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EFFECT OF THE THICK EXTRACT FROM GARDEN SPINACH LEAVES ON BILE-SECRETORY AND BILE-FORMING FUNCTION OF THE LIVER UNDER CONDITIONS OF CARBON TETRACHLORIDE LIVER LESION

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Abstract

The search for new potential drugs with choleretic action has been carried out in recent years among a large number of substances of different structure and origin, but the most promising are drugs of natural, mostly herbal origin.

The aim of this research was to study the activity of bile-secreting and bile-forming function of rat liver under conditions of its carbon tetrachloride lesion and after the use of the thick extract from garden spinach leaves.

The studies were performed on white male rats kept on the standard diet of the vivarium of Ternopil National Medical University. Animals were divided into 4 groups, three of which were affected by carbon tetrachloride, one served as intact control. One of the groups of affected rats received the cholagogue flamin at the dose of 250 mg/kg body weight, another on the background of carbon tetrachloride liver lesion was exposed to action of thick extract from garden spinach leaves at the dose of 150 mg/kg body weight.

It was established that after the lesion of rats with carbon tetrachloride decreases the rate of bile secretion and its volume, which is most pronounced on the 10th day of development of carbon tetrachloride hepatitis. The use of the thick extract from garden spinach leaves led to the restoration of biliary function of the liver, although its effectiveness was somewhat inferior to the reference drug flamin. After the use of both flamin and the studied extract in the serum of rats with carbon tetrachloride liver lesion the content of bile acids and bilirubin decreased during all periods of the experiment (4, 7 and 10 days after exposure to carbon tetrachloride). At the same time the content of bile acids in bile increased.

The results of the research make it expedient to further study the thick extract from garden spinach leaves as a cholagogue in order to create on its basis new drugs with hepatoprotective action.

Keywords: *thick extract from garden spinach leaves, white rats, carbon tetrachloride hepatitis, bile-secreting and bile-forming function of the liver, flamin*

Introduction

According to the WHO, about 29 million people in European countries suffer from chronic liver and biliary tract diseases. In diseases of the hepatobiliary system is often impaired not only exocrine function, but also other liver functions, which adversely affect the course of the disease. In this regard, there is a need to use drugs that stimulate the function of the liver and bile ducts.

Treatment of liver lesions of various etiologies and its protection requires pharmacotherapy, which includes the use of drugs that would act comprehensively, due to hepatoprotective, antioxidant, anti-inflammatory and other mechanisms, so replenishment of the pharmaceutical market with such drugs becomes one of the main directions of the pharmaceutical industry [1,2] .

Significant impairment of bile-secretory function of the liver is observed in most lesions of the hepatobiliary system, which lead to significant changes in its ability to secrete bile and worsen due to damage to the small bile ducts. Dysfunction of the biliary tract of the liver is accompanied by destructive changes in the cell membranes of hepatocytes. Therefore, modern therapeutic regimens involve the use of drugs with hepatoprotective and hepatoregenerative properties [3]. A significant role is given to preparations from plant raw materials.

According to the literature, garden spinach has a diverse content of biologically active substances. First of all, it is flavonoids, vitamins, minerals and carboxylic acids [4, 5]. Some authors have studied the effect of carotenoids and phenolic compounds on the antioxidant and anti-inflammatory properties of spinach extracts in an experiment [6]. Phenolic compounds of spinach have been shown to have high antioxidant activity, while carotenoids have higher anti-inflammatory activity.

Considering diverse chemical composition of all organs of spinach and its widespread use in folk medicine, it was expedient to study the hepatoprotective and choleretic properties of the thick extract from garden spinach leaves in a model of toxic liver lesion with carbon tetrachloride.

It is known that the modeling of toxic hepatitis in laboratory animals is accompanied by the development of characteristic clinical, pathological and histomorphological features, hyperenzymemia of hepatospecific enzymes, impaired lipid metabolism, decreased synthetic, conjugate, elimination functions of the liver, development of intrahepatic cholestasis on the background of activation of free radical processes [7]. Therefore, this model was chosen by us for further research.

The aim of our research – to study the activity of bile-secreting and bile-forming function of rat liver under conditions of its carbon tetrachloride lesion and after the use of the thick extract from garden spinach leaves.

Methods

The experimental part of the research was performed on white rats kept on the standard diet of the vivarium of Ternopil National Medical University. The animals in the experiment were divided into 4 groups: 1 - intact rats, 2 - affected rats (CCl_4), 3 - affected + extract correction, 4 - affected + flamin correction.

Toxic hepatitis was simulated in white male rats by liver injury with carbon tetrachloride (50% oil solution at the dose of 1,0 ml/kg body weight). Thick extract from garden spinach leaves (TESL) was used at a dose of 150 mg/kg body weight, which we previously established as the minimum effective dose [8], the reference drug was a cholagogue flamin at a dose of 250 mg/kg body weight.

Bile-secretory and bile-forming function of the liver were evaluated on the 4th, 7th and 10th days of toxic hepatitis. Under thiopental anesthesia (60 mg/kg), the common bile duct was catheterized in rats and bile was collected in hourly portions over 3 hours [9]. Hepatic secretory function was assessed by the rate of bile secretion per hour of observation (mg/min/100) and the total amount of bile throughout the experiment (ml/100) [10]. Animals were removed from the experiment under thiopental anesthesia. Blood samples were taken for further research.

Bile-forming function of the liver was assessed by the content of total bile acids [11] and total bilirubin in the blood, for the determination of which a standard set of reagents "Philisit-Diagnostics" was used.

Animal keeping and experiments were carried out in accordance with the regulations of "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [12].

The results of the research were subjected to statistical analysis [13] using the statistical program Statistica 6.0 using the parametric Student's t-test and the non-parametric Wilcoxon test for related samples. Changes were considered probable at $p \leq 0,05$.

Results

It is known that bile plays an important role in maintaining the stable functioning of the body, and bile acids, as specific secretory components of liver parenchymal cells, are the most important part of it. They play an important role in digestive processes and are involved in a number of metabolic processes. Bile acids affect intestinal motility, activate lipase and colipase, exhibit antiseptic properties, stabilize and maintain the colloidal state of lipid substances of bile and cholesterol and stimulate the secretion of bilirubin with bile [7].

Modeling of toxic hepatitis in laboratory animals is accompanied by the development of intrahepatic cholestasis against the background of activation of free radical processes [11].

We conducted studies to establish the effect of TESL on the indicators of bile-secretory and bile-forming functions of the liver of rats with carbon tetrachloride hepatitis. Significant impairment of bile-secretory function of the liver of animals affected by carbon tetrachloride was noted. This is evidenced by a probable ($p < 0,05$) decrease in the rate of secretion and volume of bile throughout the study period. The maximum decrease in these parameters was observed at the end of the experiment (10 days) and was 46% for bile volume and 36,9% for bile secretion rate relative to the level of intact control animals (Table 1).

The use of TESL and flamin led to a probable ($p < 0,05$) increase in bile volume of experimental animals on the 7th and 10th day of the study. For TESL, the increase was 12,7% on the 7th and 24,2% on the 10th day of the experiment relative to the level of affected animals. Flamin showed a slightly higher efficiency on this indicator and it was 25,3% and 36,8%, relative to the level of affected animals, in the same period of the study.

The use of these corrective agents also led to a probable ($p < 0,05$) increase in the rate of bile secretion in animals affected by carbon tetrachloride (Figure 1).

As can be seen from Figure 1, for TESL a probable ($p < 0,05$) increase in the rate of bile secretion in animals affected by carbon tetrachloride was observed on the 7th and 10th day of the study. Flamin showed this activity in all terms of the study. On the last day of the experiment (10th day), the effectiveness of the extract and the reference drug was approximately the same and almost reached the level of intact control rats.

In addition to impaired bile production, which plays an important role in toxic hepatitis, an important factor is the delay in bile outflow, resulting in the development of cholestasis. This causes the development of cholemia - intoxication with bile components that enter the blood, especially bilirubin and bile acids [14].

Throughout the study period, a probable ($p < 0,05$) increase in the content of bile acids in the serum and their decrease in the bile of affected animals was observed (Table 2). At the end of the study (10 days), the increase in the content of bile acids in the serum was 33,4 %, the decrease in their content in bile – 33,6 %.

The use of corrective factors had a positive effect on the content of bile acids in the serum. TESL led to a probable ($p < 0,05$) changes on the 10th day of the study and a decrease in serum bile acid content was 21,7% during the study period (relative to the level of affected animals). Flamin was more effective in changing the dynamics of this indicator in the serum and showed a probable ($p < 0,05$) decrease on the 7th (20,5%) and 10th (30,5%) days relative to the level of affected animals.

The effect of TESL and flamin on the content of bile acids in the bile of affected animals was also positive and led to an increase in this indicator (Figure 2). The use of flamin slightly exceeded the effectiveness of TESL for this indicator and was probable ($p < 0,05$) in all terms of the study.

On the 10th day of the experiment, the content of bile acids in bile increased 1,4 times relative to the level of affected animals. However, TESL also

caused probable ($p < 0,05$) changes in this indicator on the 7th and 10th day of the study. After correction with TESL, there was an increase in the content of bile acids in the bile of affected animals by 1,2 times on the 7th and 1,3 times on the 10th day of the study, relative to the level of affected animals.

A probable increase in the content of total bilirubin in the serum of animals affected by carbon tetrachloride was recorded during the whole period of the experiment. The maximum increase in this indicator (1,8 times) was on the 10th day of the study (Table 3).

The use of TESL and flamin resulted in a probable ($p < 0,05$) decrease in total serum bilirubin in tetrachloromethane-affected animals on days 7 and 10 of the study.

After the use of TESL, the reduction in total bilirubin was 31,7 % and 58,4% on the 7th and 10th day of the experiment, respectively (relative to the level of affected animals). Flamin was somewhat more effective in similar periods of the study and the reduction was 45,5 % and 69 %, respectively, compared with animals with toxic hepatitis.

Thus, there is a positive effect of TESL on the bile-secretory and bile-forming function of the liver, which may indicate the inherent choleretic properties of this herbal remedy. In our opinion, this is due to the presence in this raw material of a significant amount of carotenoids and flavonoids.

The studied thick extract from garden spinach leaves can be used in the future to create new drugs with choleretic action, which will find their use in liver disease.

Conclusions

In the conditions of tetrachloromethane liver lesions of rats, a progressive violation of the bile-secretory and bile-forming functions of the liver of animals was detected, which increased by the end of the experiment.

Used thick extract from garden spinach leaves had a positive effect on the restoration of these functions in the body of affected animals, as evidenced by increasing the volume and rate of bile secretion, reducing serum bile acids and increasing them in bile, as well as reducing total bilirubin in the serum of animals affected by carbon tetrachloride.

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Table 1. Influence of the thick extract from garden spinach leaves and flamin on biliary function of the liver of affected by carbon tetrachloride rats (M ± m; n = 6)

Groups of animals	Terms of research, days		
	4 days	7 days	10 days
	Bile volume, ml/100 g		
Intact control (IC)	0.87 ± 0.03		
Affected CCl4	0.56 ± 0.01 *	0.49 ± 0.02 *	0.47 ± 0.01 *
Affected CCl4 + TESL	0.56 ± 0.01	0.60 ± 0.01 **	0.68 ± 0.02 **
Affected CCl4 + flamin	0.59 ± 0.02	0.71 ± 0.02 **	0.79 ± 0.02 **
	The rate of bile secretion, mg/min × 100		
Intact control (IC)	5.50 ± 0.21		
Affected CCl4	4.05 ± 0.17 *	3.68 ± 0.15 *	3.47 ± 0.14 *
Affected CCl4 + TESL	4.12 ± 0.14	4.58 ± 0.17 **	4.95 ± 0.12 **
Affected CCl4 + flamin	4.65 ± 0.13 **	5.10 ± 0.11 **	5.22 ± 0.13 **

Note: Here and in the following tables * - probable changes between indicators of intact and affected by carbon tetrachloride animals ($p < 0,05$); ** - probable changes between indicators of animals affected by carbon tetrachloride and animals that received corrective factors on the background of toxic hepatitis ($p < 0,05$).

Table 2. Influence of the thick extract from garden spinach leaves and flamin on the concentration of bile acids (g/l) in the serum and bile of animals affected by carbon tetrachloride (M ± m; n = 6)

Groups of animals	Terms of research, days		
	4 days	7 days	10 days
	Serum, g/l		
Intact control (IC)	5.12 ± 0.15		
Affected CCl4	5.85 ± 0.16 *	6.52 ± 0.22 *	6.83 ± 0.14 *
Affected CCl4 + TESL	5.73 ± 0.08	6.11 ± 0.10	5.72 ± 0.12 **
Affected CCl4 + flamin	5.45 ± 0.13	5.47 ± 0.17 **	5.27 ± 0.11 **
	Bile, g/l		
Intact control (IC)	7.48 ± 0.15		
Affected CCl4	5.90 ± 0.14 *	5.45 ± 0.15 *	4.97 ± 0.13 *
Affected CCl4 + TESL	6.21 ± 0.16	6.38 ± 0.16 **	6.56 ± 0.12 **
Affected CCl4 + flamin	6.73 ± 0.13 **	6.88 ± 0.13 **	7.16 ± 0.10 **

Table 3. Influence of the thick extract from garden spinach leaves and flamin on the content of total bilirubin in the serum of animals affected by carbon tetrachloride (M ± m; n = 6)

Groups of animals	Terms of research, days		
	4 days	7 days	10 days
	Serum, µmol / l		
Intact control (IC)	7.97 ± 0.36		
Affected CCl4	10.13 ± 0.26 *	12.82 ± 0.23 *	14.40 ± 0.25 *
Affected CCl4+ TESL	9.73 ± 0.23	10.30 ± 0.25 **	9.75 ± 0.24 **
Affected CCl4+ flamin	9.57 ± 0.23	9.20 ± 0.24 **	8.90 ± 0.25 **

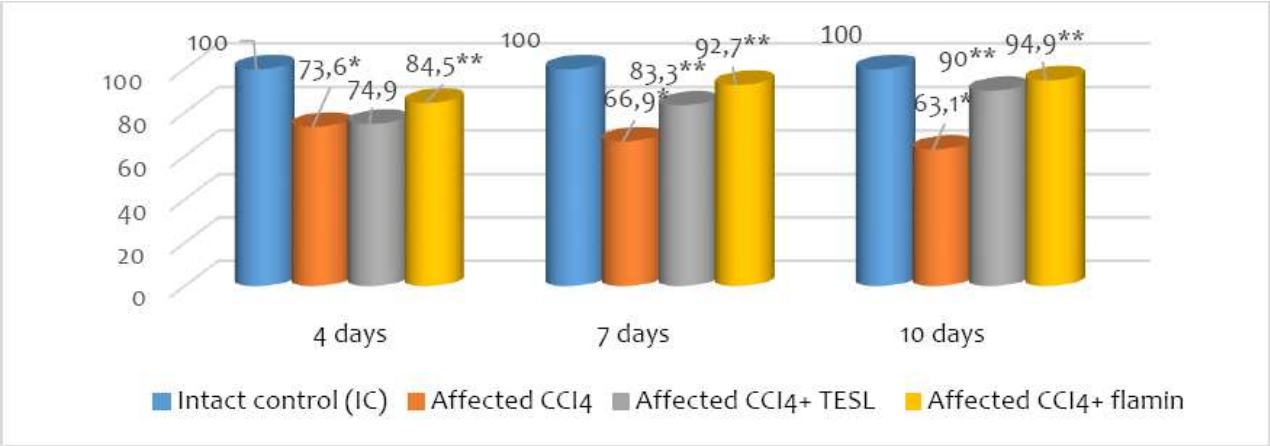

Figure 1. The effect of thick extract from garden spinach leaves and flamin on the rate of bile secretion in animals affected by carbon tetrachloride, %

Note: Here and in the following figures * - probable changes between the indicators of intact and affected by carbon tetrachloride animals (p <0,05); ** - probable changes between the indicators of animals affected by carbon tetrachloride and animals that received corrective factors on the background of toxic hepatitis

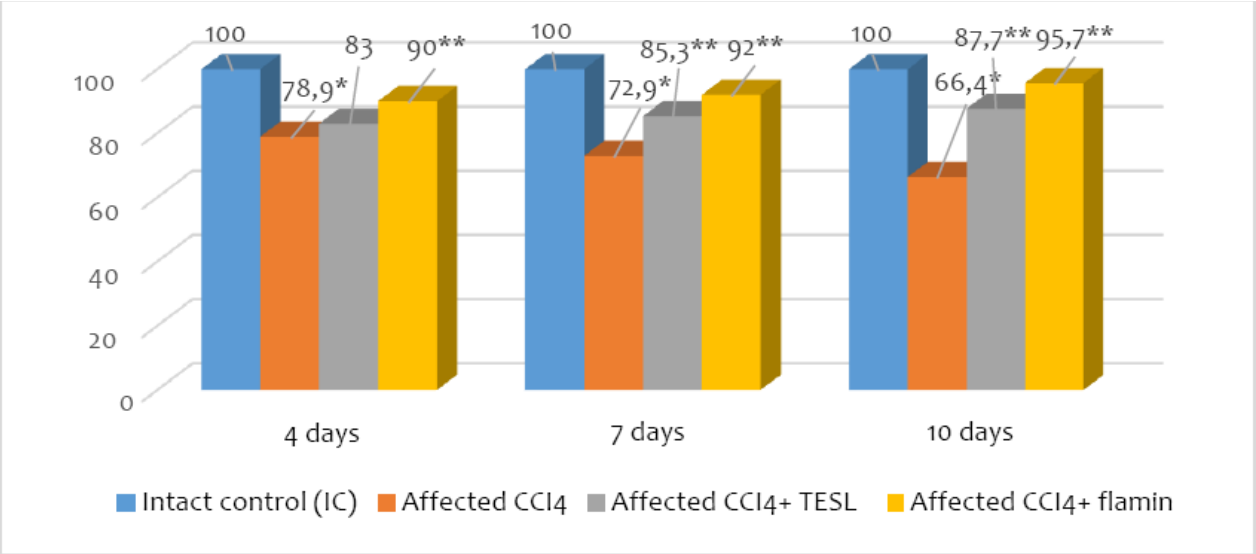

Figure 2. The effect of thick extract from garden spinach leaves and flamin on the content of bile acids in the bile of animals affected by carbon tetrachloride, %