

COMPARATIVE ASSESSMENT OF HEPATOPRO-TECTIVE ACTIVITY OF BRASSICA RAPA, NIGELLA SATIVA, SAMBUCUS NIGRA SUBSTANCES WITH C-4 AND PHOSPHOGLIV PREPARATIONS

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Abstract. *A comparative study of the hepatoprotective activity of the substances Brassica rapa, Nigella sativa, Sambucus nigra with comparison drugs C-4 and Phosphogliv in acute hepatitis induced by carbon tetrachloride (CCl₄) was carried out in experiments. The results of a comparative assessment of the hepatoprotective activity of Brassica rapa, Nigella sativa, Sambucus nigra substances with C-4 and Phosphogliv preparations showed a high degree of hepatoprotective properties of Brassica rapa substances at a dose of 20 mg/kg and Sambucus nigra at a dose of 30 mg/kg, which in the future can be used to create a biologically active additive (dietary supplement) for treatment of diseases of the hepatobiliary system.*

Key words: *substances of medicinal plants, hepatoprotector, hepatitis, carbon tetrachloride, liver, peripheral blood.*

Introduction. In recent decades, there has been a significant increase in the incidence of liver and hepatobiliary diseases in the population all over the world, which is a prerequisite for expanding the range of medicines capable of enhancing regenerative processes in the liver. The annual registration of 2-3 million new cases of viral, toxic, medicinal, alcoholic, and autoimmune hepatitis indicates the relevance of the issue of modern pharmacology, which is to improve the quality and safety of pharmacotherapy of diseases of the hepatobiliary system [1, 2].

Currently, the issue of the need for the use of hepatoprotective drugs in the complex therapy of liver diseases against the background of such common comorbid conditions as obesity, diabetes mellitus and metabolic syndrome is becoming more and more urgent [3]. To date, the problem of medicinal liver lesions is one of the most important, in the standard treatment of various groups of diseases it includes a total of 8-12 drugs with a single dose, such a number of drugs can cause the development of various forms of drug-induced hepatotoxic reactions, on average, in 28% of cases [4].

Hepatoprotectors are complex preparations, mainly of plant origin, designed to increase the resistance of the liver to toxic effects, promote the restoration of its functions, normalize or enhance the activity of liver cell enzymes and prevent the destruction of cell membranes and stimulate the regeneration of hepatocytes [3]. Timely and correct selection of hepatoprotectors based on isolated substances from medicinal plants growing in Uzbekistan in the treatment of various diseases of the hepatobiliary system will help reduce the risk of liver damage. The purpose of this study was to study the effect of the substances Brassica rapa, Nigella sativa and Sambucus nigra in comparison with C-4 (developed by the Institute of Bioorganic Chemistry of the Academy of Sciences of the Republic of Uzbekistan, preparing for patenting) and Phosphogliv (Russia) on the course of experimental acute toxic hepatitis caused in mice by carbon tetrachloride (CCl₄).

Research methodology. A model of toxic hepatitis caused by CCl₄ in mice. The study of hepatoprotective activity was conducted for a comprehensive assessment of the effectiveness of substances Brassica rapa, Nigella sativa, Sambucus nigra with hepatoprotective activity. The experiments were conducted on 48 male mice weighing 18-20 g according to the method proposed

in [5]. To obtain a model of toxic hepatitis, CCl₄, which causes necrosis of hepatocytes, was used at a dose of LD₅₀. According to preliminary experiments in rats, LD₅₀ = 2.7 ml/kg of animal weight. Next, the animals were divided into 6 groups (8 groups) to evaluate the effectiveness of the substances in comparison with control and intact mice, as well as with the comparison drug Phosphogliv at a dose of 450 mg/kg. An hour after intraperitoneal administration of CCl₄ at a dose of 20 mg/kg, the studied substances were administered intragastrically in the following doses: Brassica rapa 20 mg/kg, Nigella sativa 20 mg/kg, Sambucus nigra 30 mg/kg and Sambucus nigra 20 mg/kg, in a volume of 0.2 ml of solution, the animals of the control group were injected with saline in the appropriate volume. The animals were monitored for 6 days. Hepatitis caused by carbon tetrachloride is characterized by the development of colliquation necrosis, protein and fatty degeneration of hepatocytes localized mainly in the central zone of the renal lobule, where the maximum activity of cytochrome P-450-dependent monooxygenases and the predominant production of damaging hepatotoxin metabolites. D-galactosamine, which disrupts the synthesis of RNA and protein, causes acute hepatitis, identical in morphological and biochemical changes in the liver to viral hepatitis in humans. Studies are carried out in vivo in dynamics and after decapitation of animals under ether anesthesia a day after the last administration of potential hepatoprotectors. For further analysis, the following indicators were recorded: survival rate (%), life expectancy of dead mice (days), liver weight coefficient (ratio of organ weight in g per body weight in kg). For a comparative assessment of the results obtained, the coefficient of hepatoprotective activity – KGA was used.

Determination of biochemical parameters of blood. The surviving animals were slaughtered by decapitation, 0.5 ml of blood was taken at 3.8% citrate 1:9 and centrifuged at 3000 x gy for 10 minutes. Screening tests were conducted to identify the hepatoprotective properties of potential drugs: animal survival, body weight change and liver mass coefficient, CAA were determined. For the purpose of a more in-depth study of the effect of experimental substances on the course of hepatitis, the biochemical parameters of mouse blood characterizing liver function were evaluated. The activity of the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) was determined by photometric kinetic method using kits from Cypress Diagnostics (Belgium) in animal serum. The analysis of peripheral blood parameters: hemoglobin content, number of erythrocytes, average erythrocyte volume (MCV), average hemoglobin content in a single erythrocyte (MCH), average hemoglobin concentration in erythrocyte mass (MCHC), number of reticulocytes, platelets, leukocytes was carried out on an automatic hematology analyzer Dymind DH36, Shenzhen Dymind Biotechnology Co., Ltd, China). Statistical processing of the results was carried out using the Microsoft Office software package. The data are presented in the form of an average value (M) and a standard error of the average value (m). To test statistical hypotheses about the difference between the studied groups, the Student's criterion was used.

Analysis and results. Generalized data from experiments to determine the hepatoprotective activity of the studied substances and comparison drugs are presented in Table 1. The development of toxic hepatitis in animals of the control group was characterized by a decrease in survival rate to 50% (in the intact group – 100%), a decrease in body weight by an average of 1.3 g and an increase in liver mass coefficient by 19.69%. The life expectancy of dead mice in the group of substances Brassica rapa, Nigella sativa, Sambucus nigra and comparison drugs was 6 days, only in the group with the substance Sambucus nigra at a dose of 20 mg /kg this indicator was 5.25 days. Carbon tetrachloride intoxication was accompanied by hyperfermentemia, indicating the destruction of hepatocytes and the development of cholestasis compared with the intact group (ALT and AST by 1.75 and 2.4 times, respectively, Table 2). The introduction of

Brassica rapa, Sambucus nigra substances and comparison drugs to experimental animals led to a decrease in the hepatotoxic effect of carbon tetrachloride: the survival rate of rats increased to 100%; animal body weight increased, liver mass coefficient decreased by 1.3 times, but in the case of Nigella sativa and Sambucus nigra substances at a dose of 20 mg/kg, these indicators remain unchanged as and in the case of model pathology. Based on the data obtained, the final indicator - KHA of the studied substances and comparison drugs was 1, which means a high degree of hepatoprotective activity of substances and drugs, except for substances Nigella sativa and Sambucus nigra at a dose of 20 mg /kg, which was 0.63 and 0.42, respectively, which means an average degree of hepatoprotective activity of substances.

Table 1

The effect of substances and preparations of plant origin on the course of experimental acute toxic hepatitis, screening tests ($M \pm m$, $n=6$)

The Monitoring Group, the dose	Survival rate (%)	Life expectancy of dead mice (days)	Liver mass ratio, mg/g	Coefficient of changes in animal body weight, g	KHA
Intact	100	6	64,71± 2,52	(+) -1,25±0,24	1
Control	50	3,75	84,4 ± 2,85	(-) 1,3 ± 0,37	0
C4, 20 mg/kg	100	6	64,68 ± 2,25	(+) -2 ± 0,65	1,07
Phosphogliv, 110 mg/kg	100	6	63,3±1,7	(+) -1,75±0,43	1,07
<i>Brassica rapa</i> , 20 mg/kg	100	6	60,80 ± 1,18	(+) -0,25±0,13	0,95
<i>Nigella sativa</i> , 20 mg/kg	100	6	95,41± 5,86	(+) -1,5 ± 1,30	0,63
<i>Sambucus nigra</i> , 30 mg/kg	100	6	64,29 ± 0,80	(+) -2,0 ± 0,74	1,08
<i>Sambucus nigra</i> , 20 mg/kg	75	5,25	80,41 ± 4,85	(-) 0,5 ± 0,14	0,42

The introduction of the studied substances and comparison drugs stimulated the normalization of biochemical parameters of animal blood: the activity of ALT and AST significantly decreased, and there was also a tendency to decrease the activity of alkaline phosphatase. The use of Brassica rapa, Nigella sativa and Sambucus nigra substances for the treatment has led to changes in some biochemical parameters. The activity of AST decreased by 1.8 times under the action of Brassica rapa at a dose of 20 mg/kg compared with this indicator of animals in the control group. The indicators of the activity level of AST under the action of Sambucus nigra at a dose of 30 mg/kg and the comparison drug C-4 at a dose of 20 mg/kg were of the same value, which is confirmed by other studies. The activity levels of this enzyme in the groups of substances Nigella sativa and Sambucus nigra at the same dose of 20 mg /kg increased by 3.3 and 2.4 times compared with the control group. In addition, there was a decrease in the activity of alkaline phosphatase by 1.3 and 1.2 times under the action of Brassica rapa substances at a dose of 20 mg/kg and Sambucus nigra at a dose of 30 mg/kg, respectively, compared with similar indicators in mice of the control group. A change in the activity of AST indicates improved treatment, an increase in the activity of alkaline phosphatase indicates a decrease in hepatoprotective effect compared with similar indicators of animals with a high degree of hepatoprotective activity.

Therapy of animals with acute hepatitis with the studied substances was characterized by a significant improvement in the indicators of screening tests and biochemical experiments compared with similar indicators of both the control group and groups of animals receiving the studied substances *Nigella sativa* and *Sambucus nigra* at a dose of 20 mg/kg. Changes in all these parameters observed in animals treated with *Brassica rapa* substances at a dose of 20 mg/kg and *Sambucus nigra* at a dose of 30 mg/kg indicated a high degree of hepatoprotective properties of these substances.

Table 2

Biochemical parameters of peripheral blood of animals under the action of herbal preparations and comparison preparations, (M±m, n=6)

The Monitoring Group, the dose	ALT, E/L	AST, E/L	APh, E/L
Intact	34,1±2,0	20,6±1,9	93,3±4,4
Control	59,8±3,1*	49,6±2,1*	124,4±3,3*
C4, 20 mg/kg	33,5±2,3#	19,7±2,0#	96,3±1,9
Phosphogliv, 110 mg/kg	33,6±1,8#	20,4±1,8#	99,0±2,2
<i>Brassica rapa</i> , 20 mg/kg	31,4±3,9#	27,0±3,2#	94,4±2,2
<i>Nigella sativa</i> , 20 mg/kg	64,2±2,1*	68,5±1,8*#	108,3±2,2*
<i>Sambucus nigra</i> , 30 mg/kg	32,4±4,3#	19,7±3,2#	99,0±2,2
<i>Sambucus nigra</i> , 20 mg/kg	103,1±4,9*#	50,6±2,4*	104,4±4,7#

Note: * - p<0.05 - in relation to intact data , # - p<0.05 – in relation to control

Peripheral blood parameters were measured: hemoglobin content, number of erythrocytes, average erythrocyte volume (MCV), average hemoglobin content in a single erythrocyte (MCH), average hemoglobin concentration in erythrocyte mass (MCHC), number of reticulocytes, platelets, leukocytes, lymphocyte concentrations (Lym%, %), erythrocytes (RBC, 10¹²/l), platelets (PLT, 10⁹/l). Table 3 shows the results of the analyses on the 6th day of the experiment.

Table 3

Hematological blood analysis of animals with acute toxic hepatitis treated with herbal preparations (M±m, n=6)

Indicators, groups	Intact	Control	Phosphogliv, 110 mg/kg	C-4, 20 mg/kg	<i>Brassica rapa</i> , 20 mg/kg	<i>Nigella sativa</i> , 20 mg/kg	<i>Sambucus nigra</i> , 30 mg/kg	<i>Sambucus nigra</i> , 20 mg/kg
WBC, 10 ⁹ /л	15,6±1,0	0,28±0,02	15,1±0,4	15,35±0,2	15,3±0,83	10,4±0,8	15,0±0,2	10,57±2,2
Lym%, %	0,4±0,03	0,0±0,0	0,7±0,03	0,4±0,01	0,80±0,02	0,7±0,02	0,7±0,04	0,66±0,03
Gran%, %	0,3 ±0,01	0,0±0,0	0,1±0,01	0,4±0,006	0,57±0,05	0,1±0,005	0,1±0,02	0,15±0,01
Mid%, %	0,1±0,01	0,0±0,0	0,1±0,01	0,3±0,013	0,13±0,03	0,2±0,02	0,1±0,01	0,19±0,01
Lym#, 10 ⁹ /л	0,7±0,03	0,9±0,01	0,3±0,1	0,3±0,015	0,62±0,09	0,5±0,08	0,3±0,1	0,33±0,06

Gran#, 10 ⁹ /л	0,4±0,05	0,9±0,01	0,2±0,02	0,3±0,01	0,41±0,0 2	0,4±0,06	0,2±0,03	0,15±0,0 2
Mid#, 10 ⁹ /л	0,2±0,02	0,9±0,01	0,3±0,05	0,2±0,00 6	0,65±0,1 5	0,3±0,07	0,2±0,03	0,18±0,0 3
RBC, 10 ¹² /л	10,9±0,4	6,72±0,2 8	10,3±0,1	9,99±0,3 9	9,99±0,3 9	7,7±0,7	10,4±0,2	8,26±0,6 2
HGB, г/л	142,0±3, 3	120,6±2, 8	148,3±3, 6	140,3±2, 1	134,0±2, 1	110,0±7, 9	146,8±1, 4	101,3±1, 2
PLT, 10 ⁹ /л	349,8±34	237,5±65	248,0±19	225,0±22	225,0±22	106,5±10	308,5±17	141,3±2, 3

Note: Leukocytes - WBC, percentage of lymphocytes - Lym%, percentage of granulocytes - Gran%, percentage of average leukocytes - Mid%, concentration of lymphocytes - Lym#, concentration of granulocytes - Gran#, concentration of average leukocytes - Mid#, erythrocytes - RBC, hemoglobin – HGB, platelets – PL.

The percentage of lymphocytes, granulocytes, leukocytes, erythrocytes, hemoglobin under the action of *Brassica rapa* substances at a dose of 20 mg/kg and *Sambucus nigra* at a dose of 30 mg/kg was 0,57±0,05, 0,13±0,03, 0,62±0,09, 134,0±2,12, also, 0,1±0,02, 0,1±0,01, 0,3±0,1, 146.8±1.4, respectively, which is consistent with the results of the intact group. In the other studied groups of substances, i.e., in the groups under the action of *Nigella sativa* and *Sambucus nigra* at the same dose - 20 mg/kg, there is a slight decrease in these indicators.

Conclusion and recommendations. The results of a comparative assessment of the hepatoprotective activity of *Brassica rapa*, *Nigella sativa*, *Sambucus nigra* substances with C-4 and *Phosphogliv* preparations indicate a high degree of hepatoprotective properties of *Brassica rapa* substances at a dose of 20 mg/kg and *Sambucus nigra* at a dose of 30 mg/kg, which in the future can be used to create a dietary supplement for the treatment of diseases of the hepatobiliary system.

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