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MINERAL-VITAMIN COMPLEX «CUPRUM ACTIVE» EFFECT RESEARCH ON RAT'S ORAL CAVITY TISSUES IN METABOLIC SYNDROME MODELING

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Annotation. *The purpose of research - to study the influence of «Cuprum active» complex on the rats oral cavity tissues condition in experimental metabolic syndrome.*

The experiment was carried out for 1.5 months male rats. The intact group received a standard diet. In group 2, metabolic syndrome was modeled by daily administration of pork fat and 10% fructose solution to food. The rats of the 3rd group additionally received «Cuprum active» supplement. The duration of the experiment was 70 days.

Mineral-vitamin complex «Cuprum active» in the simulation of metabolic syndrome restored the level of glucose, uric acid and improved the functioning of the liver of rats. In the periodontal bone tissue, the complex increased the content of glycosaminoglycans and hydroxyproline, showed anti-inflammatory effect and antioxidant properties, and also reduced the number and depth of carious lesions in rats.

Key words: *rats, metabolic syndrome, «Cuprum active» complex, serum, oral mucosa, bone tissue.*

Metabolic syndrome (MS) is a complex of pathological disorders such as abdominal obesity, fasting hyperglycemia, hyperuricemia, dyslipidemia, which are prerequisites for the progression of periodontal disease.

Among patients with metabolic disorders, inflammatory diseases of the periodontal complex are widespread. Obese people are usually hypersensitive to inflammation and, therefore, more susceptible to the development of periodontal disease in the presence of periodontal pathogens. Cytotoxins and adipose tissue hormones play a key role in the association of MS and periodontal disease [1].

In the regulation of most biochemical processes occurring in a living organism, significant role is played by microelements. The regulatory functions of microelements can be comparable to the regulatory role of hormones, and the consequence of their chronic deficiency is the risk of hormonal disorders. Microelements lack leads to pathological conditions, mainly due to changes in the synthesis of enzymes in which they are composed [2]. In various pathologies and external dental procedures, such as orthodontic teeth movement and implantology role of microelements especially important

One of the important microelements in the body is cuprum (Cuprum), which biological role is associated with its participation in enzyme proteins synthesis and activation. Cuprum activates cytochrome oxidase, ascorbate oxidase, tyrosinase, phenol

oxidase, etc. [3].

Cuprum is mainly concentrated in the muscles, bones, kidneys, and brain. The average Cuprum content in mammals is 0.001-0.009 % by weight [4]. Also takes part in blood formation – it is necessary for the synthesis of heme, from which hemoglobin is formed, as well as neurotransmitters – substances that transmit nerve signals. Cuprum ions facilitate the transfer of excitation in the brain [5].

It's deficiency increases the propensity to diabetes development, because enzyme insulinase, catalyzing the destruction of insulin, is inactivated [2]. When cuprum metabolism is disturbed, lipid peroxidation increases, which accelerates the aging process. Decreases the activity of the antioxidant ceruloplasmin with a lack of cuprum.

Cuprum plays a significant role in formation of connective tissue - the walls of blood vessels, cartilage, ligaments, which form the basis of musculoskeletal system, skin. Also affects bone mineral metabolism, which plays an important role in the odrodontic movement of teeth.

Purpose of this research was to study «Cuprum Active» effect complex on the tissues of the oral cavity of rats in simulating the metabolic syndrome state.

Materials and methods. In the experiment were taken white rats-males 1.5-2 months of age. The intact group consisted of 6 individuals (I group). Animals of this group received standard food (feed + a mixture of barley and wheat) and had free access to drinking water. In the 2nd (control group), 7 rats were modeled by MS with the help of 2 components: daily oral administration of supplemental pork fat intake at the rate of 40 % of the average weight of the rats in the group; instead of drinking water – 10 % fructose ad libitum. In the 3rd group, 7 rats on the background of the MS simulation received biologically active additive «Cuprum Active» (produced by TOV "Elit-farm", Ukraine) 1 tablet was dispense on 7 rats 5 times a week in the morning. Complex contains of 1 tablet weighing 250 g: Cuprum (in the form of L-aspartin) – 1.5 mg; iron (in the form of iron fumarate) – 1.5 mg; vitamin C – 10.0 mg; folic acid (vitamin B9) – 100.0 mcg. Excipients in the complex: lactose, milk powder, starch, calcium stearate. Before the experiment, the body weight (in g) and abdominal circumference (at the level of the mid-body, in cm) were measured in rats. The duration of the experiment was 70 days. Upon its completion, the rats were anesthetized (sedazin at the rate of 0.1 ml / kg of the rats' mass intraperitoneally), after which they were euthanized by cutting the main vessels. Serum was collected from collected blood samples. Animals after the experiment were weighed, the abdominal circumference, the mass of visceral organs (liver, kidneys, testicles) with abdominal fatty tissue were measured. Pre-separated mucous membrane of the oral cavity, isolated the upper and lower jaws also the liver. The objects of biochemical studies were serum, supernatant of liver homogenates, alveolar bone (50 mg / ml), mucous membrane of the oral cavity (25 mg / ml). The supernatant liquid was obtained by centrifuging in a PC-6 centrifuge for 15 minutes at 3000 rpm at + 4 ° C.

Rats state of the connective tissue was assessed by the content of hydroxyproline (bound, free and total) [6]; glycosaminoglycans (GAG) [7] in periodontal tissues. In the serum and tissues of rats, biochemical parameters were determined: triglycerides,

total cholesterol (cholesterol), high density lipoprotein cholesterol (HDL), glucose, uric acid, calcium, phosphorus, magnesium, sialic acids; activity of alanine aminotransferase (ALT), aspartate aminotransferase (AsAT), alkaline and acid phosphatases (ALP and CF) using unified methods commercial reagent kits produced by DAC-SpectroMed (Moldova), Felicit (Ukraine), Biolatest (Czech Republic).

The level of lipid peroxidation processes was determined by the content of malonic dialdehyde (MDA) by the thiobarbiturum method [8]. Antioxidant system state was assessed by the activity of glutathione peroxidase (GPO) [9] and catalase [10]. The elastase activity was determined by the method of p-nitrophenol-N-tret-butyl-oxy-carbonyl- α -alaninate as substrate use for elastase [11].

The isolated jaws of rats were subjected to morphometric study determine alveolar process bone resorption [12]. On the selected jaws, the number (on average per 1 rat) and the depth (in points) of the tooth decay of the rats were calculated.

Experiments results were processed by statistical methods with the determination of t-criteria for the significance of differences by Student.

Results and discussion. Mineral-vitamin complex «Cuprum Active» influence study was conducted on a model of metabolic syndrome.

During the 70 days of experiment rats were transferred normally. The increment in body weight increased significantly, after the experiment, the circumference of the middle part of the body of the rats also increased (Table 1).

Table 1

The effect of the «Cuprum Active» complex on the rats morphometric parameters (M \pm m)

Indicators	Animal groups			
	MC model		MC+ «Cuprum Active»	
	before experiment	after experiment	before experiment	after experiment
The mass of rats (g)	105 \pm 7,4	290 \pm 31,9	93 \pm 9,7	258 \pm 20,1 p>0,05
The abdominal circumference of rats (middle part of the body, cm)	13,9 \pm 0,4	17,8 \pm 1,0	12,5 \pm 0,5	17,9 \pm 0,3 p>0,05
Mass of visceral organs (g): -liver	-	10,9 \pm 0,4	-	12,6 \pm 0,8 p>0,05
- kidneys (with fat)	-	10,8 \pm 1,3	-	6,9 \pm 0,5 p<0,05
- eggs (with fat)	-	10,3 \pm 0,8	-	7,9 \pm 0,7 p<0,05

Note. p - reliability index difference compared to the group «model of MS».

Under the action of the «Cuprum Active» complex, the mass of visceral organs (kidneys and testicles with fat) was unreliable decreased by 36% and by 23%, respectively. In contrast, liver mass tended to increase by 16% as compared with the «Model of MS» group.

The «Cuprum Active» complex significantly reduced the main indicators of the

blood serum of rats, which characterize the manifestations of experimental MS - the content of triglycerides and total cholesterol. The level of HDL cholesterol in this case increased 1.5 times, not reaching, however, the level of the intact group (Table 2).

Table 2

The effect of the «Cuprum Active» complex on the biochemical parameters of blood serum of rats (M ± m; p; p1)

Indicators	Animal groups		
	intact	model of MS	MC+ «Cuprum Active»
Content: triglycerides (mmol / l)	1,53±0,03	2,22±0,02 p<0,001	1,70±0,02 p<0,001 p ₁ <0,001
- cholesterol (mmol / l)	5,31±0,09	6,31±0,03 p<0,001	5,80±0,04 p<0,001 p ₁ <0,001
- HDL (mmol / l)	4,04±0,18	1,69±0,05	2,52±0,03 p<0,001 p ₁ <0,001
- glucose (mmol / l)	2,32±0,07	5,20±0,07 p<0,001	2,53±0,10 p ₁ <0,001
- uric acid (μmol / l)	258±4,13	467±6,90 p<0,001	253±2,88 p ₁ <0,001
Activity: -ALT (mmol / l)	1,42±0,16	3,09±0,13 p<0,001	1,38±0,03 p ₁ <0,001
-AsAT (μmol / l)	0,53±0,010	0,81±0,011 p<0,001	0,59±0,03 p ₁ <0,001

Note. The confidence index p is calculated in comparison with the intact group; p₁ - with a group of «Model MS».

Under the complex influence, glucose level in the blood serum of rats decreased by 2 times, uric acid - by 1.8 times. The content of these indicators, most important for the characterization of the MC, approached the data of intact groups.

The complex improved the functional state of the rat liver – ALT activity decreased 2.2 times, AST – 1.4 times as compared with the “Model MS” group (Table 2).

A study of periodontal bone resorption revealed a tendency for its decrease by 11 %. At the same time, the number and depth of caries lesions decreased (Table 3).

The obtained data are consistent with the results of the study of mineral metabolism in periodontal bone tissues. Under the action of the complex, the activity of alkaline phosphatase phospholipids, the osteoblast marker enzyme, and the content of calcium and phosphorus significantly increased (Table 4).

The activity of acid phosphatase in the bone of the alveolar process decreased twice (Table 5), which resulted in a decrease in the resorption processes.

In the soft tissues of the periodontium, inflammatory processes were reduced under the influence of the complex, as evidenced by a 1.2-fold decrease in acid phosphatase activity in the oral mucosa (Table 5).

Table 3

Influence of the «Cuprum Active» complex on the condition of the tooth-jaw system of rats (M ± m; p)

Indicators	Animal groups	
	model of MS	MC+ «Cuprum Active»
Bone resorption of the alveolar process (%): lower jaw	36,2±1,3	32,1±1,8
		p=0,10
upper jaw	27,4±1,7	24,2±1,3
The number of carious lesions (average per 1 rat)	2,7±0,2	2,0±0,2 p=0,03
Depth of teeth caries lesions (in points)	3,0±0,3	2,1±0,3 p=0,06

Note: p - an indicator of the reliability of differences compared with the group «Model MS»

Table 4

The influence of the complex «Cuprum Active» on the state of mineral metabolism in the bones of the jaws of rats (M ± m; p)

Animal groups	Activity of AP (nmol / s g)	Content	
		calcium (mmol / g)	phosphorus (mmol / g)
model of MS	330±2,90	0,32±0,0015	1,10±0,080
MC+ «Cuprum Active»	348±7,26 p=0,05	0,35±0,0055 p<0,001	1,66±0,07 p<0,001

Note. p - reliability index difference compared to the group «model of MS».

Table 5

The effect of the «Cuprum Active» complex on the activity of acid phosphatase and elastase in serum and periodontal tissues of rats (M ± m; p)

Indicators	Animal groups	
	model of MS	MC+ «Cuprum Active»
	oral cavity mucos membrane	
Acid Phosphatase activity (nkat / g)	22,3±0,81	18,0±0,49
		p=0,004
	alveolar process bone	
	24,4±1,82	12,5±2,30
		p=0,004
	blood serum	
Elastase Activity (mkat / l; mkat / kg)	157±9,65	130±6,77
		p=0,04
	alveolar process bone	
	0,270±0,036	0,187±0,014
		p=0,07

Note: p - an indicator of the reliability of differences compared with the group «Model MS»

At the same time, elastase activity in bone tissue decreased by 1.4 times, and in serum – by 17 %.

The results of the influence of the «Cuprum Active» complex on the state of the extracellular matrix of the periodontal connective tissue are presented in Table 6.

The level of GAG in the oral mucosa of the rats was almost unchanged under the action of the complex. The «Cuprum Active» complex increased the content of bound hydroxyproline in the mucous membrane by 23 % and did not significantly affect the level of total hydroxyproline.

The decrease in serum sialic acids by 24 % indicates, on the one hand, the anti-inflammatory properties of the complex, and on the other, the restoration of the connective tissue intercellular matrix under its influence.

Table 6

The effect of the «Cuprum Active» complex on the state of the extracellular matrix of the periodontal connective tissue and serum of rats (M ± m; p)

Indicators	Blod serum	
	model of MS	MC+ «Cuprum Active»
	blood serum	
Content: -sialic acids (mmol / l)	2,50±0,05	1,89±0,05 p<0,001
	oral cavity mucos membrane	
Content: - GAG (mg / g)	0,95±0,01	0,94±0,01
-oxyproline (µmol / g) -free	346,0±14,2	340±10,0
- associated	60,0±3,50	74,00±2,80 p=0,012
- general	406,0±10,6	414,0±10,5
-Cuprum (mmol/g)	0,77±0,02	0,800±0,017
	alveolar process bone	
Content: - GAG (mg / g)	0,380±0,096	0,760±0,035 p=0,007
-oxyproline (µmol / g) -free	265,0±3,5	302,0±19,0 p=0,08
- associated	32,3±3,0	41,0±0,16 p=0,016
- general	297,0±14,0	343,0±15,0 p=0,05
- Cuprum (mmol/g)	0,011±0,0017	0,027±0,0015 p<0,001

Note: p - an indicator of the reliability of differences compared with the group "Model MS"

In the bones of the alveolar process of rats, in contrast to the oral mucosa, the content of GAG under the action of the complex was doubled. At the same time, the level of free hydroxyproline increased by 14 %, associated – by 27 %, total – by 15%. The detected increase in the magnesium content in the bone of the alveolar process by 2.5 times confirms the improvement in the state of the extracellular matrix of the bone tissue, since It is known that magnesium is necessary for its normal metabolism (Table 6).

The «Cuprum Active» complex significantly reduced the content of MDA in the serum of rats, which indicates its antioxidant properties. In the bone of the alveolar process, the complex significantly reduced the level of peroxide products and activated catalase and glutathione peroxidase (Table 7).

Table 7

The effect of the «Cuprum Active» complex on the content of MDA and the activity of antioxidant enzymes in blood serum and periodontal tissues of rats (M ± m; p)

Animal groups	Indicators		
	MDA content (nmol/ml; nmol/g)	Activity	
		catalases (mkat/ml; mkat/g)	Glutathione peroxidase (μ mol/s·ml; μ mol/s·g)
blood serum			
model of MS	5,80±0,30	4,47±1,23	1,21±0,70
MC+«Cuprum Active»	4,87±0,09 p=0,01	3,78±0,45 p>0,05	2,29±0,16 p<0,05
oral cavity mucos membrane			
model of MS	54,70±5,81	65,50±7,23	74,0±12,6
MC+«Cuprum Active»	60,60±1,05 p>0,05	58,60±4,36 p>0,05	60,70±8,58 p>0,05
alveolar process bone			
model of MS	4,64±0,37	9,96±1,23	30,70±5,52
MC+«Cuprum Active»	3,20±0,23 p=0,013	12,40±0,64 p=0,08	52,20±6,68 p=0,05

Note: p - an indicator of the reliability of differences compared with the group «Model MS».

Conclusions. Studies have shown that the «Cuprum Active» mineral and vitamin complex, under the conditions of the metabolic syndrome model, quite substantially negated the negative effects of its most important components in the form of normalization of glucose and uric acid levels and improvement of the functional status of rat liver.

In terms of modeling the metabolic syndrome, the «Cuprum Active» complex in the bone of the alveolar process significantly improved the condition of collagen and GAG, the basis of the intercellular matrix of connective tissue. A significant increase in the content of Mg in the periodontal bone tissue was a positive fact for its normal metabolism.

As a result of improving the mineral metabolism, the complex significantly reduced

the intensity of the caries process in rats when modeling the metabolic syndrome.

The complex reduced levels of sialic acids, peroxide products and rat serum elastase activity. The antioxidant properties of the complex are more pronounced in the periodontal bone tissue of experimental animals.

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