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Study of Therapeutic Properties of the Prototype Injection of a Hepatoprotective Drug Based on Flavolignans of *Silybum marianum*

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Abstract

This article discusses special issues related to the study of the therapeutic properties of the prototype injection of a hepatoprotective drug based on silymarin, a flavolignan extracted from Saint-Mary's-thistle (*Silybum marianum*) (hereinafter, the study drug). Studies were conducted on laboratory animals. The result of the studies showed that the study drug has apparent hepatoprotective properties. The study drug stimulates biliary function. We observed the recovery of liver proteosynthesis after administration of the study drug to sick animals.

Keywords

Silymarin; Hepatitis; Flavolignans; Saint-Mary's-thistle; *Silybum marianum*; Hepatoprotector

Introduction

Liver is the central organ of metabolism, which performs most of the chemical processes related to the metabolism of proteins, carbohydrates, lipids, vitamins, and minerals. In addition, liver is actively involved in digestion, elimination of toxic substances released from the gastrointestinal tract and entering the body from the outside, maintaining homeostasis, etc. Therefore, violation of its functional activity leads to rather considerable violations of the body functions [1]. All this makes it relevant to find effective drugs or their combinations that reduce the risk of hepatopathy and implement an effective therapy for liver pathologies associated with environmental factors. It is important for the drugs to be non-toxic and have a high bioavailability [2]. One method of increasing the bioavailability of drug substances is the use of colloidal solutions and polymeric matrices [3,4].

Flavolignans extracted from Saint-Mary's-thistle (*Silybum marianum* L. Gaerth) are among the most promising drugs that meet the requirements of the modern medicine [5].

They are powerful antioxidants and can inactivate both free radicals and reactive oxygen species within the cell. They are also able to block receptors and transport systems on the cell membrane. This allows the transfer of toxic substances into the cell, decreases macrophages activity involved in antigen presentation, decreases the production of gamma-globulins, and blocks lipoxygenase and cyclooxygenase. Thus, flavolignans provide anti-inflammatory, immunomodulatory, and anti-carcinogenic effects [6,7].

Along with the above-mentioned properties, thistle flavolignans interact with membranes of hepatocytes, and inhibit cAMP activity and calcium-dependent activation of phospholipase. They also have a cytoprotective effect on hepatocytes and induce repair of damaged liver cells, which results in improvement of the subjective state of the patient (improved appetite, general state, digestion) and normalization of clinical analyses: decrease in transaminases and bilirubin levels [8]. The above-noted features allow using flavolignans-containing preparations of Saint-Mary's-thistle in the treatment of liver diseases, such as viral and toxic hepatitis; cirrhosis; drugs, radiation, and ischemic damages; carcinogenesis; and others [9].

However, flavolignans have a low therapeutic activity due to their low solubility in both hydrophilic and lipophilic solvents. They are usually insoluble or marginally soluble in water, whereby the rate of release of these compounds and, consequently, their bioavailability and absorbability in the body are unsatisfactory. Moreover, low solubility of flavolignans in water and biological fluids does not allow using them for injection or infusion (it could greatly enhance therapeutic efficacy of these drugs) [10].

Therefore, the main objective of our research is to develop stable injectable form of silymarin-containing drug, which will increase its bioavailability and reduce its side effects.

Materials and Methods

The study object (the study drug) was a dispersed hepatoprotective drug, which is a complex of bioflavonoid isomeric compounds (flavolignans) extracted from the medicinal plant Saint-Mary's-thistle (*Silybum marianum* L.). It contains the following active substances: silymarin (12 mg), vitamin E (2 mg), and excipients. It was prepared in the laboratory of the Center for collective use "Molecular Biology" at the Department of Therapy, Obstetrics, and Pharmacology of the Federal State Educational Institution of Higher Professional Education, Saratov State Agrarian University, named after N.I. Vavilov.

In order to study the therapeutic effect of injecting drug silymarin for liver pathologies, we carried out an experiment on laboratory animals. Two groups of six white nondescript male mice were formed

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Group	Preparation	Type of animal	Weight of animals, g	Dose, mg/kg	Course, days
Group 1 (control)	Paracetamol (per os)	Mice	23.2 ± 5.7	500	1-5
Group 2 (test)	Paracetamol (per os)	Mice	25.3 ± 3.36	500	1-5
Group 1 (control)	Saline (i.m.)	Mice	26.4 ± 1.17		6-19
Group 2 (test)	The study drug (i.m.)	Mice	28.5 ± 4.25	5	6-19

Note: paracetamol 0.5 g tablets were dissolved in 5 mL of distilled water for oral administration.

Table 1: Scheme of the experiment

Number	Parameter	Unit	Group 1		Group 2	
			M	M	M	m
1.	WBC	$\times 10^9$/L	13.1	3.67	2.85	2.05
2.	LYM	$\times 10^9$/L	12.5	4.18	1.43	1.12
3.	MID	$\times 10^9$/L	0.3	1.30	0.47	0.38
4.	GRA	$\times 10^9$/L	0.3	1.78	0.82	0.89
5.	LYM	%	95	24.64	36.37	29.37
6.	MID	%	2.1	10.96	11.40	8.91
7.	GRA	%	2.9	14.62	18.90	16.89
8.	RBC	$\times 10^{12}$/L	7.32	1.18	6.37	2.73
9.	HGB	g/L	124	10.33	102.83	44.67
10.	MCHC	g/L	330	20.38	339.83	15.09
11.	MCH	Pg	16.9	1.70	16.32	1.42
12.	MCV	Fl	51.3	7.01	48.10	4.55
13.	RDW-CV	%	19.5	3.41	16.32	2.63
14.	RDW-SD	Fl	50	6.46	38.75	2.51
15.	HCT	%	37.6	4.02	30.50	13.62
16.	PLT	$\times 10^9$/L	568	50.01	757.67	579.22
17.	MPV	Fl	5.3	0.06	7.10	3.58
18.	PDW	Fl	7	0.59	7.77	6.11
19.	PCT	%	0.299	0.26	0.74	0.75
20.	P-LCR	%	2.9	1.18	17.45	26.04

Table 2: Results of the complete blood count

according to the analog method. Animals from both groups received paracetamol (it has hepatotoxic effect) daily with an interval of 1 day in the dose of 500 mg/kg body weight for 5 days before the onset of clinical symptoms of intoxication. Animals of the first group (control) did not receive any hepatoprotective drugs. The study drug was administered intramuscular to animals of the second group (test) in the dose of 5 mg/kg (by active ingredient) for 5 days after administration of the toxicant. Administration of hepatoprotectors was carried out for 14 days. On Day 20 of the experiment, animals were decapitated. Then, blood samples were collected in tubes containing anticoagulant K_3EDTA as well as in tubes with clot activator.

Maintenance and care of animals, as well as their euthanasia, was carried out in accordance with the requirements of the Ministry of Health of the Russian Federation for experimental and biological clinics and the "European Convention for the Protection of Vertebrate Animals Used for Experiments and Other Scientific Purposes."

Clinical and hematological studies were carried out according to generally accepted methods. Biochemical studies were carried out in accordance with the "Guidelines on the application of standardized methods of biochemical studies of blood, urine and milk in veterinary research" (Wiley, 2004). They were carried out on a biochemical analyzer, "MindrayBA-88A," using diagnostic systems of the company "Olvex diagnosticum." Using all the blood samples, we performed a common blood count on HaemaScreen 7 hematological analyzer. The results are presented in Table 2. Before slaughter, the animals were weighed. Liver and kidneys were withdrawn from the slaughtered animals followed by their preservation with formalin for further histological studies.

During the whole experiment, we observed the state and behavior of animals, and the dynamics of increase in body weight. We regularly conducted studies in order to assess the functional state of liver and kidneys, and studied the effect of the drug on hematologic parameters. Statistical processing was performed by Student-Fisher method.

Results and Discussion

The studies revealed that animals developed clinical signs of intoxication on Day 5 after paracetamol administration. This was manifested by physical inactivity, dullness, and dishevelment of hair. Animals showed low physical activity, mucous membranes were pale yellowish, and skin was pale and had yellowish tinge.

After the treatment of animals of the test group, we obtained the following data. Results of the complete blood count revealed that the study drug inhibits the development of inflammatory reactions induced by hepatotoxin administration. This is evidenced by the normal values of the absolute WBC number in the peripheral blood, in contrast to animals of the control group that had considerable leukocytosis caused by the increase in the number of peripheral blood lymphocytes (Table 2). Moreover, the toxicant had no negative effect on erythropoiesis as can be seen from the table. This is evidenced by parameters indicating the number of RBC that were within the physiological range both in the test and the control group of animals.

Determination of body weight of the animals showed an absence of any deviations from the physiological norm in both groups of animals.

Biochemical blood tests revealed a significant increase in liver enzymes levels, such as aspartate aminotransferase and alanine aminotransferase in the control group of mice. This indicates the cytolytic effect of toxicant on hepatocytes. Along with this, parameters of hepatic cytolysis syndrome were much lower in the test group of animals that received the study drug at the therapeutic dose. This fact may indicate a high hepatoprotective activity of the drug. In addition,

Parameter	Unit	Group 1	Group 2
GPT	U/L	108.0 ± 3.5	69.7 ± 3.06
GOT	U/L	99.0 ± 2.0	71.7 ± 1.15
GOT/GPT		0.9 ± 0.0	1.0 ± 0.05
Alkaline phosphatase	U/L	502.0 ± 6.1	290.7 ± 62.50
Glucose	mmol/L	10.1 ± 0.6	8.1 ± 1.59
Total protein	g/L	44.6 ± 4.7	71.8 ± 3.14
Albumin	g/L	21.6 ± 3.1	24.6 ± 0.49
Globulin	g/L	23.0 ± 2.0	47.2 ± 3.05
A/G		0.9 ± 0.1	0.5 ± 0.03
Urea	mmol/L	6.4 ± 1.9	6.9 ± 0.31
Creatinine	μmol/L	219.0 ± 10.4	195.0 ± 51.92
Cholesterol	mmol/L	2.0 ± 1.0	2.0 ± 0.00

Table 3: Biochemical blood parameters

there is a considerable increase in alkaline phosphatase activity in mice of the control group, whereas in animals of the test group, this component was within the physiological range and approximately two times lower than that in the control one. Given the fact that the increase in this parameter shows cholestasis causing washout of alkaline phosphatase into the blood in large quantities, it can be argued that the study drug stimulates biliary excretion and prevents cholestasis.

Along with this, the data from Table 3 shows a considerable reduction in the amount of total protein by more than 1.5-fold in the control group as compared with the test group of animals. Hence, we can assume a violation of liver proteosynthesis and a decrease in the digestibility of nutrients in the animals of the control group. At the same time, in the test group of mice, this indicator is within physiological values and indicates a positive effect of the test drug on protein metabolism in animals.

The therapeutic efficacy of the study drug is caused by its high bioavailability and specific distribution of drug colloidal solutions in the internal organs. We have previously conducted researches on the design and study of biodynamic properties of drugs being in colloidal systems. In these studies we found that bioactive substances in colloidal systems had a high bioavailability and tropism to reticuloendothelial system. In particular, we observed the most active accumulation in the liver and spleen.

Conclusion

The study drug represents the preparation of complex isomeric bioflavonoid compounds (flavolignans) extracted from Saint-Mary's-thistle and dispersed in water. Its use in case of liver damage in animals has apparent hepatoprotective properties. It is able to stimulate biliary function. We observed the recovery of liver proteosynthesis after administration of the study drug to sick animals.

References

1. Marı M, Colell A, Morales A, von Montfort C, Garcia-Ruiz C, *et al*. (2010) Redox control of liver function in health and disease. Antioxidants & Redox Signaling 12(11): 1295-1331.
2. Evdokimova OV (2002) The use of herbal medicines. Side effects and contraindications. Pharmaceutical Review 7: 21-24.
3. Staroverov SA, Volkov AA, Larionov SV, Mezhennyy PV, Kozlov SV, *et al*. (2014) Study of transmissible gastroenteritis virus antigen-conjugated immunogenic properties of selenium nanoparticles and gold. Life Science Journal 11(11): 456-460.
4. Staroverov SA, Volkov AA, Fomin AS, Laskavuy VN, Mezhennyy PV, *et al*. (2015) The usage of phage mini-antibodies as a means of detecting ferritin concentration in animal blood serum. Journal of Immunoassay and Immunochemistry 36(1): 100-110.
5. Sridar C, Goosen TC, Kent UM, Williams AJ, Hollenberg PF (2012) Silibin inactivates cytochromes P450 3A4 and 2C9 and inhibits major hepatic glucuronosyltrasferases. Drug Metabolism and Disposition 32(6): 587-594.
6. Kidd P, Head K (2005) A review of the bioavailability and clinical efficacy of milk thistle phytosome: A silybin-phosphatidylcholine complex (Siliphos). Alternative Medicine Review 10(3): 193-203.
7. Pradhan SC, Girish C (2006) Hepatoprotective herbal drug, silymarin from experimental pharmacology to clinical medicine. Indian Journal of Medical Research 124: 491-504.
8. Wu JW, Lin LC, Hung SC, Lin CH, Chi CW, *et al*. (2008) Hepatobiliary excretion of silibinin in normal and liver cirrhotic rats. Drug Metabolism and Disposition 36:589-596.
9. Loguercio C, Festi D (2011) Silybin and the liver: From basic research to clinical practice. World Journal of Gastroenterology 17(18): 2288-2301.
10. Kren V, Walterova D (2005) Silybin and silymarin - New effects on applications. Biomedical Papers 149(1): 29-41.

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