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STUDY AND DEVELOPMENT OF HEPATOPROTECTIVE AGENT

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A multicomponent plant agent under the conditional name "Pentafit" has been developed. As a result of the experiments carried out, it was found that its course administration per os at a dose of 300 mg/kg to nonlinear rats with experimental liver injuries has antihepatotoxic, hepatoprotective and membrane stabilizing effect.

Keywords: antihepatotoxic plant agent, toxic liver injury, preclinical studies, antihepatotoxic action, hepatoprotective activity

Toxic liver injuries hold a leading place in the structure of morbidity and mortality of the population, primarily due to increase of the number of alcohol intoxications, uncontrolled large-scale use of medicines, environmental pollution, including water and food, with foreign chemical compounds [1].

The market for herbal medicines with proven anti-hepatotoxic activity is currently not too large, and the problem of effective therapy is far from being resolved. Despite the use of sufficiently active preventive measures and constantly improved treatment methods, even with the complex use of highly effective anti-hepatotoxic agents, complicated forms of toxic hepatitis are found in 26–42% of cases, and 15–25% of patients have a problem of resistance of toxic hepatitis to the most modern therapeutic effects [2].

In this regard, it is important to search for means that can increase the liver's resistance to the damaging effects of toxins and stimulate detoxification processes [3].

The purpose of the study is to determine the pharmacological activity in the development of an optimal method for obtaining a medicine that has antihepatotoxic activity.

MATERIALS AND METHODS

The object of the study was a plant composition under the conditional name "Pentafit", consisting of roots and rhizomes of elfwort (*Inula helenium* L.), common centaury herb (*Centaurium erythraéa* Rafn.), flowers of common tansy (*Anacétum vulgáre* L.), fruits of sweet-brier (*Rosa* sp.) and hawthorn berries (*Crataegus* sp.).

The components of the medicinal plant collection were selected taking into account the multifactorial mechanisms of the development of diseases of the hepatobiliary system and correspond to the principles of pharmacological regulation of the digestive system functions [3–5].

According to the literature, there is information about the anti-inflammatory, antispasmodic, choleretic and hepatoprotective effects of biologically active substances that are part of the roots and rhizomes of elfwort, common centaury herb, flowers of common tansy, fruits of sweet-brier and hawthorn berries [4–7]. In connection with the above, within the frames of the set task, the study of the anti-hepatotoxic

activity of this plant composition is promising and predictable.

To confirm the anti-hepatotoxic effect, based on data on the chemical composition, we have proposed the following method of obtaining. The medicinal plant collection containing 15% of common centaury herb, 10% of flowers of common tansy, 25% of roots and rhizomes of elfwort, 27.5% of fruits of sweet-brier and 22.5% of hawthorn berries is extracted triply with 45–55% ethyl alcohol while constantly stirring at temperature of 60–70°C for 2 hours. The combined extracts are evaporated under vacuum, separated and dried.

The resulting extract contains polysaccharides, flavonoids, carotenoids, organic acids, vitamins, macro – and microelements, essential oils and other natural compounds. **Pentafit** was standardized by the sum of flavonoids, equivalent to luteolin-standard. The content of the sum of flavonoids is regulated as at least 1%. The presence of this spectrum of biologically active substances proposes the potential anti-hepatotoxic activity of the resulting extract. The method for obtaining an agent that has anti-hepatotoxic activity is protected by patent No. 2689379 [8].

The work was performed in accordance with the Federal law "On medicines" and the Guidelines for conducting preclinical studies of medicines. Experiments were performed on 120 non-linear male rats with initial weight of 180–200 g. The animals were obtained from the Scientific Center for Biomedical Technologies of Russia and kept in a vivarium with free access to food and water. Pharmacological studies were performed according to the Rules of works using experimental animals, the Rules adopted by the European Convention for protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986), Order of MOH No. 199н dated 01.04.2016 "On approval of rules of good laboratory practice". The study design was approved by the VILAR Bioethical Commission (Protocol No. 7 dated October 1, 2018).

The hepatoprotective activity of the extract under the conditional name "Pentafit" at a previously selected dose of 300 mg/kg and the comparator agent, Karsil (Sofarma JSC, Bulgaria) at an isoeffective dose of 50 mg/kg was studied in conditions of a model of chronic experimental tetrachloromethane hepatitis.

To assess the antitoxic function of the liver the duration of hexenalum sleep was recorded as the duration of rats staying in the lateral position after abdominal injection of hexenal (MedPro Inc. Ltd, Latvia) at a dose of 60 mg/kg weight under the recommendation of Hatsura V.V. [9].

Liver damage was caused by intragastric administration of 50% oil solution of carbon tetrachloride (CCl4) to rats (Reachim, Russia) in the amount of 0.4 ml per 100 g of animal weight once a day for 4 days [10].

The functional validity of the rat liver monooxygenase system was evaluated by the amount of cytochrome P450 in the microsomal fraction of the liver. The content of this enzyme was measured using a Shimadzu spectrophotometer (Japan) by the Omura T. and Sato R. method [11]. Microsomes from animal liver tissue were isolated under the recommendation of I.I. Karuzina and A.I. Archakov [12]. The amount of protein in microsomes was determined using the Lowry method [13]. The rate of inactivation of reduced cytochrome P_{450} was recorded at temperature of 37°C every 3 minutes for 30 minutes.

Statistical processing of the obtained data was performed using the Statistica 10.0 software package (USA) [14]. The differences were considered as significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Therapeutic efficiency of "Pentafite" was determined with intragastric course administration (1 per day) of the extract in form of aqueous

solution at a previously established experimental therapeutic dose of 300 mg/kg for 10 days in case of tetrachlormethane hepatitis in rats starting from the 2nd day after the first administration of the damaging agent. As a comparator agent, the plant hepatoprotector Carsil was used at an isoeffective dose of 50 mg/kg.

The influence of "Pentafit" on the duration of hexenalum sleep in rats with carbon tetrachloride hepatitis was studied. The results of the experiments are presented in Table 1.

Previously, non-linear rats were divided into groups: intact (20 rats); control (20 rats); experimental 1 (20 rats); experimental 2 (20 rats). "Pentafit" at a dose of 300 mg/kg was injected to Experimental 1 animals into the stomach via a probe for 10 days in case of carbon tetrachloride hepatitis in rats, starting from the 2nd day after the first administration of the damaging agent. Experimental 2 rats were treated with the reference standard Carsil at an isoeffective dose of 50 mg/kg according to a similar scheme. Animals of the control group took equi-volume amounts of water purified according to a similar scheme. Animals of the intact group served as additional control.

From Table 1 it follows that when administrating "Pentafit" the duration of hexenalum sleep in rats was decreased on the 7th and 14th days of the experiment by 29% and 27%, respectively, indicating a stimulation of the liver detoxification function with a studied extract in the conditions of the tetrachlormethane hepatitis model. The comparator agent Carsil had a less pronounced effect, reducing the duration of hexenalum sleep on the 7th and 14th days of the experiment by 14%.

In case of tetrachloromethane damage to the liver of rats, the administration of "Pentafit" at an experimental therapeutic dose of 300 mg/kg had a favorable effect on the detoxifying function of the liver.

The effect of course administration of "Pentafit" at an experimental therapeutic dose of 300 mg/kg on the state of the liver monooxygenase system of non-linear male rats with toxic hepatitis was studied. The results of the experiments are presented in Table 2.

Experiments were performed on nonlinear rats, which were divided into groups: intact (10 rats); control (10 rats); experimental 1 (10 rats); experimental 2 (10 rats). "Pentafit" at an experimental therapeutic dose of 300 mg/kg was injected to Experimental 1 animals into the stomach via a probe for 7 days in case of carbon tetrachloride hepatitis in rats, starting from the 2nd day after the first administration of the damaging agent. Experimental 2 rats were treated with

Table 1

THE INFLUENCE OF "PENTAFIT" ON THE DURATION OF HEXENALUM SLEEP IN RATS WITH TOXIC CCL_4-HEPATITIS, M±M

Groups of animals	Duration of hexenalum sleep, s	
	7 days	14 days
Intact (H_2O), n=20	838±75	1103±78
Control (CCl_4 + H_2O), n=20	1470±118	1294±55
Experimental 1 (CCl_4 + "Pentafit" 300 mg/kg), n=20	1038±73*	943±70*
Experimental 2 (CCl_4 + Carsil 50 mg/kg), n=20	1268±212	1121±102

*Note: hereinafter: * – differences are statistically significant between the data of the control and experimental groups when P≤0,05*

Table 2

EFFECT OF "PENTAFIT" ON THE STATE OF THE LIVER MONOOXYGENASE SYSTEM IN CASE OF EXPERIMENTAL CCL_4-HEPATITIS IN RATS (DAY 7)

Groups of animals	Content of Cytochrome P_{450} in nmol/mg of protein	% inactivation of Cytochrome P_{450} for 30-minute incubation	The amount of MDA in μM /mL of serum x min.
Intact (H_2O), n=10	0,79±0,04	21,2±2,0	3,99±0,40
Control (CCl_4 + H_2O), n=10	0,39±0,06	58,7±1,3	5,76±0,10
Experimental 1 (CCl_4 + "Pentafit" 300 mg/kg, n=10	0,60±0,08*	18,1±0,9*	3,89±0,60*
Experimental 2 (CCl_4 + Carsil 50 мг/кг), n=10	0,53±0,07*	18,1±1,1*	4,49±0,40

the reference standard Carsil at an isoeffective dose of 50 mg/kg according to a similar scheme. Animals of the control group took equi-volume amounts of water purified according to a similar scheme. Animals of the intact group served as additional control.

When evaluating the state of the liver monooxygenase system on the 7th day of the experiment in case of toxic hepatitis in rats, it was found that the use of "Pentafit" at the specified dose significantly increased the amount of cytochrome P450 in the liver microsomes.

54% increase in the key enzyme of the monooxygenase system responsible for liver detoxification function was accompanied by slowdown in the rate of inactivation of this enzyme due to stabilization of membrane structures. The comparator agent Carsil also had an effect on the state of the liver monooxygenase system in toxic hepatitis. "Pentafit" reduced the amount of MDA in the blood serum of rats by 32%, which indicates its membrane-stabilizing activity due to the content of BAS of phenolic nature.

Thus, the course administration of "Pentafit" at an experimental therapeutic dose of 300 mg/kg to rats with tetrachloromethane hepatitis has an anti-hepatotoxic and membrane-stabilizing effect.

CONCLUSION

According to the results of the experiments, it was found that the course administration per os of the received multicomponent agent under the conditional name "Pentafit" at an experimental therapeutic dose of 300 mg/kg to non-linear rats with experimental liver damage has an anti-hepatotoxic, hepatoprotective and membrane-stabilizing effect. The pharmacotherapeutic effect of "Pentafit" in toxic liver damage is due to the presence of a complex of biologically active substances, primarily compounds of phenolic nature [3,5].

The obtained study results prove the feasibility of using the resulting multi-component agent "Pentafit", containing biologically active substances of phenolic nature, in the prevention and complex treatment of liver diseases.

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