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HEPATOPROTECTIVE EFFECT OF LECITHIN-BASED PREPARATION ON EXPERIMENTAL ANIMAL LIVER TOXICITY

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SUMMARY

Data on assessment of lecithin preparation effect on liver toxicity experimentally induced in laboratory animals are presented. Experiments were carried out in rats kept under similar conditions that had been subjected to quarantine in animal facilities and demonstrated no apparent disease manifestations. The animals were divided into groups based on paired counterparts' principle. Acute toxic damage of liver was induced by single administration of carbon tetrachloride (0.3 mg/kg of body weight). The rats demonstrated the following apparent signs of intoxication on day 2 after administration: anorexia, depression, dyspnea, fever. Per os administration of rape lecithin at a dose of 1 ml/kg of body weight one hour before hepatotoxicant administration and then daily for subsequent 30 days reduces intoxication manifestations and improves survivability of the rats. Pharmacological effect of lecithin includes biochemical characteristics improvement and functional state of liver of laboratory animals with experimentally induced acute hepatitis. Obtained data may provide grounds for application of lecithins as hepatoprotectors of natural origin in the form of biologically active supplements or active ingredients of pharmaceuticals intended for hepatic disease treatment in animals.

Key words: lecithin, phospholipids, hepatoprotectors, animals, toxic damages of liver, experimentally induced hepatitis.

INTRODUCTION

Widespread hepatopathies, as well as diagnostic complexity and variability of therapeutic approaches cause a great interest of veterinary specialists in the study of this problem. The function variety of liver as the main biochemical laboratory of the organism determines the high probability of occurrence of early metabolic disorders of various organs and systems and hepatopathologies. In connection with this, with regard to diseases of the hepatobiliary system there is still a high need for hepatoprotective agents which increase the resistance of the liver to the action of chemical agents and normalize its metabolism under conditions of straining detoxification. The decisive factors in favor of the choice of drugs of this type are safety, good tolerability and the possibility of long-term administration [6, 7].

Over the past few years, positive experience has been accumulated in the study and use of lecithin-containing drugs for hepatitis, dystrophy, and cirrhosis [1, 3]. Lecithin is the common name for a group of fat-like substances that are a mixture of phospholipids (65–75%) with triglycerides and a small number of other components. Lecithin is the main structural component of all cell membranes, that maintains the constancy of the internal cell environment and participates in all energy transformations and metabolic reactions. Emulsifying properties of lecithin allow it to provide the optimal chemical composition of bile, inhibit the formation of cholesterol gallstones, dissolve the already formed solid fat deposits on the walls of the gallbladder and in the bile ducts [2, 3].

One of the most important properties of lecithin is the protection of cells from toxicants, which is partially accomplished by inhibiting the processes of lipid peroxidation. Phospholipids, restoring the "wrapping" of polyunsaturated fatty acids in the membrane of hepatocytes, limit access of oxygen to them, thereby reducing the rate of nucleation of free radicals. Lecithin strengthens the walls of the cell membrane of hepatocytes, promotes the regeneration of liver tissue, and also helps it cope with detoxification of the body from poisons and xenobiotics [4, 5].

All these properties determine a wide range of biological and pharmacological effects of lecithin, which makes it important to search for promising approaches to pharmacological correction of liver damage by development of essential phospholipid-based preparations.

The aim of the research is to evaluate the efficacy of the lecithin preparation in the experimental toxic damage of the liver of laboratory animals caused by carbon tetrachloride.

MATERIALS AND METHODS

The experiments were carried out on random-bred laboratory rats with an average body weight of 205.7 ± 3.1 g. The animals that were under quarantine in the vivarium of the Krasnodar Scientific Research Veterinary Institute and did not have apparent disease manifestations were used in the experiment. The rats were kept under similar conditions on a usual diet.

To obtain statistically reliable results, the groups were formed according to the principle of paired analogues. The experiment was carried out in accordance with the principles specified in the Convention for the Protection

Table 1
Content of macro- and micronutrients in rapeseed lecithin

Indicator	Quantity
Phospholipids, g/100 g	62.0
including phosphatidylcholines	16.0
phosphatidylethanolamines	15.5
phosphatidylserines	9.5
phosphatidic acids	9.5
phosphatidylinositols	10.0
Biphosphatidylglycerols	1.5
Polyunsaturated fatty acids, g/100 g	
linoleic acid $C_{18:2}$ (omega-6)	15.2
linolenic acid $C_{18:3}$ (omega-3)	3.1
Ratio of omega-6 : omega-3 fatty acids	5:1
Mineral substances, g/100 g	6.81
Tocopherols (vitamin E), mg/100 g	56.9
including a-tocopherol	14.0
b+g-tocopherols	36.9
d- tocopherol	6.0
Phytosterols, mg/100 g	650
including b-sitosterol (provitamin D)	445
Macro elements, mg/100 g	
potassium	640
magnesium	400
calcium	710
phosphorus	2380
Micro elements, mg/100 g	
iron	5.3
copper	0.2

Table 2
Effect of lecithin on body weight dynamics of laboratory rats experimentally infected with hepatitis ($M \pm m$)

Group	Body weight (g)		
	Background	Day 15	Day 30
Experimental group 1 – CCl_4 + lecithin	205.8 ± 3.3	206.8 ± 2.4*	229.5 ± 1.8**
Experimental group 2– CCl_4	206.5 ± 2.4	199.2 ± 1.1	203.7 ± 2.9
Control (intact)	204.7 ± 3.5	218.7 ± 4.2	231.0 ± 2.5

Differences are reliable (* $p \leq 0,05$; ** $p \leq 0,001$) compared to animals receiving CCl_4 without treatment.

of Vertebrate Animals Used for Experimental or Other Scientific Purposes (Strasbourg, France, 1986).

The study of the efficacy of the lecithin-based drug was performed using the experimental model of acute hepatitis in rats caused by tetrachloromethane, in accordance with the "Methodological guidelines for the study of hepatoprotective activity of pharmacological substances" (Instructions on experimental (preclinical) study of new pharmacological substances, R. U. Khabriev (editor), 2005). Acute toxic liver damage was modeled by a single intraperitoneal injection of carbon tetrachloride (CCl_4) at a dose of 0.3 mg/kg of body weight.

Laboratory rats were divided into three groups with 10 animals in each: the 1st – the experimental group, the 2nd – the positive control and the 3rd – the intact control. The lecithin-based drug was daily administered to animals of group 1 *per os* an hour prior to hepatotoxicant administration and then daily for subsequent 30 days at a dose of 1 ml/kg of animal body weight; animals in group 2 were left untreated after receiving the bait; group 3 consisted of healthy animals that were given vegetable oil in a volume equivalent to that of the hepatoprotector according to a similar scheme.

Efficacy of the hepatoprotective property of the drug was assessed on the basis of the survival rate of laboratory rats, gravimetric body weight, clinical signs, and the degree of change in biochemical syndromes observed in liver lesions: cytolysis was assessed on the basis of serum aminotransferase activity (ASAT, ALT); disturbances in the bile dynamics were evaluated based on the terms of serum alkaline phosphatase (AFP), bilirubin and cholesterol levels.

Blood samples for biochemical analysis were collected from animals in each group on day 15 after the start of the experiment and in 24 hours after the last administration of the lecithin composition. Laboratory studies were carried out on an biochemical analyzer Vitalab Flexor using ELITech Clinical Systems kits.

The object of research is a hepatoprotective drug based on rapeseed lecithin, the composition of which is presented in Table 1.

The statistical processing of the results was carried out using special software packages; the study of quantitative characteristics was conducted by comparing sample means from two populations and determining Student's t-test criteria and significance level (p).

RESULTS AND DISCUSSION

The conducted studies showed that when rats in the first two groups receieved CCl_4, the animals in group 2 (non-treated) showed apparent signs of intoxication on day 2 of the experiment: anorexia, depression, dyspnea, fever. On day 3 the death of one rat was recorded. Wherein in rats of experimental group 1, which were additionally given lecithin, clinical manifestations of intoxication were less pronounced and were recorded later – on day 4 of the experiment at 100% survivability.

Gravimetric studies showed that throughout the experiment rats in group 2 showed loss of body weight, whereas the animal weight in the group where the hepatoprotector was administered, had a positive dynamics, though did not reach the parameters of the intact group at the end of the experimental period. When calculating the percentage increase in body weight of animals in groups 1 and 2, the difference was 12.7% with a high degree of reliability ($p \leq 0.001$) (Table 2).

Biochemistry blood test showed that in case of acute intoxication with carbon tetrachloride there is a significant change in the parameters of the hepatocyte metabolic condition (Table 3).

The administration of CCl_4 to animals caused significant changes in enzyme activity being the markers of the

Table 3
Effect of lecithin on biochemical parameters of blood of laboratory rats experimentally infected with hepatitis ($M \pm m$; $n = 5$)

Parameter	Experimental group 1 CCl_4 + lecithin		Experimental group 2 CCl_4		Control (intact)	
	Day post intoxication					
	15	30	15	30	15	30
Total protein, g/l	88.5 ± 3.25*	81.4 ± 2.28**	93.3 ± 3.06	74.1 ± 3.83	82.4 ± 1.92	83.9 ± 2.11
Мочевина, mMol/L	6.83 ± 0.27	7.12 ± 0.17*	6.64 ± 0.32	3.82 ± 0.41	7.25 ± 0.55	7.38 ± 0.37
AST, Units/L	194.9 ± 7.6**	125.7 ± 6.1***	257.6 ± 5.8	174.3 ± 7.6	95.5 ± 3.5	106.4 ± 5.0
ALT, Units/L	158.4 ± 4.2***	90.8 ± 3.9**	209.6 ± 8.9	125.0 ± 6.2	57.3 ± 4.8	63.5 ± 3.9
ALP, Units/L	703.9 ± 17.0*	648.5 ± 9.7*	711.0 ± 16.4	1107.2 ± 25.3	576.5 ± 13.5	585.0 ± 17.0
Cholesterol, mMol/L	2.13 ± 0.22**	1.25 ± 0.13**	7.83 ± 0.09	5.45 ± 0.18	1.53 ± 0.03	1.48 ± 0.02
Total bilirubin, μM/L	20.7 ± 3.5***	16.3 ± 2.7***	43.7 ± 4.3	30.9 ± 2.9	9.4 ± 1.2	10.5 ± 0.9

Differences are reliable (* $p \le 0.05$; ** $p \le 0.01$; *** $p \le 0.001$) compared to animals receiving CCl_4 without treatment.

functional state of the liver. In group 2 (without treatment) there was a reliable 2.7-fold increase in the activity of AST by the middle of the experiment, and the difference with intact animals was 1.6 times at the end of the experiment. The activity of ALT increased even more: on day 15 – by 3.7 times and on day 30 - by 2 times compared to the data obtained in the control group of rats.

AST activity in group 1 animals that received the lecithin-based drug was 32.2% lower than the levels of the same indicator in group 2 animals by the middle of the experiment ($p \le 0.01$), and 38.7% – by the end of the experiment ($p \le 0.001$), but it exceeded the values obtained in the control group. The ALT levels were similar: on day 15 of observations the difference was 32.3% ($p \le 0.001$) and on day 30 – 37.7% ($p \le 0.01$) compared to group 2 rats, but the indicator value remained higher than in intact animals.

As regards parameters of bile formation, all animals in the experimental groups demonstrated hyperbilirubinemia, wherein the total bilirubin concentration in the blood of the animals of the group without treatment exceeded the levels of group 1 rats by 1.9 times at the end of the experiment. The level of alkaline phosphatase in group 2 increased by 1.89 times at the end of the observations and in group 1 - only by 10.9%. These results indicate presence of a cholestatic syndrome caused by the biliary excretory dysfunction of the liver and bile duct lesions (intrahepatic cholestasis).

Administration of CCl_4 led to protein-synthetic dysfunction of the liver which was confirmed by a 13.2% decrease in the total protein content in group 2 animals as compared with healthy rats. The use of lecithin phospholipids allowed to minimize the development of metabolic disturbances. The difference between groups 1 and 2 was 3.1%.

The results of biochemical studies of blood serum of rats allow us to assert that during intoxication of animals with CCl_4 there is an increase in activity of aminotransferases that indicates a damage to the membranes of hepatocytes, as well as the death of liver cells under the influence of hepatotoxicanth which leads to the release of intracellular substances into the blood and lymph. This process is accompanied by intrahepatic cholestasis and a protein-synthesizing dysfunction of the liver. The use of lecithin phospholipids improves the parameters of biochemical constants of homeostasis and liver functional status of experimental rats against toxic damage.

CONCLUSION

Thus summarizing the study results of the effect of lecithin on experimental liver toxicity of laboratory animals, it can be claimed that it has pronounced hepatoprotective properties that contribute to a reduction in the total toxic, cytolytic and cholestatic manifestations of the damaging effect of the toxicant.

The obtained data can serve the basis for the application of rapeseed lecithins as promising hepatoprotectors of natural origin with the possibility of using them as biologically active additives or active substance of medicinal products intended for treatment of liver diseases in animals.

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