



# Antitoxic Effects of Chicory Herb Extract Fractions in Rats with Toxic Hepatitis

Irina A. Lupanova\*, Olga L. Saybel, Elena N. Kurmanova, Ekaterina V. Ferubko, Irina A. Martynchik, Praskovia G. Mizina

All-Russian Research Institute of Medicinal and Aromatic Plants, Moscow, Russian Federation

**Aim.** To evaluate the antitoxic activity of chicory herb extract fractions in rats with toxic hepatitis.

**Materials and methods.** There were 64 male rats were divided into groups of 6 animals that received the whole chicory herb extract, its fractions, or the reference drug (RD) and 2 groups did not receive any drug. The Silimar substance (a dry purified extract obtained from the fruits of milk thistle [*Silybum marianum*]) was chosen as RD. Animals from one of the groups that did not receive the drug (control group) and the groups that received drugs were injected with CCl<sub>4</sub> to simulate toxic liver damage. The last group of animals received neither drugs nor CCl<sub>4</sub> (intact animals). The changes in the activity of biotransformation enzymes (cytochrome P<sub>450</sub> [cytP<sub>450</sub>] and glutathione transferase [GT]) in hepatocytes and serum liver enzymes (AST, ALT and AP) were assessed.

**Results.** There was a decrease in cytP<sub>450</sub> and GT activity in the liver microsomes of control rats (having CCl<sub>4</sub> hepatitis without treatment) compared with intact animals. These parameters were more in the animals that were administrated with chicory herb extract, its fractions, and RD than in control animals. The administration of the whole extract, aqueous fraction, and RD led to an increase in the activity of cytP<sub>450</sub> and GT enzymes almost to the level of the same indicators in intact animals. Pronounced grown in the activity of serum liver enzymes (ALT, AST and AP) were found after CCl<sub>4</sub> injection. Administration of common chicory herb fractions before toxic liver damage caused a decrease in the grown of the activity of these enzymes. The greatest inhibitory effect on the grown of the activity of these liver enzymes had RD, aqueous and butanolic fractions of chicory herb. Despite a significant decrease in hyperfermentemia found in groups of animals treated with fractions of chicory herb, AST, ALT and AP activities did not reach the level that was in the intact animal group.

**Conclusion.** The aqueous and butanolic fractions of the chicory herb extract had the highest antitoxic activity in experimental animal toxic liver injury.

**Keywords:** *Cichorium intybus*; chicory herb extract; fractions; antitoxic activity; toxic hepatitis model

**Conflict of interest:** the authors declare no conflict of interest.

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## Сравнительное изучение антитоксических свойств фракций экстракта травы цикория обыкновенного у крыс с токсическим гепатитом

И.А. Лупанова\*, О.Л. Сайбель, Е.Н. Курманова, Е.В. Ферубко, И.А. Мартынич, П.Г. Мизина

ФГБНУ «Всероссийский научно-исследовательский институт лекарственных и ароматических растений», Москва, Российская Федерация

**Цель.** Оценить антитоксическую активность фракций веществ различной полярности экстракта травы цикория обыкновенного (*Cichorium intybus* L.) у крыс с токсическим гепатитом.

**Материалы и методы.** 64 крысы-самца были разделены на группы по 6 животных, получавших экстракт травы цикория целиком, его фракции или препарат сравнения, и 2 группы не получали никакого препарата. В качестве препарата сравнения была выбрана субстанция силимара (сухой очищенный экстракт, полученный из плодов расторопши пятнистой (*Silybum marianum*)). Животным одной из групп, не получавших препарат (контрольная группа), и групп, получавших препараты, вводили CCl<sub>4</sub> для имитации токсического поражения печени. Последняя группа животных не получала ни препаратов, ни CCl<sub>4</sub> (интактные животные). Оценивали изменения активности ферментов биотрансформации (цитохрома P450 (цит P450) и глутатионтрансферазы (ГТ)) в гепатоцитах и сывороточных ферментов печени (АСТ, АЛТ и ЩФ).

**Результаты.** Выявлено снижение активности цит Р450 и ГТ в микросомах печени крыс контрольной группы (с гепатитом, индуцированным  $CCl_4$ , без лечения) по сравнению с интактными животными. Эти показатели были больше у животных, которым вводили экстракт травы цикория, его фракции и препарат сравнения, чем у контрольных животных. Введение цельного экстракта, водной фракции и контрольного препарата привело к повышению активности ферментов цит Р450 и ГТ практически до уровня аналогичных показателей у интактных животных. Выраженное повышение активности сывороточных ферментов печени (АЛТ, АСТ и ЩФ) обнаруживалось после введения  $CCl_4$ . Введение фракций травы цикория обыкновенного до токсического поражения печени вызывало снижение роста активности этих ферментов. Наибольшее нивелирующее действие на рост активности этих печеночных ферментов оказали препарат сравнения, водная и бутанольная фракции травы цикория. Несмотря на достоверное снижение гиперферментемии, выявленное в группах животных, получавших фракции травы цикория, активность АСТ, АЛТ и ЩФ не достигла уровня, который был в группе интактных животных.

**Вывод.** Водная и бутанольная фракции экстракта травы цикория обладали наибольшей антитоксической активностью при токсическом поражении печени экспериментальных животных.

**Ключевые слова:** *Cichorium intybus*, экстракт травы цикория, фракции веществ различной полярности, антитоксическая активность, модель токсического гепатита

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## Introduction

To date, some progress has been achieved in the diagnosis and treatment of liver pathology of various origins. First of all, the successes are associated with the treatment of chronic hepatitis B and C, as well as with the active introduction of orthotopic liver transplantation into clinical practice. However, despite these facts, there is an increase in morbidity and mortality from chronic liver diseases. Damage that occurs during pathological processes in liver can lead to serious disorders of metabolism, immune response, detoxification and antimicrobial functions [1]. The pathogenesis of toxic and drug-induced liver injury is based on the direct effect of the toxicant on liver cells and its indirect effects on intracellular metabolism. Metabolic exposure to chemical agents leads to mitochondrial dysfunction, tissue hypoxia along with progressing adenosine triphosphate (ATP) deficiency, activation of free radical oxidation and consequently development of membrane and cytotoxicity.

Xenobiotics in the liver undergo biotransformation with the formation of less toxic metabolites, which are further transferred by transport proteins and eliminated from the body. Thus, the liver is involved in maintaining the biochemical homeostasis of the body, which directly depends on the state of the liver enzyme systems. A change in liver enzyme activity leads to a decrease in the liver detoxification function, as well as intensity in phospholipid biosynthesis in cell membranes, which leads to violation of liver function [2]. Hepatoprotectors used currently are often not effective enough, they can contribute to the increment of cholestasis and enzymatic hyperactivity of liver cells [3].

In this regard, the development of new hepatoprotective drugs for the correction of metabolic disorders in liver failure is relevant.

Several groups can be distinguished among the preventive and therapeutic agents that have a hepatoprotective effect and increase the resistance of hepatocytes to pathological influences:

- agents of animal origin (the detoxifying effect is due to the presence in the composition of hydrolyzed amino acids, low molecular weight metabolites and fragments of liver growth factors, due to which the functional activity of hepatocytes is restored);
- agents of plant origin (inhibit lipid peroxidation, having antihypoxic activity);
- essential phospholipids (phospholipids are directly integrated into the phospholipid bilayer of the damaged liver cell membranes, which leads to the restoration of the barrier function of this bilayer);
- bile acids (enhance the elimination of free radicals and other toxic metabolites from cells, stimulate the regeneration processes there);
- biologically active food supplements and homeopathic preparations (made based on raw materials of plant, animal and mineral origin and therefore have a complex effect on hepatocytes) [4].

The use of medicinal plants for the prevention and treatment of such lesions has a long history and has several advantages over other pharmacotherapy kinds, because herbal medicines have sufficient efficacy, a wide action spectrum, low toxicity, and therefore possibility of their long-term use.

Common chicory (*Cichorium intybus* L.), a representative of the *Asteraceae* family, is a promising source of hepatoprotective agents. This wild plant as

Table 1. BAS contents in the fractions of common chicory herb extract

Таблица 1. Содержание БАВ во фракциях экстракта травы цикория обыкновенного

	Total extract Суммарный экстракт	Ethyl acetate fraction Этилацетатная фракция	Butanolic fraction Бутанольная фракция	Alcoholic fraction Спиртовая фракция	Aqueous fraction Водная фракция
Mass fraction, % Массовая доля, %	100	11.24	15.25	20.0	53.21
Esculetin, % Эскулетин, %	0.40 ± 0.01	4.38 ± 0.15	0.83 ± 0.03	0.88 ± 0.04	0.00
Chicoryin, % Цикориин, %	1.40 ± 0.06	4.05 ± 0.14	5.25 ± 0.19	0.26 ± 0.01	0.00
Chicoric acid, % Цикориевая кислота, %	2.52 ± 0.08	1.24 ± 0.05	3.74 ± 0.15	0.53 ± 0.02	3.98 ± 0.16
Cafftaric acid, % Кафтаровая кислота, %	0.18 ± 0.007	0.08 ± 0.003	0.17 ± 0.008	0.09 ± 0.004	0.56 ± 0.02
Chlorogenic acid, % Хлорогеновая кислота, %	0.17 ± 0.008	5.88 ± 0.23	4.92 ± 0.17	0.53 ± 0.02	0.40 ± 0.02

a weed is widely distributed in Russia excluding the north regions [5].

Dried and roasted roots of cultivated common chicory are used to make healthy drinks and as a coffee substitute, young leaves are added to salads and vegetable dishes. In folk medicine in different countries, the herb and roots of the plant are used to alleviate violations of the functions of the liver and kidneys, in diabetes mellitus, as a choleric, antispasmodic, anti-inflammatory and hypoglycemic agent [6].

Based on the results of phytochemical studies and biological screening, the scientists of All-Russian Research Institute of Medicinal and Aromatic Plants (ARIMAP) have developed a method for obtaining a substance that is a dry common chicory herb extract. *In vivo* experiments confirmed hepatoprotective activity of this substance [7, 8].

Phenolic metabolites, namely oxycoumarins (esculetin and its chicoryin glycoside), hydroxycinnamic acids (chicory, chlorogenic, cafftaric acids and other derivatives of caffeic, coumaric, ferulic and shikimic acid) and flavonoids (glycosides of quercetin, kaempferol and luteolin) were identified in the obtained extract by high-performance liquid chromatography with tandem mass spectrometry detection (HPLC-MS/MS).

To identify a group of biologically active substances (BAS) that determine the antitoxic activity of a dry extract of common chicory herb, we fractionated this substance and obtained fractions of the substances of different polarity.

The aim of this study was to evaluate the antitoxic activity of chicory herb extract fractions through their effect on the activity of biotransformation enzymes (cytochrome P<sub>450</sub> and glutathione transferase) and enzymes marking of the morphofunctional state of hepatocytes in rats with toxic hepatitis.

## Materials and methods

Common chicory herb stocked at flowering stage in Rybnovsky district of the Ryazan region in 2019 has been used as raw material.

The crushed raw materials were extracted triply with ethyl alcohol 70 % in a ratio of 1:10 at a temperature of 60 ± 5 °C. The combined extract was concentrated on a rotary evaporator to an aqueous residue and treated sequentially with ethyl acetate, then with n-butanol. The resulting extracts were concentrated and dried until the solvent was completely removed obtaining ethyl acetate (EA) and butanolic (BU) fractions, respectively. After that, the aqueous residue was poured into a threefold volume of ethyl alcohol 96 % and left for 24 hours. The formed precipitate was separated by filtration and dried obtaining aqueous fraction (AQ). The supernatant solution was concentrated to dry residue formation and dried, it was alcohol fraction (AL).

According to our previous studies, it was found that the dominant components of common chicory herb dry extract are esculetin and chicoryin, as well as chicoric, chlorogenic, cafftaric acids. The content of these compounds was also determined in the studied fractions (table 1).

The Silimar substance, which is a dry purified extract obtained from the fruits of milk thistle (*Silybum marianum* (L.) Gaerth of the *Asteraceae* family) was chosen as the reference drug (RD).

Pharmacological studies were carried out in accordance with the Decision of the EAEC Council dated November 3, 2016 No. 81 "On Approval of the Rules for Good Laboratory Practice of the EAEU", the National Standard of the Russian Federation GOST 33044-2014 "Principles of Good Laboratory Practice", "Guidelines for preclinical studies of drugs" [9]. The research design was approved by the

Bioethical Commission of the FSBSI ARIMAP, protocol No. 42 dated 05.02.2021.

To study the antitoxic properties of common chicory herb fractions, we used a model of experimental toxic hepatitis in rats simulated by tetrachloromethane ( $\text{CCl}_4$ ) injection [9]. The experimental animals were kept in the vivarium of the FSBSI ARIMAP on a standard diet. Before the start of the experiment, the animals were quarantined for 14 days. In the experiments, 64 outbred male rats weighing 200–250 grams each were used. All the animals were the same age. The use of animals in the experiment was carried out in accordance with the recommendations of the European Convention for the Protection of Vertebrate Animals used for Experiments for Scientific or Other Purposes (1986). The experimental animals were divided into eight groups of 8 animals each. The animals of first (I) group were intact (healthy) rats, which were used to determine the initial background of liver functional state; the second (II) group – control animals, which before the injection of  $\text{CCl}_4$  for five days were injected intragastrically with an equivalent amount of purified water in a regimen similar to the experimental groups; groups from third to seventh (III–VII) were experimental ones, the rats received the test substances, namely the common chicory herb extract and different fractions of it for five days at a dose of 100 mg/kg; the eighth (VIII) group includes animals that received for five days the Silimar substance (reference drug) at a dose of 100 mg/kg, suspended in 1 % starch gel.

Liver toxic damage was caused by a single subcutaneous injection of a 50 %  $\text{CCl}_4$  oil solution (produced by Component-Reaktiv LLC, Russia) at a dose of 0.4 ml per 100 g of body weight, one hour after the last injection of the test substances. 48 hours after  $\text{CCl}_4$  injection, blood samples were taken from the tail vein of rats. On the eighth day of the experiment, the rats were euthanized in a  $\text{CO}_2$  chamber.

Biochemical studies were performed by conventional methods using CS-T240 biochemical analyzer (Dirui, China). Liver functional state was characterized by the enzymatic activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), as well as the contents of total bilirubin, glucose and cholesterol in the blood serum of rats.

Microsomes were isolated from the livers of intact animals, as well as of animals with experimental toxic hepatitis, which did not receive (control) and received samples of the chicory herb extract, fractions of it, and the reference drug. The liver samples were frozen at  $-80^\circ\text{C}$  for subsequent isolation of enzymes. The microsomal fraction from frozen liver samples was isolated by differential centrifugation method [10]. The protein content in the microsome suspension was determined by Lowry method [11] in presence of 0.1 % sodium deoxycholate. The content of  $\text{cytP}_{450}$  was determined by spectrophotometry, according to the method of Omura and Sato [12],

by recording the differential absorption spectra of the CO complex reduced by sodium dithionite using Shimadzu MPS-2000 spectrophotometer (Japan). The rates of demethylase and hydroxylase reactions of  $\text{cytP}_{450}$  were determined by spectrophotometry, in the kinetic mode, by a decrease in absorption at 340 nm due to the oxidation of NADPH in the presence of  $\text{cytP}_{450}$  substrates, namely aniline, 3  $\mu\text{M}$ . The concentration of microsomes in the sample was 1 mg protein/mL. As a buffer solution, 0.1 M K-phosphate (pH 7.4) was used.

The activity of glutathione transferase was calculated by measuring the increase in the optical density of conjugation products at the wavelength of 340 nm [13].

As a result of the first detoxification phase, cytochrome  $\text{P}_{450}$  ( $\text{cytP}_{450}$ ) increases solubility of a hydrophobic substances, and these molecules may lose biological activity or becomes more active [14]. The conjugation of molecules with glutathione by glutathione transferase (GT) may lead to a decrease these toxic potential but may increase it [15].

The contents of biologically active substances in the obtained fractions were determined by HPLC-UV using LC-2030C 3D chromatograph (Shimadzu, Japan) equipped with a diode array detector. Chromatography was carried out on a Luna 5 $\mu\text{m}$  C18 100 Å column (250×4.6 mm), at a column thermostatting temperature of  $30^\circ\text{C}$ , a mobile phase flow rate of 1 ml/min, and an injection volume of the test solution of 10  $\mu\text{l}$ . As a mobile phase, solvent systems of 0.2 % formic acid solution (A) and acetonitrile (B) were used in a gradient elution mode: (0–20 min – 10 % B, 20–30 min – 10–25 % B, 30–40 min – 40 % B, 40–44 min – 60 % B, 44–48 min – 80 % B, 48–60 min – 10 % B).

Statistical data processing was carried out using the licensed software package for statistical analysis Statistica 13 (StatSoft, USA). To assess the significance of differences between samples having a normal distribution, the parametric Student's t-test, and for other distributions, the Mann-Whitney U-test were used. The mean value (M) and the standard error of the mean (m) were calculated. Differences between the compared values were considered significant at a probability level of 95 % or more ( $p < 0.05$ ).

## Results

Table 2 showed a decrease in  $\text{cytP}_{450}$  aniline hydroxylase and GT activity and  $\text{cytP}_{450}$  content in the liver microsomes of control rats (having  $\text{CCl}_4$  hepatitis without treatment) compared with intact animals. However, these parameters were more in the animals that were administrated with chicory herb extract, its fractions, and RD than in control animals. It indicates that the tested substances possess significant detoxifying activity. The degree of GT activation increased in the following order: EA fraction < BU fraction < AL fraction < RD < whole

extract < AQ fraction. It should be noted that the administration of the whole extract, AQ fraction, and RD led to an increase in the content of cytP<sub>450</sub> and the activity of cytP<sub>450</sub> and GT enzymes almost to the level of the same indicators in intact animals. This proves a property of this plant to activate the detoxifying liver function [15].

Pronounced changes in the activity of liver enzymes and significant ( $p < 0.05$ ) violations of metabolic processes (decrease in glucose and cholesterol blood levels) were found after CCl<sub>4</sub> injection (Table 3). Administration of common chicory herb fractions in rats before toxic liver damage caused a decrease in the growth of the activity of AST, ALT and AP. The greatest inhibitory effect on growth of the activity of liver enzymes had RD (AST by 15 %, ALT by 33 % and AP by 14 %,  $p < 0.05$ ), AQ (by 20.5 %, 40.9 % and 11.8 %, respectively;  $p < 0.05$ ) and BU (by 18.2 %, 33.2 % and 17.1 %, respectively;  $p < 0.05$ ) fractions. Despite a significant decrease in hyperfermentemia found in groups of animals treated with fractions of common chicory herb, AST, ALT and AP activities did not reach the level that was in the intact animal group. The revealed effect of fractions on the activity of liver enzymes is consistent

with the previously results obtained for the common chicory herb extract [7].

Hypoglycemia in rats with CCl<sub>4</sub> intoxication may be probably associated with impaired processes of glycogenolysis and gluconeogenesis in the liver [2]. In rats with CCl<sub>4</sub> intoxication treated with fractions of the common chicory herb extract, an increase in the concentration of glucose and cholesterol was noted. The greatest influence comparable to the effect of the RD, had AQ (glucose content increased by 10.2 %, cholesterol increased by 28.8 %;  $p < 0.05$ ), and BU (by 16.2 % and 16.7 %, respectively;  $p < 0.05$ ) fractions.

## Discussion

From the data presented above, it can be seen that the AQ and BU fractions (that contain predominantly chicory and chlorogenic acids) of common chicory herb had the maximum hepatoprotective effect in this model.

According to the literature data, the hepatoprotective effect of many herbal drugs is associated with antioxidant activity [16]. The role of hydroxycinnamic acids in the manifestation of the antioxidant

**Table 2.** The results of studying the effect of common chicory herb fractions on the content of cytP<sub>450</sub> and on the activity of key biotransformation enzymes in a CCl<sub>4</sub> hepatitis model in rats ( $M \pm m$ )

**Таблица 2.** Результаты изучения влияния фракций травы цикория обыкновенного на содержание цитP<sub>450</sub> и на активность ключевых ферментов биотрансформации в условиях модели CCl<sub>4</sub> гепатита у крыс ( $M \pm m$ )

Test variant, $n = 8$ Вариант опыта, $n = 8$	cytP <sub>450</sub> content (nM/mg protein) Содержание цитP <sub>450</sub> (нмоль/мг белка)	Hydroxylation of aniline, nm NADPH/nmol цит P <sub>450</sub> Гидроксилирование анилина, нмоль НАДФН/нмоль цит P <sub>450</sub>	GT activity, nm of product/mg/min Активность ГТФ, нмоль продукта/мг/мин
Intact Интakтные	26.25 ± 1.22	14.60 ± 0.65	351.4 ± 26.7
Control (CCl <sub>4</sub> hepatitis) Контрольные (CCl <sub>4</sub> гепатит)	13.30 ± 0.62	4.90 ± 0.43	153.4 ± 15.2
Extract, 100 mg/kg Экстракт, 100 мг/кг	22.13 ± 1.05*	9.11 ± 0.44*	450.5 ± 21.9*
AL, 100 mg/kg СП, 100 мг/кг	13.92 ± 0.50	7.90 ± 0.87*	335.0 ± 26.5*
EA, 100 mg/kg ЭА, 100 мг/кг	16.24 ± 0.60*	6.90 ± 0.50*	231.5 ± 19.5*
BU, 100 mg/kg БУ, 100 мг/кг	18.36 ± 0.60*	8.70 ± 0.80*	320.9 ± 27.6*
AQ, 100 mg/kg ВО, 100 мг/кг	20.71 ± 1.34*	8.90 ± 0.65*	473.5 ± 28.5*
Silimar, 100 mg/kg Силимар, 100 мг/кг	23.62 ± 1.25*	12.00 ± 1.43*	381.2 ± 22.9*

Note: Hereinafter ± statistical significance of differences in comparison with intact control,  $p < 0.05$ , \* in comparison to the hepatitis model,  $p < 0.05$

Примечание: Далее и везде ± статистическая значимость различий в сравнении с интактным контролем,  $p < 0,05$ , \* относительно модели гепатита,  $p < 0,05$ .

**Table 3.** The results of studying the effect of common chicory herb fractions on the activity of liver enzymes and the serum level of glucose and cholesterol in rats with  $CCl_4$  intoxication ( $M \pm m$ )

**Таблица 3.** Результаты изучения влияния фракций травы цикория обыкновенного на активность ферментов-маркеров морфофункционального состояния гепатоцитов и содержание глюкозы и холестерина в сыворотке крови крыс с интоксикацией  $CCl_4$  ( $M \pm m$ )

Rat groups, <i>n</i> = 8 Группы крыс, <i>n</i> = 8	AST, U/L АСТ, U/л	ALT, U/L АЛТ, U/л	AP, U/L ЩФ, U/л	Bilirubin total, $\mu$ M/L Билирубин общ, мкмоль/л	Glucose, mm/L Глюкоза, ммоль/л	Cholesterol, mm/L Холестерин, ммоль/л
Intact Интактные	96.8 $\pm$ 6.5	55.9 $\pm$ 5.0	393.2 $\pm$ 3.5	2.4 $\pm$ 0.26	5.3 $\pm$ 0.46	1.9 $\pm$ 0.14
Control ( $CCl_4$ hepatitis) Контрольные ( $CCl_4$ гепатит)	143.9 $\pm$ 12.0	111.0 $\pm$ 6.4	545.6 $\pm$ 8.1	2.8 $\pm$ 0.04	4.4 $\pm$ 0.08	0.66 $\pm$ 0.07
AL, 100 mg/kg СП, 100 мг/кг	134.8 $\pm$ 2.1*	84.2 $\pm$ 2.9*	543.2 $\pm$ 4.8*	2.46 $\pm$ 0.08*	4.5 $\pm$ 0.36	0.74 $\pm$ 0.07*
EA, 100 mg/kg ЭА, 100 мг/кг	129.2 $\pm$ 3.1*	93.1 $\pm$ 1.7*	484.0 $\pm$ 6.9*	2.4 $\pm$ 0.4*	4.6 $\pm$ 0.08*	0.79 $\pm$ 0.08*
BU, 100 mg/kg БУ, 100 мг/кг	117.8 $\pm$ 2.4*	74.1 $\pm$ 1.7*	452.0 $\pm$ 6.8*	2.5 $\pm$ 0.06*	5.1 $\pm$ 0.13*	0.77 $\pm$ 0.02*
AQ, 100 mg/kg ВО, 100 мг/кг	114.5 $\pm$ 4.5*	65.6 $\pm$ 3.1*	481.0 $\pm$ 16.2*	2.4 $\pm$ 0.03*	4.8 $\pm$ 0.08*	0.85 $\pm$ 0.02*
Silimar, 100 mg/kg Силимар, 100 мг/кг	113.70 $\pm$ 3.94*	65.56 $\pm$ 1.23*	479.00 $\pm$ 8.80*	2.34 $\pm$ 0.08*	4.88 $\pm$ 0.03*	0.92 $\pm$ 0.04*

Note: \*  $p < 0.05$  (compared to control).

Примечание: \* относительно модели гепатита,  $p < 0,05$ .

effect of chicory extracts is also described by other researchers.

A. Epure et al. (2021) showed that antioxidant activity of chicory extract is determined by presence of flavonoids and phenolic acids, substantially by chicory acid. At the same time, it was noted that activity of phenolic compounds *in vivo* is more than *in vitro* [17].

The lyophilized extract of chicory seeds that was studied by J. Milala et al. (2009) showed the highest antioxidant properties among the extracts of various parts of this plant; the content of polyphenolic compounds in it was 10 % including 71 % dicaffeoylquinic acids [18].

O.E. Hussein et al. (2020) found that the protective effect of chicory acid in liver injury after administration of methotrexate to animals is associated with attenuation of oxidative stress and inflammation and up-regulation of Peroxisome Proliferators'-Activated Receptor  $\gamma$  (PPAR $\gamma$ ) and Nrf2/HO-1 signaling [19].

The different scientific studies demonstrates that chlorogenic acid has very valuable effects including anti-inflammation and antioxidant [20]. For example, Naveed et al. (2018) reported that chlorogenic acid in mice fed a high-fat diet and obese mice highly decreased the expression of macrophage

biomarker genes in adipose tissue (such as Cd11c, Cd11b, Cd68, and F4/80) and pro-inflammatory mediator genes (such as MCP-1 and TNF- $\alpha$ ) in macrophages. Furthermore, the researchers found that chlorogenic acid inhibited the hepatic PPAR $\gamma$ , which promotes the fatty acids uptake into liver cells [21]. Hence, it was proposed that chlorogenic acids contribute scavenging reactive oxygen species generated in cells as a result of a high-fat diet, and this suppresses the expression of inflammation, resulting in the reduction of insulin resistance, fat accumulation, and body weight, while inhibition of PPAR $\gamma$  prevents the liver steatosis [19].

## Conclusion

The aqueous and butanolic fractions of chicory herb extract showed maximal antitoxic properties in the experimental model of toxic liver injury. It manifested in a significant trend towards the normalization the disorders of proteins, carbohydrates and lipids metabolisms. These fractions contain predominantly chicoric and chlorogenic acids; most probably, these compounds play dominant role in the antitoxic effect of the fractions, as well as the total extract as a whole.

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**Information about the authors**

**Irina A. Lupanova** — Cand. Sci. (Biol.), Head of pre-clinical studies center, All-Russian Research Institute of Medicinal and Aromatic Plants.

Contact information: lupanova@vilarnii.ru;  
117216, Moscow, Grin str., 7–1.  
ORCID: <https://orcid.org/0000-0001-8183-2877>

**Olga L. Saybel** — Cand. Sci. (Pharm.), Head of the center for Chemistry and Pharmaceutical Technology All-Russian Research Institute of Medicinal and Aromatic Plants.

Contact information: saybel@vilarnii.ru;  
117216, Moscow, Grin str., 7–1.  
ORCID: <https://orcid.org/0000-0001-8059-5064>

**Elena N. Kurmanova** — research scientist of experimental pharmacology department, All-Russian Research Institute of Medicinal and Aromatic Plants

Contact information: kurmanova@vilarnii.ru;  
117216, Moscow, Grin str., 7–1.  
ORCID: <https://orcid.org/0000-0002-9243-5268>

**Ekaterina V. Ferubko** — Dr. Sci. (Med.), head of experimental pharmacology department All-Russian Research Institute of Medicinal and Aromatic Plants.

Contact information: ferubko@vilarnii.ru;  
117216, Moscow, Grin str., 7–1.  
ORCID: <https://orcid.org/0000-0003-1949-2597>

**Irina A. Martynchik** — research scientist of experimental pharmacology department, All-Russian Research Institute of Medicinal and Aromatic Plants.

Contact information: martynchik@vilarnii.ru;  
117216, Moscow, Grin str., 7–1.  
ORCID: <https://orcid.org/0000-0001-7081-5545>

**Praskovia G. Mizina** — Dr. Sci. (Pharm.), Prof., deputy director, All-Russian Research Institute of Medicinal and Aromatic Plants.

Contact information: pg-mizina@vilarnii.ru;  
117216, Moscow, Grin str., 7–1.  
ORCID: <https://orcid.org/0000-0001-7494-2365>

**Сведения об авторах**

**Лупанова Ирина Александровна\*** — кандидат биологических наук, руководитель Центра доклинических исследований ФГБНУ «Всероссийский научно-исследовательский институт лекарственных и ароматических растений».

Контактная информация: lupanova@vilarnii.ru;  
117216, Москва, ул. Грина, д. 7, стр. 1.  
ORCID: <https://orcid.org/0000-0001-8183-2877>

**Сайбель Ольга Леонидовна** — кандидат фармацевтических наук, руководитель Центра химии и фармацевтической технологии ФГБНУ «Всероссийский научно-исследовательский институт лекарственных и ароматических растений».

Контактная информация: saybel@vilarnii.ru;  
117216, Москва, ул. Грина, д. 7, стр. 1.  
ORCID: <https://orcid.org/0000-0001-8059-5064>

**Курманова Елена Николаевна** — научный сотрудник отдела экспериментальной фармакологии ФГБНУ «Всероссийский научно-исследовательский институт лекарственных и ароматических растений».

Контактная информация: kurmanova@vilarnii.ru;  
117216, Москва, ул. Грина, д. 7, стр. 1.  
ORCID: <https://orcid.org/0000-0002-9243-5268>

**Ферубко Екатерина Владимировна** — доктор медицинских наук, заведующая отделом экспериментальной фармакологии ФГБНУ «Всероссийский научно-исследовательский институт лекарственных и ароматических растений».

Контактная информация: ferubko@vilarnii.ru;  
117216, Москва, ул. Грина, д. 7, стр. 1.  
ORCID: <https://orcid.org/0000-0003-1949-2597>

**Мартынчик Ирина Анатольевна** — старший научный сотрудник отдела экспериментальной фармакологии ФГБНУ «Всероссийский научно-исследовательский институт лекарственных и ароматических растений».

Контактная информация: martynchik@vilarnii.ru;  
117216, Москва, ул. Грина, д. 7, стр. 1.  
ORCID: <https://orcid.org/0000-0001-7081-5545>

**Мизина Прасковья Георгиевна** — доктор фармацевтических наук, профессор, заместитель директора по научной работе ФГБНУ «Всероссийский научно-исследовательский институт лекарственных и ароматических растений».

Контактная информация: pg-mizina@vilarnii.ru;  
117216, Москва, ул. Грина, д. 7, стр. 1.  
ORCID: <https://orcid.org/0000-0001-7494-2365>

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\* Corresponding author / Автор, ответственный за переписку