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A Hepatoprotective Influence of Ecdisten Under Conditions of Alcohol Intoxication In Rats

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ABSTRACT

The preparation of ecdisten, created on the basis of a natural compound – the phytoecdysteroid ecdysterone, isolated from *Rhaponticum carthamoides* and *Ajuga turkestanica*, has been tested as a hepatoprotective agent in rats with alcohol intoxication. It was found that ecdisten effectively eliminates the symptoms of hepatocyte cytolysis and cholestasis, normalizes the glycogen-synthesizing function of the liver, maintains homeostasis of energy production, reduces hypoproteinemia, inhibits lipid peroxidation, inhibits liver fatty degeneration, and has a beneficial effect on the ratio of phospholipid fractions. Ecdisten normalizes bile secretion processes in conditions of alcoholic hepatitis, optimizes the chemical composition of bile.

Keywords

Ecdisten, experimental alcoholic hepatitis, pharmacotherapeutic action.

Introduction

In recent years, the search for new effective agents, contributing to the elimination of the effect to the organism of various toxic factors becomes important. This especially applies to identifying means, eliminating the consequences of excessive alcohol exposure. This problem seems relevant due to the fact that alcohol intoxication of the organism, especially of a long-term nature, is often accompanied by serious disorders in the hepatobiliary system [1, 2]. We have previously shown that promising substances in this regard can be various compounds isolated from local plant materials: flavonoids and polyprenols [3, 4, 5]. Continuing research in this direction, the herbal preparation Ecdysten was studied under appropriate conditions, created at the Institute of Chemistry of Plant Substances of the Academy of Sciences of Uzbekistan on the basis of the phytoecdysteroid - ecdysterone, isolated from *Rhaponticum carthamoides (Willd.), Iljin* and *Ajuga turklestanica (Rgl.)* [6]. This preparation activates protein-synthesizing processes in the organism, optimizes the course of reactions of carbohydrate and lipid metabolism, exhibits a general strengthening and adaptogenic effect in many pathological conditions, including some forms of liver damage [6, 7]. All of this served as the basis for an experimental assessment of the possibility of using ecdisten also as a pharmacy-corrective agent for the disturbed metabolic-functional state of the hepatobiliary system during alcohol intoxication of the organism.

The aim of the research: To evaluate the possible hepatoprotective effect of ecdisten in alcohol intoxication in an experiment to laboratory animals.

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Methods

Materials and methods: male rats, weighing 180-200 g were used in the experiment. All animals were kept in stationary vivarium conditions on a normal diet with free access to water. Experiments with them were carried out adhering to the general principles of ethics in accordance with the requirements of the "European Convention for the Protection of Vertebrate Animals Used for Scientific Purposes" (Strasbourg, 1986). Alcoholic hepatitis in rats was caused by the introduction through a tube into the stomach of 40% ethanol, 0.7 ml. per 100 g of body weight once a day for 21 days. Ecdisten (5 mg / kg) was administered orally during the entire observation period, starting from the first day of the experiment (6 hours after the introduction of ethanol). On the 14^{th} - 21^{st} days, some of the animals were subjected to instant decapitation. The pharmacotherapeutic efficiency of ecdisten as a hepatoprotective agent in alcohol intoxication was judged by the content of total protein in the blood serum (determined by refractometric), the activity of alanine aminotransferase, aspartate aminotransferase [8] and alkaline phosphatase [9], as well as total cholestine [10].
The content of malondialdehyde [11], glycogen [12], adenine nucleotides [13], and total lipids [14] were determined in the liver tissue. The main fractions of phospholipids were obtained by separation of the lipid extract by thin layer chromatography on silica gel [15]. In another part of the animals under barbamil anesthesia (1% solution intraperitoneal at a dose of 1 ml per 100 g of body weight) through a catheter inserted into the common bile duct, bile was collected for 4 hours, in which the concentration of bile acids was determined [16] , cholesterol [17] and bilirubin [18]. Based on the obtained data, the cholato-cholesterol ratio was calculated.
The results were statistically processed using the Student's t-test.

Results

As can be seen from the presented data in the table, the introduction of ethanol to rats led to serious violations of the metabolic-functional state of the liver. The development of hepatocyte cytolysis syndrome was indicated by an increase in the activity of serum enzymes alanine aminotransferase (on the 14^{th} day at 154.3% and on the 21^{st} day by 282.6%) and aspartate aminotransferase (on the 14^{th} day at 28, 6 and 21^{st} day by 67.5%). The development of the same phenomena of cholestasis was evidenced by an increase in these periods of alkaline phosphatase by 168.9 and 197.3%, as well as cholesterol by 101.7 and 107.6%. All these changes occurred against the background of a significant decrease in the total protein content in the blood serum (table). Directly in the liver tissue, a pronounced fatty infiltration was revealed (the lipid content in the liver increased on the 14^{th} and 21^{st} day of observation by 23.3 and 52.2%), a significant activation of lipid peroxidation processes – the content of malondialdehyde increased on the days of observation by 133.3 and 153.8%. The accumulation of lipid peroxidation products has a labializing effect on lysosomes, which leads to the release of phospholipase A [19]. This, apparently, was one of the main factors of the imbalance found in the experiments in the content of individual phospholipid fractions, characterized by a sharp increase in lysophospholipids (Fig.1). The latter are extremely toxic products leading to the destruction of hepatocyte membranes, which, of course, is accompanied by a violation of their functional state. In addition, alcohol intoxication was accompanied by inhibition of the glycogen-synthesizing function of the liver and a significant deterioration in the state of the energy metabolism of hepatocytes (table 1). The secretion of

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bile was suppressed, the content of bile acids, cholesterol and bilirubin in it decreased (table 1).

In the same case, when rats were injected with ecdisten along with ethyl alcohol, pathological changes in the parameters under consideration were not so significant or did not differ at all from the norm. So, on the 14th and 21st day of observation, the activity of alanine aminotransferase in the experiment was lower than the control values in terms of observation periods of 41.1 and 72.2%, and the activity of aspartate aminotransferase by 15.2 and 38.8%. The activity of alkaline phosphatase was 45.2 and 63.2% lower than the control, and the serum cholesterol level under the action of ecdisten was only 29.2% in the first observation period, and 2.6% higher in the second than in inactive animals (below control by 35.9 and 50.6%).

Also, in animals that were injected with ecdisten, such a pronounced fatty liver infiltration was not observed. The content of total lipids on the 14th and 21st days of observation was below to corresponding control by 14.1 and 30.8% (table). It should be noted that in animals that received ecdisten, activation of lipid peroxidation was not observed as sharp as in the control. The content of malondialdehyde in the liver of experimental rats after 2 and 3 weeks of observation was only 16.7 and 7.7% higher than the corresponding indicator in intact animals. Providing an antioxidant effect, ecdisten prevented the development of disorders in phospholipid metabolism, first of all, it significantly inhibits the formation of lysophospholipid fractions, which have a detergent effect on liver tissue. The content of lysophosphatidylcholine, lysophosphatidylethanolamine, lysocardiolipin and lysophosphatidic acids was 36.0 after 2 weeks of ecdisten administration; 22.0; 43.0 and 18.0%, and after 3 weeks at 61.04; 42.0; 54.0 and 20.0% lower than the corresponding control (and at the last observation period, these indicators in experimental and intact animals practically did not differ).

Attention is also drawn to the fact that ecdisten, especially by the 3rd week of administration, significantly prevented the decrease in the biosynthesis of phosphatidylcholine and phosphatidylethanolamine, which play an important role in maintaining the normal functioning of cell membranes (Fig.1.). The ability of ecdisten to maintain the metabolism of hepatocytes at a high level under conditions of alcohol intoxication was also evidenced by its glycogen-saving action, restoration of the ratio of high-energy phosphorus compounds to the values of intact animals, and elimination of hypoproteinemia.

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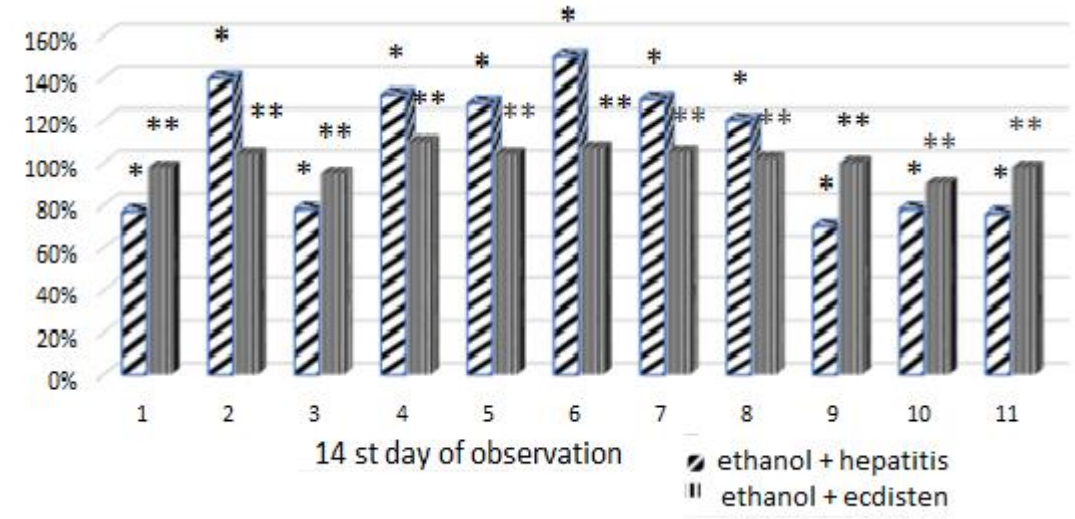
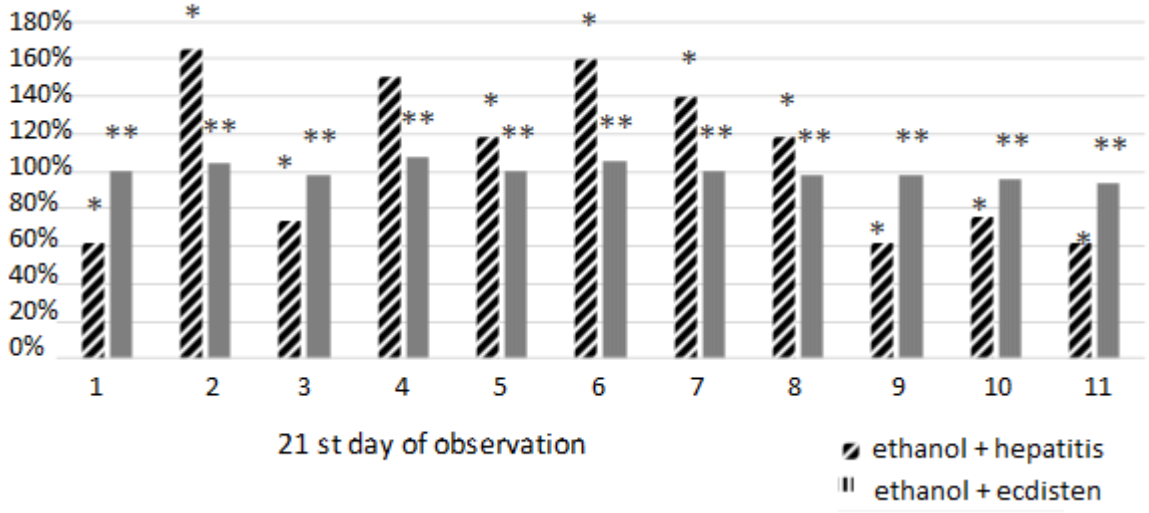


A.



B.

Fig.1. A and B. Changes in the phospholipid spectrum of the liver in rats with ethanol hepatitis (bars with oblique shading) and treated with ecdisten (bars with straight shading) against the background of ethanol in % to the corresponding indicators of intact animals: 1-phosphatidylcholine; 2-lysophosphatidyl choline; 3-phosphatidylethanolamine; 4-lysophosphatidylethanolamine; 5-cardiolipin; 6-lysocardiolipin; 7- phosphatidic acid; 8-lysophosphatidic acid; 9-phosphatidylserine; 10-phosphatidylipositol; 11-sphingomyelin.

* - Reliable to indicators of intact animals; ** - in relation to the corresponding control ($p < 0.05$).

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Table 1

The influence of ecdisten on some parameters reflecting the metabolic-functional state of the liver during the development of ethanol hepatitis in rats (M ± m, n = 6)

Experimental conditions	Intact animals	Observation days			
		14 st		21 st	
		control (ethanol)	ethanol + ecdisten	control (ethanol)	ethanol + ecdisten
Blood serum					
Alanine aminotransferase, mM pyruvic acid / ml / hour	0.92±0.06	2.34±0.16[1]	1.38±0.12[1,2]	3.52±0.24[1]	0.98±0.08[2]
Aspartate aminotransferase, mM pyruvic acid / ml / hour	1.54±0.08	1.98±0.09[1]	1.68±0.04[2]	2.58±0.20[1]	1.58±0.03[2]
Alkaline phosphatase, U / l	148.0±14.2	398.0±22.6[1]	218.0±12.2 [1,2]	440.0±24.2[1]	162.0±10.2[2]
Protein, g%	7.3±0.16	5.8±0.14[1]	6.8±0.18[1]	5.4±0.06[1]	7.0±0.22[2]
Cholesterol, mg%	68.4±4.6	138.0±13.6[1]	88.4±5.2[1,2]	142.0±14.4[1]	70.2±5.4[2]
Liver					
Total lipids, mg%	4270.0±253.0	5264.0±198.0[1]	4524.0±286.0	6498.0±370.0[1]	4498.0±226.0[2]
Malondialdehyde nmol / mg protein	0.78±0.05	1.820±0.09[1]	0.910±0.08[2]	1.980±0.09[1]	0.840±0.06[2]
Glycogen, mg%	1980.0±50.2	840.0±32.6[1]	1452.0±42.6[1,2]	726.0±22.4[1]	1900.0±54.6[2]
ATP, μM / g tissue	2.95±0.07	2.15±0.04[1]	2.89±0.06[2]	1.98±0.02[1]	2.90±0.06[2]
ADP, μM / g tissue	0.85±0.03	0.76±0.02[1]	0.82±0.02	0.71±0.01[1]	0.82±0.02[2]
AMP, μM / g tissue	0.68±0.02	0.82±0.03	0.67±0.02[2]	0.88±0.03[1]	0.67±0.01[2]
The total amount of bile, mg / 100 g in 4 hours	1085.0±57.6	806.0±33.6[1]	956.2±34.6[2]	638.6±22.4[1]	1012.0±52.4[2]
Bile acids, mg%	1374.0±56.2	792.0±32.6[1]	1036.0±48.2[1,2]	632.0±28.4[1]	1298.0±52.4[2]
Cholesterol, mg%	26.2±0.68	20.4±0.56[1]	24.4±0.62[2]	19.2±0.60[1]	26.0±0.72[2]
Bilirubin, mg%	22.2±0.72	14.6±0.62[1]	20.4±1.6	13.4±0.54[1]	22.6±2.2[2]
Cholato-cholesterol ratio	52.4±3.8	38.8±2.6[1]	42.4±3.2	32.9±2.2[1]	49.9±3.6[2]

Note. 1 - Significantly to the indices of intact animals, 2 - significantly to the corresponding control (the level of reliability is taken as $p < 0.05$)

Ecdisten promoted the restoration of bile secretion processes in rats with alcoholic liver damage, normalized the concentration of bile acids and cholesterol in bile, and positively influenced the hepatic link of bilirubin metabolism. It should also be noted that in rats treated with ecdisten, the calculated value of the cholatocholesterol coefficient on the 14th day of observation and, especially, on the 21st day of observation was significantly higher than in the control. This indicated a decrease under its influence of cholesterol-stabilizing properties of bile, leading to an increase in cholesterol resistance in it [20] and a decrease in the possibility of gallstones formation [21].

Thus, the obtained data can significantly expand the area of practical use of ecdisten as an effective hepatoprotective agent in hepato-biliary pathology that develops against the background of long-term alcohol consumption.

Conclusion

The preparation of ecdisten, administered to rats against the background of alcohol intoxication, largely prevents metabolic processes in the liver, contributes to the normalization of bile secretion and optimizes its chemical composition.

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