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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF HAIR-VEIN ARGIMONY (AGRIMONIA PILOSA) EXTRACT ON TOXIC HEPATITIS MODEL

E.N. Kurmanova, *Research Scientist of the Department of Experimental and Clinical Pharmacology, All-Russian Research Institute of Medicinal and Aromatic Plants (VILAR), Moscow, Russia, kurmanova1968@yandex.ru*
L.B. Strelkova, *Candidate of Medicine, All-Russian Research Institute of Medicinal and Aromatic Plants (VILAR), Moscow, Russia*
E.V. Ferubko, *Candidate of Medicine, Head of the Department of Experimental and Clinical Pharmacology, All-Russian Research Institute of Medicinal and Aromatic Plants (VILAR), Moscow, Russia,*
O.P. Sheichenko, *Candidate of Chemical Sciences, Leading Researcher of the Department of Phytochemistry and Standardization, All-Russian Research Institute of Medicinal and Aromatic Plants (VILAR), Moscow, Russia*

The effect of Hair-Vein Argimony herb dry extract on the state of the rat liver in experimental toxic hepatitis was studied. The pharmacological properties of the extract were studied during its intragastric administration to laboratory animals for 3 days. Experimental hepatitis in rats was caused by a single subcutaneous injection of 50% oil solution of carbon tetrachloride an hour after the last administration of the test substance and the Carsil reference medication. Microsomal fraction was isolated from the liver of animals and the content of microsomal protein, as well as the rate of monooxygenase reactions catalyzed by cytochrome P450 – p-hydroxylation of aniline, N-demethylation of dimethylaniline reflecting the activity of the detoxifying microsomal system of the liver were determined. Taking into account all the studied parameters, the hepatoprotective and detoxifying properties of the extract were evaluated in comparison with the Carsil standard medication. As a result of the study, it was found that the treatment of hepatitis with the extract of Hair-Vein Argimony at doses of 50 mg/kg and 100 mg/kg normalizes the content of microsomal protein, and cytochrome P450 content and anilination and demethylation rates in liver microsomes increase compared to control. Therefore, dry purified extract of Hair-Vein Argimony at the studied doses has a hepatoprotective effect against detoxifying enzyme systems of cytochrome P450 in the rat liver under the conditions of a model of carbon tetrachloride hepatitis.

Keywords: *Hair-Vein Argimony* herb dry extract, experimental toxic hepatitis, hepatoprotective activity, antitoxic effect

The significant prevalence of liver diseases, the special severity of their course, the hepatotoxic effect of a number of medicines, and the insufficient nomenclature of domestic hepatotropic agents used in medical practice, specify the significance of the search for new medicines that have hepatoprotective effect [1].

Currently, there is a search for sources of new effective biologically active substances from plant raw materials for creation of medicines based on them with a wide spectrum of action, low toxicity and the associated possibility of their long-term use [2].

A promising object for the development of new medicines for the prevention and treatment of liver lesions is the genus *Agrimonia* of the *Rosaceae* family. The representative of this genus is the *Hair-Vein Argimony* (Agrimonia pilosa Ledeb.). Extracts from the aboveground part of the plant have anti-inflammatory, antimicrobial, tonic, diuretic, hemostatic for uterine and pulmonary bleeding, astringent, anthelmintic, antitumor effects, regulate the function of the liver and gallbladder [3]. The chemical composition of the aboveground part of the *Hair-Vein Argimony* (herb) includes flavonoids (rutin, quercetin, kaempferol, hyperoside, luteolin, luteolin-7-glucoside), phenol-carboxylic acids (caffeic, chlorogenic, ellagic) [4].

Purpose of the study: to study the effect of *Hair-Vein Argimony* herb dry extract on the liver of rats in experimental toxic hepatitis to create a new herbal medicine.

MATERIALS AND METHODS

Hair-Vein Argimony herb dry extract was prepared in the All-Russian Research Institute of Medicinal and Aromatic Plants (VILAR). The Department of Experimental and Clinical Pharmacology conducted its pharmacological study. The effect of the extract on the state of the rat liver under experimental toxic hepatitis was studied. Experimental toxic hepatitis caused by a single subcutaneous injection of carbon tetrachloride to rats was used [5]. The study was performed according to the Rules of laboratory practice in the Russian Federation (the Order of the Ministry of Health of Russia No. 199н dated 01.04.2016, the National Standard of the Russian Federation GOST 33044-2014 "Principles of good laboratory practice"), the Guidelines for Preclinical Studies of Medicines (2012) and in accordance with the Federal law No.61-FZ dated 12.04.2010 (as amended on 28.11.2018) "On circulation of medicines". The studies were approved by the Bioethical Commission (Protocol No. 4 of 24.09.2018). When conducting an experiment on a model of carbon tetrachloride hepatitis, white non-linear male rats with body weight of 200–250 g in the number of 50 individuals were used. Animal producer is Andreevka Branch of the BTSC (Biomedical Technology Science Center) of the FMBA (Federal Medical and Biological Agency) of Russia (Moscow region). The animals were kept in the vivarium of the VILAR on a standard diet.

The pharmacological properties of the *Hair-Vein Argimony* extract were studied during its intragastric administration to laboratory rats. The experimental animals were divided into 5 groups of 10 individuals: the first group – intact animals; the second group – control animals, in which an experimental toxic hepatitis was reproduced; the third, fourth and fifth groups – experimental animals, which additionally received the *Hair-Vein Argimony* extract at doses of 50 mg/kg, 100 mg/kg and the Carsil standard medication at a dose of 100 mg/kg, suspended in 1% starch suspension. The extract and Carsil were pre-administered once a day for 3 days to experimental groups of laboratory animals, at the same time the control rats received an equivalent volume of 1% starch suspension. Experimental hepatitis in animals was caused by a single subcutaneous injection of 50% carbon tetrachloride oil solution at a dose of 0.4 ml per 100 g of animal weight an hour after the last administration of the test substance and the reference medicine. After 48 hours, the rats were euthanized in a CO_2 chamber and the liver was removed for further examination.

Microsomal fraction was isolated from the liver of animals using the method of differential centrifugation [6]. In the microsomal fractions of the liver of experimental groups of animals, the content of microsomal protein was determined [7], as well as the rate of monooxygenase reactions catalyzed by cytochrome P_{450} – p-hydroxylation of aniline, N-demethylation of dimethylaniline (DMA), reflecting the activity of the detoxifying microsomal system of the liver. Taking into account

all the studied parameters, the hepatoprotective and detoxifying properties of the *Hair-Vein Argimony* extract were evaluated in comparison with the Carsil standard medication.

Statistical processing of the obtained data was performed using the Statistica 10.0 software package (USA). The sampling is symmetric. The significance of differences between samples with a distribution approaching the normal one was assessed using the Student's t-test. The differences were considered as significant at $p \leq 0,05$.

The work was carried out on the subject: "Preclinical studies of individual fractions, substances and medicinal products made of medicinal plant raw materials", topic code No. 0576-2019-0009.

RESULTS AND DISCUSSION

The results of the study of the hepatoprotective and detoxifying properties of the *Hair-Vein Argimony* extract in comparison with the Carsil standard medication in experiments on animals with reproduced CCl4 hepatitis are shown in Tables 1 and 2.

Table 1 contains the results on the effect of the *Hair-Vein Argimony* extract on the content of cytochrome P450 microsomal protein in the suspension of rat liver microsomes in toxic hepatitis.

Based on the results obtained, it was found that in experimental carbon tetrachloride acute hepatitis, the content of microsomal protein in the liver microsomes definitely increased by 28%, but the content of cytochrome P_{450} decreased by 53%. It is not impossible that the compensatory mechanisms of toxic damage to liver tissue influence on the protein content in microsomes. It is known that hepatitis negatively affects the liver cells due to the formation of lipid peroxidation products, which has an effect on the content of cytochrome P_{450} [1,2].

Table 1

EFFECT OF *HAIR-VEIN ARGIMONY* EXTRACT ON THE CONTENT OF MICROSOMAL PROTEIN IN TOXIC HEPATITIS

Groups of animals, n= 10	Content of microsomal protein, protein mg/liver g, M±m	Experiment/ control, %	Content of cytochrome P_{450}, cyt. P_{450} nM/protein mg, M±m	Experiment/ control, %
Intact	2.25±0.14	72	0.544±0.020	153
Control (experimental CCl_4 hepatitis)	3.11±0.11	100	0.355±0.018	100
Experimental 1 (*Hair-Vein Argimony* extract, 50 mg/kg)	2.10±0.13*	67	0.471±0.014*	133
Experimental 2 (*Hair-Vein Argimony* extract, 100 mg/kg)	2.08±0.09*	68	0.557±0.010	156
Experimental 3 (Carsil, 100 mg/kg)	1.88±0.07*	60	0.784±0.022*	220

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

The results of the study have revealed that the treatment of hepatitis with *Hair-Vein Argimony* extract at doses of 50 mg/kg and 100 mg/kg normalizes the content of microsomal protein, and the content of cytochrome P_{450} in liver microsomes increases by 33% and 56%, respectively, compared to control. When the Carsil standard medication is administered to animals, the content of microsomal protein is normalized, and the content of cytochrome P_{450} increases by 2.2 times.

Table 2 presents the results of the evaluation of the effect of the *Hair-Vein Argimony* extract and the Carsil standard medication on the hepatoprotective and detoxifying function of the liver microsomes. The activity of the detoxifying microsomal system of the liver was evaluated by the rate of enzymatic reactions catalyzed by cytochrome P_{450}, namely, by the reaction of demethylation with the type I substrate – dimethylaniline and by the reaction of p-hydroxylation with the type II substrate – aniline.

As we can see from the presented data, in the control, as a result of the toxic effect of carbon tetrachloride on the liver, the specific enzymatic aniline hydroxylase and demethylase activity of cytochrome P_{450} decreased.

The administration of the *Hair-Vein Argimony* extract to animals at a dose of 50 mg/ml and 100 mg/ml against the background of acute toxic hepatitis showed the hepatoprotective activity of the *Hair-Vein Argimony* herb extract against detoxifying enzyme systems of rat liver cytochrome P450. It was found that in the treatment of hepatitis with the *Hair-Vein Argimony* herb extract at a dose of 50 mg /kg and 100 mg/kg, the anilination

Table 2

EVALUATION OF THE EFFECT OF THE *HAIR-VEIN ARGIMONY* EXTRACT ON THE ACTIVITY OF CYTOCHROME P450 IN MICROSOMAL FRACTIONS OF THE LIVER IN EXPERIMENTAL HEPATITIS

Groups of animals, n=10	Activity of cytochrome $P_{450,}$ M±m			
	Aniline hydroxylation NADPH nM/cyt. P_{450} nM per minute	Experiment/ control, %	Demethylation of DMA, NADPH nM/cyt. P_{450} nM per minute	Experiment/ control, %
Intact	1.84±0.04	127	1.67±0.05	123
Control (experimental CCl_4 hepatitis)	1.45±0.03	100	1.35±0.04	100
Experimental 1 (*Hair-Vein Argimony* extract, 50 mg/kg)	1.68±0.04*	116	1.90±0.04*	141
Experimental 2 (*Hair-Vein Argimony* extract, 100 mg/kg)	1.75±0.05*	121	2.00±0.06*	148
Experimental 3 (Carsil, 100 mg/kg)	1.92±0.03*	132	2.33±0.06*	172

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

rate increases by 16% and 21%, respectively, and the demethylation rate increases by 41% and 48%, respectively. This indicates the enzymatic activity of the cytochrome P450 demethylation centers and the inducing effect of BAS contained in the *Hair-Vein Argimony* extract on the liver monooxygenase system. In this experiment, the Carsil standard medication (100 mg/kg) showed hydroxylase and demethylase activity exceeding the activity of the *Hair-Vein Argimony* extract.

Thus, the *Hair-Vein Argimony* dry extract at the studied doses has the less pronounced hepatoprotective effect against detoxifying enzyme systems of cytochrome P450 of the rat liver in the conditions of the experimental carbon tetrachloride hepatitis, than that of the Carsil standard medication.

CONCLUSIONS

It was experimentally concluded that the *Hair-Vein Argimony* herb dry extract in the conditions of simulation of carbon tetrachloride hepatitis in rats has hepatoprotective and detoxifying properties at doses of 50 and 100 mg/kg; at the same time, the dose-dependent effect of the extract was noted. The activating effect of the *Hair-Vein Argimony* dry extract at a dose of 100 mg/kg on the detoxifying microsomal systems enzymes of cytochrome P450 of the rat liver was established.

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