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EXPERIMENTAL PHYTOCORRECTION OF ACUTE D-GALACTOSAMINE HEPATITIS IN WHITE RATS

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ABSTRACT

The aim of our research was in defining hepatoprotective properties of new complex plant supplement on a rat model of D-galactosamine hepatitis. The new complex drug supplement is a dry extract consisting of Hypecoum erectus L., Hedysarum dauricum, Glycyrhhiza uralensis Fisch., Calendula officinalis and Scutellaria baicalensis. Intensity of the main pathogenetic syndromes was evaluated by biochemical tests, lipid peroxidation grade and morphological research. The use for the complex extract resulted in correction of functional state of the liver, inhibition of cytolysis and cholestasis, delay of LPO and enhancing synthetic function of liver manifested in albumin and fibrinogen increase.

Keywords: acute experimental hepatitis, hepatoprotective drugs, complex drug of medicinal plants.

Introduction. A necessity in finding new hepatoprotective drugs and supplements is dictated by the growing demand: increasingly widespread liver pathologies tending to chronic forms being caused by viruses, toxic agents including some medication [8, 9, 10]. Under these conditions, medicinal plants are of interest considering the wide range of their therapeutic effect, low toxicity and the possibility of gaining amplified effect by combining active components of complex plant supplements [1- 4].

The aim was in determining pharmacotherapeutical effectiveness of new complex medicinal supplement on a rat model of acute D-galactosamine toxic hepatitits.

Materials and methods of research. The researched plant supplement is a dry extract consisting of dry extracts of *Hypecoum erectum* L.; Papaveraceae grass, dry extract from *Hedysarum alpinum* L.; Leguminosae grass, dry extract of *Glycyrrhiza uralensis* Fisch.; Leguminosae roots, dried flower extract of *Calendula officinalis* L.; Compositae and dry root extract of *Scutellaria baicalensis* Georgi; Lamiaceae in proportion of 5:5:4:4:2.

Compounded preparations were performed using HPLC with UV-detector on MiLiChrom A-02 by ECONOVA (Милихром А-02, Эконова), Novosibirsk, Russia with column ProntoSIL-120-5-C18 AQ (2 × 75 mm, ∅ 5 μm; Metrohm AG, Herisau, Switzerland); mobile phase: 0.2 M $LiClO_4$ with 0.006 M $HClO_4$ (A), MeCN (B). During the separation process profile of gradient elution was set to 0–40` 5–100% B, 40–43` 100% B with speed of 100 μl/min, temperature set to 35°C with UV-detection at a wavelength of 210 nm. Concentration of substances was measured with commercially available samples (Sigma-Aldrich). Detected components included: glycyrrhizic acid 2.06 ± 0.04%, baikalin 1.85 ± 0.04%, protopin 1.09 ± 0.03%, mangipyrin 0.68 ± 0.02%, typhaneoside and narcissin combined 0.27 ± 0.01%.

The pharmacotherapeutical effective-

ness of complex plant supplement was studied after inducing acute hepatitis in rats by D-galactosamine infusion. The chosen model of acute liver damage is known to be close to viral hepatitis in its morphological and biochemical features [5].

D-galactosamine was injected intraperitoneally in dose of 400 mg/kg [5]. The researched supplement was injected intragastrically in doses 100 (group 1), 200 (group 2), 300 (group 3) mg/kg one hour before D-galactosamine injection and then daily for 14 days. *Carsil (Silibinin)* obtained from the milk thistle plant *Silybum marianum (L.) Gaertn.* was used as a reference agent in a dose of 100 mg/kg daily likewise. Control group of white rats was injected with D-galactosamine but instead of pharmacological agents received an equal volume of distilled water. Intact group received only distilled water.

Functional state of the liver was evaluated by biochemical markers: Alanine aminotransferase (ALT) and aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGTP), cholesterol, total protein (reagents “Abris” and “Vital” on analyser “VitaRay”). Lipid peroxidation intensity was determined with Malondialdehyde (MDA) and conjugated dienes in serum [7]. In order to analyze the morphofunctional state of liver a set of histological, histochemical and histoensymological methods was applied [5].

The pharmacotherapeutical effectiveness of complex plant supplement was studied on 7th, 14th and 21st days of experiment. Data were analyzed using MedCalc version 18.5 statistical software [10]. Values were considered statistically significant when P<0.05.

Results and discussion. The use of D-galactosamine model did not cause acute lethality in experimental animals. General condition, reaction and moveability, appetite of the rats remained intact. There was no weight loss during first 7 days of the experiment in D-galactosamine groups while the animals of the intact group have gained 20-25 g.

Biochemical tests performed on D-galactosamine-exposed groups demonstrated drastic changes of the functional state of the liver (Table 1). A significant deviation of biochemical parameters was observed in the groups of rats with D-galactosamine-induced liver toxicity pointing to the development of the main pathogenic syndromes of liver damage: cytolysis, cholestasis, mesenchymal inflammatory reaction combined with hypoalbuminemia and hypocoagulation. The intensity of lipid peroxidation was evaluated by malondialdehyde and diene conjugates concentration in blood serum of the experimental animals [9] wherein in the experimental groups signs of lipid peroxidation were significant.

On the 7th day of the experiment in the control group of the animals activity of AST and ALT was amplified by 3,1-3,3 in comparison with the intact animals testifying to the expressed cytolysis that had developed from an early date. Albumin concentration was decreased by 14,9% (down to 31,4-34,0 g/l in 6 rats of the control group) reflecting remarkable change in the functional state of hepatocytes while the level of total proteins remained on the same levels due to globulin fraction growth. Furthermore, level of fibrinogen and prothrombin index dropped by 18,9% and more (Table 1).

In the comparison group (Carsil) on the according dates ALT and AST decrease was 7,32-10,25% . In the groups of rats receiving the studied plant supplement there was a more pronounced decrease of the activity of aminotransferases.

The degree of cholestasis severity was measured by compex evaluation of cholesterol concentration, ALP activity and GGTP in blood serum. As it is known, lipid metabolism values in rats’ serum are lower than in human serum due to the functions of α-muricholic, and β-muricholic acids that force cholesterol metabolism [9]. However, despite that fact in the experiment cholesterol level was still slightly growing because of the liver damage. In the comparison group on the 7th day of the trial ALP activity and GGTP level dropped by 9-10%. As a result of using the complex drug supplement a decrease in cholesterol levels was more compelling than a decrease of it in the comparison group (Tab. 1). Additionally a decrease by 17,5-18,5% of ALP and GGPT was demonstrated in the tested drug supplement group. Furthermore in the group of the drug supplement in dose 200 mg/kg demonstrated more substantial decrease in ALP, GGTP and cholesterol levels (in 6 out of 8 animals). In the given period of the experiment after injection with the studied supplement relevant supression of peroxidation intensity manifested in a decrease of malondialdehyde and diene conjugates concentration by 18-24%

Pathomorphological study showed that in liver of animals from the control group on the 7th day of the observation after injection with D-galactosamine

Table 1

Blood serum biochemical markers dynamics in D-galactosamine induced liver damage on rat model on the 7th day of the experiment (M±m, n=8)

Markers	Intact group	Groups with D-galactosamine induced liver damage				
		Control+ H2O	Comparison group + Carsil	Group 1 + 100 mg/kg complex supplement	Group 2 + 100 mg/kg complex supplement	Group 3 + 100 mg/kg complex supplement
ALT, U/l	58.6±5.4	152.9±9.2	141.7±9.7	132.6±8.2	123.5±7.9*	125.4±6.9
AST, U/l	73.5±4.7	242.7±10.2	217.8±11.4	205.7±9.1*	198.5±11.3*	203.3 ±9.2*
Cholesterol, µmol/l	1.82±0.13	3.52±0.19	3.05±0.12	2.97±0.13	2.63±0.14*	2.90±0.12
ALP, U/l	308±21	742 ±41	667±43	612.0±27	604.7±25*	619±21*
GGTP, U/l	7.13±0.31	23.94±2.10	21.83±1.34	19.45±0.89*	19.75±0.94*	18.38±1.25
Albumin, g/l	42.5±2.2	33.5±1.4	37.3±2.1	38.5±1.3*	39.2±1.2*	38.9±1.7*
Total protein, g/l	72.9±3.9	64.3±2.4	64.5±4.1	65.7±3.8	68.7±3.9	66.7±2.1
Globulin, g/l	30.4±2.9	30.8±1.7	27.2±2.6	27.2±2.1	29.5±1.9	27.8±2.1
Fibrinogen, g/l	2.43±0.13	1.51±0.11	1.82±0.19	1.97±0.12*	2.03±0.14*	1.99±0.11*
Prothrombin index (PI) %	84.4±4.7	50.3±2.4*	57.1±3.7	59.9±2.9*	64.7±3.8*	63.8±3.4*

Note. In the Tables 1-2 * - the differences are statistically significant between the control and experimental groups at P <0.05; n is the number of animals in the group

changes, characterized by dystrophic and necrobiotic transformation of hepatocytes, mesenchymal-inflammatory effect coupled with activation of macrophages, accumulation of lymphocytes in portal tracts and deterioration of bile tract cells were present. The use of the complex drug supplement in doses from 100 mg/kg to 300 mg/kg and the comparison drug Carsil had limited the grade of necrobiotic processes and mesenchymal-inflammatory effects induced by D-galactosamine. Thus, in the observation group on the 7th day moderate granular degeneration of hepatocytes prevailed. In the group of dose 100 mg/kg hepatocytes affected by hydropic dystrophy accounted for 2/3 of periportal lobe while in other drug supplement groups fractions of transformed hepatocyres were scarce and situated periportal. The data of morphometric research demonstrated that number of necrotic hepatocytes was lower in 1,9 times in average (compared to the data of the control group).

On the 14th day of the experiment after D-galactosamine-induced damage the animals demonstrated weight loss in comparison with the intact group by 7-10%, lowered appetite and moveability. Biochemical parameters reflected persistence of the main features of liver damage in experimental animals. After injection with Carsil a remarkable decrease of amynotranispherases was observed.

During the second week of the experiment signs of cholestasis were still apparent. As a result of injection with the complex drug supplement ALP, GGTP and cholesterol were lower than that of the comparison group: the group of the studied supplement demonstrated decrease in ALP by 15,2% in dose 100 mg/kg and by 17,84% in the group of the dose 200 mg/kg while in Carsil group ALP was decreasing by 9,16%. On the 14-th day of the experiment hypoalbuminemia in the groups of the studied supplement was significantly less expressed than in the control gruop while coagulation parameters were normalizing as a result of increased synthetic ability of the liver. The grade of lipid peroxidation was decreasing.

On the 14-th day of the observation in animals' liver of the control group the expression of structural changes decreased in comparison to the previous control point. Dystrophic hepatocytes were found locally but not diffusely. In three specimens of the control group moderate hydropic dystrophy was still observed. In liver of the animals from experimental groups was observed moderate expansion of sinusoidal spaces, local venocapillary erythrostasis, increased blood filling of some vessels and granular degeneration of hepatocytes. Hepatocytes with lipid degeneration were scarce in the several portal tracts. In the results of morphometric research the number of fatty transformed hepatocypes was 5 times lower than that of the control group. As a result of delayed destruction of hepatocytes in the experimental groups significant signs of reparation were observed. Thus, in the group of studied supplement injected in dose 100 mg/kg and in the comparison group was found increased number of hypertrophic hepatocytes and binucleate hepatocytes (by 22%), while in groups with doses 200 mg/kg and 300 mg/kg the increase was 31% in comparison with the control group.

On the 3rd week of the experiment in the control group deviation of biochemical markers was less evident than that of the intact animals with ALT and AST close to those of the intact group. At the same time in the groups treated with the studied plant supplement relevant decrease in cholestasis was observed. The pathomorphological study of the specimens taken on the 3rd week of the trial showed no insignificant deviations.

Conclusion. After experimental D-galactosamine induced liver damage use of the comparison drug influenced the pathological processes by decreasing cytolysis and cholestasis on the 2nd week of the experiment while levels of albumin and globulin and parameters of coagulation remained constant. The use of the studied complex plant supplement had led to decrease of biochemical markers deviations. The grade of cytolysis in the experimental group №2 (dose of the complex drug supplement 200mg/kg) was significantly lower starting from the first control point (7 days after the beginning of the experiment) with ALT and AST dropping by 15-18% compared to the comparison drug at 7-10%. Based on the complex evaluation of cholesterol, ALP and GGTP levels the experiment had shown that the use of the drug supplement leads to significant growth of albumin, fibrinogen and prothrombin index levels evident of the improvements in synthetic function of the liver starting from the first point of the control. The use of the studied plant supplement contributed to a decrease in lipid peroxidation, inhibited the accumulation of malonialdehyde and diene conjugates with significant differences observed in the early periods of the experiment, on

Table 2

Blood serum biochemical markers dynamics in D-galactosamine induced liver damage on rat model on the 14th day of the experiment (M±m, n=8)

Markers	Intact group	Groups with D-galactosamine induced liver damage				
		Control+ H2O	Comparison group + Carsil	Group 1 + 100 mg/kg complex supplement	Group 2 + 100 mg/kg complex supplement	Group 3 + 100 mg/kg complex supplement
ALT, U/l	58.6±5.4	128.2±6.9	116.7±5.3*	103.6±5.4*	104.1±5.8*	105.1±4.9*
AST, U/l	73.5±4.7	207.0±9.4	189.7±7.1*	172.9±8.7*	169.9±8.3*	170.5±8.2*
Cholesterol, µmol/l	1.82 ±0.13	2.97±0.16	2.52±0.11*	2.44±0.15*	2.41±0.14*	2.43±0.15*
ALP, U/l	308±21	585.5±25	532.4±17*	496.5±18*	481.5±24*	497.7±17*
GGTP, U/l	7.43±0.31	18.82±0.8	16.31±0.7*	15.8±0.82*	15.43±0.72*	15.15±0.76*
Albumin, g/l	42.5±2.2	35.3±1.7	38.8±1.9	40.9±1.2*	40.5±1.1*	41.8±1.3*
Total protein, g/l	71.8 ±3.9	66.2±2.9	67.3±4.7	68.8±3.8	71.8±5.6	70.9±3.7
Globulin, g/l	29.34±2.9	30.9±3.7	28.5±2.8	27.9±2.1	31.3±1.4	29.1±2.1
Fibrinogen, g/l	1.93±0.11	1.97±0.11	2.05±0.10	2.51±0.12*	2.47±0.13*	2.53±0.12*
Prothrombin index (PI) %	84.4±4.7	50.3±2.4	57.1±3.7	59.9±2.9*	64.7±3.8*	63.8±3.4*

the 7th and 14th days of the observation.

Combined with ability to inhibit lipid peroxidation and apparent limitation of dystrophic and necrobiotic signs and lowered inflammatory intensity, improved reparation was observed. Consequently, the researched complex drug supplement demonstrated pronounced hepatoprotective effect in D-galactosamine-induced liver damage. Dose elevation to 200 and 300 mg/kg did not lead to the significant improvement.

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