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# INVESTIGATION OF THE HEPATOPROTECTIVE EFFECT OF THE COMMON CAT'S FOOT HERB DRY EXTRACT

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## Abstract

The present study was conducted with the aim to further use common cat's foot herb not only in folk medicine to treat liver diseases but in official medicine too. The research of the hepatoprotective activity of the common cat's foot herb dry extract was performed on a model of acute toxic hepatitis caused by tetrachloromethane (CCl4). The pathology was modeled by the intragastric introduction of 50% oil solution of alcohol – CCl4 at a dose of 0.7 ml/100 g mass to rats for 2 days (days 8 and 9 of the experiment). The common cat's foot extracts (25 mg/kg and 50 mg/kg) and comparator drug – Silibor (100 mg/kg) were administered 1, 2 hours prior to the administration of alcohol – CCl4, during 7 days. The results of the study showed that the use of the common cat's foot herb dry extract and its administration at doses of 25 and 50 mg/kg had a positive effect on the course of toxic hepatitis. But the studied extract has a more pronounced hepatoprotective effect at a dose of 50 mg/kg. Therapeutic and prophylactic administration of the common cat's foot herb dry extract showed moderate antioxidant activity. The obtained results can be used in a further preclinical study of the common cat's foot herb dry extract to create on its basis of new hepatoprotective medicines.

**Keywords**: common cat's foot, dry extract, herb, hepatoprotective activity, Antennaria dioica Gaertn.

# Introduction

Throughout many years plants using not only as a source of the meal but also in the fight against diseases [1, 2]. One of the directions of modern pharmaceutical science for herbal medicinal products production is the use of plant raw materials. Plant metabolites are close to metabolites of the human body, and the main effect of the use of plant remedies is to regulate impaired metabolic processes. The use of medicinal plants (MP) in folk and scientific medicine has a centuries-old tradition [3]. The searching for plants with a long history of usage, minor side effects and high tolerability, regardless of the age of patients are the objects of interest in our society [4]. Herbal remedies have a milder effect, have a fairly wide range of pharmacological activity, practically do not cause addictions compared to synthetic drugs, and also go well with food and synthetic medicines. Due to the presence in plants of many groups of biologically substances (BAS) various active with pharmacological actions, plant remedies can be used for the treatment of many diseases [5].

The BAS complex, which is formed in a living cell of plants, has a great resemblance to the human body, so the components of MP are more easily assimilated by the body and have fewer side effects. Unlike synthetic drugs, plant medicines cause fewer complications, especially allergic ones, so they can be prescribed for a long time, especially for the rehabilitation of patients.

Considering that today both in Ukraine and all over the world the problem of liver damage is becoming more urgent, and drugs, including natural origin, are not enough for the treatment of liver pathologies, the research and expansion of the nomenclature of choleretic and hepatoprotective drugs due to new and little-studied species of medicinal plants is relevant.

One way to increase the amount of hepatoprotective plant medicines is to study those, which are more widely used in folk medicine and are potential sources of valuable BAS.

The typical plants for the treatment of diseases are of families Lamiaceae, Asteraceae, Fabaceae, Rosaceae, Apiaceae, Poaceae, Boraginaceae [6]. Common cat's foot (Antennaria dioica (L.) Gaertn.) is a perennial plant in the family *Asteraceae* [7]. This family includes around 23,000 species in the world and is the largest family of flowering plants [8-10]. *Antennaria dioica* Gaertn. has hemostatic and astringent properties, used to treat respiratory and biliary ailments, and also as a hepatoprotective [11-13].

Infusion of herbs or flowers treats liver disease, inflammation of the liver and gallbladder, jaundice [14]. When jaundice, it is recommended to wash with a decoction of herb [15]. The use of infusions of common cat's foot proved to be effective in the expressed functional disorders of the gallbladder activity and bile ducts, especially those accompanied by hypertonic form of dyskinesia, and in chronic cholecystitis. Decoctions of the inflorescences (flowers) of the plant are used as a choleretic agent for the treatment of hepatitis and cholecystitis. Powder from common cat's foot flowers is used as a choleretic agent in folk medicine.

In Romanian folk medicine, common cat's foot is traditionally used to treat diseases of the liver, gallbladder, upper respiratory tract [12], and as a binder and hemostatic agent [16, 17].

Despite the sufficiently wide arsenal of hepatoprotective medicines, the problem of effective treatment of liver diseases remains unresolved. Thus, the purpose of the study was to investigate the hepatoprotective effect of the dry extract obtained from aerial part of Antennaria dioica Gaertn.

## Methods

## Plant Materials

The object of the study was to select the common cat's foot herb (Antennaria dioica (L) Gaertner), which was harvested during the flowering period in the Vyzhnytsia district, Chernivtsi region (N 48°13'23.2" E 25°11'42.0"), in 2017 [6]. Raw material was dried in a shade under tents; laid out in a thin layer (2-3 cm) on paper and periodically fliped. The herb was dried using a conventional method and stored in paper bags in a dry, protected from direct sunlight place [18].

Preparation of extract. About 500 g of dried raw material was powdered. It was taken in extractor and extracted using 50 % ethanol as a solvent [19].

The extract was concentrated under vacuum and dried by rotator evaporator under reduced pressure.

## Animal models

The 45 white nonlinear male rats weighing 200-250 g were used as the experimental animals. The animals were kept in room having temperature  $22 \pm 2$  ° C, and relative humidity of 44-55 % under 12/12 hour light and dark cycle with standard laboratory diet and water given ad libitum.

Pharmacological studies have been conducted in accordance with the rules and requirements of the "General Principles for the Work on Animals" approved by the I National Congress on Bioethics (Kyiv, Ukraine, 2001 and agreed with the provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Council of Europe No 123, Strasbourg 1985), and the Law of Ukraine "On the Protection of Animals from Cruelty" of 26.02.2006 [20, 21]. The removal of animals from the experiment was carried out under light inhalation (ether) anesthesia by decapitation.

Acute toxicity studies: were performed by the method of V.B. Prozorovskyi [22] on 10 white nonlinear rats of both sexes weighing 170-190 g, which were divided into groups of 5 animals (females and males) in each group. Animals (5 males and 5 females) were intragastrically administered the common cat's foot herb dry extract at a maximum dose of 5000 mg/kg. To calculate the average lethal dose ( $LD_{50}$ ) after 14 days, the mortality rate in each group was determined according to the method of probit analysis of lethality curves according to V.B. Prozorovsky [22].

Before intragastric administration, rats were fasted ovemight. During the experiment, the animals had free access to water, to food – after 4 hours [23].

Throughout the study, we monitored the survival of the experimental animals, the consumption of food and water, as well as the clinical manifestations of intoxication (if they occur): the overall condition, changes in body position, skin condition, mucous membranes and some symptoms (miosis, tear, diarrhea, changes in urine and faeces, drowsiness, convulsions, etc.). All animals in the event of death should have been autopsied and macroscopically analyzed for abdominal organs in order to establish that the animal did not die due to manipulation errors and to determine the probable cause of death.

At the end of the study period (14 days), the animals were removed from the experiment according to the rules and regulations of bioethics: rats were decapitated under mild ether anesthesia. After that, an autopsy, macroscopic examination of the internal organs of the animals was performed and internal organs (brain, heart, kidneys, liver, spleen) were weighed to determine their mass ratio.

## Hepatoprotective activity

The study of hepatoprotective activity of the common cat's foot herb dry extract was performed on the model of acute toxic hepatitis caused by alcohol –  $CCl_4$  in comparison with the known hepatoprotective agent, which is widely used in the clinic, Silibor (produced by "Zdorovia" Ltd., pharmaceutical company, Kharkiv, Ukraine) [24].

The experiments were conducted on 45 white nonlinear male rats weighing 200-250 g [23]. Animals were divided into 5 groups of 9 animals in each: group 1 – intact control; group 2 – control pathology, animals that were injected intragastrically with a 50 % oil solution of alcohol - $CCl_4$  at a dose of 0.7 ml/100 g mass; groups 3, 4 and 5 are animals that received the common cat's foot herb dry extract 1, 2 hours prior to the administration of alcohol – CCl<sub>4</sub>, respectively, in doses of 25 mg/kg and 50 mg/kg, and Silibor comparator drug 100 mg/kg. Tested remedies were administered in animals prophylactically for 7 days. Control pathology animals were treated with an equivalent volume of drinking water (1 ml/100 g mass). Hepatotoxin was administered daily for 2 days (day 8 and day 9 of the experiment). 24 hours after the last injection of alcohol – CCl<sub>4</sub>, the animals were removed from the experiment, the liver was excluded, which was weighed and its weight factor was calculated, and blood was collected for biochemical study.

To assess the functional state of the liver the biochemical parameters were determined: in serum – the activity of alanine aminotransferase enzymes (ALT) and aspartate aminotransferase (AST) (using standard sets of the Phyllis-Diagnostics production, Ukraine), for differential diagnostics, the De Ritis ratio (AST/ALT, norm 1.3) was used, which indicates the nature of the damage, since these enzymes exhibit organ specificity and which, when the liver is affected, is less than 1. The content of total lipids, cholesterol, HDL, LDL was determined by the sets of Lashema brand. The intensity of the lipid peroxidation (LPO) processes and the state of the natural antioxidant system (AOS) were determined by the content of TBA reactants [25] and reduced glutathione (RG) in the liver tissue [26].

Statistical processing of the results was conducted using generally accepted statistical methods, by calculating the arithmetic mean  $(\overline{x})$  and average errors  $(S\overline{x})$ . The reliability of the obtained results was evaluated according to the criterion of reliability of Student. The difference in probability values was p>0.95 (significance level p). To process the experimental data obtained were used "Statistica v.10.1" and "Microsoft Excel".

#### **Results and Discussion**

The study of acute toxicity of the common cat's foot herb dry extract with intragastric administration to mice

Acute toxicity of the common cat's foot herb dry extract was studied in adult rats in females and males. The experiment used 10 adult rats (5 males and 5 females) with a body weight of 170-190 g. The maximum dose of the toxicity class 4 (low-toxic substances) was selected as the limiting indicator for determining acute toxicity, taking into account the way of administration, namely intragastric – 5000 mg/kg.

After single intragastric administration of test specimens to rats of both sexes, no deaths of experimental animals were recorded during the entire observation period. No abnormal appearance and toxic effects of the test specimens were observed after the introduction of the test specimen and until the end of the observation period. All animals were active, had smooth wool and clear skin, and their eating behavior was unchanged. The lack of lethality in animals suggests that the value of LD<sub>50</sub> with enteral administration of

the extracts exceeds the maximum dose used in the experiment, ie in rats  $LD_{50}$ > 5000 mg/kg.

At the end of the observation period (14 days), an autopsy was performed and a macroscopic examination of the internal organs. During the autopsy, all animals had a neat wool, unchanged mucous membranes of natural openings. Subcutaneous lymph nodes normal in size and to the touch, in the peritoneal cavity unchanged serous peritoneum were observed. In appearance, the liver, kidneys and adrenal glands without visible signs of pathology - the color, shape, size of organs were normal. No nodular formations were noted. The pancreas was gravish pink. The spleen was fullblooded, resilient. Gastric mucosa was with pronounced relief of folds. The organ retained its characteristic anatomical structure. The intestinal mucosa was normal. Testes, prostate had normal appearance. In the thoracic cavity, all the organs were located anatomically correct. The heart muscle in the section was dark red, homogeneous. The lungs were airy, the pleura leaves were unchanged. Thymus gland (thymus) was without features. The lymph nodes of the thoracic and peritoneal cavities were not altered in appearance.

The calculation on subsequent analysis of the masses indices of animals' internal organs showed that the use of the common cat's foot herb dry extract did not lead to their change and the indicators were within the physiological norm (Table 1).

No changes in the internal organs of animals were observed at intragastric administration of the common cat's foot herb dry extract at a dose of 5000 mg/kg; their indicators were within the physiological norm, which gives the right to characterize the investigated extract according to the classification of substances by toxicity, as lowtoxic, therefore further establishment of the mean lethal dose of the common cat's foot herb dry extract was considered inappropriate [27].

Results of the study of the common cat's foot herb dry extract hepatoprotective activity

Today, many drugs are often used to successfully treat many diseases, which, together with high efficacy, have a number of side effects, including hepatotoxicity. In contrast to the lungs and kidneys, which also suffer from the toxic effects of intravenously and orally administered drugs, liver damage occurs more frequently with enteral administration, which is related to the peculiarities of the blood supply to the liver and its metabolism.

Complex therapy of liver diseases of different genesis requires the use of safe multifunctional remedies – hepatoprotectors, which contribute to the preservation and repair of damaged liver tissue [28]. Many years of medical practice have proven that herbal remedies for the treatment of a number of diseases are not inferior to synthetic analogs, and because of the absence of side effects and contraindications have many advantages [29].

Despite the sufficiently wide arsenal of hepatoprotective agents, the problem of effective treatment of liver diseases remains unresolved, which makes it urgent to search for or create new effective agents.

Study of hepatoprotective activity of the common cat's foot herb dry extract (CCDE) was performed on a model of acute toxic hepatitis caused by  $alcohol - CCl_4$ . The results of the studies are given in Tables 2-4.

The data in Table 2 show that oral administration of alcohol –  $CCl_4$  to rats caused a clear activation of the LPO process and was accompanied by a plausible, relatively intact control, accumulation of TBA reactants in the liver. The depletion of the hepatic pool of reduced glutathione (Table 2) indicates a decrease in the power of the animal body's antioxidant protection. Under the influence of alcohol –  $CCl_4$ , the formation of cytolysis syndrome was observed, one of the markers of which is considered to be a significant increase in the activity of the enzymes ALAT and ASAT, resulting in a decrease in the Ritis ratio twice, indicating severe liver damage (Table 3).

A significant decrease in the level of HDL was found in the model of alcohol –  $CCl_4$  liver lesions in determining the lipid spectrum, which confirms the violation of the synthetic function of the liver under pathology. Other studied indicators of significant changes in pathology were not found.

The results of the study showed that in tissues of the liver (Table 2), CCDE at a dose of 25 mg/kg and

50 mg/kg contributed to the inhibition of the processes of lipid peroxidation (POL) – the level of TBA-reactants decreased by 1.2 and 1.4 times, however, the pool of reduced glutathione (RG) remained at levels of disease control (DC). At the same time, the level of ALAT in relation to the DC group decreased (Table 3). However, lipid metabolism was not significantly affected by the extract in these doses.

According to the results of the studies, the administration of CCDE at doses of 25 and 50 mg/kg and the comparator drug had a positive effect on the course of toxic hepatitis. In the groups receiving CCDE and Silibor, 100 % survival was recorded, while the DC group had only 78 % survival. Changes in coefficient of the liver weight in the groups (Table 4) that received the medication were found to be authentic with respect to the normal control (NC) group.

Prophylactic use of the comparator drug Silibor in the dose of 100 mg/kg had a positive effect on the processes of LPO – the values of the content of TBAreactants and RG in the liver did not differ from the values of NC. The analysis of the obtained data showed that under the influence of Silibor in serum the activity of ALAT decreased, as a result of which the Ritis ratio increased, the content of HDL significantly increased and the level of LDL decreased, which indicates the antiatherogenic properties of the agent. Under the influence of Silibor, the survival rate was also 100 %. However, the coefficient of the liver weight remained increased (Table 4).

Thus, the data obtained indicate that CCDE has a pronounced hepatoprotective effect at a dose of 50 mg/kg. Therapeutic and prophylactic administration of CCDE at this dose contributed to 100 % survival of animals, preservation of lipidosynthetic function. In addition, CCDE showed moderate antioxidant properties.

#### Conclusions

The conducted researches allow us to state, that the common cat's foot herb dry extract at a dose of 50 mg/kg has a hepatoprotective effect, which is realized due to the membrane-stabilizing and antioxidant properties of the extract's biologically active substances. The obtained results can be used in a further preclinical study of the studied raw material's dry extract to create new hepatoprotective agents on its basis.

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**Table 1.** Mass coefficients of rats' internal organs in the study of acute toxicity of the common cat's foot herbdry extract in the intragastric way of administration,  $\overline{X} \pm S_{\overline{x}}$ 

Group of animals	Mass coefficient of an organ							
	liver	kidneys		lungs	Adrenal	heart	spleen	thymus
		left	right	141185	glands			,
Female rats	3.66	0.33	0.33	0.73	0.027	0.036	0.38	0.154
	±0.16	±0.01	±0.01	±0.02	±0.005	±0.01	±0.03	±0.020
Male rats	3.61	0.33	0.33	0.67	0.031	0.038	0.37	0.152
	±0.13	±0.01	±0.01	±0.03	±0.001	±0.01	±0.02	±0.013

All values are expressed in mean ± S.E.M., n=5, \* p < 0.05 significant

**Table 2.** The effect of the common cat's foot herb dry extract and Silibor on indicators of the LPO/AOS system in liver homogenate in rats,  $\overline{X} \pm S \overline{x}$ 

Index	NC	DC	DC+CCDE, 25 mg/kg	DC+CCDE, 50 mg/kg	DC+Silibor, 100 mg/kg
TBA, μmol/g	52.52 ± 3.41	88.76 ± 5.60*	74.49 ± 2.32 */**	65.13 ± 4.50	65.69 ±7.10
RG, μmol/g	3.57 ± 0.15	2.70 ± 0.11 *	2.71 ± 0.23 *	2.94 ± 0.10 *	3.16 ± 0.15

Values are mean ± S.E.M. (n=9)

The Kruskal-Wallis test and Mann-Whitney test

\*- deviations are authentic for normal control (NC) group values, when p<0.05

\*\* – deviations are authentic regarding disease control (DC) group values, when p<0.05

Schummats, $x \pm y_x$						
Index	Index NC		DC+CCDE,	DC+CCDE,	DC+Silibor,	
			25 mg/kg	50 mg/kg	100 mg/kg	
ALAT, mmol/ŀh	0.61 ± 0.05	5.52 ± 0.22*	2.65 ± 0.24*/**	2.82 ± 0.03*/**	3.12 ± 0.21*/**	
ASAT, mmol/ŀh	0.84±0.05	3.31 ± 0.19*	2.54 ± 0.04*/**	2.52 ± 0.06*/**	2.81 ± 0.11*/**	
Ritis ratio (ASAT/ALAT)	1.38 ± 0.07	0.60 ± 0.05*	0.95 ± 0.06*/**	0.89±0.05*/**	0.90±0.10*/**	
Cholesterol, mmol/l	1.91±0.15	1.03±0.16*	1.21±0.16	1.35±0.22	1.36±0.25	
HDL, mmol/l	2.34±0.12	0.89±0.08*	1.11±0.08*	1.08±0.08*	1.52±0.26*/**	
Common lipids, g/l	1.65 ± 0.28	1.46 ± 0.35	1.05 ± 0.40	1.75 ± 0.18	1.10 ± 0.25	
LDL, g/l	0.90 ± 0.03	0.73 ± 0.14	0.88 ± 0.19	0.85 ± 0.14	0.65 ± 0.06*	

**Table 3.** The effect of the common cat's foot herb dry extract and Silibor on biochemical parameters of blood serum in rats,  $\overline{X} \pm S_{\overline{x}}$ 

Values are mean ± S.E.M. (n=9)

The Kruskal-Wallis test and Mann-Whitney test

\*- deviations are authentic for normal control (NC) group values, when p<0.05

\*\* – deviations are authentic regarding disease control (DC) group values, when p<0.05

**Table 4.** The effect of the common cat's foot herb dry extract and Silibor on survival on the coefficient of liver weight on a model of acute alcohol –  $CCl_4$  liver damage in rats,  $\overline{X} \pm S_x^-$ 

Index	NC	DC	DC+CCDE,	DC+CCDE,	DC+Silibor,
			25 mg/kg	50 mg/kg	100 mg/kg
Survival of animals, %	100	78	100	100	100
Coefficient of liver weight	2.76 ± 0.10	4.28 ± 0.23*	4.68 ± 0.31*	4.29 ± 0.16*	4.25 ± 0.39*

Values are mean ± S.E.M. (n=9)

The Kruskal-Wallis test and Mann-Whitney test

\*- deviations are authentic for normal control (NC) group values, when p<0.05