	Chronic inflammation of the musculoskeletal system
Tablets “Assilix”	Germany	White willow extract, colloidal anhydride, corn starch, micro-crystalline cellulose, lactose monohydrate, magnesium stearate, gum arabic, sucrose, talc	Anti-inflammatory action, rheumatoid diseases

As seen from the table 1, drugs with white willow bark extract are available in 7 countries, among which the US is the leader (3 drugs). In this case, the main action of drugs is anti-inflammatory.

White willow is successfully used in homeopathic medicine. In homeopathy, the willow bark is used, which is collected in the morning from three-year shoots. Based on it, prepared on 85% of alcohol, a phyto is made in a ratio (1:3). Then the solution of 35% alcohol used for gout, arthritis, rheumatism is received[14].

Willow bark is also widely used in home cosmetics and cosmetics industry. It is very popular in hair care. Active substances in its composition, mainly salicin, improve the processes of the skin of the head exchange, they are regulators of the allocation of fat in the skin, hair grows better and faster. Due to the properties of regulating fatty processes in the skin, it is used to make creams and lotions for the face and body. They are used for excessive

[14]Blumenthal M. The Complete German Commission E Monographs. Austin, TX: American Botanical Council. Boston: Integrative Medicine Communications; 1998. 684 p.

oily skin and have anti-inflammatory action. At home, a lotion for oily skin can be prepared.

In folk medicine, there are many prescriptions for the use of willow bark.

– When the disease of the joints and rheumatism, the bark of the tree is used: pour 1 tablespoon of crushed bark with 1 glass of boiling water and infuse for 30 minutes in a water bath, strain the hot decoction and bring to the original volume. Take 1 tablespoon 3-5 times a day before meals.

– When gastritis, inflammation of gastrointestinal tract: pour 1 tablespoon of crushed bark with 200 ml of water and boil for 30 minutes, strain and bring the decoction to the original volume. Take 1 tablespoon 4 times a day before meals.

– When diarrhea: pour 1 tablespoon of crushed bark with 400 ml of water, boil for 30 minutes, and bring with boiling water to the original volume. Take 2 tablespoons 3–4 times a day.

– In case of varicose veins it is recommended to take the bath with the decoction of the willow bark. Pour a glass of dry crushed bark into boiling water, infuse for half an hour, add in a basin with hot water and lower the feet there, do the procedure until the water is warm. The use of such baths is effective at high sweating of the legs, various skin diseases, including fungal infections, boils, trophic ulcers, whitish inflammations.

– To heal the wounds, sprinkle them with powder of dry willow bark. However, the powder can be used internally: 1 g, drink with water, 3 times a day after meals.

– Young branches of willow are used when headache. They are slightly soaked and applied to the forehead, fastened with a bandage.

Treatment with willow bark has certain cautions. It is not recommended to use a decoction of the willow bark at a stomach ulcer, gastritis with high secretion of acid, with viral infections. The use of a drug with a white willow bark can cause Reye's syndrome - a life-threatening defeat of the liver and brain[15].

1. Sage as a source drug development

Another object of interest to us is sage (Salvia officinalis, Fig. 3).

There are over 900 species of sage, but only some of them have healing properties. Salvia officinalis shows the highest concentration of essential

[15]Болотов Б.В. Травник академика Болотова. Спб.: Питер, 2012. 128 с.

oils in it, which determines the medicinal properties of the plant. This does not mean that other species of plant do not have beneficial properties. However, the concentration of essential oil in them is lower, therefore, and the use is not so effective[16][17].

Fig. 3. ***Sage***

In the writings of Italian scholars, it is noted that in ancient Egypt, sage was called "Sacred Grass" and the most useful drug that healed infertility among women. The Latin name Salvia means "save the health", "be healthy". From ancient times, sage was considered a cure for all diseases. This fact was recorded in the writings of Galen and Pliny the Elder. Water infusion of sage leaves is known as "Greek tea".

In the Middle Ages the plant was used as a means of hygiene of the oral cavity. Boccaccio mentions that sage rubbed gums in the fight against stomatitis. The spectrum of its physiological effect on the body is so wide

[16] Gora J., Lis A. Essential oils from sage. Aromatherapy. 2008. No 4 [14]. P. 5–12.

[17] Bowles E. J. The Chemistry of Aromatherapeutic Oils. South Australia: Grif n Press, 2008. P. 37, 85, 98, 122, 168.

that in different countries in different epochs and in our time the plant is called “sacred grass”, “savior of life”, “grass of immortality” (Fig. 4)[18].

Fig. 4. ***Sage leaves***

It is a perennial plant that likes heat, is resistant to drought, a height of 20–70 cm. The odor is strong, specific.

Sage comes from Asia Minor and the Mediterranean, which grows on the southern slopes. In our lands it is cultivated in gardens, backyards, flower gardens.

For harvesting, the leaves and tops of the stem are collected during flowering. Raw material is dried in the room with professional ventilation.

The main active substances of the sage are phenolic compounds (in particular, up to 3 % of flavonoids and tannins) and terpenoids. Anthocyanin salvanin, 3,5-diglycoside cyanidine, dolphinidine and pellagonidine, flavonoidal aglycones, gentvquinin, 6-methoxygencquinin, hypsidulin, luteolin, 6-methoxylutheolin, circioliol, etc. were isolated from the above-ground part, and only glycosides were cinarozid[1920].

Out of some Central Asian species of sage, petolinarin, acetylpectolinarin, as well as apigenin, luteolin, chrysoeryol and their glycosides (cosmoses,

[18] Юрчак Л.Л. Культура шавлії мускатної у лісостепах України. К.: Знання, 1997. 367 с.

[19] Прибилова Г. Ф. Хімічне вивчення деяких видів шавлії. *Біологія та фармація*. 2010. Т. 5, No 5. С. 49–52.

[20] Cuvelier M. E., Richard H., Berset C. Antioxidative activity and phenolic composition of pilot-plant and commercial extracts of sage and rosemary. J. Amer. Oil Chem. Soc. 2008. Vol. 73. P. 645–652.

cyanoside, thermosensor) were isolated. The presence of phenolcarboxylic acids is proved: caffeic, chlorogenic, neoclorogenic, cryptochlorogenic. There are many bicyclic monoterpenoids in the essential oil (1,5–2,5%), namely: tunion (22–37%), cineol (8-15%) and camphor (in some species up to 30%), there is also borneol (5–8%), bornil acetate, β-pinene and camphene. In addition, the leaves contain di- and triterpenoids[21].

One of the first ones was bicyclic diterp[22]enoid sclareol, later - carnosol and cleavage. The bitterness of the raw material is given by diterpen of picrosalvin[2324].

– Vitamin B1 (thiamine) regulating the entire human nervous system and metabolism;

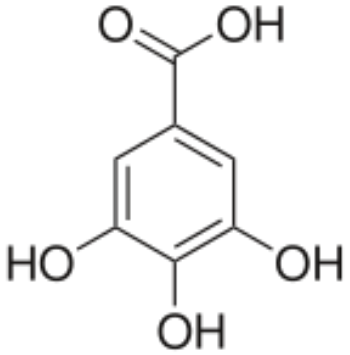
– Vitamin P (flavonoids), which strengthens the walls of capillaries and blood vessels and reduces their permeability;

– Vitamin C (ascorbic acid), which increases the resistance of the body to pathogenic microorganisms;

– Vitamin PP (nicotinic acid) involved in the development of energy for the biological processes in the body;

– Tannins with hemostatic and anti-inflammatory actions. Tannins are used as an antidote.

Terpenoids



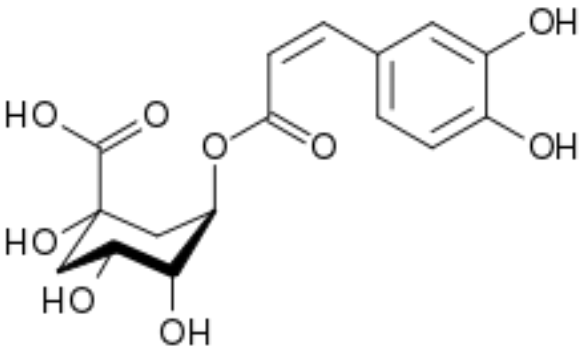
[21] Прокопенко С. В. Хімічні дослідження видів роду шавлія. *Фармація.* 2009. No 6. С. 48.

[22] Tada M., Okuno K., Chiba K., [et al.] Antiviral diterpenes from Salvia officinalis. Phytochem. 1994. No 35. P. 539–541.

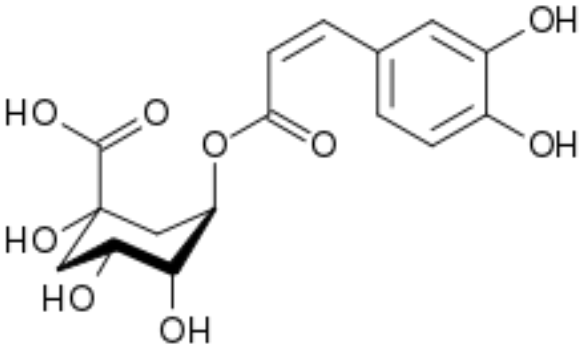
[23] Simić D., Vuković-Gačić B., Knežević-Vukčević J. [et al.] Antimutagenic effect of terpenoids from sage (Salvia of cinalis L.). J. Environ. Path. Tox. Oncol. 2007. No 16 (4). P. 293–301.

[24] Lu Y., Foo L. Y. Flavonoid and phenolic glycosides from Salvia officinalis. *Phytochem*. 2010. No 55. P. 263–267.

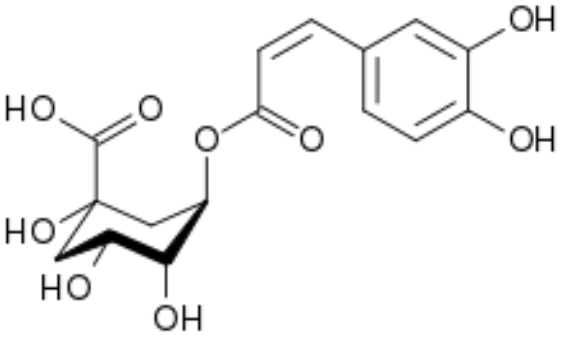
Chlorogenic acid



Cyneroside



Tannin



The plant has an expectorant effect, so sage is part of the herbal species for the treatment of bronchitis. It possesses diuretic properties to treat kidney disease. Anti-inflammatory properties of sage help to treat angina, inflammation of the gums and inflammation on the skin.

Antimicrobial and antifungal properties are successfully used for the treatment of skin diseases. Sage has a beneficial effect on the work of the brain. It helps preserve the clarity of mental processes and improve memory[25].

[25] Hooriuchi K., Shiota S. [et al.] Potentiation of antimicrobial activity of aminoglycosides by carnosol from salvia of cinalis. Biol. Pharm. Bull. 2007. No 30. P. 287–290.

Therefore, using the herb is good for people who have devoted their lives to creativity. It will not be superfluous to have sage for children, who have a high mental load in connection with school education.

In phytotherapy and folk medicine, the sage is a part both of the internal (infusions, decoctions, tinctures, teas, pills, lozenges, syrups, and soaps), and external (concentrates for lotions, baths, ointments, gels, creams, gynecological phytotepanes, shampoos) dosage forms[26].

Domestic scientists conduct experimental research on the expediency of using the extract of sage in suppositories with antiseptic and anti-inflammatory action, in particular in the treatment of hyperplasia of the prostate gland, and phyto-tampoons in gynecological diseases. The composition of the leaves of sage includes salvinium – a natural antibiotic – which is able to stop the development of staphylococci, to get rid of boils and to remove inflammation of the lymph nodes.

Aqueous extracts of sage (infusions, decoctions) are used mainly as antidiabetic, anti-inflammatory, antidiarrheal, general-purpose, antimicrobial, astringent and those that improve digestion and reduce sweating (in tuberculosis, climacteric period). Alcoholic tinctures are more effective as antiseptic, antispasmodic (in diseases of the digestive tract) and in the treatment of diabetes and gynecological diseases. It is proved that sage leaves are useful for the elderly, as it promotes memory improvement, strengthening the nervous system, and decreasing the tremor of the hands[27].

For this purpose, tincture and infusion of sage are widely used in France and Germany.

Angiogenesis (the process of blood vessel formation) plays a key role in the growth and maturation of tumors. It is important for the growth, spread of metastases or invasion into organs and tissues.

Studies in the field of oncology have shown that a certain therapeutic dose of sage is able to suppress the formation of blood vessels in the tumor in the body of the animal and humans.

1. When inflammations of the lungs, bronchus and upper respiratory tract, the decoction of the sage is not prepared not on the water, but on milk (1 tablespoon of sage on 1 cup of milk). But here it is important that the milk should not be hot, but cold, and then it is brought to a boil together with

[26] Болотов Б.В. Травник академика Болотова. Спб.: Питер, 2012. 128 с.

[27] Ніженковська І. В., Цуркан О. О., Седько К. В. Шавлія лікарська – сучасні аспекти застосування (Огляд літератури). *Фітотерапія.* 2014. № 2. С. 58–61.

leaves of sage under the hood, allowed to infuse for 10 minutes and filter. Such decoction should be drunk at night. If the inflammatory processes are associated with the respiratory system, then use sage leaves for steam inhalations. To do this, boil the handful of leaves for 5 minutes on low heat and inhale a couple, covered with a towel.

2. Pour 1 teaspoon of sage leaves with a glass of boiling water, allow standing 5–7 minutes and use it as a tea every day. But every 3 months it is better to make a lunar break. This tea is also soothing removes the fever and brush the blood.

3. To prepare the oil, use the entire aboveground part of the plant – leaves, stems and inflorescences. Leaves and flowers are allowed to dry in a shady room, and in the evening they are stacked in a glass bowl and poured with vegetable oil – the basis. Put the vessel in the dark and warm place for two weeks.

4. During the infusion, the oil must be stirred periodically. After two weeks stop stirring and leave to infuse oil for another 4 weeks. It is important that there are should be no temperature differences in the room: they negatively affect the useful properties of sage. When the oil is sprayed, it is filtered from the smallest impurities of the grass using a gauze fabric, folded up twice – three times.

5. Massage with the use of sage oil helps to get rid of tides during climax and high sweating. Oil can lubricate small wounds, grease and burns due to its antiseptic properties. If you smear with sage, your blood vessels will be strengthened and the level of the content of pathogenic bacteria in the oral cavity will decrease. Properties of sage help to get rid of gingivitis - gum inflammation. Essential oil of sage cannot be prepared at home. It also cannot be used in its pure form.

6. An infusion made from the plant has rejuvenating properties. Courses should be conducted within a month 3 times a year. For preparation take 1 tablespoon of fresh or 2 tablespoons of dry leaves of the plant and pour them with 1 tablespoon of boiling water Put everything on a water bath and hold for 10 minutes. To improve the taste, add 1 teaspoon of honey or a piece of lemon. Drink the infusion by 1 glass on an empty stomach an hour before meal.

7. Many women after pregnancy, severe stress, beriberi, and with other problems complain about the appearance of serious hair problems. Sage can help them. To prepare the healing decoction, take 2 tablespoons of dry

plants and pour them with 300 ml of hot water. Put everything on a small fire and bring to a boil, remove and leave for cooling. Strain and use a decoction for rinsing hair after washing.

In homeopathy, a prescription for preparation oil is used in the treatment of gastrointestinal inflammations, tonsillitis, and bronchitis.

Sage extract is gaining popularity in the Ukrainian pharmaceutical market. Liquid extract of sage leaves is dispensed in vials of 30 ml.

Extract of sage has a powerful antiseptic, anti-inflammatory, bundle, vitro and diuretic action. The drug is prescribed for complex therapy of gastritis with reduced acidity, gastrointestinal ulcers, and biliary tract dysfunction, as well as flatulence, colitis, diabetes mellitus, and urinary tract infections. In addition, the sage extracts are effective in the treatment of diseases of the upper and lower respiratory tract, cow infection, tuberculosis[28].

Externally, the extract is used for angina, pharyngitis, nasal passages over the periosteum, stomatitis, long wounds (including purulent), eczema, burns. The wrap with the use of the drug is used for radiculitis, osteochondrosis, inflammation of the joints, arthrosis, neuritis. Recommendations for use: 5–7 drops per water, 2–3 times daily before meals. If necessary, the dose can be doubled.

Consequently, decoctions and tincture of sage are not recommended for women during pregnancy. The plant promotes estradiol and reduces progesterone. This can cause severe contractions of the uterus, which may lead to miscarriage. Sage increases arterial pressure. Decoction or tea from this plant reduces lactation, which negatively affects such symbiosis as mother and child. In pregnancy, the decoction of sage can be used only externally – to prevent varicose veins.

It should also be noted that sage is dangerous to the health of those who suffer from thyroid function disorders. While regular usage of this plant, it should be considered that every 3 months, a break for at least 20 days should be done, so that the body was able to clear itself of resins and tannins, which are part of the sage.

CONCLUSIONS

Consequently, as a result of the research, it can be concluded that medicinal plants such as white willow and sage are a promising raw material

[28] Седько К. В. Оптимізація методів фармакохімічного дослідження препаратів шавлії. *Укр. наук.-мед. журн.* 2012. № 4. С. 369.

for the development of new drugs for internal and external use. Nevertheless, the existing complex of active ingredients of both plants needs the deep research and study of their interaction, potentiation and cross-effects.

SUMMARY

The purpose of our work was to analyze literary sources about medicinal plant raw materials of willow white and sage farms in order to create new drugs on their basis. It was found that there are about 12 thousand plants that have healing properties and are used in both traditional and folk medicine. In this case, medicinal plants are often combined with other treatment methods. White willow and sage were investigated as the possible sources of active ingredients for the further development of new domestic dosage forms. It was established that both plant show powerful therapeutic potential and a wide range of biologically active substances.

REFERENCES

1. Akao, T., Yoshino, T., Kobashi, K., and Hattori, M. Evaluation of salicin as an antipyretic prodrug that does not cause gastric injury. *Planta Med.* 2002. Vol. 68(8). P. 714–718.

2. Baricevic D. Sosa S., Della Loggia R. Topical anti-in ammatory activity of Salvia of cinalis L. leaves: the relevance of ursolic acid. *J. Ethnopharmaco.,* 2009. No 75 [2–3]. P. 125–132.

3. Bisset N.G. Herbal Drugs and Phytopharmaceuticals. Stuttgart, Germany: Medpharm Scientific Publishers, 2004. P. 534–536.

4. Blumenthal M. The Complete German Commission E Monographs. Austin, TX: American Botanical Council. Boston: Integrative Medicine Communications; 1998. 684 p.

5. Bowles E. J. The Chemistry of Aromatherapeutic Oils. South Australia: Grif n Press, 2008. P. 37, 85, 98, 122, 168.

6. Cuvelier M. E., Richard H., Berset C. Antioxidative activity and phenolic composition of pilot-plant and commercial extracts of sage and rosemary. *J. Amer. Oil Chem. Soc.* 2008. Vol. 73. P. 645–652.

7. Gora J., Lis A. Essential oils from sage. *Aromatherapy.* 2008. No 4 [14]. P. 5–12.

8. Hooriuchi K., Shiota S. [et al.] Potentiation of antimicrobial activity of aminoglycosides by carnosol from salvia of cinalis. *Biol. Pharm. Bull.* 2007. No 30. P. 287–290.

9. Lu Y., Foo L. Y. Flavonoid and phenolic glycosides from Salvia officinalis. *Phytochem*. 2010. No 55. P. 263–267.

10. Simić D., Vuković-Gačić B., Knežević-Vukčević J. [et al.] Antimutagenic effect of terpenoids from sage (Salvia of cinalis L.). *J. Environ. Path. Tox. Oncol*. 2007. No 16 (4). P. 293–301.

11. Tada M., Okuno K., Chiba K., [et al.] Antiviral diterpenes from Salvia officinalis. *Phytochem*. 1994. No 35. P. 539–541.

12. Болотов Б.В. Травник академика Болотова. Спб.: Питер, 2012. 128 с.

13. Мазнев Н.И. Золотая книга лекарственных растений. 15-е изд., доп. М.: ООО «ИД РИПОЛ Классик», ООО Издательство «ДОМ. XXI век», 2008. 621 с.

14. Ніженковська І.В., Цуркан О.О., Седько К.В. Шавлія лікарська – сучасні аспекти застосування (Огляд літератури). *Фітотерапія*. 2014. № 2. С. 58–61.

15. Прибилова Г.Ф. Хімічне вивчення деяких видів шавлії *Біологія та фармація*. 2010. Т. 5, No 5. С. 49–52.

16. Прокопенко С. В. Хімічні дослідження видів роду шавлія. *Фармація*. 2009. No 6. С. 48.

17. Седько К.В. Оптимізація методів фармакохімічного дослідження препаратів шавлії. *Укр. наук.-мед. журн*. 2012. № 4. С. 369.

18. Чухно Т. Большая энциклопедия лекарственных растений. М.: Эксмо, 2007. 1024 с.

19. Юрчак Л.Л. Культура шавлії мускатної у лісостепах України. К.: Знання, 1997. 367 с.

Information about authors:

Postoy V. V.,

PHD student of the Pharmaceutical Technology of Drugs Department of the National University of Pharmacy

4, Valentynska st., Kharkiv, 61002, Ukraine

Mykhailyk D. O.,

Student of the 2nd year, group 1 of the Pharmacy faculty National University of Pharmacy,

member of SSS of the Pharmaceutical Technology of Drugs Department of the National University of Pharmacy

4, Valentynska st., Kharkiv, 61002, Ukraine

CAPSULES: HISTORY AND MODERN DIRECTIONS OF DEVELOPMENT

Semchenko K. V., Bysaga Ye. I.
Scientific supervisor: **prof. Vyshnevska L. I.**

INTRODUCTION

The history of pharmacy includes ages of hard work on the search for medicinal substances, studying and improving the technological methods of preparation of various dosage forms, developing methods for quality control of the obtained products.

To date, according to generalized data from various sources, 82 dosage forms are known[1]:

– Solid: briquettes, inlays, gloves, granules, adsorbing sponges, dragees, dry extracts, capsules, caramels, cachets, lamellae, medical crayons, lozenges, pellets, pearls, pills, powders, poultices, powders, rectocapsules, effervescent salts, salt tablets, tablets pessaries, suppositories;

– Liquid: applications, balms, medical wines, decoctions, aromatic waters, ear drops, gels, glycerols, drops, showers (washings), liquid extracts, elixirs, emulsions, irrigation, enemas, swallows, droplets, lemonade, lintulose, lotions, magmas, soaps, mixtures, drinks, tinctures, infusions, nasal drops, oils, eye drops, eye lotions, pigments, foams, throat rinsing, throat rinses, liquids, solutions, syrups, mucus, alcohols, suspensions, shampoos;

– Semi-solid: bougies, cones, creams, balls, liniments, ointments, ovules, ophthalmic ointments, pastes, patches;

– Parenteral and inhalation: aerosols, injections, inhalations, vapors, sprays.

Capsules deserve special attention among the existing variety of dosage forms, since the encapsulation of drugs remains a popular method of drug administration. From the point of view of the practicality of application in the pharmaceutical compounding, hard gelatin capsules are much more convenient than tablets and powders due to the combination of the

[1] Решение Коллегии Евразийской экономической комиссии от 22 декабря 2015 г. № 172 «Об утверждении номенклатуры лекарственных форм».

possibility of varying the composition of the drug and the accuracy of dosing.

The wide opportunities for prescribing medicinal substances in the form of capsules have led to an increase in their production and consumption. So, abroad, among the doses of industrial production, capsules occupy the 3rd place after tablets and ampouleous solutions.

Capsules exhibit a sufficiently high level of bioavailability and have a number of advantages: good appearance; easiness of usage (easy to swallow); on average, the therapeutic effect of the content is detected after 5–10 minutes after administration; the gelatin shell is impervious to volatile liquids, gases, and air oxygen (which is very important for storing products that are easily oxidized); placement in the shell is convenient for the dispensing of coloring substances and substances that have an unpleasant taste and smell. Also, capsules are very promising for use in pediatrics and gerontology.

Therefore, the purpose of our study is a retrospective analysis of the development of capsules as a dosage form and the study of the latest trends in their development and improvement.

1. Capsules as a dosage form

Capsules (Lat. *capsula* – case, box) is a solid dosage form with a soft or hard shell containing one dose of one or more active ingredients[2].

Depending on the place of action capsules are divided into sublingual, gastrointestinal, intestinal-soluble, rectal, vaginal. A separate group consists of capsules with adjustable speed and completeness of release of the active substance. Modified release is achieved by introducing into the contents or the shell (or in both at the same time) the appropriate excipients that are designed to change the rate or place of active substances release.

Some types of capsules have independent names: tubatine is a special pediatric medicinal form that is soft gelatin capsules with an “elongated neck” designed for young children who cannot swallow the tablets; when the neck is stuck, the child absorbs the contents of the capsules; spansula is a solid gelatin capsule for internal use, containing a mixture of microcapsules (micronutrient) with a fatty shell and the same time release of medicinal

[2] Сайко І.В. Капсули. *Фармацевтична енциклопедія: електрон. наук. фахове вид.* URL: https://www.pharmencyclopedia.com.ua/article/3436/kapsuli (дата звернення 8.10.2018 р.).

substances; meduda is a solid gelatin capsule containing microcapsules with a film coating[3].

The advantages of capsules as a dosage form are[4]:

– precision of dosing – modern equipment ensures high accuracy of filling capsules with a mixture of active substances with minimal losses;

– high performance – depending on the equipment used and the technological process features, it is possible to receive up to 250 thousand capsules per hour;

– high bioavailability – studies conducted by a number of scientists (Eckert, Lindwald, et al.) showed that capsules tend to break down faster than tablets or dragees, and their content is absorbed faster and more easily in the human body due to the lack of compression stage;

– expanding indications for use – in some cases, capsules, as dosage forms, help to detect new types of pharmacological activity that did not manifest at the same doses in other forms (for example, the group of scientists at the scientific laboratory of the Italian company "Pharmagel" found that the thetazempaam tablets in a dose of 20 mg exhibited tranquilizing properties, while the same dose in capsules gives a hypnotic effect and allows it to be used as a soporific);

– high stability – the shell of capsules provides a high enough tightness and isolation of labile components of the content from adverse environmental factors, which often avoids the need to use antioxidants or stabilizers, or reduce their number;

– correcting ability – the shell of capsules also helps to hide the unpleasant smell or taste of medicinal substances;

---

[3] Державна Фармакопея України: в 3 т. / Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів». 2-е вид. *Харків: Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів».* 2014. Т. 1. 1127 с.

Державна Фармакопея України: в 3 т. / Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів». 2-е вид. *Харків: Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів»*. 2014. Т. 2. 724 с.

Державна Фармакопея України: в 3 т. / Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів». 2-е вид. *Харків: Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів»*. 2014. Т. 3. 724 с.

[4] Никитюк В.Г., Шемет Н.А. История, преимущества и современная классификация желатиновых капсул. *Провизор.* 1999. № 2.: електрон. наук. фахове вид. URL: http://www.provisor.com.ua/archive/1999/N4/nikituk.php?part_code=40&art_code=1467 (дата звернення 16.10.2018 р.).

– minimizing the possibility of manufacturing errors – the possibility of applying different colors, as well as labeling, reduces the risk of errors and the replacement of drugs in the production process;

– high aesthetics – it is achieved due to the application of various dyes when receiving capsules shells;

– the possibility of giving to the medicinal products the certain properties – this can be demonstrated the most clearly by the example of the creation of so-called intestinal soluble capsules (resistant to gastric juice, but easily destroyed in the medium of the small intestine), as well as capsule-retard (with prolonged release of the drug), which can be achieved by different technological methods;

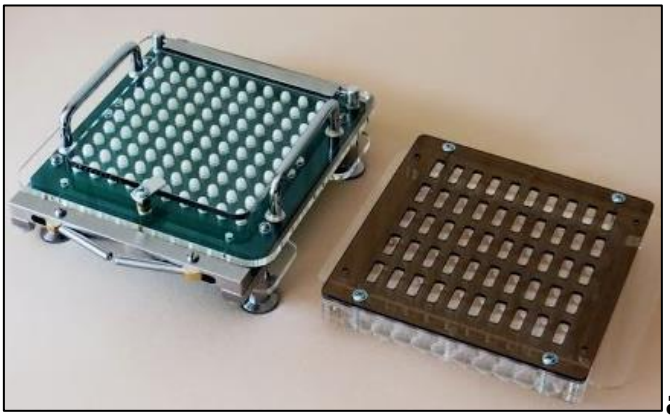
– gentle technological regimes – technological methods of encapsulation allow to avoid undesirable effects of moisture and pressure on many substances.

Nevertheless, capsules have such disadvantages as incompatibility with a number of active substances (highly soluble salts (e.g., iodides, bromides or chlorides), usually are not recommended to dispense in hard gelatin capsules[5]. Their rapid release can cause irritation of the stomach due to the formation of high concentration of the drug in the localized areas[6].

Capsules are divided into hard (Lat. capsulae durae operculatae) and soft (Lat. capsulae molles). The solid capsule shell consists of two pre-fabricated parts, in which the active ingredient (substance) is encapsulated, usually in a solid state. Soft capsules are obtained by placing active substances (usually oleaginous solutions) in the soft, even elastic shell in the process of their production. Solid capsules are actively used in the preparation of extemporal drugs, while soft capsules are mostly produced in industrial conditions. Equipment for the preparation of capsules in pharmacies, small-scale production and in industry is represented in Fig. 1 and 2

[5] Pharmaceutical Compounding and Dispensing. 2nd ed. J. F. Marriott, K. A. Wilson, Ch. A. Langley, D. Belcher. *London: Pharmaceutical Press*, 2010. 288 p.

[6] R.K. Parikh PhD. Thesis. *Pharm. Technology LMCP-AHD.* Chapter 1. 2003. P. 3–36.



a

b

c

Fig. 1. Equipment for the preparation of capsules in pharmacies and small-scale production: a – manual capsule machine for hard capsules; b – machine for small-scale and laboratory production of tablets and capsules; c – capsulator for the production of soft gelatin capsules in small quantities

Fig. 2. Automatic capsule machine NJ-200 by Minipress

Gelatin for the preparation of solid capsules shells is obtained by hydrolysis of collagen derived from the connective tissue, bones, skin and tendons of animals. This high-molecular compound in hydrolysis gives 18 amino acids, among which glycine and alanine predominate. Gelatin may vary in terms of its chemical and physical properties, depending on the source of the collagen and the method of extraction. There are two main types of gelatin. Type A is obtained by acid hydrolysis mainly from pig skin. Type B is obtained by alkaline hydrolysis mainly from animal bones. These two types can be differentiated according to their isoelectric points (4.8–5.0 for type B and 7.0–9.0 for type type A); they also differ in viscosity and film-forming characteristics.

Any type of gelatin can be used, but often the combinations of both types are used to optimize the characteristics of the shell: bone gelatin promotes stability, whereas gelatin from pig skin provides ductility and transparency.

Excipients for the expansion of gelatin are cheap and easily accessible materials that can be used to reduce the quality of gelatin needed for the preparation of capsules and to provide the cheap gelatin with appropriate physical properties.

Attempts to replace gelatin in the production of solid gelatin capsules led to the invention of the subsequent substitutes.

1. Modified gelatin or combined gelatin: crystalline cellulose, high amylose starch (Hylon VII), natural calcium powder, dissolved in sodium metaphosphate, succinic gelatin, gelatin capsule with a surface coating to provide slip, antistatic and printing qualities during production steps. First of all, modified gelatin or combined gelatin is not restored after welding.

2. Substitutes of natural gelatin of vegetable origin are classified into derivatives of cellulose, starches, proteins, etc. They have a certain range of molecular mass, most of them are protected by patents. At one time, the properties of hydroxypropyl starch, whey protein, vegetable protein, and starch gel hydrolyzate with or without plasticizers were studied. Of all other such materials, hydroxypropylmethylcellulose was better for the production of commercially available capsules. However, such shells are much more expensive than conventional hard gelatin capsules, making their use inappropriate from an economic point of view, although studies to improve them are actively conducted in many countries around the world.

3. Various materials, other than those specified in paragraphs 1 and 2, are classified as either natural or synthetic:

а) natural materials (chitosan, agar, alginates and dextran): most of them are used for specific content, but not as a substitute for traditional hard gelatin capsules;

б) synthetic materials (polyvinyl alcohol, polyvinyl ether, polyamide, poly-D-l lactic acid, polyglutamic acid and salt, hydroxyethyl cellulose in

combination with polyvinylpyrrolidone, PEG-poly (1-lysine) block copolymer: these materials cannot replace conventional hard gelatin capsules[7].

The particular attention deserves excipients that are added to the gelatin mass in the preparation of capsules shells. Their main groups are presented in Table 1.

Table 1

Excipients used in the production of gelatin capsules shells

Name	Examples	Purpose
Plasticizers	Glycerin, sorbitol, PEO-400, polypropylene, hexanthropol	Improvement of structural and mechanical properties and provision of appropriate flexibility, increase of strength and reduction of brittleness
Colorants	Eosin, erythrosine, acid red 2C, tropeoline 00, indigotin, indigo, colored sugars (ruberozum, flavorozum, cerulezum), their combinations	Identification of the product and promotion of patient compliance (for example, Buckalew and Coffield in their study found that the four colors were significantly more associated with certain therapeutic groups (white – analgesics, lilac – treatment of states with hallucinogenic effects, orange or yellow – stimulants and antidepressants)
Flavors	Essential oils, essences, ethylvaniline 0.1 %	Provide capsules pleasant smell
Opacifying agents	Titanium dioxide	Make the shell opaque. Opaque capsules can be used for light protection or to hide the contents
Preservatives	Parabens, benzoic acid and sodium benzoate, nipahin	Microbial purity and stability during storage
Water	Hot demineralized purified water	Used when preparing a solution of gelatin. The viscosity of this solution is critical for controlling the thickness of the walls of the capsule
Acid-resistant coatings	Film coatings of cellulose acetophthalate, polyvinyl acetate phthalate, phthalates of dextrin, lactose, mannitol, sorbitol	Prevent the dissolution of capsules in the stomach and obtain the intestinal-soluble form

[7] R.K.Parikh PhD. Thesis. *Pharm. Technology LMCP-AHD*. Chapter 1. 2003. P. 3–36.

To date, the pharmaceutical industry mainly uses sizes of capsule from 000 to 5 (Table 2).

Table 2

Sizes of hard gelatin capsules and the content of the active substances mixture

Capsule size	Volume of the capsule, ml	Capacity, mg Density of powder, g / ml			
		0.6	**0.8**	**1.0**	**1.2**
000	1.37	822	1096	1370	1644
00el	1.02	612	816	1020	1224
00	0.91	546	728	910	1092
0el	0.78	468	624	780	936
0	0.68	408	544	680	816
1	0.50	300	400	500	600
2	0.37	222	296	370	444
3	0.30	180	240	300	360
4	0.21	126	168	210	252
5	0.10	78	104	130	156

To predict the required capsule size, as well as to evaluate and control the powder flow and compressibility, the Carr's index is used[8]:

$$Carr'sindex = \frac{Tapped\ density - Bulk\ density}{Bulk\ density} * 100$$

It is believed that Carr < 15% provides a very good flow, 16 – 26% is good flow, 27 – 35% is sufficient flow and > 35% of the flow properties are considered unsatisfactory.

Soft gelatin capsules may also vary in capacity, although there is no clear standardization, unlike solid capsules. Seam soft capsules can hold up to 7.5 ml. The capacity of the rolls of the machine, by means of which the capsules are formed, filled and sealed, is measured in units called minim. In this case, 1 minim is equal to 0.062 ml on average, and the most pleasant size of the cells of the rolls is from 2 to 80 minim. More spacious capsules (up to 120 minim) are used in the perfume industry.

[8] Carr R.L.: Evaluating flow properties of solids. *Chem Eng*. 1965. Vol. 72. P. 163–168.

Unlike soft seamless capsules that have a strictly spherical shape, the seam capsules may differ in shape and are spherical (round), oblong, oval, in the form of rectal suppositories and tubes. If necessary, soft seam capsules of other forms can be prepared (Fig. 2).

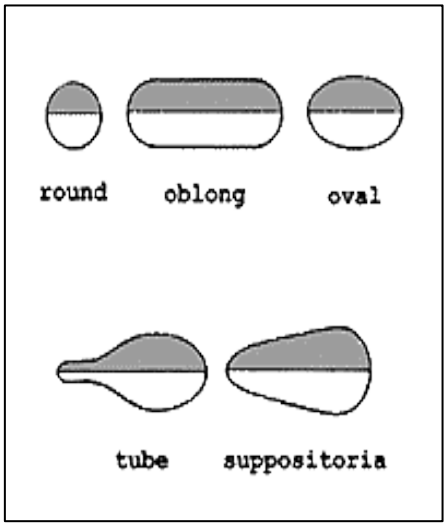


Fig. 2. ***Forms of soft gelatin suppositories***

From the point of view of industrial production, the preparation of capsules is more economical and much faster compared with the production technology of tablet formulations. Thus, the technological process of making capsules involves weighing, preparation of ingredients, mixing, filling capsules (Fig. 3), packaging and labeling, while the process of producing tablets includes significantly more operations: weighing, preparation of ingredients, mixing, granulation, drying, sieving, introduction of substances that improve sliding/mixing/sifting, if necessary, pressing, packaging and marking.

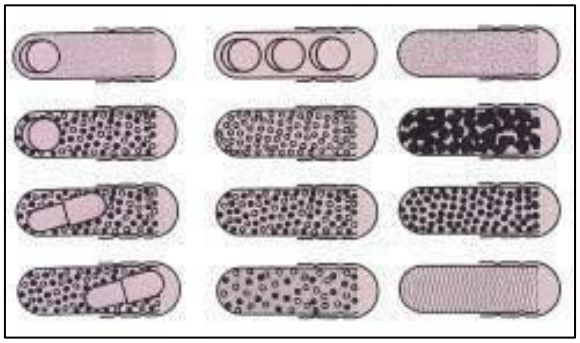


Fig. 3. Possible combinations of filling material for hard gelatin capsules [9]

[9] Бібліотека Capsugel, «Все о твердой капсуле из желатина» (1997 р.)

The quality control of capsules is carried out according to the requirements of the State Pharmacopoeia. In the vast majority of countries, quality control of capsules involves the following basic tests: description, identification, quantification, impurities[10]. Quality control in-process and control of finished products are carried out according to the following parameters: appearance, size and shape, unique identification markers, quantitative content, content of active substances, homogeneity of contents, mass homogeneity, mass variation test, time of disintegration, dissolution time, moisture penetration test, stability[1112].

2. The history of the invention and development of capsules

The first reports of capsules as dosage forms were found in Papyrus Ebers, dated approximately 1500 BC. The following mention was dated 1730, when the Venetian pharmacist de Pauli prepared a captive capsule to "hide" the unpleasant taste of pure terpentine.

In fact, historically, the first hard gelatin capsules were actually designed for liquid drugs. Thanks to the gelatin capsule it was possible to form a new dosage form for liquid balsamic copaivae, which was used during the Napoleonic wars as a cure for vascular diseases. This was very important because the substance causes serious nausea if taken orally.

In 1833, a patent was issued in Paris for pharmacists Mothes and DuBlanc, who used the original method of obtaining gelatin capsules – immersion of leather bags with mercury into gelatin melt. Originally, patent owners sold both empty and filled soft gelatin capsules, but in 1837 the sale of empty capsules shells was stopped. However, the demand for empty capsules led to several attempts to overcome the patented technology of their production, which in turn led to the invention of pills covered with gelatin (in 1838, M. G. Garout received a French patent for the production of a gelatinous pill), and hard gelatin capsule[13].

---

[10] Tangri P., Mamgain P., Shaffi, Verma A.M.L., Lakshmayya. In process quality control: A review. Int. J. of Ind Pharma and Bio Science. 2014.Vol. 1, p. 48–49.

[11] Haleem R.M., Salem M.Y., Fatahallah F.A., Abdelfattah L.E. Quality in the pharmaceutical industry - A literature review. URL: http://www.sciencedirect.com/science/article/pii/S1319016413001114 (дата звернення 8.10.2018 р.)

[12] Md. Sahab Uddin, Abdullah Al Mamun, Mamunur Rashid, Md. Asaduzzaman In-Process and Finished Products Quality Control Tests for Pharmaceutical Capsules According to Pharmacopoeias *British Journal of Pharmaceutical Research*, 2016. Vol. 9(2). P. 1–9.

[13] Dorvault S.L.M. L'officine ou rèpertoire gènèral de pharmacie pratique. *Paris, Vigot Fères*, 1923. 504 p.

The first hard gelatin capsule was invented by J.C. Lehuby, to whom the French patent was issued in 1846. Such a capsule resembled a modern hard gelatin capsule, because it consisted of two telescopic parts, cap and body. According to the Lehuby patent, the capsule shells were made from starch or tapioca with the addition of syrup, although it was subsequently proposed to add carrageenan (1847) and a mixture of carrageenan and gelatin (1850) to the approved patent. The first person who described the gelatin capsule from two parts was J. Murdock, to whom a British patent was issued in 1848, and who is often considered as the inventor of a modern hard gelatin capsule.

In 1872 the French scientist Limousine developed a device for the production and filling of smooth two-section capsules.

In 1874, Hubel from Detroit designed an industrial apparatus for dipping capsules and received capsules in large quantities for the first time. He also proposed a capsule size numbering system: 00 – the largest, 5 – the smallest. Interestingly, in Germany and Austria, the numbering was the opposite[14].

In 1888, John B. Russell patented a method for production gelatinous coatings, which was put on stream by the firm "Park, Davis & Co.", Detroit[15]. In 1895, Arthur Colton, on behalf of the company, patented a machine with a productivity of 6 to 10 thousand products. At the same time, the company produced soft capsules from gelatin film[16].

The industrial filling of solid gelatin capsules was significantly developed in the 1950–1960s, when the speed of the capsule machines allowed producing 20–25 thousand capsules per hour. Active studies to improve the production line have allowed to increase the production of capsules to 250 thousand per hour.

3. Modern directions of development and improvement of capsules

Modern pharmaceutical companies produce capsules in accordance with the requirements of GMP, constantly working on improving both the dosage form itself and its production methods.

The main directions of capsules improvement are related to:

– overcoming the disadvantages of conventional capsules;

– the achievement of a modified release of drugs;

[14] Feldhaus F. M. Zur Gedchichte der Arzneikapsel. *Dtsch. Apoth*. 1954. Ztg. 94 (16), 321 p.

[15] La Wall C. H. 4000 years of pharmacy, an outline history of pharmacy and the allied sciences. J. *B. Lippincott Comp., Philadelphia/London/Montreal,* 1940.

[16] Словарь современного русского литературного языка. М., 1991. Т. 1; *Техническая энциклопедия*. М., 1930.

– encapsulation of various types of material;
– modified application[17].

A study on improving the capsule shell is aimed at:
– improvement of shell properties;
– providing physical opacity;
– moisture protection;
– protection against microbial contamination;
– protection from light and oxygen;
– improvement the compatibility of the filler material with the capsule shell.

Depending on the origin, new capsule shells are divided into animal and non-animal.

Animal capsule shells include capsules from gelatin and PEGs and Coni-Snap® (OceanCaps) capsules (Press-fit® gelatin Capsules, LiCaps®, Posilock, Minicasule, DBcaps® capsules)[18].

Gelatin and PEG capsules reduce the fragility of standard gelatin capsules when exposed to a mixture of substances with a low moisture content, making capsules more compatible with hygroscopic compositions or mixtures of moisture-sensitive ingredients.

OceanCapsTM. These are capsules made of fish gelatin. They contain completely natural sea additives. These shells are used in 40% of dietary supplements in France, Germany and the UK.

Capsules of non-animal origin offer a wide range of gelatin substitutes and are becoming increasingly popular among vegetarians and Asian countries population. Today, such capsules are made of hypromellose (HPMC capsules), polysaccharides (PULLULAN capsules), polyvinyl acetate (capsules PONDAC), starch, cellulose raw materials (VCaps®, offered directly for vegetarians).

Improvement and search of innovations in the system of encapsulation is carried out in directions[19]:

---

[17] Твердые капсулы: история постоянных изменений и усовершенствований / *Фармацевтическая отрасль*. 2013. № 2 (37).

[18] Bhavisha Rabadiya A review: capsule shell material from gelatin to nonanimal original material. *IJPRBS*. 2013. Vol. 2(3). P. 42–71.

[19] Stegemann S. Hard gelatin capsules today – and tomorrow. 2nd ed. // *Capsugel Library*. 2002. 23 p.

– port capsule technology (contains a separator between layers of immediate release of active substances and a layer with a medicinal substance and an energy source, Fig. 4);

– “hydrophilic sandwich” capsule (capsule inside the capsule in which the intercapsular space is filled with a layer of hydrophilic polymer);

– L-OROS® (provides controlled release of non-aqueous liquid ingredients, Fig. 5);

– PULSINCAP (used for pulsatile delivery of medicinal substances; consists of an insoluble body capsule and a soluble capsule cap);

– the newest technology for filling fluid chewable soft capsules;

– INNERCAP technology (Fig. 6; the form consists of a primary emulsion capsule, a pH-coated tablet, a clear, filled capsule and a granule capsule);

– GALACTICLES (consist of a mixture of galactolecetin and one or more other lipids, for example, mono-, di- and/or triglycerides, a novel delivery system for more oral bioavailability).

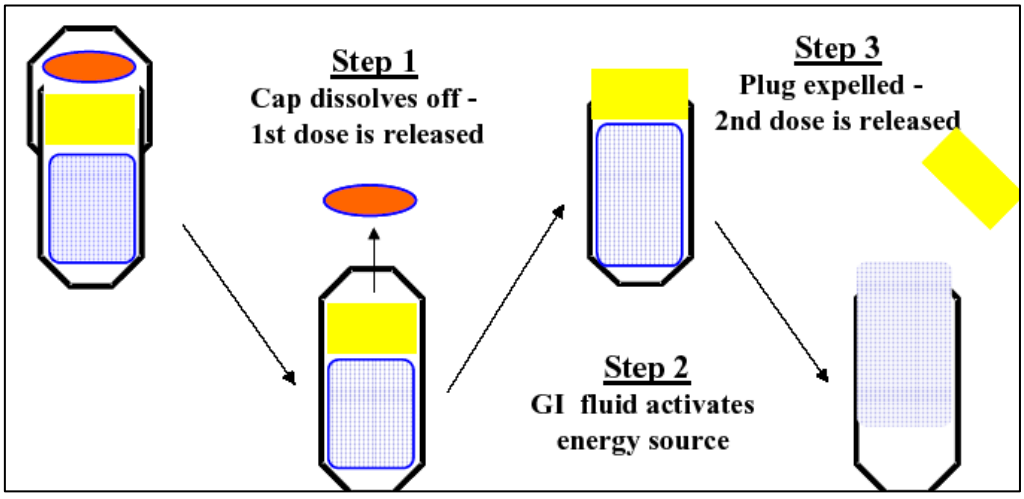
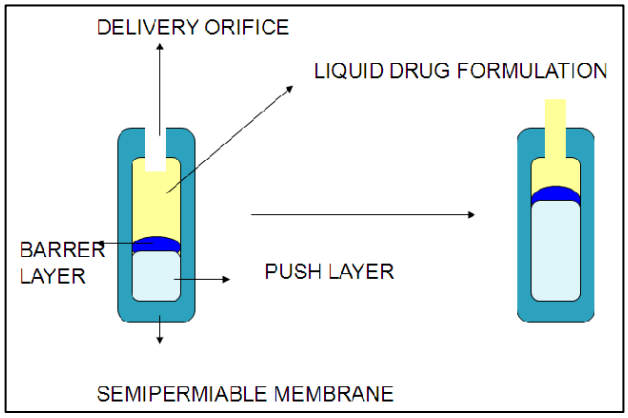
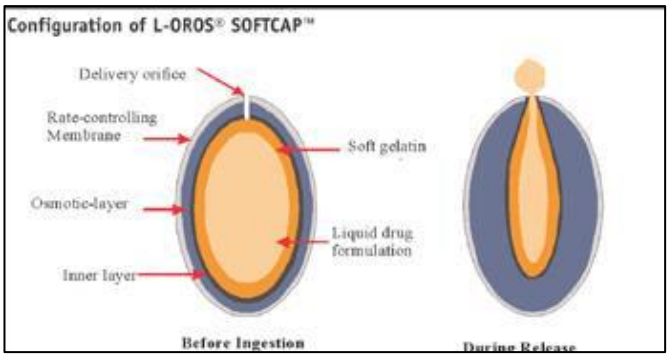


Fig. 4. Mechanism of active substances release from PORT capsules



a



b

Fig. 5. Mechanism of active substances release from L-OROS hard (a) and soft (b) capsules

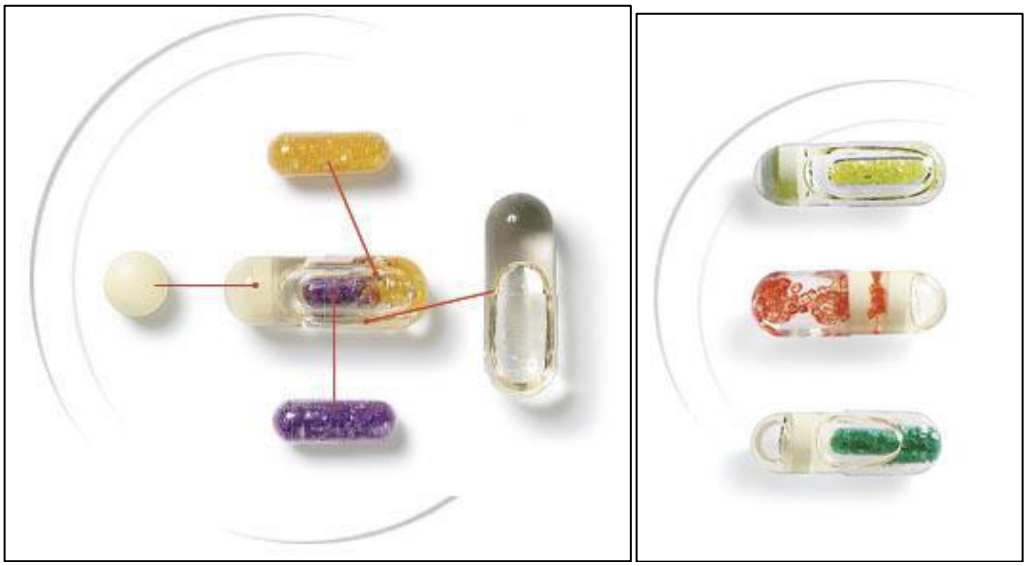


Fig. 6. Capsules obtained by INNERCAP technology

Special attention is paid to the latest technology of making soft gelatin capsules, offered by Banner's VersetrolTM Technology. The drug is introduced into a lipophilic or hydrophilic matrix, which is placed in the soft shell of the gelatin capsule. The technology is universal, since depending on the physical and chemical properties of the drug, an emulsion or suspension can be developed. Applying a combination of lipophilic and hydrophilic matrices, it is possible to achieve the desired release profile[20] (Fig. 7).

	1st Generation	2nd Generation	3rd Generation	4th Generation	5th Generation
Structure					
Concept	2 layers capsules Development of encapsulator Reduction of shell ratio	3 layers capsules Encapsulate hydrophilic substances Mass production	3 layers capsules Development of acid resistance shell Application to ethical drugs	4 layers capsules Control of release	Development of Biocapsule Incubation and cultivation in capsule
Development of function	Improve stability of content Encapsulate liquid	Mixture with products Burst impact	DDS Improvement of compliance	Deepen DDS Functional Development of 4 layers capsules	Semipermeable membrane Biotechnology
Shell Function	Solubility	Freezing resistance Heat resistance	Acid resistance Control of release	Solution in mouth + Solution in stomach Control of release	Semipermeable membrane
Content	Flavor Functional oil	Hydrophilic flavor Fruit juice exstract	Hydrophilic substance Bifidus powder	Functional oil	Lactobacillus Yeast DNA. cell
Application	Chewing gum Health food Tooth paste Instant noodle	Crystal Dew Capsule JINTAN Ice cream	Bifina Constipation OTC Solmiran	Twin clean Plum Freshener	Biotechnology

Fig. 7. Generations of capsules made by Banner's VersetrolTM Technology

CONCLUSIONS

The retrospective analysis of the development of capsules as a dosage form made it possible to trace the main milestones of their development to the present form of hard and soft capsules. Thus, it has been established that

[20] *PharmaBioWorld*, October 2009, Vol. 8, Is. 3, p. 71–77.

capsules are one of the few medical forms that have been preserved since ancient times.

The development of capsules in their present form dates back to 1833 in France. It was exactly this technology that became the basis for the development of capsules preparation both in pharmaceutical and industrial conditions.

The study of the technological aspects of the capsules preparation has shown that this dosage form has significant advantages over many other dosage forms, especially for oral administration, which suggests the expedient development of new drugs in the form of capsules. Uniform requirements for quality control in-process and quality control of the finished product allow the production of high quality medicinal products.

To date, active research is being conducted on the improvement and innovation of capsules in four main areas: overcoming the disadvantages of conventional capsules; achievement of modified release of drugs; encapsulation of various types of active substances; modified application.

Interestingly, the latest capsule production technologies have allowed using some of the former gelatin substitutes formerly rejected due to the high cost of capsule production, poorer stability, etc. (for example, cellulose and starch derivatives).

Thus, the results of the conducted retrospective and bibliographic analysis allow us to conclude that the development of new domestic drugs in the form of capsules for the needs of the population of Ukraine in modern domestic medicinal products according to the import substitution program is urgent and rational.

SUMMARY

Capsules are one of the most commonly used dosage forms. It has gone a long way in improving both the form itself and the way of its obtaining. The presented retrospective analysis allowed tracing the main stages of development of capsules from the first patent to the present days. The main technological aspects of capsules preparation are given. The peculiarities excipients used in technology of capsule shells are described. The newest directions of innovation and improvement of the composition of the capsule shell and the development of new technologies for their production are described. The findings obtained allowed to conclude the especial prospects of capsules usage in developing of new drugs.

REFERENCES

1. Bhavisha Rabadiya A review: capsule shell material from gelatin to nonanimal original material. *IJPRBS*. 2013. Vol. 2(3). P. 42–71.
2. Carr R.L.: Evaluating flow properties of solids. *Chem Engg*. 1965. Vol. 72. P. 163–168.
3. Dorvault S. L. M. L'officine ou rèpertoire gènèral de pharmacie pratique. *Paris, Vigot Fères*, 1923. 504 p.
4. Feldhaus F. M. Zur Gedchichte der Arzneikapsel. *Dtsch. Apoth*. 1954. Ztg. 94 (16), 321 p.
5. Haleem R.M., Salem M.Y., Fatahallah F.A., Abdelfattah L.E. Quality in the pharmaceutical industry – A literature review. Available: http://www.sciencedirect.com/science/article/pii/S1319016413001114 (дата звернення 8.10.2018 р.)
6. La Wall C. H. 4000 years of pharmacy, an outline history of pharmacy and the allied sciences. J. *B. Lippincott Comp., Philadelphia/London/Montreal*, 1940.
7. Md. Sahab Uddin, Abdullah Al Mamun, Mamunur Rashid, Md. Asaduzzaman In-Process and Finished Products Quality Control Tests for Pharmaceutical Capsules According to Pharmacopoeias *British Journal of Pharmaceutical Research*, 2016. Vol. 9(2). P. 1–9.
8. *PharmaBioWorld*, October 2009, Vol. 8, Is. 3, p. 71–77.
9. Pharmaceutical Compounding and Dispensing. 2nd ed. J. F. Marriott, K. A. Wilson, Ch. A .Langley, D. Belcher. *London: Pharmaceutical Press*, 2010. 288 p.
10. R.K. Parikh PhD. Thesis. *Pharm. Technology LMCP-AHD*. Chapter 1. 2003. P. 3–36.
11. Stegemann S. Hard gelatin capsules today – and tomorrow. 2nd ed. *Capsugel Library*. 2002. 23 p.
12. Tangri P., Mamgain P., Shaffi, Verma A.M.L., Lakshmayya. In process quality control: A review. *Int. J. of Ind Pharma and Bio Science*. 2014.Vol. 1, p. 48–49.
13. Державна Фармакопея України: в 3 т. / Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів». 2-е вид. *Харків: Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів».* 2014. Т. 1. 1127 с.

14. Державна Фармакопея України: в 3 т. / Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів». – 2-е вид. *Харків: Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів»*. 2014. Т. 2. 724 с.

15. Державна Фармакопея України: в 3 т. / Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів». 2-е вид. *Харків: Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів»*. 2014. Т. 3. 724 с.

16. Никитюк В. Г., Шемет Н. А. История, преимущества и современная классификация желатиновых капсул. *Провизор.* 1999. № 2.: електрон. наук. фахове вид. URL: http://www.provisor.com.ua/archive/1999/N4/nikituk.php?part_code=40&art_code=1467 (дата звернення 16.10.2018 р.).

17. Решение Коллегии Евразийской экономической комиссии от 22 декабря 2015 г. № 172 «Об утверждении номенклатуры лекарственных форм».

18. Сайко І.В. Капсули. *Фармацевтична енциклопедія: електорн. наук. фахове вид.* URL: https://www.pharmencyclopedia.com.ua/article/3436/kapsuli (дата звернення 8.10.2018 р.).

19. Словарь современного русского литературного языка. М., 1991. Т. 1; *Техническая энциклопедия*. М., 1930.

20. Твердые капсулы: история постоянных изменений и усовершенствований. *Фармацевтическая отрасль*. 2013. № 2 (37).

Information about authors:
Semchneko K. V.,
Candidate of Pharmaceutical Sciences,
Associate Professor of the Pharmaceutical Technology of Drugs Department
of the National University of Pharmacy
4, Valentynska st., Kharkiv, 61002, Ukraine
Bysaga Ye. I.,
Candidate of Pharmaceutical Sciences,
Associate Professor of the Department of Pharmaceutical Disciplines
of Uzhhorod NationalUniversity,
1, Naridna st, Uzhhorod, 88000, Ukraine

SURGICAL TREATMENT BY THE MENINGIOMS OF THE BACK CRANIAL FOSSA OF PARATRUNCAL LOCALIZATION WITH THE USE OF A COMBINED METHOD

Chmut V. O., Lakhtin P. V.

INTRODUCTION

Paratruncal meningioms make up 15–20% of all neoplasms of the cranial cavity[1]. A high percentage indicates the need to find ways to solve this problem - the early diagnosis and surgical treatment of posterior fossa meningioms. Topographic and anatomical location of paratruncal meningiomas often complicates their early diagnosis[2]. In the clinical picture at an early stage of the disease, mild and slowly increasing focal symptoms are noted, which also complicates the early diagnosis and treatment of this pathology[3]. The use of modern methods of neuroimaging diagnostics: magnetic resonance imaging (MRI), magnetic resonance angiography (MRA), spiral computed tomography (CT), computerized control and analysis of the functional activity of the brain stem using the DX-NT32

[1] Practical neurosurgery / B.V. Gaidar, V.A. Khilko, V.E. Parfenov and others: / ed. B. V. Gaidar. SPb. Hippocrates, 2002. 648 p.; Gusev E.I. Neurology and neurosurgery. T. 2. Neurosurgery / E.I. Gusev, A.N. Konovalov, V.I. Skvortsova: ed. A.N. Konovalova, A.V. Kozlova. M.: GEOTAR-Media, 2015. 408 p.; Outcome of the elderly patients undergoing intracranial meningioma resection – a systematic review and meta-analysis / M.T. Poon, L.H. Fung, J.K. Pu, G.K. Leung // Br. J. Neurosurg. 2014. Vol. 28, No. 3. P. 303–309; Hallinan J.T. Dilemmas and diagnostic conditions in meningioma / J.T. Hallinan, A.N. Hegde, W.E. Lim. *Clin. Radiol*. 2013. Vol. 68, No. 8. P. 837–844; International Society of Neuropathology-Haarlem consensus guidelines for grading / D.D. Louis, A. Perry, P. Burger. et.al P. Wesseling. *Brain Parhology*. 2014. Vol. 24, No. 5. P. 429–435.

[2] Practical neurosurgery / B.V. Gaidar, V.A. Khilko, V.E. Parfenov and others: / ed. B. V. Gaidar. SPb. Hippocrates, 2002. 648 p., Anatomical and topographic features of the substrates of supratentorial petroclavic meninges. M.I. Shamayev, R.M. Trosh, D.M. Tsyurup. *Clinical Surgery*. 2012. № 1. P. 49–51; Operational approaches in surgery of the skull and brain. B.V. Gaidar, V.E. Parfenov, D.A. Gulyaev, etc. *Bulletin of the Russian Military Medical Academy*. 2011. № 2 (34). pp. 210–213; Meningiomas: knowledge base, the outcome, and uncertainties / L. Rogers, I. Barani, M. Chamberlain. et.al. *J. Neurosurg*. 2015. Vol. 122, No. 1. P. 4–23.

[3] Trofimova T.N. Radiodiagnostics and Therapy of Head and Neck Diseases: National Guidelines / T.N. Trofimova. M.: GEOTAR-Media, 2013. 888 p.; Evaluation of the effectiveness of surgical treatment of patients with epidermoid tumors of the posterior cranial fossa / R.M. Trosh, A.N. Lisyany, V.V. Gudkov, P.M. Onishchenko. *Ukrainian Neurosurgical Journal.* 2000. № 2. p. 60–64., Hallinan J.T. Dilemmas and diagnostic conditions in meningioma / J.T. Hallinan, A.N. Hegde, W.E. Lim. *Clin. Radiol.* 2013. Vol. 68, No. 8. P. 837–844.

system, greatly facilitates diagnosis in the early stages of development of paratruncal meningiomas of the posterior cranial fossa with the establishment of the exact localization of the tumor, size, density and degree of vascularization[4].

The presence of a tight connection between a tumor capsule and a pre-underlying brain stem often forces subtotal removal. Radical removal of a tumor is often impossible because of its close proximity to the vital parts of the brain and adhesion to neurovascular structures[5].

Endoscopic assistance with simultaneous removal of the meningioma of the posterior cranial fossa and vascular decompression of the trigeminal nerve root / V.N. Shimansky, V.V. Karnaukhov, T.A. Sergienko. *Questions of neurosurgery*. 2014. № 4. P. 70–81; Practical neurosurgery / B.V. Gaidar, V.A. Khilko, V.E. Parfenov and others: / ed. B. V. Gaidar. SPb. Hippocrates, 2002. 648 p., Anatomical and topographic features of the substrates of supratentorial petroclavic meninges M.I. Shamayev, R.M. Trosh, D.M. Tsyurup. *Clinical Surgery*. 2012. № 1. P. 49–51., Koval G. Yu. Features of radial manifestations of meningiomas / G.Yu. Koval, S.A. Grabovetsky Radial diagnostics. Radiation therapy. 2010. № 3–4. p. 9–16; Dikan I.M. Grid-technology: radial diagnostics. I. M. Dikan, B. A. Tarasyuk, S. B. Sinyuta. *Radiology, radiation therapy*. 2013. № 2–3. pp. 57–60; Trofimova T.N. Radiodiagnostics and Therapy of Head and Neck Diseases: National Guidelines / T.N. Trofimova. M.: GEOTAR-Media, 2013. 888 p., Operational approaches in surgery of the skull and brain. B.V. Gaidar, V.E. Parfenov, D.A. Gulyaev, etc. *Bulletin of the Russian Military Medical Academy*. 2011. № 2 (34). pp. 210–213., Evaluation of the effectiveness of surgical treatment of patients with epidermoid tumors of the posterior cranial fossa / R.M. Trosh, A.N. Lisyany, V.V. Gudkov, P.M. Onishchenko. *Ukrainian Neurosurgical Journal*. 2000. № 2. p. 60–64., Gusev E.I. Neurology and neurosurgery. T. 2. Neurosurgery / E.I. Gusev, A.N. Konovalov, V.I. Skvortsova: ed. A.N. Konovalova, A.V. Kozlova. M.: GEOTAR-Media, 2015. 408 p.; Vranic A. New insights into meningioma: from genetics to trials / A. Vranic, M. Peyre, M. Kalamarides. *Curr. Opin. Oncol*. 2012. Vol. 24, No. 6. P. 660–665; Positron emission tomography imaging of meningioma in clinical practice: review of literature and future directions / J.F. Cornelius, K.J. Langen, G. Stoffels. et.al. *Neurosurgery*. 2012. Vol. 70, No. 4. P. 1033–1041; Outcome of the elderly patients undergoing intracranial meningioma resection – a systematic review and meta-analysis / M.T. Poon, L.H. Fung, J.K. Pu, G.K. Leung. *Br. J. Neurosurg*. 2014. Vol. 28, No. 3. P. 303–309; Hallinan J.T. Dilemmas and diagnostic conditions in meningioma / J.T. Hallinan, A.N. Hegde, W.E. Lim. *Clin. Radiol*. 2013. Vol. 68, No. 8. P. 837–844; Experimental and Clinical Medicine: Modern methods of Investigations in early diagnostics and treatment of paratruncal meningiomas/ V.I. Sipituy, S.A. Pagalov, I.A. Kytovoi, V.A. Chmut, V.N. Kycun, O.A. Storchak, A.V. Genkin .2016, № 4(73), P.120

[5] Evaluation of the effectiveness of surgical treatment of patients with epidermoid tumors of the posterior cranial fossa / R.M. Trosh, A.N. Lisyany, V.V. Gudkov, P.M. Onishchenko. *Ukrainian Neurosurgical Journal*. 2000. № 2. p. 60–64., Gusev E.I. *Neurology and neurosurgery*. T. 2. Neurosurgery / E.I. Gusev, A.N. Konovalov, V.I. Skvortsova: ed. A.N. Konovalova, A.V. Kozlova. M.: GEOTAR-Media, 2015. 408 p., Vranic A. New insights into meningioma: from genetics to trials / A. Vranic, M. Peyre, M. Kalamarides. *Curr. Opin. Oncol*. 2012. Vol. 24, No. 6. P. 660–665; Positron emission tomography imaging of meningioma in clinical practice: review of literature and future directions / J.F. Cornelius, K.J. Langen, G. Stoffels. et.al. Neurosurgery. 2012. Vol. 70, No.

In the scientific literature there are enough works confirming the effectiveness of endoscopy in surgery of tumors of the posterior cranial fossa[6]. The main advantage of this method is the possibility of revision of the "blind" zones for the microscope. Unfortunately, the comparison of the results of treatment of patients with and without endoscopic assistance was carried out only in isolated cases. This circumstance may partly explain the lack of clearly formulated indications for the use of endoscopic assistance, depending on the type of tumor. According to[7], in the [8]group using this

4. P. 1033–1041; Outcome of the elderly patients undergoing intracranial meningioma resection - a systematic review and meta-analysis / M.T. Poon, L.H. Fung, J.K.Pu, G.K. Leung. *Br. J. Neurosurg*. 2014. Vol. 28, No. 3. P. 303–309.

[6] Endoscopic assistance with simultaneous removal of the meningioma of the posterior cranial fossa and vascular decompression of the trigeminal nerve root / V.N. Shimansky, V.V. Karnaukhov, T.A. Sergienko. *Questions of neurosurgery*. 2014. № 4. P. 70–81., Practical neurosurgery / B.V. Gaidar, V.A. Khilko, V.E. Parfenov and others: / ed. B.V. Gaidar. SPb. Hippocrates, 2002. 648 p., Anatomical and topographic features of the substrates of supratentorial petroclavic meninges M.I. Shamayev, R. M. Trosh, D. M. Tsyurup. that i. *Clinical Surgery*. 2012. № 1. P. 49–51., Koval G. Yu. Features of radial manifestations of meningiomas / G. Yu. Koval, S. A. Grabovetsky Radial diagnostics, Radiation therapy. 2010. № 3–4. p. 9–16., Dikan I. M. Grid-technology: radial diagnostics. I.M. Dikan, B.A. Tarasyuk, S.B. Sinyuta. *Radiology, radiation therapy*. 2013. № 2–3. pp. 57–60., Operational approaches in surgery of the skull and brain. B.V. Gaidar, V.E. Parfenov, D.A. Gulyaev, etc. Bulletin of the Russian Military Medical Academy. 2011. № 2 (34). pp. 210–213., Experimental and Clinical Medicine: Modern methods of Investigations in early diagnostics and treatment of paratruncal meningiomas / V.I. Sipituy, S.A. Pagalov, I.A. Kytovoi, V.A. Chmut, V.N. Kycun, O.A. Storchak, A.V. Genkin .2016, № 4(73), P.120.

[7] Hallinan J.T. Dilemmas and diagnostic conditions in meningioma / J.T. Hallinan, A.N. Hegde, W.E. Lim. *Clin. Radiol*. 2013. Vol. 68, No. 8. P. 837–844.

[8] Koval G.Yu. Features of radial manifestations of meningiomas / G.Yu. Koval, S.A. Grabovetsky Radial diagnostics, Radiation therapy. 2010. № 3–4. p. 9–16., Dikan I.M. Grid-technology: radial diagnostics. I.M. Dikan, B.A. Tarasyuk, S.B. Sinyuta. Radiology, radiation therapy. 2013. № 2–3. pp. 57-60; Trofimova T.N. Radiodiagnostics and Therapy of Head and Neck Diseases: National Guidelines. M.: GEOTAR-Media, 2013. 888 p.; Evaluation of the effectiveness of surgical treatment of patients with epidermoid tumors of the posterior cranial fossa / R.M. Trosh, A.N. Lisyany, V.V. Gudkov, P.M. Onishchenko. *Ukrainian Neurosurgical Journal*. 2000. № 2. p. 60–64., Gusev E.I. Neurology and neurosurgery. T.2. Neurosurgery / E.I. Gusev, A.N. Konovalov, V.I. Skvortsova: ed. A.N. Konovalova, A.V. Kozlova. M.: GEOTAR-Media, 2015. 408 p., Vranic A. New insights into meningioma: from genetics to trials / A. Vranic, M. Peyre, M. Kalamarides. Curr. Opin. Oncol. 2012. Vol. 24, No. 6. P. 660–665., Positron emission tomography imaging of meningioma in clinical practice: review of literature and future directions / J.F. Cornelius, K.J. Langen, G. Stoffels. et.al. *Neurosurgery*. 2012. Vol. 70, No. 4. P. 1033–1041; Hallinan J.T. Dilemmas and diagnostic conditions in meningioma / J.T. Hallinan, A.N. Hegde, W.E. Lim. Clin. Radiol. 2013. Vol. 68, No. 8. P. 837–844; International Society of Neuropathology-Haarlem consensus guidelines for grading / D.D. Louis, A. Perry, P. Burger. et.al P. Wesseling. *Brain Parhology*. 2014. Vol. 24, No. 5. P. 429–435; Meningiomas: knowledge base, the outcome, and

method, both the more radical interventions and fewer relapses in the postoperative period were noted[9]. The authors[10] have not obtained a statistically significant difference in the frequency of preservation of the function of the facial nerve. We have worked out an algorithm for endoscopic assistance in removing paratruncal plumes. The deformed brain retains its position throughout the operation and thus creates the space necessary for the inspection. The use of the endoscope made it possible to detect tumor fragments in tanks of the cerebral-cerebellar angle, which were inaccessible for inspection with an operating microscope.

When meningiomas and neuromas with paratruncal growth were removed, the use of endoscopic techniques made it possible to assess the degree of invasion of tumor tissue into the bone structures, to establish the source of bleeding, which often falling out of the microscope "field of view".

The radicality of the carried out operations depended not only on the size of the tumor, its connection with brain structures, but also on the degree to which the formation was incorporated into the surrounding tissues. The use of endoscopic assistance was an effective way to detect the remains of a tumor, and in some cases it was the endoscopic inspection that allowed changing the tactics of the operation due to the increased risk of injury to the cranial nerves with further removal of the tumor.Contradictory literature data served as the basis for a more in-depth study of the results of early diagnosis and surgical treatment of meningiomas and neurinomas of the posterior cranial fossa with paratruncal growth[11].

---

uncertainties / L. Rogers, I. Barani, M. Chamberlain. et.al. *J. Neurosurg.* 2015. Vol. 122, No. 1. P. 4–23; Experimental and Clinical Medicine: Modern methods of Investigations in early diagnostics and treatment of paratruncal meningiomas / V.I. Sipituy, S.A. Pagalov, I.A. Kytovoi, V.A. Chmut, V.N. Kycun, O.A. Storchak, A.V. Genkin .2016, № 4(73), P.120.

[9] Dikan I.M. Grid-technology: radial diagnostics. I.M. Dikan, B.A. Tarasyuk, S.B. Sinyuta. Radiology, radiation therapy. 2013. № 2–3. pp. 57–60.

[10] Dikan I. M. Grid-technology: radial diagnostics. I.M. Dikan, B.A. Tarasyuk, S.B. Sinyuta. Radiology, radiation therapy. 2013. № 2–3. pp. 57–60.

[11]Koval G.Yu. Features of radial manifestations of meningiomas / G.Yu. Koval, S.A. Grabovetsky Radial diagnostics, Radiation therapy. 2010. № 3–4. p. 9–16; Trofimova T.N. Radiodiagnostics and Therapy of Head and Neck Diseases: National Guidelines. M.: GEOTAR-Media, 2013. 888 p.; Evaluation of the effectiveness of surgical treatment of patients with epidermoid tumors of the posterior cranial fossa / R.M. Trosh, A.N. Lisyany, V.V. Gudkov, P.M. Onishchenko. *Ukrainian Neurosurgical Journal.* 2000. № 2. p. 60–64., Gusev EI. Neurology and neurosurgery. T.2. Neurosurgery / E.I. Gusev, A.N. Konovalov, V.I. Skvortsova: ed. A.N. Konovalova, A.V. Kozlova. M.: GEOTAR-Media, 2015. 408 p., Vranic A. New insights into

The purpose of this study was to study the modern methods of examination in the early diagnosis and surgical treatment of paratruncal meningiomas. Expansion of radical operation. Prevention of intraoperative vital complications. Reduced operation time. Reduced mortality. Reducing the degree of disability. Reduction in terms of rehabilitation.

Determination of the effectiveness of the application of a new diagnostic intraoperative method for recording the bioelectric activity of brain stem structures.

MATERIAL AND METHODS

111 cases of surgical interventions for paratruncal meningiomas of the posterior cranial fossa were analyzed under an agreement with the Institute of Neurosurgery named after Acad. A.P. Romodanova NAMS of Ukraine (Kiev).

Of the 111 patients aged 25 to 65 years, there were 35 men, and 76 women. All patients underwent clinical and instrumental examination, including neurological, somatic, neuro-ophthalmological, and encephalographic examination. The diagnosis was clarified using CT, MRI, MRA.

During the operation, a new minimally invasive method of computer control and analysis of the functional activity of the brain stem was used using the DX-NT32 system, which allows to visually assess the brain stem activity, as well as correct the volume and course of the surgical intervention. Computer monitoring was performed before surgery, intraoperatively and after surgery with dynamic observation. The findings on the functional activity of stem structures were based on the data of an encephalogram, represented by fronto-temporal leads, based on the presence

---

meningioma: from genetics to trials / A. Vranic, M. Peyre, M. Kalamarides. *Curr. Opin. Oncol.* 2012. Vol. 24, No. 6. P. 660–665; Positron emission tomography imaging of meningioma in clinical practice: review of literature and future directions / J.F. Cornelius, K.J. Langen, G. Stoffels. et.al. Neurosurgery. 2012. Vol. 70, No. 4. P. 1033–1041., Hallinan J.T. Dilemmas and diagnostic conditions in meningioma / J.T. Hallinan, A.N. Hegde, W.E. Lim. Clin. Radiol. 2013. Vol. 68, No. 8. P. 837–844; International Society of Neuropathology-Haarlem consensus guidelines for grading / D. D. Louis, A. Perry, P. Burger. et.al P. Wesseling. Brain Parhology. 2014. Vol. 24, No. 5. P. 429–435; Meningiomas: knowledge base, the outcome, and uncertainties / L. Rogers, I. Barani, M. Chamberlain. et.al. *J. Neurosurg.* 2015. Vol. 122, No. 1. P. 4–23., Experimental and Clinical Medicine: Modern methods of Investigations in early diagnostics and treatment of paratruncal meningiomas/ V.I. Sipituy, S.A. Pagalov, I.A. Kytovoi, V.A. Chmut, V.N. Kycun, O.A. Storchak, A.V. Genkin .2016, № 4(73), P.120.

of functional fronto-pons-cerebellar and temporal-pons-cerebellar connections.

Analyzed the spectral power, the average frequency and the index of the delta and theta bands, the average EEG frequency in general, the value of the coefficient “slow-fast” temporal shifts of the “slow” waves, determined by cross-correlation analysis, the average coherence levels in four frequency ranges and EEG in whole, as well as the value of the common coherence function.

RESULTS AND ITS DISCUSSION

The total removal of meningiomas with paratruncal growth was made in 39 patients [(35.1 ± 0.5)%], subtotal in 63 [(56.7 ± 0.5)%], partial – in 9 [(8.1 ± 0 ,five)%]. Total postoperative mortality was 7 patients [(6.3 ± 0.4)%]. With a total removal of 39 (35.1 ± 0.5%) patients, the fatal outcome was 4 [(3.6 ± 0.2)%], with Subtotal - 2 [(1.8 ± 0.5)%] patients, with partial – in 1 (0.9 ± 0.5%).

The anatomical integrity of the cranial nerves is preserved in 101 patients [(91 ± 5.4)%]. After the performed operations, good results were observed in 79 cases [(71.2 ± 4.3)%], satisfactory – in 26 [(23.4 ± 1.4)%], unsatisfactory – in 6 [(5.4 ± 0 , 3)%] cases. Functional outcomes were assessed on the Karnofsky scale, out of 111 patients in terms of up to 10 years, 70 [(63.1 ± 3.8)%] returned to the previous type of activity, in 25 [(22.5 ± 5.4)%] – activity partially restricted.

Before the operation, an abnormal slow theta activity was recorded on the EEG, along with alpha and beta waves in all leads, due to the rising influence of the reticular formation of the trunk, with a predominance in the occipital region. In particular, the means of detailed mathematical analysis of EEG make it possible to identify changes in its pattern, even in cases that are difficult for visual expert assessment. In this regard, it was of interest to apply a complex of this kind of EEG analysis methods in patients with verified focal processes in order to determine the optimal set of parameters studied.

In particular, the tumors were characterized both by an increase in the spectral power of the “slow-wave components” in the temporal leads, and by a steady increase in the values of the M / B coefficient, a decrease in the frequency of the delta waves and the presence of a delay in the delta waves in the ipsilateral occipital and parietal leads to 10 ms, the contralateral

frontal-temporal leads – from 15 to 40 ms in relation to the temporal leads on the tumor side. Delays during the occurrence of the delta and theta waves between the leads during meningiomas of the posterior cranial fossa, especially the cerebral-cerebellar angle, could not be detected. Stable high values of the M / B coefficient in the temporal region were recorded, which was clearly seen when mapping this indicator for various functional samples during the study. Attention is drawn to the fact that the peak values of the coherence function in these cases were in the range of "near" beta activity.

During the operation, abnormal high-amplitude slow delta and theta waves was recorded in the fronto-temporal leads on the EEG, as well as irritative, activity (Fig. 1a, b, c).

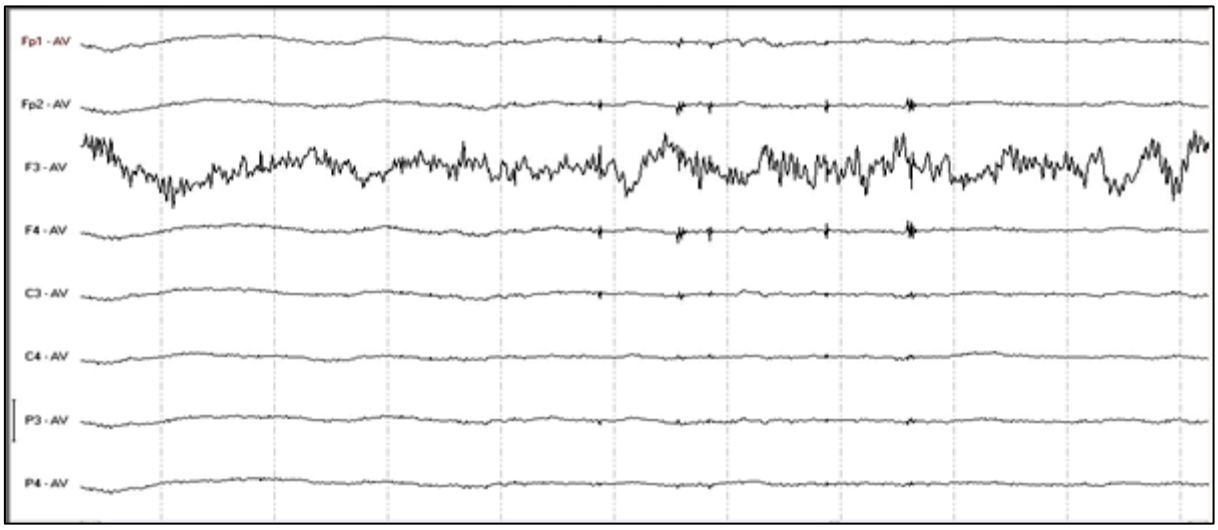


Fig. 1a Dysrhythmia biocurrents of the brain stem I st.

On the subaccorticogram, an acute form of alpha-like activity is recorded, as well as slow-wave theta and delta-like activity.

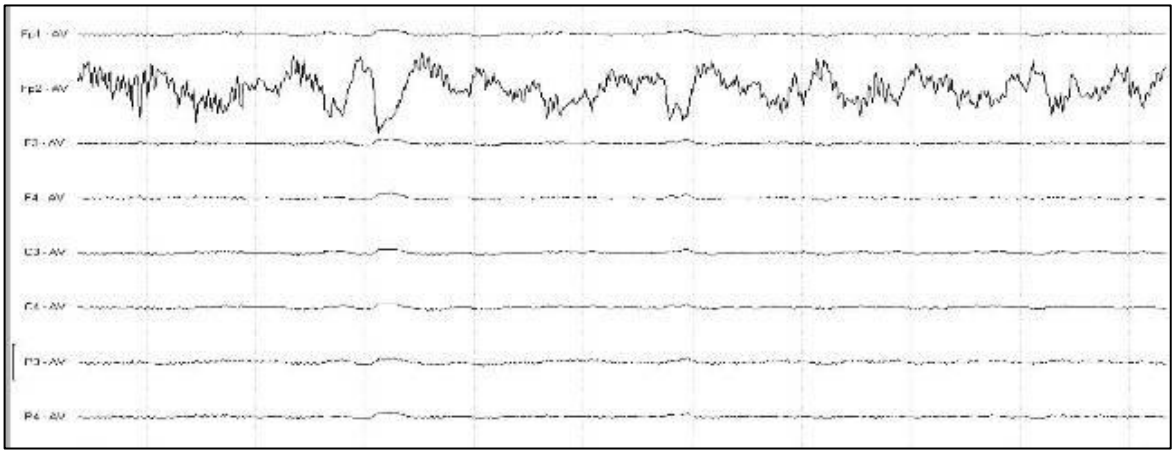


Fig.1b. Second degree dysrhythmia of brain stem biocurrents

An increase in the index and amplitude of the slow theta and delta-like activity is noted on the electro-subcorticogram.

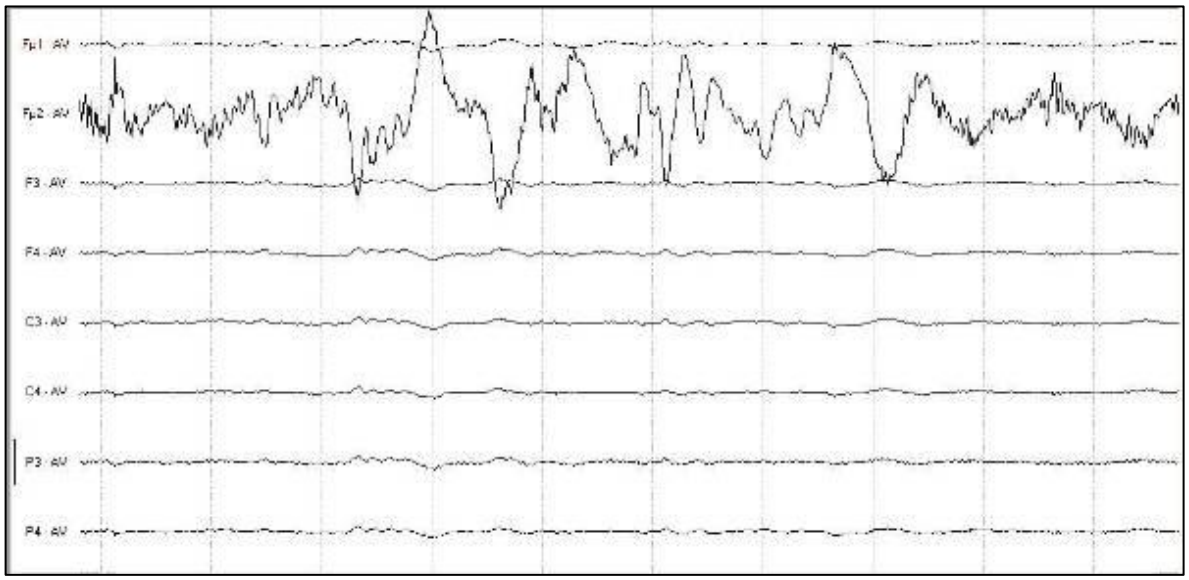


Fig. 1в. The third degree dysrhythmia biocurrents of the brain stem

The electroaccorticogram shows a significant increase in the amplitude of sharp in shape slow delta-like waves.

After the tumor was removed, a clear dynamics was observed on the EEG: the index decreased, the amplitudes of pathological theta and delta waves increased, the index of low-amplitude high-frequency beta activity increased, isolated and alpha-like vibrations were recorded as patterns.

Before the tumor was removed, its exact location, size, density, degree of vascularization, degree of displacement of the brain stem with the help of CT, MRI, MPA were determined.

An example of MRI verification of the ventrolateral paratruncal meningioma of the localization of the posterior cranial fossa is shown in Fig. 2

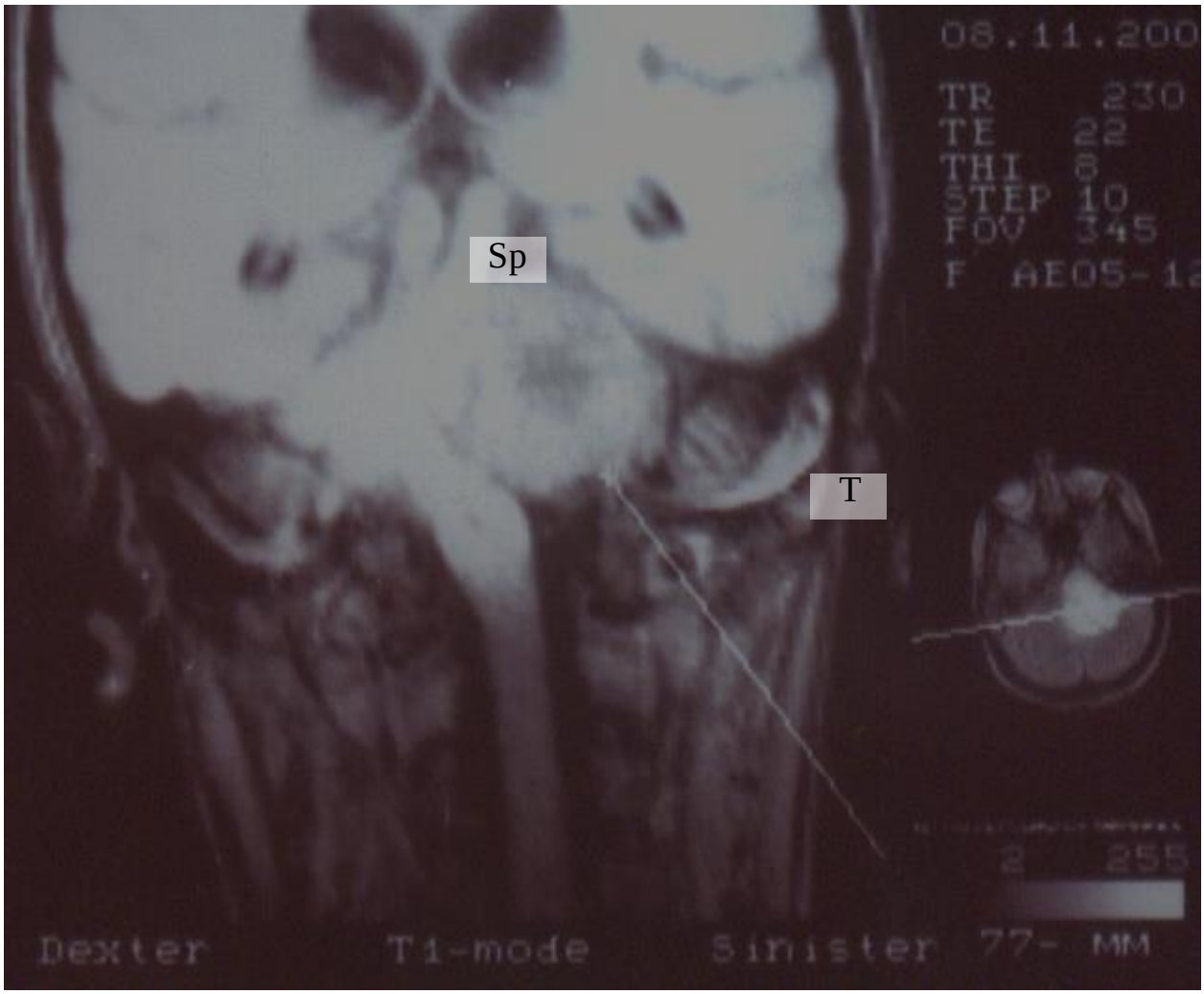


Fig. 2. Ventromedial paratruncal meningioma of the localization of the posterior cranial fossa MRI T1 – WI, T2 – WI sagittal section. T-tumor, Sp-brainstem

MRI in the vascular mode allows to detect the displacement of the main vessels by the tumor, as well as to determine the source and degree of blood supply to the paratruncal tumor, to conduct a differential diagnosis with aneurysm or arteriovenous malformation (Fig. 3. 4).

The interrelationship of the main arterial vessels with the tumor tissue is a defining moment for making a decision about tumor resection. It is also very important to restore venous outflow from the posterior cranial fossa. The permeability of the jugular vein, sigmoid and transverse sinuses and confluence is evaluated. Three degrees of tumor vascularization were identified:

1st minor,

2nd moderate

3rd is abundant.

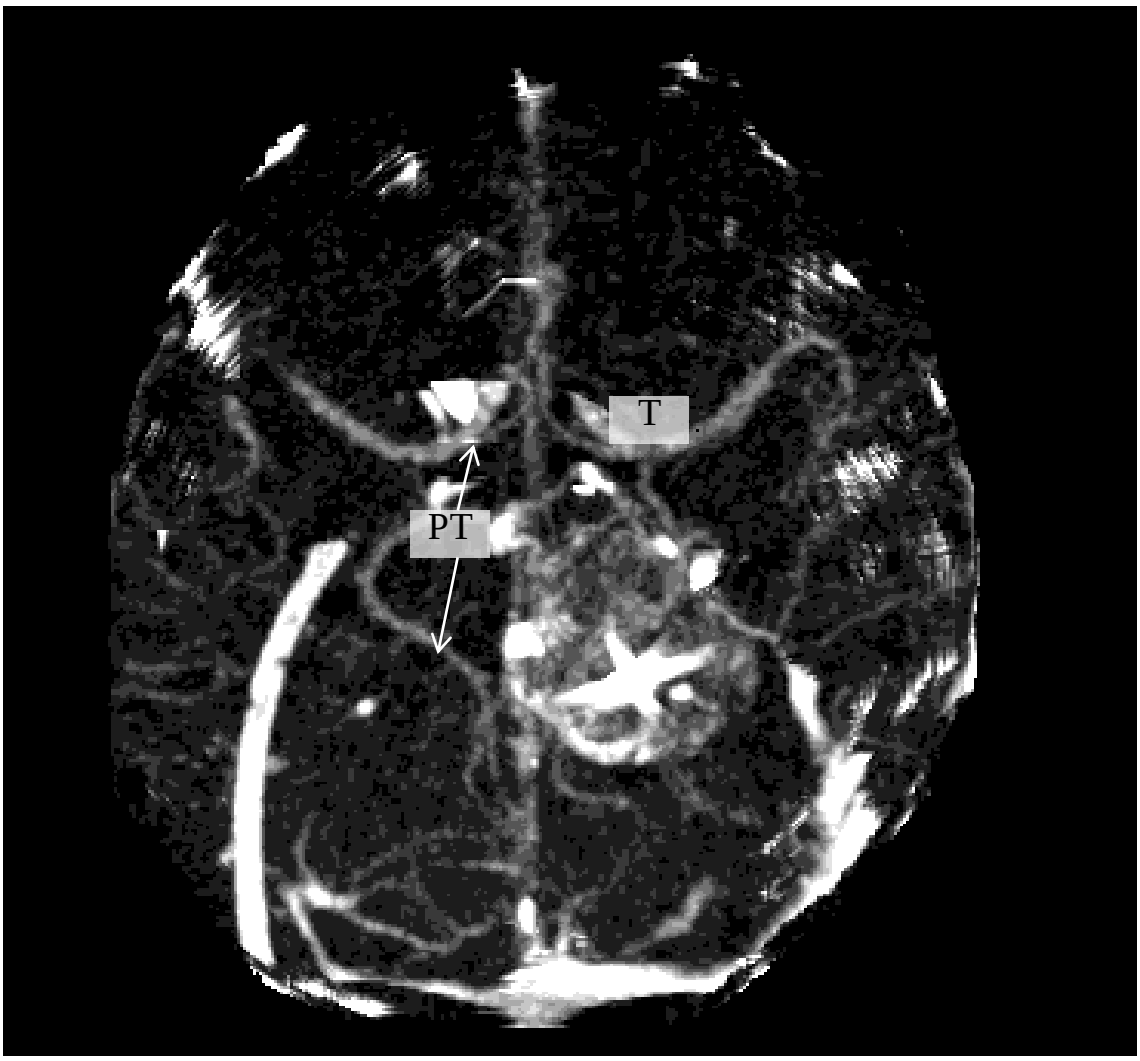


Fig. 3. Ventromedial meningioma of the parastolic localization horizontal section. T – tumor, PT – paratumorosis vessels.

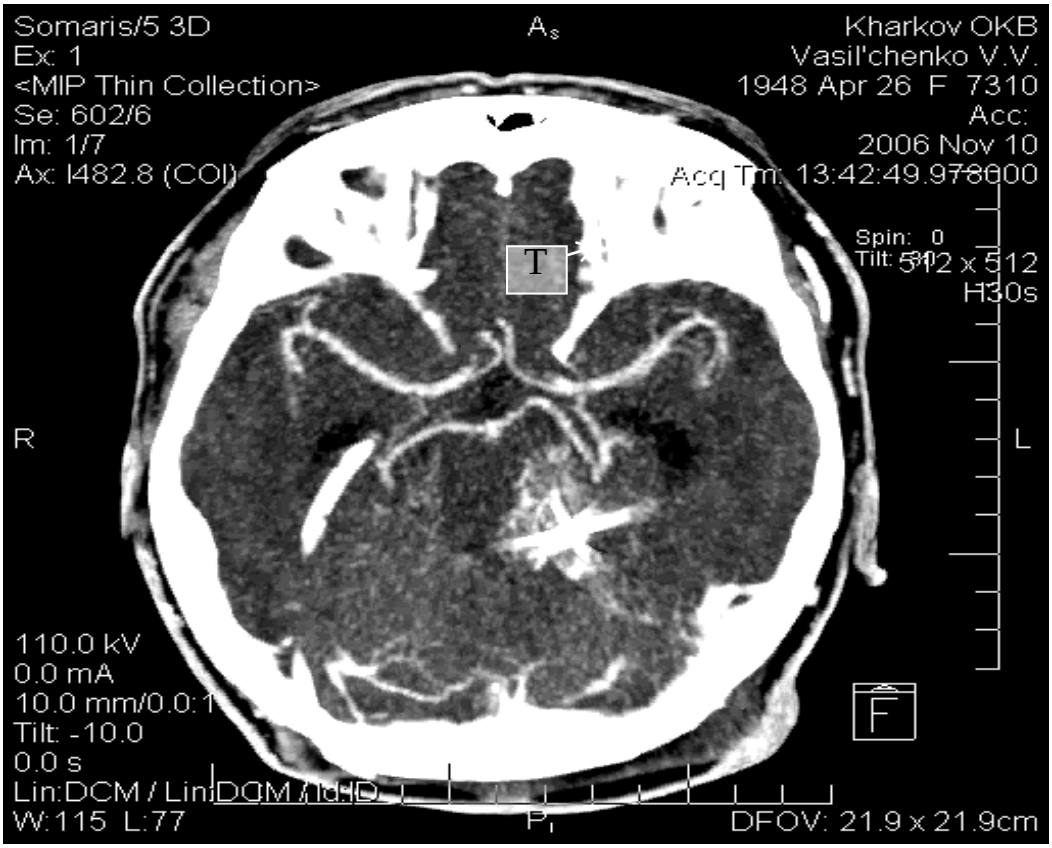


Fig. 4. Ventromedial meningioma of paratruncal localization

MRI angiography in the arterial and venous phase allows determining the degree of compression by the tumor of the jugular vein, sigmoid and transverse sinuses on the affected side, as well as the volume of the contralateral venous drainage, and consequently, assess the likely risk of ligation and resection of these vascular structures.

During vascular diagnosis, intracranial aneurysms or other vascular anomalies can also be detected. Giant aneurysm of the posterior-inferior cerebellar artery can cause the syndrome of the large foramen of the occipital bone and must be taken into account in the differential diagnosis with extracerebral tumors of the posterior cranial fossa.

According to angiography, the tumor is supplied with blood from the anterior and posterior branches of the vertebral artery, the anterior sheath artery, the dorsal sheath artery from the meningo-hypophysial trunk, and the posterior spinal artery. Rarely the blood supply of the tumor was carried out from the pool of the internal carotid artery.

The anterior meningeal branch of the vertebral artery departs from the distal portion of the vertebral artery, passes medially to the entrance to the

vertebral canal, continuing up the medial surface. The artery ends in small branches in the dura mater at the level of the large foramen occipital bone. In normal settings of angiograms, the proximal part of the anterior sheath artery is 1.0–1.5 cm long and 0.5 mm in diameter. Pathological changes significantly increase in size.[12]

Additional data on the relationship between blood vessels, tumors and bone structures are provided by CT-cerebral angiography[13]. These include the following:

– displacement of the vertebral, posterior-inferior cerebellar and anterior spinal arteries;

– an increase in the diameter and size of the anterior meningeal branch of the vertebral artery;

– the presence of a "shadow" of the tumor on the CT angiograms with possible destruction of the bone structures.

The tumor was removed with the use of microsurgery under continuous control of endoscopic video monitoring and intraoperative computer encephalography, which made it possible to expand the radical nature of the operation, reduce its duration, prevent vital intraoperative complications, signaled by the computer encephalographic system DX-NT32.

Depending on the size of the tumor, they were divided into four types: the 1st – small, up to 10 mm, in 20 patients [(18.01 ± 1.1)%]; 2nd – medium size, from 10 to 30 mm, in 35 patients [(31.5 ± 1.9)%], 3rd – large, from 30 to 50 mm, in 40 patients [(36.0 ± 2.2)]%, the 4th – gigantic, 50–60 mm, in 16 patients [(14.4 ± 0.9)%].

Topographic-anatomical location of the tumor, its size, density, degree of invasion and vascularization in all our observations were determined using CT, MRI, MPA. Depending on the data obtained, operational access was selected: I - median bilateral suboccipital, in 92 patients [(82.9 ± 4.9)%], II - unilateral suboccipital craniotomy with tentoriotomy, in 19 patients [(17.1 ± 1, 0)%].

---

[12] Koval G. Yu. Features of radial manifestations of meningiomas / G. Yu. Koval, S. A. Grabovetsky Radial diagnostics, Radiation therapy. 2010. № 3-4. p. 9–16., Dikan I. M. Grid-technology: radial diagnostics. I. M. Dikan, B. A. Tarasyuk, S. B. Sinyuta // Radiology, radiation therapy. 2013. № 2–3. pp. 57-60.

[13] 4. Koval G. Yu. Features of radial manifestations of meningiomas / G. Yu. Koval, S. A. Grabovetsky Radial diagnostics, Radiation therapy. 2010. № 3-4. p. 9–16.

5. Dikan I. M. Grid-technology: radial diagnostics. I. M. Dikan, B. A. Tarasyuk, S. B. Sinyuta // Radiology, radiation therapy. 2013. № 2–3. pp. 57-60.

When selecting meningiomas, topographic-anatomical features and degree of brain stem displacement were taken into account. In 40 patients [(36.0 ± 2.2)%] with large and in 16 patients [(14.4 ± 0.9)%] with giant tumors with severe hydrocephalus, the first stage of the operation was Torkildsen ventriculocystinal anastomosis.

The operation was performed under constant video endoscopic monitoring and initially formed a panoramic, and then sighting, video endoscopic picture[14]. The tumor was visualized, its size, attitude to the cranial nerves were evaluated; the presence of blood vessels welded to the tumor capsule was determined, and the degree of deformation and dislocation of the brainstem was also detected (Figure 5.6).

Fig. 5. Video endoscopic monitoring

[14] Endoscopic assistance with simultaneous removal of the meningioma of the posterior cranial fossa and vascular decompression of the trigeminal nerve root / V.N. Shimansky, V.V. Karnaukhov, T.A. Sergienko. *Questions of neurosurgery*. 2014. № 4. P. 70–81.

After removal of the tumor, occlusion was eliminated, the liquor outflow from the fourth ventricle was restored. The operation ended with plasty of the dura mater with a cryopreserved membrane, bone defect and titanium plate (Fig. 6,7,8).

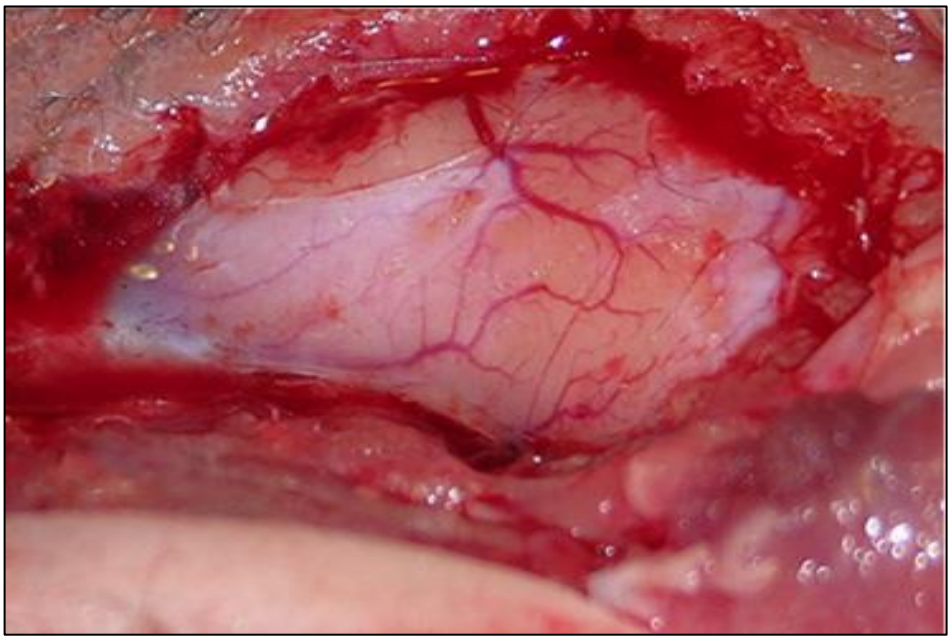


Fig. 6. Bone postoperative defect prepared for plasty

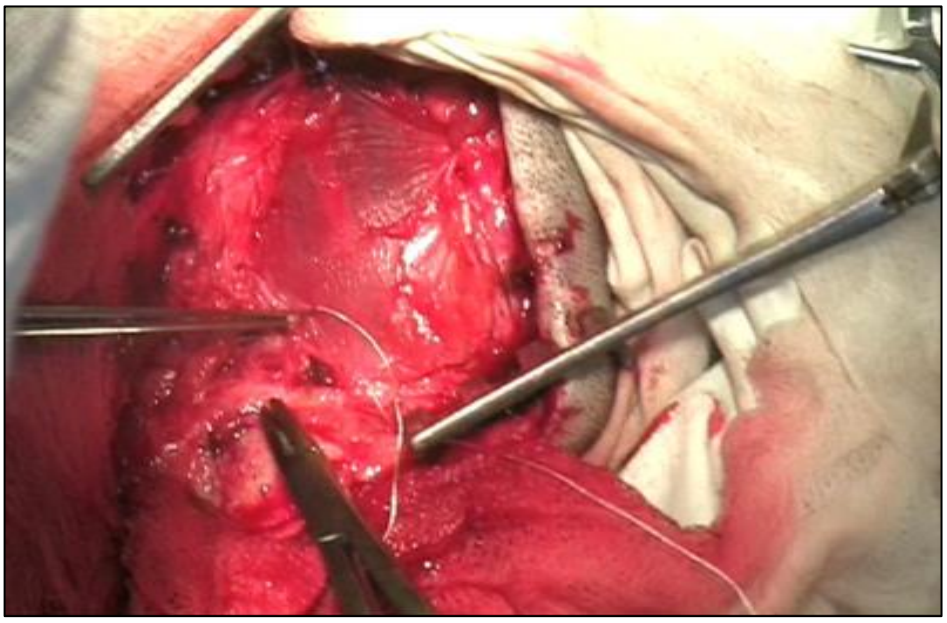


Fig. 7. Plastic defect of the dura mater by cryopreserved membrane

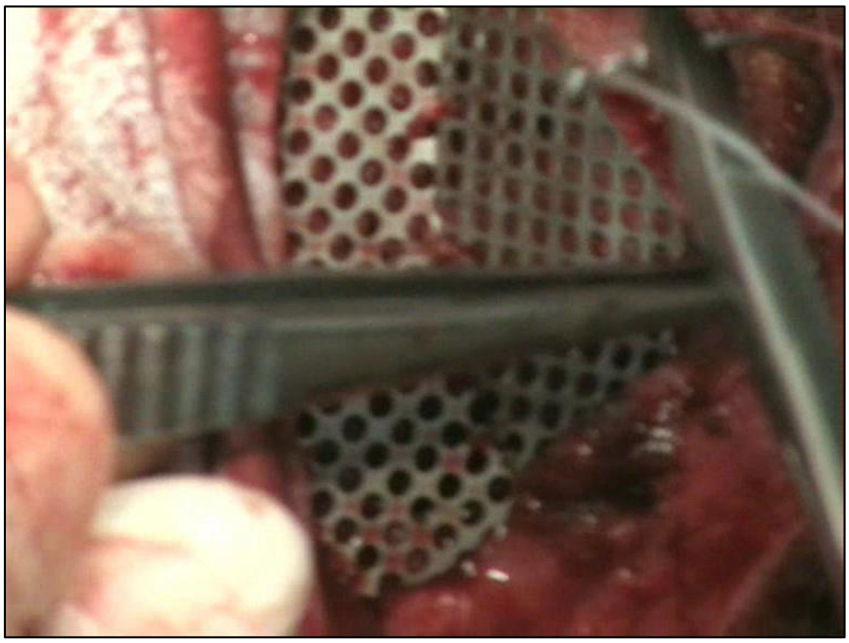


Fig. 8. Plasty of bone postoperative defect with a titanium plate.

After surgery, the neurological symptoms regressed.

According to the degree of dislocation of the brain stem, several options are distinguished: 1st degree – 1-2 mm, 2nd – 3-4 mm, 3rd – 5-7 mm. The topographic-anatomical relationship of the tumor with the brain stem and the structure of the posterior cranial fossa was clarified according to intraoperative data six [(5.4 ± 0.3)%], ill people in which there was a development of ischemic disorders in the brainstem with edema and dislocation after surgical treatment. The above can predict the course of the disease, prevent severe complications during surgery to reduce postoperative mortality. The identified risk factors for the development of complications after the elimination of tumors in this group of patients can be taken into account when choosing surgical tactics and promoting the development of ways to prevent them.

CONCLUSIONS

1. The use of CT, MRI, MR-angiography methods allow to determine the exact localization, size of formations, density, degree of invasion, vascularization, as well as the degree of displacement of the brain stem by the tumor.

2. Microsurgical removal of the tumor with video endoscopic monitoring is a highly effective method that allows you to carry out radical operations, reduce mortality, shorten the operation period and the postoperative rehabilitation period.

3. The use of neurophysiological intraoperative monitoring of corticoencephalographic potentials using the neurophysiological computer system DXNT-32 allows to correct the course of the operation, which eliminates vital complications.

SUMMARY

On the basis of application of modern methods of neuroimaging of CT, MRI and MRA defined basic, necessary for the successful operation parameters of meningiomas of the posterior fossa with paratruncal growth. The method of tumor removal with the use of video endoscopy allowed to achieve radical surgery, to reduce the extent of brain trauma and surgical complications, reduce the time of operation. The use of advanced minimally invasive surgical approaches can dramatically remove the tumor without the trauma of the brain stem. Intraoperative EEG monitoring the functional activity of the brain stem has reduced mortality by preventing vital intraoperative complications.

REFERENCES

1. Endoscopic assistance with simultaneous removal of the meningioma of the posterior cranial fossa and vascular decompression of the trigeminal nerve root / V.N. Shimansky, V.V. Karnaukhov, T.A. Sergienko. Questions of neurosurgery. 2014. № 4. P. 70–81.

2. Practical neurosurgery / B.V. Gaidar, V.A. Khilko, V.E. Parfenov and others: / ed. B. V. Gaidar. SPb. Hippocrates, 2002. 648 p.

3. Anatomical and topographic features of the substrates of supratentorial petroclavic meninges M.I. Shamayev, R.M. Trosh, D.M. Tsyurup. that i. Clinical Surgery. 2012. № 1. P. 49–51.

4. Koval G.Yu. Features of radial manifestations of meningiomas / G.Yu. Koval, S. A. Grabovetsky Radial diagnostics, Radiation therapy. 2010. № 3-4. p. 9–16.

5. Dikan I.M. Grid-technology: radial diagnostics. I.M. Dikan, B.A. Tarasyuk, S.B. Sinyuta. Radiology, radiation therapy. 2013. № 2–3. pp. 57–60.

6. Trofimova T.N. Radiodiagnostics and Therapy of Head and Neck Diseases: National Guidelines / T.N. Trofimova. M.: GEOTAR-Media, 2013. 888 p.

7. Operational approaches in surgery of the skull and brain. B.V. Gaidar, V.E. Parfenov, D.A. Gulyaev, etc. Bulletin of the Russian Military Medical Academy. 2011. № 2 (34). pp. 210–213.

8. Evaluation of the effectiveness of surgical treatment of patients with epidermoid tumors of the posterior cranial fossa / R. M. Trosh, A. N. Lisyany, V. V. Gudkov, P. M. Onishchenko. *Ukrainian Neurosurgical Journal*. 2000. № 2. p. 60–64.

9. Gusev EI. Neurology and neurosurgery. T. 2. Neurosurgery / E.I. Gusev, A.N. Konovalov, V.I. Skvortsova: ed. A.N. Konovalova, A.V. Kozlova. M.: GEOTAR-Media, 2015. 408 p.

10. Vranic A. New insights into meningioma: from genetics to trials / A. Vranic, M. Peyre, M. Kalamarides. *Curr. Opin. Oncol*. 2012. Vol. 24, No. 6. P. 660–665.

11. Positron emission tomography imaging of meningioma in clinical practice: review of literature and future directions / J.F. Cornelius, K.J. Langen, G. Stoffels. et.al. Neurosurgery. 2012. Vol. 70, No. 4. P. 1033–1041.

12. Outcome of the elderly patients undergoing intracranial meningioma resection - a systematic review and meta-analysis / M.T. Poon, L.H. Fung, J.K.Pu, G.K. Leung. *Br. J. Neurosurg*. 2014. Vol. 28, No. 3. P. 303–309.

13. Hallinan J.T. Dilemmas and diagnostic conditions in meningioma / J.T. Hallinan, A.N. Hegde, W.E. Lim. Clin. Radiol. 2013. Vol. 68, No. 8. P. 837–844.

14. International Society of Neuropathology-Haarlem consensus guidelines for grading / D.D. Louis, A. Perry, P. Burger. et.al P. Wesseling. Brain Parhology. 2014. Vol. 24, No. 5. P. 429–435.

15. Meningiomas: knowledge base, the outcome, and uncertainties / L. Rogers, I. Barani, M. Chamberlain. et.al. *J. Neurosurg*. 2015. Vol. 122, No. 1. P. 4–23.

16. Experimental and Clinical Medicine: Modern methods of Investigations in early diagnostics and treatment of paratruncal meningiomas / V.I. Sipituy, S.A. Pagalov, I.A. Kytovoi, V.A. Chmut, V.N. Kycun, O.A. Storchak, A.V. Genkin. 2016, № 4(73), P. 120.

Information about authors:
Chmut V. O.,
Candidate of Medical Sciences, Associate Professor
Department of Neurosurgery
Kharkiv National Medical University
4, Nauky Ave., Kharkiv, 61022, Ukraine
Lakhtin P. V.,
Senior Researcher
Department of Neuropathology and Neurosurgery
Kharkiv Medical Academy of Postgraduate Education
58, Korchagintsev Street, Kharkiv, 61000, Ukraine

NOTES

The project was implemented with the support of

The Center for Ukrainian-European Scientific Cooperation is a non-governmental organization, which was established in 2010 with a view to ensuring the development of international science and education in Ukraine by organizing different scientific events for Ukrainian academic community.

The priority guidelines of the centre for ukrainian-european scientific cooperation

1. International scientific events in the EU

Assistance to Ukrainian scientists in participating in international scientific events that take place within the territory of the EU countries, in particular, participation in academic conferences and internships, elaboration of collective monographs.

2. Scientific analytical research

Implementation of scientific analytical research aimed at studying best practices of higher education establishments, research institutions, and subjects of public administration in the sphere of education and science of the EU countries towards the organization of educational process and scientific activities, as well as the state certification of academic staff.

3. International institutions study visits

The organisation of institutional visits for domestic students, postgraduates, young lecturers and scientists to international and European institutes, government authorities of the European Union countries.

4. International scientific events in Ukraine with the involvement of EU speakers

The organisation of academic conferences, trainings, workshops, and round tables in picturesque Ukrainian cities for domestic scholars with the involvement of leading scholars, coaches, government leaders of domestic and neighbouring EU countries as main speakers.

Contacts:

Head Office of the Center for Ukrainian-European Scientific Cooperation:
88017, Uzhhorod, 7, Malovnycha Str., Office 2
+38 (066) 421 55 76
info@cuesc.org.ua

www.cuesc.org.ua

Izdevniecība "Baltija Publishing"
Valdeķu iela 62 – 156, Rīga, LV-1058

Iespiests tipogrāfijā SIA "Izdevniecība "Baltija Publishing"
Parakstīts iespiešanai: 2018. gada 20. decembris
Tirāža 150 eks.