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Hepatoprotective Efficiency of the Preparation Based on Lecithin at Medicinal-Induced Liver Damage in Laboratory Animals.

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ABSTRACT

The article presents the results of studying of the efficiency of lecithin-containing preparation esvelan at experimental medicinal-induced liver damage in laboratory animals, which was caused by intragastric administration of tetracycline hydrochloride with the use of hepatoprotector in different doses. The data obtained in the model experiment revealed that during intoxication with tetracycline in rats, an increase in the activity of aminotransferases, indicating damage of the membranes of hepatocytes, is observed, intrahepatic cholestasis develops and the synthetic protein function of liver is disrupted. The use of esvelan improves biochemical homeostasis as well as functional and structural state of the liver of rats on the background of toxic damage.

Keywords: veterinary pharmacology, hepatoprotectors, lecithin, liver, laboratory animals

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INTRODUCTION

The problem of medicinal hepatotoxicity is currently particularly relevant, taking into account the tendency for a constant increase in the number of registered side effects in various pharmaceutical preparations. Most often, medicinal-induced liver damage is associated with antimicrobial and non-steroidal anti-inflammatory preparations. Substances that have a negative effect on the hepatobiliary system and the ability to block the hepatocyte enzyme system include antibiotics of the class of polyketides - tetracyclines [2, 7].

Accordingly, tetracycline liver damage in animals is widely used in pharmacological experiments in the study of preparations of the hepatoprotective profile.

Over the past years, in case of hepatopathies of various origins a positive experience has been accumulated in the study and application of preparations, which include lecithins. Lecithins – is the name of a group of lipoid substances, representing a mixture of phospholipids (65-75%) with triglycerides. One of the most important properties of lecithin is the protection of cells from toxicants that is partially realized through inhibition of lipid peroxidation processes. Phospholipids, restoring the structure of polyunsaturated fatty acids in the membrane of hepatocytes, reduce the 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 to cope with the detoxification of the body from xenobiotics [3, 6].

The aim of the research was to evaluate the effectiveness of lecithin-containing preparation esvelan at experimental medicinal-induced liver damage in laboratory animals.

METHODOLOGY OF RESEARCH

The object of the research is the complex hepatoprotective preparation esvelan, which in 1 ml contains: lecithin (equivalent to the PPh fraction) – 100 mg; silymarin (equivalent to silibinin) – 20 mg; methionine – 30 mg; dihydroquercetin – 2 mg; excipients – the rest.

In the study of the pharmacological properties of esvelan, experimental liver damage in animals was caused by intragastric administration of tetracycline hydrochloride as a suspension with Tween-80 (1:10) at a dose of 0.5 g/kg of body weight daily for 7 days to laboratory rats [5]. The experiments were carried out on outbred rats, which were divided into five groups of 10 animals each.

When poisoning, the rats of the first three groups one hour before the use of the antibiotic and for the next 7 days got esvelan orally in different doses, the scheme of the experiment is presented in Table 1.

Table 1 – Scheme of the experiment on the study of the pharmacological properties of esvelan at experimental liver damage in animals (M±m; n=10)

Groups	Experimental conditions
1 – experimental	tetracycline + esvelan at a dose of 0.3 ml/kg of body weight
2 – experimental	tetracycline + esvelan at a dose of 0.5 ml/kg of body weight
3 – experimental	tetracycline + esvelan at a dose of 0.7 ml/kg of body weight
4 – control	animals with liver damage with tetracycline, without treatment
5 – intact group	healthy animals, the parameters of the blood system and metabolism of which have been studied to calculate the “normal values”

During the experiment, a daily clinical examination of animals was made; the weighing was carried out 3 times – background one, in the middle and at the end of the experiment.

One day after the final administration of the hepatoprotector (the 15th day of the experiment), five animals from each group were sampled for blood for laboratory analysis. The degree of liver damage was evaluated by the activity of enzymes (AST – aspartate aminotransferase, ALT – alanine aminotransferase and ALP – alkaline phosphatase) in the blood serum, taking into account changes in the content of protein and

carbohydrate metabolism, as well as indicators of lipid peroxidation products. Laboratory tests were conducted on the Vitalab Flexor biochemical analyzer using the ELITech Clinical Systems kits.

The level of lipid peroxidation (LP) was determined by a number of indicators — diene conjugates (DC), ketodienes (KD) and malondialdehyde (MDA) in the thiobarbituric acid test using the method of Andreeva L.I. (1988).

Statistical data processing was performed using the Statistica v. 6. The significance criteria were determined according to the Student's table.

RESULTS

As a result of the experiments it was determined that the death of animals was not registered in any group. In the control group of rats (without treatment) the following manifestations of toxicosis were revealed during the clinical examination: depression during the second half of the experimental period; decreased motor activity and reactions to external stimuli; ruffled appearance, dullness and pollution of fur; visible mucous membranes with a weak degree of icterus. Water consumption was in the normal range, feed consumption was reduced.

A clinical examination of the rats of the first three experimental groups (with hepatoprotector) revealed the following: the animals were a bit depressed; feed and water consumption was not reduced; the fur was matt, clean, without alopecia, with visible pink mucous membranes. Specific symptoms of poisoning were not revealed.

The dynamics of body weight of laboratory rats participating in the experiment are presented in Table 2.

Table 2 – The dynamics of body weight of rats in the study of the preparation esvelan at experimental liver damage (M±m; n=10)

Group	Body weight, g		
	initial	on the 7th day	on the 14th day
1 – experimental	197.3±2.18	189.0±2.53	186.9±1.84
2 – experimental	201.4±3.26	193.0±2.55	190.6±1.77
3 – experimental	189.7±1.49	184.8±2.42	181.1±3.54
4 – control	192.6±3.44	181.5±3.15*	168.5±2.57**
5 – intact group	186.7±2.56	192.5±1.53	194.7±2.86

Note: the differences are significant (*p≤0.05; **p≥0.01) in comparison with the animals of the intact group

Gravimetric studies showed that in rats of the fourth group (receiving only tetracycline) the loss of body weight was recorded throughout the experiment (a significant difference at the end of the experiment was 14.3%), whereas in the groups with the use of esvelan, although the body weight of rats decreased by the end of the experimental period, the changes were minor. When calculating percentages, the difference in body weight between the background data and the indicators at the end of the experiment in average over the groups was: in the 1st experimental group – 5.7%; in the 2nd experimental group – 5.6%; in the 3rd experimental group – 4.7%. In the group of intact animals a positive growth of body weight was determined with an increase of weight of 4.3%.

Simulation of toxic liver damage in rats, caused by the administration of tetracycline, led to the development of hepatocyte destruction. Thus, in the activity of excretory (ALP) and indicator (AST, ALT) enzyme markers of the state of liver an increase in the concentration of these enzymes indicating liver damage was revealed (Table 3).

Table 3 – Influence of esvelan on the activity of blood enzymes in rats at experimental liver damage (M±m; n=5)

Group	Indictor, U / l		
	ALP	AST	ALT
1 – experimental	428.2±11.7	118.7±3.9	40.3±2.4*
2 – experimental	421.2±9.9	111.4±4.5*	37.4±3.1**
3 – experimental	418.3±8.5	109.4±6.3**	39.0±2.5*
4 – control	470.2±16.3	139.8±5.7	53.7±3.6
5 – intact group	390.5±15.6	96.8±4.2	29.6±2.8

Note: the differences are significant (*p≤0.05; **p≥0.001) in comparison with the animals of the 4th group

In the level of enzymes giving the evidence of the development of cytolysis, in the group of rats without treatment an increase in the activity of AST by 1.5 times and an increase in the activity of ALT by 1.8 times relative to intact animals was revealed. In the experimental groups with the treatment with esvelan the difference in the content of enzymes relative to the control (4th group) was: for AST – 1st group by 17.8%, 2nd group by 25.5% (p≤0.05) and 3rd group by 27.8% (p≥0.001); for ALT – 1st group by 33.2% (p≤0.05), 2nd group by 43.6% (p≥0.001) and 3rd group by 37.7% (p≤0.05). Thus, in general in groups, in animals with experimental medicinal-induced liver damage was revealed the ALT dominant that belongs to cytoplasmic enzymes and its level increases in mild forms of hepatocytes damage.

Increased activity of ALP in the experimental groups by 9.6% (1st group), by 7.7% (2nd group) and by 7.1% (3rd group) relative to the intact group indicates a slight development of cholestatic disorders in animals whereas in the control group without treatment the difference was 20.4%.

The study of one of the key indicators of pigment metabolism – blood bilirubin, which is quite informative in relation to the functional state of liver, allowed us to determine a significant increase in the content of this compound in rats with experimental liver damage. Thus, the concentration of total bilirubin blood serum of rats treated with tetracycline in a toxic dose at the end of the experiment was significantly higher than the data of intact animals: 1st group 1 – in 1.54 times (p≥0.01); 2nd group – in 1.4 times (p≤0.05); 3rd group – in 1.48 times (p≤0.05); 4th group – in 2.1 times (p≥0.001). The maximum difference between the group with the treatment and the group without the use of hepatoprotectors was 56.3% that indicates the hepatoprotective properties of esvelan.

The results of studies of a number of parameters of protein metabolism indicate violations of protein metabolism in rats with experimental liver damage (Table 4).

Table 4 – Influence of esvelan on protein and carbohydrate metabolism in rats at experimental liver damage (M±m; n=5)

Group	Indicator		
	Total protein, g/l	Urea, mmol/l	Glucose, mmol/l
1 – experimental	68.9±1.23*	7.86±0.34	5.38±0.19
2 – experimental	70.3±0.98*	7.95±0.25	5.76±0.37
3 – experimental	69.7±0.17**	8.11±0.13*	5.42±0.21
4 – control	63.8±2.14**	6.97±0.36**	4.95±0.48
5 – intact group	73.6±1.95	9.15±0.27	5.99±0.32

Note: the differences are significant (*p≤0.05; **p≥0.001) in comparison with animals of the intact group

By the end of the experiment, in the blood serum of animals of 1-4 groups the total protein content was lower by 6.8%, 4.7%, 5.6% and 15.4%, respectively in groups, compared with the intact group of rats. Similar changes were recorded in the concentration of urea, with a maximum decrease by 31.2% in the group without treatment. The significant difference in carbohydrate metabolism between groups was not deter-

mined. The above data allow us to state that with the toxic influence of antibiotics on the organism of experimental rats, animals showed a pronounced impairment of the protein-synthetic function of liver, whereas the preventive effects of the hepatoprotector significantly reduced the pathological processes in hepatocytes.

The metabolic disorders of the organism of the experimental animals, accompanying by the development of liver damage, led to the activation of lipid peroxidation processes, which resulted in an increase in the concentration of lipid peroxidation products (Table 5).

Table 5 – Influence of esvelan on the concentration of lipid peroxidation products in rats at experimental liver damage (M±m; n=5)

Group	Indicator		
	$DC_{(232)}$, AU	$KD_{(273)}$, AU	$MDA_{(537)}$, µM/l
1 – experimental	0.18±0.01**	0.18±0.04	2.48±0.15*
2 – experimental	0.16±0.03*	0.16±0.01*	2.34±0.24*
3 – experimental	0.15±0.05***	0.15±0.05**	2.37±0.11**
4 – control	0.22±0.02	0.19±0.03	3.23±0.26
5 – intact group	0.14±0.01	0.12± 0.02	1.58±0.19

Note: the differences are significant (*p≤0.05; **p≥0.01; ***p≥0.001) in comparison with the animals of the 4th group

The analysis of the obtained data indicates that the preparation esvelan has antioxidant properties, which was manifested in a decrease in the concentration of lipid peroxidation products in the experimental groups in comparison with rats without treatment: for DC – 1st group – by 22.2% (p≥0.01), 2nd group – by 37.5% (p≤0.05) and 3rd group – by 46.7% (p≥0.001); for KD – 1st group – by 5.6%, 2nd group – by 18.7% (p≤0.05) and 3rd group – by 26.7% (p≥0.01); for MDA – 1st group – by 30.2% (p≤0.05), 2nd group – by 38% (p≤0.05) and 3rd group – by 36.3% (p≥0.01) .

CONCLUSION

An analysis of the scientific literature revealed that a large number of scientific publications are devoted to the problem of the pathological effects of various pharmaceutical preparations on liver. In this regard, it seemed to us relevant to experimentally substantiate the efficiency of using lecithin-containing preparation esvelan at medicinal-induced liver damage in laboratory animals.

The obtained results allow us to state that at intoxication with tetracycline in rats, there is an increase in the activity of aminotransferases, indicating damage of the membranes of hepatocytes, as well as the death of liver cells under the action of an antibiotic, leading to the release of intracellular substances into the blood and lymph. This process is accompanied by intrahepatic cholestasis and impaired protein synthetic liver function. The use of hepatoprotector esvelan improves the parameters of biochemical constants of homeostasis and the functional state of liver of the experimental rats on the background of toxic damage.

The data obtained in the model experiment on the high antioxidant activity of the studied preparation are in agreement with the existing ideas about the mechanisms of the antioxidant action of components of esvelan, such as phospholipids, silymarin and dihydroquercetin [1, 4, 8].

Thus, the use of hepatoprotector esvelan in different doses for medicinal-induced liver damage with tetracycline in rats was accompanied by inhibition of free radical oxidation processes, normalization of biochemical parameters while increasing the stability of the structures of the damaged organ.

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