PHARMACIA

Pharmacia 68(2): 485–492
DOI 10.3897/pharmacia.68.e64159

Research Article

Study of laxative and hepatoprotective activity of extracts obtained from *Prunus domestica* fruits

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Received 11 February 2021 ♦ **Accepted** 23 April 2021 ♦ **Published** 14 June 2021

Citation: Seniuk I, Al-Sahlanee BJA, Bakri Bakri AA, Kravchenko V, Shovkova O (2021) Study of laxative and hepatoprotective activity of extracts obtained from *Prunus domestica* fruits. Pharmacia 68(2): 485–492. https://doi.org/10.3897/pharmacia.68.e64159

Abstract

The experimental work focused on the study of the pharmacological properties of extracts obtained from the *Prunus domestica* fruits to create a prospective laxative drug with moderate hepatoprotective properties. *Prunus domestica* fruit extracts have been shown to have a pronounced laxative and moderate hepatoprotective effect. Extract containing fibers at a dose of 200 mg/kg was selected as the most active extract for laxative and hepatoprotective activity among all extracts from the *Prunus domestica* fruits. It was conventionally named "Prunophyte". Studies of the specific pharmacological action of "Prunophyte" extract in a model of comorbid functional constipation on the background of combined alcoholic liver disease in rats showed that "Prunophyte" at a dose of 200 mg/kg had positive dynamics in the treatment of constipation on the background of subacute liver disease. In some cases it exceeded the effects of combination therapy with drugs "Silibor" at a dose of 25 mg/kg and "Senadexin" at a dose of 14 mg/kg. "Prunophyte" extract, in contrast to "Senodexin", did not cause signs of diarrhea in animals, which may be a beneficial feature of this drug in subsequent clinical use. This drug has shown that it can be a promising alternative to a one-time complex treatment with herbal laxatives and hepatoprotectors, which will avoid polypragmatism in the treatment of comorbid conditions in gastroenterology associated with functional constipation and liver dysfunction.

Keywords

Alcoholic hepatitis, comorbid functional constipation, hepatoprotective activity, intestinal motility, laxative properties, loperamide-induced constipation, liver markers, protein metabolism, *Prunus domestica*

Introduction

The search for drugs that normalize the motor function of the digestive tract (prokinetics) has attracted the attention of researchers for centuries (Strahl 1851), as motility disorders underlie the pathogenesis of many diseases such as gastroesophageal reflux disease, hepatitis, gastric ulcer, irritable bowel syndrome (IBS), cancer, etc. (Black et al. 2020; Oka et al. 2020). Irritable bowel syndrome which can manifest itself in the form of functional constipation, remains an urgent problem of modern medicine. It has a significant negative impact on the quality of life and social function of many people. In addition, it is now known that IBS can be combined with the development of serious diseases and cause an increase in mortality. Irritable bowel syndrome necessitates a significant increase in health

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care costs – direct (due to the actual IBS and related diseases) and indirect (due to an increase in the number of days of temporary incapacity for work) (Buono et al. 2017; Frändemark et al. 2018). Constipation is not a nosological form of the disease or a separate symptom. This is a polyetiological, multifactorial symptom complex of general and gastrointestinal disorders. In recent years, there has been a steady increase in the incidence of constipation in patients of all ages. They are diagnosed in 35% of the adult population and in 3% of children who visit a doctor; and in 10–25% of patients with chronic diseases of the digestive system (Red`kin et al. 2020). Pathogenesis and correction of constipation are of considerable scientific and practical interest, which is reflected in many modern publications (Ford et al. 2018; Ballou et al. 2019; Ford et al. 2020; Lenhart et al. 2020; Shin et al. 2020) and in internationally agreed documents: Roman II, III, IV (1999, 2006, 2016) consensus, WGO: Practice Guideline: Constipation (OMGE2005, WCOG2017, WCOG2019). Therefore, the search for new laxatives of plant origin with a long and safe period of use is a topical issue in modern medicine.

Prunus domestica, which is widespread in our country and is well known for its medicinal properties and applications in folk medicine drew our attention. (Igwe et al. 2016; Preeti et al. 2017). The variety of pharmacological effects (laxative, hepatoprotective, anti-inflammatory, antioxidant, membrane-stabilizing, etc.) and the absence of single-component drugs based on *Prunus domestica*, indicates the feasibility of pharmacological studies of these herbal raw materials as promising to create an effective and safe laxative for treatment intestinal tract with impaired liver function.

Materials and methods

The fruits of *Prunus domestica L.* (family *Rosacea*, breed "*Ugorka*"), which is widely cultivated in Ukraine as a fruit and berry crops, were the object of the study. The materials of the study were extracts obtained from the *Prunus domestica* fruits: PEF (extract containing fiber) and PEPC (extract containing polysaccharide complex). The extracts were obtained at the Department of Chemistry of Natural Compounds of NUPh by the original method of extracting biologically active compounds from plant materials. According to the results of phytochemical analysis of Prunus domestica fruits, it was found that PEF contains 59.23% of neutral sugars as products of hydrolysis of homopolysaccharides. The chemical composition of PEF extract also includes organic acids (succinic, fumaric, chicory, oxalic, citric, ascorbic, hydroxycinnamic acids), anthocyanins (cyanidin-3-rutinoside, cyanidin-3-glucoside), sum of polyphenolic compounds, amino acids (glutamic, aspartic acids, proline, alanine, valine, leucine, phenylalanine, histidine, lysine, arginine). It is brown powder with a faint specific odor, and PEPC extract is a light brown powder without odor. The studied extracts were standardized by the content of neutral sugars. The presence of homopolysaccharides in PEF extract and heteropolysaccharides in PEPC extract was determined by their hydrolysis, which resulted in the formation of neutral sugars, quantitative content of which was determined by chromato-mass spectrometry (chromato-MS) (Upyr et al. 2018).

Tiered screening studies to identify the most therapeutically effective phytosample for laxative and hepatoprotective activity involved the use of doses in the range of 25–200 mg/kg, which based on the experimentally detected biological activity of studied extracts at different doses and proven low toxicity of extracts.

The study of the laxative effect of PEF and PEPC extracts was performed on a model of intestinal peristalsis damage in mice induced by barium chloride (Chornoivan et al. 2010). It was used the method of monitoring the passage rate of the contrast mass through the intestines of mice. A reference drug, oral drops "Picolax" (OAO "Farmak", Kyiv, Ukraine), was selected according to the probable mechanism of action of extracts from the *Prunus domestica* fruits. The active substance of "Picolax" is sodium picosulfate, which is a laxative of local action of the triarylmethane group. The reference drug was used at a dose of ED_{50}, which is 0.3 ml/kg. The percentage of intestinal length was used as an integral indicator of the intestinal peristalsis force (L, %) (Yakovleva et al. 2001).

The next stage of experimental research was a screening study of hepatoprotective activity of extracts from the *Prunus domestica* fruits: PEF and PEPC. The hepatoprotective effect of PEF and PEPC extracts was determined under conditions of alcoholic liver damage in rats (Stefanov 2002). Hepatoprotector with polyphenolic composition – tablets "Silybor" (Pharmaceutical Company "Zdorovya", Kharkiv, Ukraine) was used as a reference drug, which chemical composition was similar to the phytochemical composition of the studied extracts. Its chemical composition is similar to the phytochemical content of the studied extracts. In studies, "Silybor" was used at a dose of 25 mg/kg, which was equivalent to ED_{30} of this hepatoprotective agent (Drogovoz et al. 1998). In decapitated animals, blood was collected to obtain serum, the body was prepared to remove liver tissue (Mironova 2012). After that, the obtained samples were studied biochemical and functional indicators of the liver. Liver status, biliary function and cytolytic syndrome were assessed by enzyme activity: Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) and Gamma-Glutamyl Transpeptidase (GGT). Enzyme activity was determined using standard sets of reagents TOV NVP "Fylisit-Diagnostika" (Ukraine). A unified dinitrophenylhydrazine method proposed by S. Reitman and S. Frenkel used to determine the ALT activity (Kamyshnikov 2009). The expression of cholestatic syndrome was assessed by the activity of indicator enzymes – ALP and GGT, which was determined by kinetic method using test kits "Lachema" (Czech Republic). The content of lipid oxidation products (LOPs) was determined in the liver homogenate: diene conjugates (DC), lipid hydroperoxide (LH) and products reacting with TBA (TBA-AP). Determination of DC content in the liver homogenate was per-

formed by the method of Stalnaya I.D. in modification of Skornyakova V.I. (1997) The DC content of the liver homogenate sample was calculated in μmol/g tissue. The LH content was determined by a standard biochemical method using a set of reagents "Lipid Hydroperoxide (LPO) Assay Kit" № 705002 (Cayman chemical, Estonia) and expressed as nmol/g. Determination of the TBK-AP level was performed by the method of Uchiyma M & Michara M in the modification of Volchegorskiy IA (1997) by the test with TBA. The content of TBA-Active products in the sample was calculated in μmol/g of liver tissue (Karpishchenko 1997).

Studies of the specificity and probable mechanisms of action of the PEF extract selected by the laxative and hepatoprotective activities at a dose of 200 mg/kg (conventionally named "Prunophyte") were carried out on a model of comorbid functional constipation combined with alcoholic liver damage in rats (Choi et al. 2014). The following drugs were used as reference drugs: "Silybor" tablets at a dose of 25 mg/kg and "Senadexin" tablets (active ingredient – senna leaf extract, 70 mg; PAT "Lubnipharm", Ukraine) at a dose of 14 mg/kg (in terms of content of calcium sennosides A and B). The choice of reference drugs was based on the similarity of their chemical composition with the chemical set of BASes in "Prunophyte"extract, as well as the mechanism of action of the first, which probably coincides with the possible mechanism of action of the studied extract. The equivalent dose for animals of the reference drugs "Silybor" and "Senadexin" was calculated from the average daily dose for humans and the interspecific difference between body weight and surface area (Nair et al. 2016). Given the lack of data on drug interactions of both reference drugs, to maintain equal experimental conditions, both drugs were administered during one period, but due to the gradual introduction of drugs between the administration of drugs took 30 minutes. In addition, this scheme allows to reproduce the features of patient compliance to avoid polypragmatism on the background of comorbid conditions. (Kucheryavyy et al. 2016; Nair et al. 2016). Experimental subacute combined alcoholic liver damage was induced in animals of the experimental groups. The animals were then induced functional constipation by daily intragastric administration of "Loperamide hydrochloride" (PhC Zdorovya, Ukraine) at a dose of 3 mg/kg for 6 days. Evaluation of intestinal motor activity in rats was determined by the method of Sagar L (2005) which was modified (Choi et al. 2014) and adapted for this species of laboratory animals. The condition of the liver was assessed by biochemical parameters in the blood serum. It was determined the content of total protein (g/l), urea (mmol/l), activity of ALT (μcat/l) and ALP (μcat/l) by unified colorimetric methods using sets of TOV NVP "Phylisit-Diagnostica" (Ukraine).

All experimental studies were carried out in accordance with the general ethical principles of animal experiments regulated by the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg 1998) and the Law of Ukraine № 3447- IV, 21.02.2006 "On protection of animals from cruel treatment", Order of the Ministry of Education and Science, Youth and Sports of Ukraine № 249, 01.03.2012 "Procedure for conducting experiments and experiments on animals by scientific institutions". The Commission on Bioethics of NUPh (prot. № 01, 02.10.2019) did not reveal any violations of moral and ethical norms during experimental research.

Statistical analysis

All data expressed as Mean ± sem and data entered and analyzed using statistical package "Statistica 6.0". The nonparametric Student's t-test used to determine difference between groups. Values of $p \leq 0.05$ was considered as statistically significant.

Results and discussion

Extract containing fibers and extract containing polysaccharide complex from the *Prunus domestica* fruits were selected as the most active among the four investigated extracts according to the results of previous experimental studies of laxative activity in the model of loperamide-induced constipation (Seniuk 2017). To confirm the laxative activity of the selected extracts from the *Prunus domestica* fruits PEF and PEPC and determine among them more effective with the appropriate dose, it was studied their effect on the passage rate of contrast mass through the intestines of mice in the model of intestinal peristalsis damage with barium chloride.

Intragastric administration of barium chloride to mice led to spasm of the smooth muscle fibers of the stomach and intestine, resulting in delayed evacuation of gastric contents and, consequently, a reduction in the length of the intestinal contrast mass (Table 1). The percentage of intestine with contrast mass from the total length of the intestine of the control pathology group was significantly reduced by 26% compared with intact control, indicating a slowing of intestinal motility (Table 1). Administration of PEF and PEPC at doses of 75 mg/kg, 100 mg/kg, 200 mg/kg and the reference drug "Picolax" at a dose of 0.3 ml/kg eliminated spasm of smooth muscles of the stomach and intestines (Marcdante et al. 2015), as evidenced by the lengthening of the path of contrast mass passage in the intestine of mice to the level of

Table 1. The effect of extracts from *Prunus domestica* fruits on the passage rate of the contrast mass through the intestines in mice (n = 7).

Group of animals	Dose	L, %
Intact control	-	67.41±2.29
Control pathology	-	59.87±2.34*
Picolax	0.3 ml/kg	75.76±2.36**
PEF	75 mg/kg	69.21±2.61**
	100 mg/kg	73.00±3.37**
	200 mg/kg	77.26±2.37 */**
PEPC	75 mg/kg	68.76±2.27**
	100 mg/kg	75.73±2.13**
	200 mg/kg	71.96±2.86**

Notes: L – the length of the intestine of mice filled with a contrast mass, %; * – $p \leq 0.05$ versus intact control group; ** – $p \leq 0.05$ versus control pathology group; n – number of animals in the group.

intact control (Table 1). Moreover, in the case of using the PEF extract, the intensiy of the laxative activity had a clear dose-dependent nature: the intensity of the action increased with increasing dose. At a dose of 200 mg/kg, in contrast to the doses of 75 and 100 mg/kg, the contrast pathway was statistically significantly higher than in the intact control group. Thus, the PEF extract showed a laxative activity at a dose of 200 mg/kg (29%), which exceeded the maximum activity of the PEPC extract at a dose of 100 mg/kg (26%) and was at the level of the drug "Picolax" (27%). The proven laxative effect of the studied extracts is probably due to the presence of homopolysaccharides (59%) in the chemical composition of PEF and heteropolysaccharides (61%) in PEPC chemical composition (Luo et al. 2017). Extract containing polysaccharides complex showed the maximum laxative effect at a dose of 100 mg/kg, which was at the level of the reference drug "Picolax", and was significantly inferior to PEF extract at a dose of 200 mg/kg compared with the control pathology.

Thus, in a model of spasm of the gastrointestinal tract of mice caused by barium chloride, PEF extract at a dose of 200 mg/kg showed a pronounced laxative effect and slightly exceeded the activity of the reference drug "Picolax". Separate studies of the laxative properties of the dried plums show that the laxative effect is realized due to the content of oxyphenisatin, which directly interacts with receptors on the enterocyte membrane and acts as a contact laxative. The high content of sorbitol and chlorogenic acid in the dried plums also contributes to the realization of the laxative effect by increasing the osmotic pressure (Attaluri et al. 2010; Jabeen 2011).

According to modern notions, the syndrome of hyperlipoperoxidation is considered as one of the leading pathogenetic factors that plays the role of a nonspecific mechanism of cell damage at the membrane level and plays a key role in the development of various diseases, including diseases of the hepatobiliary tract (Vladimirov et al. 1972).

The purpose of screening studies of hepatoprotective activity of extracts from the *Prunus domestica* fruits PEF and PEPC was to identify the most effective among them with the appropriate maximum therapeutic dose. The results of screening studies of hepatoprotective activity (Table 2) revealed the PEF extract as the most active and determined the dose of 200 mg/kg as its effective dose. PEPC extract at a dose of 200 mg/kg significantly reduced ($p \leq 0.05$) the activity of ALT, AST, ALP and GGT by 18%, 19%, 10% and 11%, respectively, compared with control pathology. PEF extract at a dose of 200 mg/kg significantly ($p \leq 0.05$) reduced the activity of same enzymes by 30%, 30%, 31% and 29%, respectively (Table 2), compared with control pathology. PEF extract at a dose of 200 mg/kg was more effective, and its effectiveness was close to the reference drug "Silybor", which reduced the activity of ALT, AST, ALP and GGT by 27%, 24%, 18% and 17% ($p \leq 0.05$) respectively, compared with control pathology.

Agents that due to antioxidant properties reduce the manifestations of oxidative imbalance in cells, inhibit the formation of LOPs, thereby increasing the stability of membranes at all organization levels and normalize intracellular metabolism are used in the complex pathogenetic therapy of the so-called "free radical pathologies" (Pak et al. 1991). Therefore, further experimental studies were aimed at studying the effect of the PEF and PEPC studied extracts on the markers of lipoperoxidation (DC, TBA-Active Products, LH). In the alcohol intoxication with 40% ethanol solution for 7 days in animals significantly increased LOPs in the liver homogenate: DC and TBA-AP by 1.8 times, the LH content by 2 times compared with intact control. In animals treated with "Silybor", the DC content in liver tissue was significantly reduced by 21.6%. The content of TBA-AP and LH also decreased, but their content did not differ significantly from similar indicators of the control pathology group (Table 3). All studied parameters in liver homogena-

Table 2. The effect of extracts from the *Prunus domestica* fruits on the activity of specific hepatic enzymes in the serum on the model of alcoholic hepatitis (n = 10).

Group of animals	ALT, μkat/L	AST, μkat/L	ALP, μkat/L	GGT, μkat/L
Intact control	0.56±0.10	1.41±0.12	3.68±0.21	2.56±0.19
Control pathology	1.17±0.15 *	2.54±0.26*	6.21±0.39*	5.97±0.42*
Silybor, 25 mg/kg	0.85±0.09 */**	1.93±0.14*/**	5.08±0.18*/**	4.93±0.24*/**
PEF, 100 mg/kg	0.94±0.12*	2.11±0.30 *	5.59±0.35 *	5.28±0.35*
PEF, 200 mg/kg	0.82±0.08*/**	1.80±0.09*/**	4.31±0.16*/**	4.25±0.26*/**
PEPC, 100 mg/kg	1.15±0.09*	2.48±0.15 *	6.14±0.29 *	5.83±0.37*
PEPC, 200 mg/kg	0.96±0.07*/**	2.05±0.15*/**	5.61±0.18*/**	5.30±0.30 *

Note: * – $p \leq 0.05$ versus intact control group; ** – $p \leq 0.05$ versus control pathology group; n – number of animals in the group.

Table 3. The effect of extracts from the *Prunus domestica* fruits on the content of LOPs in the liver homogenate of rat on the model of alcoholic hepatitis (n = 10).

Group of animals	DC, μmol/g	TBA-AP, μmol/g	LH, nmol/g
Intact control	7.63±0.65	35.28±2.62	79.53±4.52
Control pathology	13.92±0.96*	63.15±3.19*	160.44±8.98*
Silybor, 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*

Note: * – $p \leq 0.05$ versus intact control group; ** – $p \leq 0.05$ versus control pathology group; n – number of animals in the group.

te in animals treated with PEF and PEPC extracts at a dose of 100 mg/kg did not differ significantly from those in the control pathology group. PEF at a dose of 200 mg/kg had a normalizing effect on all markers of the intensity of the LOPs formation. Its introduction during the experiment to animals led to decreasing of DC content by 29.3%, TBA-AP – by 21.3% and LH – by 22.6% in the liver homogenate. PEF activity exceeded the studied effect of PEPC extract at a dose of 200 mg/kg and was at the level of the reference drug "Silybor". PEPC at a dose of 200 mg/kg, as well as "Silybor", was able to significantly affect only the DC content, reducing its level by 11.8% compared with control pathology. Other parameters in the liver homogenate of these animals did not differ statistically from similar indicators in animals of the control pathology group (Table 3). In the Jabeen's study (2011) of the pharmacological properties of plum juice, normalization of the activity of marker liver enzymes ALT and ALP in serum was observed. Experimental studies by Soni et al. (2011) showed that the use of *Prunus domestica* extracts at doses of 150 mg/kg and 300 mg/kg was accompanied by normalization of ALT, AST, ALP and LOPs content, which the authors associate with pronounced antioxidant activity of extracts, as hepatotoxic effects of paracetamol and tetrachloromethane is primarily due to the activation of oxidative stress in hepatocytes.

Thus, among the studied extracts (PEF and PEPC), the greatest efficiency in the conducted biochemical studies was shown by PEF extract at a dose of 200 mg/kg. Its effectiveness in percentage terms was equal to the reference drug "Silybor". The anticytolytic and antioxidant effects of PEF extract at a dose of 200 mg/kg were probably associated with the presence of hydroxycinnamic acids (0.27%) and anthocyanins (27 mg/kg) in its chemical composition (Tattis et al. 2016). Considering the screening results, the most promising object for further in-depth pharmacological study is PEF at a dose of 200 mg/kg, which was conventionally named "Prunophyte".

The final stage of experimental research was the study of the specificity and probable mechanism of action of "Prunophyte" extract in the model of comorbid functional constipation combined with alcoholic hepatitis in rats. The use of "Loperamide hydrochloride" for 5 days led to almost identical disorders of the gastrointestinal tract in healthy animals (negative control group) and in animals with subacute alcoholic liver damage (control pathology group). Functional constipation was observed, which was reflected in a decrease in the number of defecations per day due to inhibition of intestinal motility, the percentage of water in the fecal boluses, absorption and increased secretion of water in the intestinal cavity (Table 4).

Treatment with the extract "Prunophyte" caused a laxative effect without provoking secretory diarrhea (the number of fecal boluses is 30ru vs. 18ru in control pathology, $p \leq 0.05$). After administration of "Prunophyte", the studied stool parameters did not differ significantly from the indicators of intact control (Table 4). In the reference group, treatment of animals with "Silybor" and "Senadexin" contributed to a marked change in defecation parameters (fecal bolus number – 41ru), which confirms a pronounced laxative effect of "Senadexin". Treatment with reference drugs led to increased number of defecations and the total average weight of fecal bolus due to the increase in fluid content compared with animals from negative control and control pathology groups. Administration of "Prunophyte" was accompanied by a significant reduction in the percentage of water in the bolus by 2.1 times compared with the reference group, $p \leq 0.05$. In the case of the use of reference drugs, the percentage of water increased by 2.6 times compared with negative control, $p \leq 0.05$ (Table 4). Fecal masses in the animals of the reference group were poorly formed and difficult to separate, which indicated the development of secretory diarrhea. The use of "Prunophyte" throughout the experiment contributed to the normalization of intestinal motility, which did not differ statistically from the intact control. "Prunophyte" extract has a mild laxative effect (68% vs. 50% of control pathology) mainly due to stimulation of intestinal motility, significantly increasing the distance traveled by contrast mass by 1.3 times compared the negative control ($p \leq 0.05$). The studied extract significantly exceeded the effect of reference drugs by 1.1 times compared with negative control, control pathology and reference groups, $p \leq 0.05$ (Table 5).

In an experimental study of gastrointestinal motility parameters, it was shown that "Loperamide hydrochloride" significantly inhibited motility in the small intestine and caused stagnation of fecal masses in the large intestine in the groups of negative control and control pathology (Table 5). It should be noted that the combination of model pathologies in a complex disorder contributed to a moderate insignificant suppression of peristalsis compared with an isolated model of constipation, which is consistent with the literature (Dong-Sung et al. 2019).

Combination therapy with "Silybor" and "Senadexin" improved gastrointestinal motility, but a lower mean relative distance of contrast mass through the small intestine

Table 4. The effect of "Prunophyte" on the parameters of daily bowel movements of experimental animals with subacute alcoholic liver damage combined with loperamide-induced constipation on the 5th day of the experiment (n = 8).

Group of animals	N, ru	M_W, g	M_D, g	% of water
Intact control	32.1±3.2	6.51±0.64	3.98±0.44	39.50±2.27
Negative control	21.5±2.6*	4.48±0.29*	3.31±0.21	25.93±1.31*
Control pathology	18.5±2.3*	5.01±0.35	3.70±0.29	26.43±1.48*
Prunophyte, 200 mg/kg	29.8±3.5*/**/#/##	5.84±0.58*/**/#/##	3.88±0.43	33.00±2.67*/**/#/##
Silibor 25 mg/kg + Senadexin 14 mg/kg (reference group)	40.9±4.0**/#	13.69±1.38*/**/#	4.43±0.51	67.61±1.28*/**/#

Note: N – the number of fecal bolus, ru; M_W – wet mass of feces, g; M_D – dry mass of feces, g; * – $p \leq 0.05$ versus intact control group; ** – $p \leq 0.05$ versus negative control group; # – $p \leq 0.05$ versus control pathology group; ## – $p \leq 0.05$ versus reference control group; n – number of animals in the group.

Table 5. The effect of "Prunophyte" on intestinal motility parameters in experimental animals with subacute alcoholic liver damage combined with loperamide-induced constipation for 30 minutes (n = 8).

Group of animals	L_t, cm	D_C, cm	I_{RD}, %	N, ru
Intact control	102.34±2.87	71.41±3.48	69.70±2.66	4.0±0.60
Negative control	100.35±2.95	54.66±2.41*	54.41±1.50*	6.63±0.60*
Control pathology	104.25±3.82	50.25±2.15*	48.33±1.57*	5.88±0.58*
Prunophyte, 200 mg/kg	100.63±2.95	68.51±4.32**/#/##	67.91±3.33**/#	3.63±0.50**/#/##
Silibor 25 mg/kg + Senadexin 14 mg/kg (reference group)	103.50±2.93	63.65±3.27**/#	61.58±2.84*/**/#	1.88±0.40*/**/#

Note: L_t – total length of the small intestine, cm; D_C – distance passed by contrast mass, cm; I_{RD} – an indicator of the relative distance passed by the contrast mass, %; N – the number of fecal bolus in the colon, ru; * – $p \leq 0.05$ versus intact control group; ** – $p \leq 0.05$ versus negative control group; # – $p \leq 0.05$ versus control pathology group; ## – $p \leq 0.05$ versus reference control group; n – number of animals in the group.

(61%) compared with intact control (69%) was noted (Table 5). The laxative effect of this therapy was characterized by increased secretion and decreased fluid reabsorption in the large intestine. Thus, secretory diarrhea caused by "Senadexin" compensated for constipation. This was indicated by a significant ($p \leq 0.05$) decrease in the number of fecal bolus in the lumen of the colon, even in comparison with intact control (2.0ru vs. intact control – 4.0 ru, $p \leq 0.05$) (Table 5).

In the model of loperamide-induced constipation in the negative control group, the distance of the contrast mass was significantly reduced by 1.3 times compared the intact control, the number of fecal bolus in the colon was significantly reduced by 1.5 times. Treatment with "Prunophyte" throughout the experiment contributed to the normalization of all studied parameters of intestinal motility, which did not differ statistically from similar parameters in the group of intact control ($p \leq 0.05$). Thus, the extract "Prunophyte" significantly by 1.3 times increased the distance covered by the contrast mass compared with the negative control. It significantly exceeded the effect of reference drugs by 1.1 times compared with the negative control, control pathology and reference groups. Regarding the number of fecal bolus in the colon, when "Prunophyte" was administered there was a significant decrease by 1.8 times compared with the negative control and a significant increase by 3.5 times compared the reference group. Therefore, according to the obtained experimental data, it is obvious that the studied extract shows a mild laxative effect mainly due to the improvement of intestinal motility (Table 5). Combination therapy with "Silybor" and "Senadexin" also helped to improve gastrointestinal motility. However, the lower than intact controls average relative path of the contrast mass through the small intestine and a significantly smaller number of residual fecal bolus in the lumen of the large intestine were registered. Also, this may indicate episodic diarrhea on the background of small intestine motility depression observed in some patients with liver cirrhosis (Ilinskiy et al. 2018; Appleby et al. 2020). On the other hand, less motility of the small intestine may be caused by the development of tolerances to sennosides, which is their characteristic feature during long-term therapy. Another probable cause may be an increase in the content of Loperamide in the blood due to greater suppression of liver function (Levitt et al. 2018). The laxative effect of this therapy was characterized by an increase in secretion and a decrease in fluid reabsorption in the large intestine. Thus, secretory diarrhea (under the action of "Senadexin") compensated for constipation. This was indicated by the most probable decrease in the number of fecal bolus in the lumen of the large intestine, even in comparison with intact control ($p \leq 0.05$). In addition, it should be noted that fecal masses in the colon of the reference group were amorphous, poorly textured and formed and separated only by areas of peristalsis, which is also a functional feature of diarrhea (Table 5). In the model of loperamide-induced constipation combined with subacute alcoholic liver damage in the control group there was a significant decrease in total protein by 1.3 times, an increase in urea by 2.1 times and a significant increase in ALT (2.5 times) and ALP (2.6 times) compared with intact control. It reflects the destructive processes of liver tissue and biliary tract (Table 6). When "Prunophyte" was administered there was a significant decrease in urea content (36%) compared to the control pathology, and exceeded the effect of drugs of the reference group (22%). "Prunophyte" significantly increased the total protein content by 1.2 times and was at the level of the reference drugs, which increased the total protein content by 1.1 times compared with the control pathology (Table 6). Combination therapy and monotherapy with "Prunophyte" extract contributed to the improvement of liver function, which was mediated by positive changes in the serum markers content. "Prunophyte" significantly reduced ALT activity by 1.7 times and ALP activity by 1.4 times compared with control pathology. It should be noted that the hepatoprotective effect of "Prunophyte" was more pronounced than in the reference group. It reduced ALT activity by 1.2 times and ALP activity 1.06 times compared with the reference group (Table 6), which has already been proven in previous experimental studies of the hepatoprotective properties of the extract indifferent doses in comparison with "Silybor".

In the study of the functional state of the liver by biochemical parameters, it was demonstrated that dual pathology contributes to a significant disorder of protein-synthesizing and detoxifying functions of the liver, which in turn led to cytolysis of hepatocytes. Thus, in the control pathology group there was a significant decrease in total protein content by 21.53% and an increase in urea concentration by 13.92% compared with intact control. Treatment with" Prunophyte" led to a significant decrease in urea content (36%) compared to the control pathology,

Table 6. The effect of "Prunophyte" on the content of markers of the functional state of the liver in the serum of rats with subacute alcoholic liver damage combined with loperamide-induced constipation (n = 8).

Group of animals	Total protein, g/L	Urea, μmol/L	ALT, μkat/L	ALP, μkat/L
Intact control	73.14±4.10	6.78±0.32	0.61±0.03	2.34±0.31
Negative control	65.10±2.44	8.35±0.21*	0.63±0.06	5.83±0.27
Control pathology	57.28±1.68*/**	13.92±0.53*/**	1.55±0.10*/**	6.20±0.30*/**
Prunophyte, 200 mg/kg	68.65±2.41#	9.18±0.69*/#	0.93±0.08*/**/#	4.53±0.15*/**
Silibor 25 mg/kg + Senadexin 14 mg/kg (reference group)	66.08±2.23#	10.93±0.60*/**/#	1.10±0.09*/**/#	4.82±0.12*/**

Note: p ≤ 0.05 versus intact control group; ** – p ≤ 0.05 versus negative control group; # – p ≤ 0.05 versus control pathology group; n – number of animals in the group.

and exceeded the detoxifying effect of the reference drugs (22%). The studied extract significantly increased the total protein content by 1.2 times and was at the level of the reference drugs, which increased the total protein content by 1.1 times compared to the control pathology. The use of "Loperamide hydrochloride" in an isolated negative control did not lead to such severe hepatic impairment, but the urea content in the serum of these animals was significantly higher by 24.1% than in the intact control (p ≤ 0.05). The obtained experimental data correlate with the data on the partial excretion of urea in the feces (except for the main route – in the urine) (Levitt et al. 2018), thus due to constipation may decrease the overall rate of excretion (Table 6). Combination therapy ("Silybor"+"Senadexin") and monotherapy with "Prunophyte" probably contributed to the improvement of liver function, mediated by positive changes in markers of liver damage: total protein, urea, ALT and ALP in blood serum (p ≤ 0.05 vs control pathology). However, it should be noted that the hepatoprotective effect of "Prunophyte" was more pronounced than in the reference group, which has already been shown in previous experimental studies of the hepatoprotective properties of extract at different doses compared to "Silybor".

Conclusion

In the study of the laxative effect of PEF and PEPC extracts from the *Prunus domestica* fruits on the model of intestinal peristalsis damage with barium chloride, the activity of PEF extracts at a dose of 200 mg kg exceeded the activity of PEPC extract and the reference drug "Picolax".

In the model of alcoholic liver damage in the study of the hepatoprotective effect of PEF and PEPC extracts, it was found that the PEF extract at a dose of 200 mg/kg was more effective according to biochemical studies. Compared with animals of the control pathology group, PEF extract reduced the activity of ALT, AST, ALP, GGT, reduced the content of DC, TBA-AP, LH, urea and increased the content of total protein. PEF extract at a dose of 200 mg/kg was not inferior to the reference drug "Silybor" and was selected for further research as a more effective extract by laxative and hepatoprotective activity. It was conventionally named "Prunophyte".

The model of loperamide-induced constipation confirmed a moderate (mild) laxative effect of PEF extract at a dose of 200 mg/kg ("Prunophyte") without provoking secretory diarrhea in contrast to the comparison drug "Senadexin", increasing the distance passed by contrast mass and reducing the number of fecal bolus. The laxative effect of "Prunophyte" extract is realized mainly through the strengthening of intestinal motility. The hepatoprotective effect of "Prunophyte" is realized due to protein-synthesizing (increased total protein), detoxifying (decreased urea), anticytolytic (decreased ALT activity) and bile-secretory (decreased ALP activity) properties.

"Prunophyte" extract can be a prospective alternative to a one-time complex treatment with herbal hepatoprotectors and laxatives, which will avoid polypragmatism in the treatment of comorbid conditions in gastroenterology associated with functional constipation and liver disease.

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