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EXPERIMENTAL STUDIES ON THE HEPATOPROTECTIVE PROPERTIES OF HERBAL SUBSTANCES

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INTRODUCTION

Liver diseases include a wide range of pathologies: from fatty hepatosis (steatosis) and hepatitis to cirrhosis liver cirrhosis and hepatocellular carcinoma. They are widespread throughout the world and have a high incidence of hepatitis. widespread throughout the world and have a high social relevance[1]. Liver failure, especially its severe form caused by cirrhosis, is the 12th leading cause of death in the world. liver failure, especially the severe form caused by cirrhosis, is the 12th leading cause of death in the world[2]. Cirrhosis not only causes impairment of liver function. liver function disorders, but also cause hepatic encephalopathy syndrome. encephalopathic syndrome, which is cognitive and psychomotor disorders. Hepatic encephalopathy can lead to Disability[3]. Over the past 10 years non-alcoholic fatty liver dysfunction has become non-alcoholic fatty liver dysfunction has become the leading chronic liver disease type, occurring in over 30 % of the population[4]. The development of fatty hepatosis is statistically associated with diabetes mellitus, cardiovascular disease, obesity. Its occurrence is provoked by the use of drugs medications and exposure to toxic compounds. Steatosis has been found to be associated with other chronic diseases such as sleep apnoea, colorectal cancer, osteoporosis, psoriasis and endocrine disorders[5]. Non-alcoholic fatty liver dystrophy is a is a nonspecific response of hepatocytes to toxic effects. The main characteristic of this condition is an excessive accumulation of fat in the liver. In severe fatty dystrophy, fat cysts can be observed. Fat cysts and

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connective tissue overgrowth can be observed. tissue overgrowth can be observed which leads to functional impairment of the liver and associated systemic pathologies. liver and related systemic pathologies.

Multiple signalling and metabolic pathways, involved in the regulation of liver function provide a choice of therapeutic targets[6]. Various agents are used as hepatoprotective compounds in complex therapy: antibiotics (Neomycin, Paromycin, Metronidazole, Vancomycin, Rifaximin)[7] and disaccharides (Lactulose, Lactitol) with low absorption capacity[8], natural amino acids and nitrogen metabolites (ornithine aspartate, branched chain amino acids)[9], modulators of bacterial intestinal microflora (probiotics, synbiotics)[10], bile acid derivatives acids and β-agonists of thyroid hormone receptors[11]. However, the currently available tools for prophylaxis and treatment of liver pathologies of various severity and ethology of liver pathology include mainly symptomatic medications of a rather broad range of action. a wide range of action, a considerable part of which are either not recommended for long term use or not approved for use in a number of countries. Among medicines with high evidence of efficacy and safety of long-term hepatoprotectors we can distinguish Essential phospholipids (EPP), ursodeoxycholic acid (UDHL), milk thistle preparations, ademetionine[12]. Natural polyphenols are widely used now as antioxidant pharmacological substances that have a general anti-inflammatory, neuro- and cardioprotective actions, modelling autophagy and protecting mitochondria from pathological events by inducing signalling pathways

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of cell survival[13]. The success of polyphenols application polyphenols for the treatment of diseases with complex ethology (neurodegenerative disorders of various origins, autoimmune, allergic, cancer and prion diseases) is due to their direct effect on the cells of the body's defence systems organism and induction of apoptosis in cells of key tissue[14]. Polyphenols of plant origin have influence on oxidative stress, lipid metabolism, insulin resistance and inflammation, which are the most important pathological processes in aetiology of liver diseases[1]. Positive effects of some polyphenols effect of some polyphenols on functional state of liver in model of toxic hepatitis, induced by various hepatotoxicants. Thus, natural flavonoid quercetin protected liver from dysfunction induced by tetrachlormethane (CCl_4).

The authors attributed the mechanism of its effect to antioxidant action, as well as the inhibition of a number of reactions with participation of NF-κB, which resulted in decrease of the level of hepatic inflammatory cytokine secretion[15]. A topical problem of contemporary medicine is the development of innovative, safe and therapeutically effective drugs for the treatment of hepatobiliary system diseases. Liver pathologies are one of the most common causes of morbidity and mortality. Toxic liver injuries constitute an important problem[16, 17, 18, 19]. Drug injuries of the liver constitute a significant part of all adverse reactions of the human organism associated with the use of drugs [20, 21, 22, 23, 24, 25, 26]. Moreover, diseases caused by functional liver disorders are a challenge for medical researchers.

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In spite of the fact that the scientific researches in the field of the creation of new hepatorenal drugs are carried out continuously, the field of these drugs is still limited. Thus, the search for effective hepatoprotectors remains an urgent and promising task.

Natural polyphenols have been considered for the search of new phytobiotics with potential hepatoprotective properties, since this group of herbal compounds exhibits a wide spectrum of pharmacological activity and plays an important role in the regulation of oxidative balance in the human organism[27, 28, 29].

The results of the analysis of literature data concerning the phytochemical composition and use of domestic plums and grapes in folk medicine became the basis for experimental research and the creation of medicinal products with hepatoprotective properties[30, 31, 32, 33, 34, 35].

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The studies were devoted to the investigation of antioxidant and anticytolytic properties in liver pathology and to the determination of the most therapeutically active dose for the indicators of lipid peroxidation (LPO) and the activity of the cytolytic enzyme alanine aminotransferase (ALT) of plant substances, which are inhibited by polyphenol compounds: extracts from the leaf of the domestic plum and the fruit of the domestic plum, as well as grape seed oil.

1. Phytochemical characteristics of the studied substances

The object of the study were extracts from the Plum (*Prunus domestica* L., fam. *Rosaceae*) leaves and fruits of the *Ugorka* variety, obtained using an original technique for extracting biologically active substances from raw materials. The extracts were obtained and standardized (by the content of neutral sugars) by the scientist of the Department of Chemistry of Natural Compounds of National University of Pharmacy.

The dry extract from the leaves of the *Prunus domestica* for physic-chemical properties is a bulk mass of dark brown colour with a characteristic smell. It is well soluble in water, chloroform, diethyl ether, 96 % and 40 % ethanol. The phytochemical composition of the extract includes polyphenolic compounds: phenolcarboxylic acids, oxystilbene derivatives, coumarins, flavones, flavonols, flavanones, isoflavonoids and their derivatives[36, 37, 38, 39]. Phenolcarboxylic acids are represented by chlorogenic, caffeic, rosmarinic and salicylic acids. Esculin, gerniarin, esculetin belong to the composition of coumarins. Among flavones and their derivatives luteolin, apigenin, vitexin-O-rhamnoside, cinaroside, baicalin are identified; among flavonols and their derivatives – quercetin-3,4-isoremnetin

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diglucoside, robinin, rutin, piperoside, isoflavonoids (sophoricoside); among flavanones – naringenin (first identified); among derivatives of oxystilbene – resveratrol.

According to the phytochemical analysis, plum fruits extract PEF extract contained homo- and heteropolysaccharides, the sum of phenolic compounds (anthocyanins and hydroxycinnamic acids), organic acids, proteinogenic amino acids, while PEPC extract contained a heteropolysaccharide complex, bound amino acids and organic acids[40, 41].

Flavonoids, carotenoids, tocopherols, chlorophylls, phytosterol, essential fatty acids (palmitic, stearic, oleic, linoleic) belong to the composition of grape oil[42 43 44].

2. Research methods

Investigation of the antioxidant properties of the extract from the leaves of the domestic plum in the *in vitro* system was carried out on the model of spontaneous LOP in the homogenate of the liver[45]. The experimental animals were divided into six experimental groups: intact control; pathology control (animals were intraperitoneally injected once with 50 %-olive solution of tetrachloromethane at a dose of 1.0 ml/100 g body weight); the third, fourth and fifth groups of animals were administered the studied extract at the doses of 25,50 and 100 mg/kg, respectively, on the basis of the liver tetrachloromethane injury; the sixth group of animals received the preparation of "Silibor" at the dose of 25 mg/kg.

For the preparation of 25 % homogenate on 0.1 M tritium-chloride buffer (pH=7.0), the extract of the recovered pike liver tissue was used. The extract was added to the homogenate at the rate of 0.5, 1.0 and 2.0 mg per 1 g of

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liver tissue in the form of a thinly dispersed suspension dispersed with Tween-80. The 0.2 ml aliquots were added to the samples with 3 ml of Tris-chloride buffer (pH=7.4). The incubation was carried out at 37 °C for 10, 15 and 20 h in a water thermostat with post-shaking. The reaction was stopped by adding 1.0 ml of 40 % trichloroacetic acid solution, and in the incubation medium the content of TBA-active products (TBA-AP), which are the kinetic products of peroxidative degradation of polyunsaturated carboxylic acids that are components of membrane phospholipids, was determined. Determination of the TBA-AP content was carried out by the thiobarbituric acid reaction and by the subtraction photometric method. α-Tocopherol (a natural antioxidant) was used as a comparator drug and was added to the homogenate as a 0.5 % emulsion dispersed with Tween-80 at a rate of 1.0 mg per 1.0 g of liver tissue. The doses of the extract introduced into the incubation medium were calculated on the basis of the amount of the extract that was injected through the portal vein into the liver during a single oral administration of the extract from leaves *Prunus domestica* at doses of 25, 50 and 100 mg/kg, respectively. Similarly, the dose of α-Tocopherol, which corresponded to ED_{50} and became 50 mg/kg, was calculated[46].

Screening studies of the antioxidant and anticytolytic activity of the extract from leaves *Prunus domestica in vivo* conditions were carried out on a model of severe tetrachloromethane hepatitis in crickets. The key role of the liver in the degradation of xenobiotics is determined by the inhibition of the initiation of free radical oxidation by the enzyme systems of hepatocytes and the liver. Therefore, liver pathologies, which are modeled in experimental studies, are considered to be the most indicative and acceptable for the clinical investigation of antioxidant properties of pharmacologically active substances[47].

The studies were conducted on 39 age-matched male pikelets weighing 180–200 g. The pathology was modelled by intraperitoneal injection of 50 % oil solution of tetrachloroethane at a dose of 1.0 ml/100 g body weight of the animal once[48]. For screening of antioxidant and anticytolytic activity of the extract from leaves *Prunus domestica*, doses of 25, 50 and 100 mg/kg were selected, which correspond to 0.05, 0.01 and 0.05 LD_{50}. The studied

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substance was administered intranasally 1 year before and 2 years after the administration of hepatotoxin.

The animals were decapitated under chloralose urethane anesthesia and biochemical parameters of blood and liver were determined.

The antioxidant activity was measured by the level of TBA-AP in liver tissue. The amount of TBK-AP in liver homogenate was determined by colorimetric method, which is based on the ability of LPO products to form at pH < 7 inhibited trimethine complexes with thiobarbituric acid, which have a maximum of glycation at 532 nm[49].

The anticytolytic activity of the extract from leaves *Prunus domestica* was investigated by the activity of the enzyme alanine aminotransferase (ALT) in the gray blood of the study animals. The activity of ALT in blood serum was determined by the unified method of *Reitman-Frenkel* using standard sets of reagents from *Simko Ltd* (Czech Republic). The method is based on the fact that after addition of 2,4-diphenylhydrazine reagent to the blood whey, transamination with the formation of glutamic and oxalic acids takes place, and the substrate is loaded into the appropriate column, the intensity of which is directly proportional to the activity of the enzyme[50]. Determination of indicators of the functional state of hepatocytes was performed on the background of alcoholic liver damage. The studies were performed on 110 outbred rats of both sexes, mass of 220–250 g.

Hepatoprotector of polyphenolic composition – "Silibor" (Produced by Pharmaceutical Company "*Health*", Kharkiv, Ukraine) was used as the reference drug because "Silibor" is a herbal preparation that is the standard for hepatoprotective activity In our investigation it was used, at a dose 25 mg/kg, which was equivalent to ED_{30} of this hepatoprotective agent[50].

The animals were divided into five groups. The first group of animals (intact control – IC) was without liver damage, the animals were injected with an appropriate volume of water. The second group of animals (control pathology – CP) had alcoholic hepatitis and the animals was administered corresponding volume of water. The third group of animals on the background of alcoholic hepatitis was administered the comparison drug "Silibor" at a dose of 25 mg/kg. The fourth and fifth groups of animals on the background of alcoholic hepatitis were administered extracts PEF and PEPC at the doses of 100 mg/kg and 200 mg/kg respectively.

Alcoholic subacute hepatitis was caused by intragastric administration of 40 % ethanol at a dose of 7.0 ml/kg for 7 days[50]. The dry extracts PEF and

[49] Стефанов А. В. Доклинические исследования лекарственных средств. Метод. рек. К. : Авитцена, 2002. 528 р.

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PEPC were administered intragastrically at doses of 100 mg/kg and 200 mg/kg.

All extracts were dissolved or suspended in 4.0 ml of purified water and administered intragastrically in 1 h after receiving the ethanol solution. Animals of IC and CP groups were administered only by purified water to reproduce the conditions of the experiment. 72 hours after the last administration of hepatotoxins, the animals were removed from the experiment under chloroform anesthesia by decapitation. 6 hours before euthanasia, the animals were denied free access to food. In decapitated animals, blood was collected to obtain serum, and the body was prepared to extract liver tissue[50]. Biochemical and functional indices of liver condition were investigated in the obtained samples.

Under the influence of ethanol, hepatocyte membranes are damaged and lipoperoxidation processes are activated under the influence of metabolites, thus, in the liver tissue, the rate of formation and content of lipid peroxidation products increases: diene conjugates (DCs), lipid hydroperoxide (LPO), and thiobarite products (TBA-AP), which were selected to evaluate the state of liver tissues in the homogenate. The state of AOS was evaluated by the content of reduced glutathione (RG) and α-Tocopherol in the tissue homogenate[51].

The content of DCs in the liver homogenate was determined by the method of *I. D. Stalnaya* in the modification of *V. I. Skornyakov*.

During the determination 4.5 ml of mixture of heptane with isopropyl alcohol (1:1) was added to the 0.5 ml of heptane layer of homogenate, shook for 10 min and 0.5 ml of purified water was added. After dividing into layers of the sample 0.5 ml from the upper (heptane) fraction was collected in a separate test tube and 2.5 ml of 96 % ethyl alcohol was added. The optical density of the sample was determined with Spectrophotometer-46 at λ 233 nm (against ethyl alcohol). The content of DC in the sample of liver homogenate was calculated in micromoles per gram (μmol/g) of tissue[52] and was calculated by the formula:

$$A = E \times K \times 0X, \text{ where}$$

E – extinction of the test sample;
K – coefficient of molar extinction 2.2×10^{-5} $M^{-1} \times cm^{-1}$;
0X – dilution of the sample.

[51] Стальная И. Д., Гаришвили Т. Г. Современные методы в биохимии. М. : Медицина, 1977. – С. 66–68.

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The content of LPO was determined by a standard biochemical method using a redox reaction with Fe^{3+} ions using the kit "*Lipid Hydroperoxide Assay Kit*" *No. 705002* (Sayman chemical, Estonia) according to the instruction manual, and then determined extinction at λ 500 nm on a microplate reader.

Determination of TBA-AP level was performed by the method of *Uchiyma M. & Michara M.* in the modification of *I. A. Volchegorsky* by the test with TBA-AP. During the reaction, 3.0 ml of 0.8 % TBA-AP solution in 3 % orthophosphate acid was added to 0.5 ml of heptane homogenate layer. The sample was kept for 45 min in a water bath, cooled and 5.0 ml of butyl alcohol was added. After 10 h, extinction was determined at λ 535 nm and 580 nm. The content of TBA-AP in the sample was calculated in micromoles per gram (μmol/g) of liver tissue[53] and was calculated by the formula:

$$A = (E_{535} - E_{580}) \times K \times 0X, \text{ where}$$

E_{535} и E_{580} – extinction at an appropriate wavelength;
K – coefficient of molar extinction $1{,}88 \times 10^{-5}$ $M^{-1} \times см^{-1}$;
0X – dilution of the sample.

The content of RG in the liver homogenate was determined by spectrophotometric method with *Ellman's reagent*[54]. The principle of the method is based on the use of a specific thiol reagent – 5,5 dithiobisnitrobenzoic acid (DTNB – *Ellman's reagent*), which is easily restored by SH substances, forming a colored complex with them. 0.5 ml of supernatant was added to the test tube, and 0.5 ml of purified water was added to the control tube. To the test and blank tubes were added 0.5 ml of 10 % trichloroacetic acid, stirred and centrifuged for 10 min at 1500 rpm. To 0.5 ml of centrifuge was added 2.0 ml of *Ellman's* solution. Incubated for 10 min at t 18–22 °C. The extinction was determined with Spectrophotometer-46 at λ 412 nm against a control sample (10.0 mm cell). The content of RG in liver tissue was calculated in terms of units by the formula:

$$C = E \times 1094, \text{ where}$$

E – extinction of the test sample;
1094 – estimated coefficient.

[53] Северин С. Ю., Соловьева Г. А. Практикум по биохимии. М. : Изд-во МГУ, 1989. С. 160–161.

[54] Кибардин С. А. Определение витамина Е в сыворотке крови. Биохимия, 1951. Т. 16. С. 511–514.

Determination of α-Tocopherol was performed by color reaction with Fe^{3+} (indicator α, α-bipyridyl); the results of the determination were corrected for the presence of cholesterol[55].

When studying the hepatoprotective properties of grapevine oil, the animals were divided into five groups: intact control; pathology control; animals treated with the substance under study; animals treated with the poisoning agent sea buckthorn oil and animals treated with the other poisoning agent, comparison drug "Silibor". Grape seed oil and grape seed oil were administered intranasally at a dose of 2.0 ml/kg one year before and two years after the introduction of the tetrachloromethane solution. The porin preparation "Silibor" was administered at a dose of 25 mg/kg (ED30) with an analogous treatment and prophylactic regimen.

The animals were removed from the experiment by decapitation and biochemical indicators of blood and liver were determined.

The influence of grape oil and the preparation of pores on the course of the LOP processes was determined by the amount of TBA-AP (kinase products of LOP) in the liver tissue.

The amount of TBA-AP in liver tissue was determined by colorimetric method, which is based on their ability to form in acidic medium inhibited trimethine complexes with thiobarbituric acid, which have a maximum of glycation at a hill length of 532 nm[45].

The amount (C, μmol/g) of the TBA-reactants was calculated according to the formula:

$$C = E \times 641, \text{ where}$$

E – extinction of the test sample;
641 – estimated coefficient.

The state of antioxidant system was evaluated by the amount of renewed glutathione in the liver tissue.

The principle of renewed glutathione content determination is based on the ability of low-molecular weight thiol spores to form with 5,5'-ditio-bis-2-nitrobenzoate a stained compound – thio-2-nitrobenzoic acid, water solution of which has the characteristic peaking at chiral length of 412 nm[45]. The solution extinction was determined on the Spectrophotometer-46. The amount of HG (C, n.u.) in the liver tissue was determined according to the formula:

[55] Халафян А. А. Statistica 6. Статистический анализ данных : учебник. 3-е изд. М. : ООО "Бином-Пресс", 2007. 512 с.

$C = E \times 1094$, where
E – extinction of the test sample;
1094 – estimated coefficient.

Experimental data were processed by variance statistics methods (mean (M), standard deviation (m) or minimum (min) and maximum (max) values of the sample) using nonparametric (t-criterion St'udent) methods of analysis. Experimental data were processed using standard software package "Statistica 6.0" using dispersion analysis algorithm ANOVA (t-criterion). The accepted significance level was $p \leq 0.05$[56, 57, 58].

Animals were kept in the same conditions, on a standard diet in accordance with the sanitary and hygiene requirements[58] in the vivarium of the Central Research Laboratory National University of Pharmacy (certificate No. 058/15 of 08.12.2015; valid until 07.12.2019).

Experimental investigations of safety of extracts from fruit of house plums were carried out in compliance with the principles of Directive 2010/63/EU of the European Parliament and the Council of Europe "On Protection of Animals Used for Scientific Purposes" (Brussels, 2010), the Law of Ukraine "On protection of animals against cruelty" No 3477-IV of 21.02.2006 with amendments and Order of the Ministry of Education and Science of Ukraine "On approval of the Order of scientific institutions conducting investigations, experiments on animals" No 249 of 01.03.2012.

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3. Study of hepatoprotective activity of extract from leaves *Prunus domestica*

The analysis of experimental data (Table 1) showed the presence of pronounced antioxidant properties of the plum leaves extract. Incorporation of the investigated extract into the incubation medium at a dose of 0.5 mg per 1.0 g of liver tissue resulted in reduction of TBA-AP content in the test samples by 46.5 % over 20 min compared with the control series.

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[58] Кожем'якін Ю. М., Хромов О. С., Філоненко М. А., Сайфетдінова Г. А. Науково-практичні рекомендації з утримання лабораторних тварин та роботи з ними. Держ. фармакол. центр. Київ : Вид. дім "Авіцена", 2002. С. 153–155.

Table 1

Influence of extract from leaves *Prunus domestica* on the outgrowth of spontaneous LPO in a model system based on liver homogenates (n=3)

Animals group	TBA-AP		
	10 min	15 min	20 min
Control (IC)	3.26±0.13	6.24±0.18	10.12±0.32
Plum extract, 0.5 mg/g	1.54±0.14*	3.45±0.31*	6.27±0.63*
Plum extract, 1.0 mg/g	0.61±0.11*/**	1.76±0.17*/**	3.31±0.45*/**
Plum extract, 2.0 mg/g	0.32±0.06*/**	0.80±0.18*/**	1.5±0.17*/**
α-Tocopherol, 1.0 mg/g	1.1±0.03*	2.56±0.25*	3.95±0.08*

Notes:

** – differences that are statistically reliable on control, $p \leq 0.05$;*

*** – differences that are statistically reliable on to reference drug, $p \leq 0.05$;*

n – number of animals in the group.

The effect of the investigated substance on the course of spontaneous oxidation by increasing its concentration in the reaction medium was characterized by a more pronounced inhibition of LPO. At the dose of 1.0 mg/g the plum leaves extract decreased the amount of TBA-AP by 75.6 %, α-Tocopherol at the dose of 1.0 mg/g – by 56.6 %. The extract was most active in inhibiting LPO at the dose of 2.0 mg/g, reducing the level of LPO products by 88.1 % for 20 min.

The content of the extract from leaves *Prunus domestica* has a relatively low content of polyphenols (12 %). Therefore, to study antioxidant activity it was appropriate to use the studied extract at higher doses. The doses selected for screening of antioxidant activity were 25, 50 and 100 mg/kg.

The analysis of experimental data (Table 2) showed that the extract from leaves *Prunus domestica* exhibited significant antioxidant activity, inhibiting TBA-AP accumulation by 32.46 % at the dose of 50 mg/kg and by 34.16 % at the dose of 100 mg/kg.

Hepatoprotective activity is a laden type of pharmacological activity, therefore the effective dose was determined taking into account two types of activity: antioxidant and anticytolytic.

The highest activity for inhibition of LPO processes of the extract from leaves *Prunus domestica* were found at a dose of 25 mg/kg. Antioxidant activity of the studied extract at the indicated dose was 53.05 %, which is more than twice as high as the activity of the drug of the comparison. “Silibor” at a dose of 25 mg/kg exhibited antioxidant activity under conditions of model pathology, which was validly received by the studied substance, decreasing the accumulation of TBA-AP in liver tissue by 23.13 %.

Table 2

Influence of extract from leaves *Prunus domestica* on the intensity of LPO and cytodestructive processes of acute toxic hepatitis in comparison with "Silibor" (n=6)

Animals group	TBA-AP, µmol/g	ALT, µmol /h•l
Intact control (IC)	30.77±0.98	0.86±0.03
Control pathology (CP)	90.81±4.66*	2.36±0.1*
Plum extract, 25 mg/g	58.96±2.61*/**/***	1.52±0.06*/**
Plum extract, 50 mg/g	70.30±4.97*/**	1.75±0.02*/**
Plum extract, 100 mg/g	71.32±4.01*/**	2.16±0.07*
Silibor, 25 mg/kg	76.92±1.31*/**	1.45±0.04*/**

Notes:

** – differences that are statistically reliable on intact control, p>0.05;*

*** – differences that are statistically reliable on control pathology, p>0.05;*

**** – differences that are statistically reliable on to reference drug, p≤0.05;*

n – number of animals in the group.

According to experimental data it was found that at a dose of 25 mg/kg the investigated extract exhibited antioxidant properties, but did not have significant anticytolytic activity compared with the reference drug. Administration of 25 mg/kg extract from leaves *Prunus domestica* to test animals was found to decrease ALT activity by 56.0 % against 60.7 % in animals that received "Silibor" at the rate of control pathology group.

With increasing dosage the anticytolytic effect of the extract from leaves *Prunus domestica* decreased: in animals that received the studied extract at a dose of 50 mg/kg the activity decreased by 40.7 % compared to non-treated animals. At a dose of 100 mg/kg the extract exhibited an anticytolitic effect of 13.3 %.

The fact that "Silibor" exhibited sufficiently high anticytolytic activity on the basis of relatively weak antioxidant effect, possibly due to the fact that the drug is able to interfere with the development of cytolytic syndrome due to its influence not only through peroxidation, but also through phospholipase mechanism, as evidenced by the data of scientific literature on the ability of silimarin to inhibit calcium-dependent activation of phospholipases, resulting in galvanic transport of Ca^{2+} ions across the membrane[59, 60].

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[60] Zhao X.-A., Chen G. M., Liu Y. et al. Inhibitory effect of silymarin on CCl_4-induced liver fibrosis by reducing Ly6Chi monocytes infiltration. *Int. J. Clin. Exp. Pathol.*, 2017. N 10 (12). P. 11941–11951.

4. Study of hepatoprotective activity of extract from fruts *Prunus domestica*

Indicators of lipid peroxidation in liver homogenate increased significantly on the background of in animal's alcohol intoxication by 40 % ethanol solution for 7 days. The DCs and TBA-AP increased in 1.8 times, the LHP level increased in 2.0 times relative to the IC.

Animals with received "Silibor" at the dose of 25 mg/kg, DCs content decreased for 21.6 % in liver tissues reliably. Other studied indicators (LHP, TBA-AP) also had trend for decrease, but their content did not differ from similar indicators in the CP group reliably (Table 3).

Table 3

Effects of Plum fruit extracts on the content of lipid peroxidation markers in rat liver tissue homogenate on the background of alcoholic hepatitis (n=10)

Animals group	DCs, µmol/g	TBA-AP, µmol/g	LPO, nmol/g
Intact control (IC)	7.63±0.65	35.28±2,62	79.53±4.52
Control pathology (CP)	13.92±0.96*	63.15±3.19*	160.44±8.98*
Silibor, 25 mg/kg	10.92±0.53*/**	57.82±2.79*	148.00±6.62*
PEF, 100 mg/kg	12.07±1.04*	60.37±2.44*	152.27±4.11*
PEF, 200 mg/kg	9.84±0.78*/**	49.71±3.51*/**	124.25±7.85*/**
PEPC, 100 mg/kg	13.57±0.86*	64.27±1.41*	153.58±9.42*
PEPC, 200 mg/kg	12.28±0.60*/**	59.75±3.54*	151.91±4.73*

Notes:

** – differences that are statistically reliable on intact control, p>0.05;*

** – differences that are statistically reliable on control pathology, p>0.05;*

n – number of animals in the group.

All investigated parameters of liver homogenate of animals, which received extracts PEF 100 mg/kg and PEPC 100 mg/kg, were at the IC level and were not reliably different from similar parameters in the CP group.

PEF extract at a dose of 200 mg/kg showed a normalizing effect on the content of the markers of the lipid peroxidation intensity on the background of the reproduced pathology.

There was a significant decrease in the content of DCs by 29.3 %, TBK-AP – by 21.3 % and LPO – by 22.6 % in the homogenate of the liver relative to CP, which exceeded the antioxidant effect of the PEPC extract at a dose of 200 mg/kg (decrease in the content DCs by 11.8 %, TBK-AP – by 5.4 %, LPO – by 5.3 %) and the reference drug "Silibor" at a dose of 25 mg/kg according to TBK-AP and LPO (decrease in content by 8.4 % and 7.8 %, respectively) and was at the level of the reference drug for reducing the content of DCs (21.2 %) (Table 4.1).

The investigated AOS markers (RG and α-Tocopherol) in rat liver homogenate decreased significantly under conditions of intoxication. Thus, the level of RG in the animals of the CP group decreased for 38 % and the content of α-Tocopherol for 41.9 %, which indicates about destructive processes in the liver tissue.

"Silibor" administrated at the dose of 25 mg/kg moderately improved the condition of the AOS of the liver, and showed a reliable increase of the content of RG for 36.9 %, and α-Tocopherol for 35.23 % compared with IC (Table 4).

Table 4

Effects of Plum fruit extracts on the content of AOS markers in rat liver tissue homogenate on the background of alcoholic hepatitis (n=10)

Animals group	RG, relative units	α-Tocopherol, μmol/g
Intact control (IC)	36.28±2.13	32.02±1.59
Control pathology (CP)	22.49±3.16*	18.59±2.66*
Silibor, 25 mg/kg	30.78±1.82*/**	25.14±1.48*/**
PEF, 100 mg/kg	24.28±2.78*	20.35±2.13*
PEF, 200 mg/kg	31.36±1.98*/**	26.43±1.85*/**
PEPC, 100 mg/kg	22.87±2.99*	20.06±1.74*
PEPC, 200 mg/kg	28.53±1.42*/**	22.38±1.45*

Notes:

** – differences that are statistically reliable on intact control, p>0.05;*

** – differences that are statistically reliable on control pathology, p>0.05;*

n – number of animals in the group.

As in the previous experiments extracts PEF and PEPC in the dose of 100 mg/kg, showed no statistically significant effect. At the same time, the state of AOS in liver tissues remained at the level of CP.

The daily administration of PEF at a dose 200 mg/kg to animals for 7 days on the background of liver injury by alcohol intoxication significantly increased the RG content for 39.4 % and α-Tocopherol for 42.2 % in a comparison with similar indicators in the liver homogenate of animals CP group.

Thus, among the studied PEF and PEPC extracts, PEF extract in a dose 200 mg/kg showed the highest efficiency in the biochemical investigation, whose percentage efficiency was at a level of "Silibor".

5. Study of hepatoprotective activity of grape seed oil

Antioxidant properties of grape seed oil were studied under conditions of acute toxic liver injury with tetrachloroethane.

Tetrachlomethane intoxication in the control group of animals was accompanied by the development of a severe peroxidation syndrome, which

was manifested by an increase in the amount of TBA-AP in the liver tissue by 2.64 times and a 30.6 % decrease in the level of renewed glutathione. Lethality among the animals of the control pathology group was 33.3 % (Table 5).

Table 5

Influence of grape seed oil on free-radical processes in the liver under conditions of acute tetrachloromethane hepatitis (n=6)

Animals group	TBA-AP, μmol/g	RG, n.u.
Intact control (IC)	33.69±4.402	33.91±3.10
Control pathology (CP)	88.94±9.57*	23.52±2.60*
Grape seed oil, 2.0 ml/kg	58.23±7.96*/**	30.27±2.04**
Sea buckthorn oil, 2.0 ml/kg	65.70±6.33*/**	30.08±1.93**
Silibor, 25 mg/kg	54.49±5.73*/**	30.81±2.44**

Notes:

** – differences that are statistically reliable on intact control, p>0.05;*

*** – differences that are statistically reliable on control pathology, p>0.05;*

n – number of animals in the group.

Prophylactic and treatment administration of grape seed oil was accompanied by curbing of peroxidative destructive processes and decrease of the amount of TBA-reactants in the liver tissue by 34.5 %. The drug "Silibor" had significantly different effect on the LPO processes processing, reducing the level of TBA-AP by 38.7 %. The lowest antioxidant activity was demonstrated by sea buckthorn oil, reducing the level of TBA-AP by 26.13 % (Table 5).

Grape seed oil and the confounding preparations (sea buckthorn oil, "Silibor") normalized the antioxidant status of cells, as evidenced by the increased level of renewed glutathione in all study groups of animals up to the level of the intact control.

Thus, the results of the research indicate that grape seed oil exhibits significant antioxidant properties due to the presence of Tocopherols and unsaturated fatty acids in its chemical composition. In terms of the severity of the antioxidant effect in the model of acute carbon tetrachloride hepatitis, grape seed oil was slightly inferior to the comparison drug "Silibor" and exceeded the effect of sea buckthorn oil. But this difference was at the level of the trend; the values of TBA-AP and RG in the three experimental groups did not differ significantly from each other.

CONCLUSIONS

1. The presented phytochemical analysis of polyphenols of the studied phytoobjects provided prerequisites for the study of pharmacological properties in the context of their impact on antioxidant and anticytolytic status of the body, which is an integral part of hepatoprotective activity.

2. Unified methods for the study of substances of plant origin with probable hepatoprotective activity are presented. Designs of experimental models in the conditions of liver pathology are described. identified key indicators of antioxidant and anticytolytic activity. a reasoned choice of reference drugs for comparative analysis of the results of experimental data.

3. It was found that the extract from leaves *Prunus domestica* in all doses exhibited a significant capacity to inhibit LPO in vitro system, and at doses of 1.0 and 2.0 mg/g did not come for the antioxidant properties of the drug of the comparison α-Tocopherol, which indicates the presence of anticytolytic and visible antioxidant activity in the substance extract of plum leaves. Analysis of experimental data allowed to determine the effective dose of the plum leaves extract. It was found that under conditions of severe tetrachloromethane hepatitis at the dose of 25 mg/kg the extract under study exhibited different antioxidant properties exceeding the activity of "Silibor" 2.3 times and had no comparison with the preparation for its anticytolytic activity.

4. Experimental data on the study of anti free radical and antioxidant properties of *Prunus domestica* extracts showed inhibitory effect on lipid peroxidation markers and stabilizing effect on hepatocyte AOC markers in studies with useing of PEF extract at the dose of 200 mg/kg on the background of alcoholic liver damage. Thus, an effective dose of the PEF extract was established, which was 200 mg/kg. The anti free radical and antioxidant effects of the PEF extract at a dose 200 mg/kg exceeded the corresponding effects of the PEPC extract at the doses tested on the background of alcoholic liver damage. According to the anti free radical properties, the PEF extract was slightly higher than the drug "Silibor" at 25 mg/kg and was at its antioxidant effect level. The anti free radical and antioxidant properties of the PEF extract are probably related to the presence in its chemical composition of the amount of phenolic compounds (anthocyanins and hydroxycinnamic acids). Considering the results of screening, the most promising subject for further in-depth pharmacological study is an extract derived from Plum fruits containing fiber (including the amount of phenolic compounds) at a dose of 200 mg/kg.

5. In the model of carbon tetrachloride hepatitis, grape seed oil showed antioxidant activity, which was more pronounced in relation to the antioxidant effect of the comparison drug sea buckthorn oil.

SUMMARY

Most drugs used in toxic hepatopathies act quite selectively, providing mainly regeneration of hepatocyte membranes (Essenciale, Epler, Lipostabil) or their parenchyma (Cytidine, Methylmethionine sulfonium chloride, Orotic acid, Calcium pangamate, Methionine, Uridine, Cyanocobalamin). At the same time, multifactorial toxic liver damage requires multilevel protection from drugs, which only few hepatoprotectors have (Legalon, Silibor, Hepaton). Characteristic features of modern hepatoprotectors are: origin from plant materials and content of polyphenolic compounds (flavonoids, flavolignans, cinnamic acids, etc.) having antioxidant activity. Currently, the share of effective domestic hepatoprotectors in the Ukrainian pharmaceutical market is small and amounts to only 15 % of similar foreign drugs. Thus, there is a need to search for new agents that increase the liver resistance to toxic damage. At the same time, preference should be given to herbal remedies with generally low toxicity combined with sufficient efficacy and breadth of therapeutic action as well as pharmacoeconomic component (cheap herbal raw materials).

For the first time, an experimental study of the hepatoprotective properties of extracts derived from the leaves and fruits of Plum, as well as grape seed oil, phytochemical, whose unique composition is rich in polyphenolic compounds.

According to experimental studies of the antioxidant and cytolytic activity of selected plant substances, it was confirmed that their potential hepatoprotective activity, which was at the level and in some cases exceeded the activity of the reference drugs used.

A promising area of further experimental research is the in-depth study of the mechanisms of implementation of antioxidant and anticytolytic properties for the development of potential, therapeutically active drugs, which normalize the functional state of the hepatobiliary system.

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