Recent studies have shown a clear tendency for an increase in gastroenterological diseases. Every tenth visitor to the pharmacy has symptoms of indigestion despite the absence of obvious causes, such as abdominal pain, bloating and flatulence, accompanied by constipation or diarrhea or their «alternation». The most common complaints they seek from a doctor are a disorder or insufficient bowel movements, which is a manifestation of irritable bowel syndrome. It is a functional disease that is increasingly affecting young people.

Stress, poor nutrition, the pursuit of success, a tough competitive environment, the desire to meet certain standards of beauty, debilitating diets for weight loss, abuse of appropriate pills to reduce body weight – all these are factors that can trigger the development of constipation and disorders of the functional state of the liver [1, 2, 3]. Constipation is often accompanied by a decrease in immunity due to the formation of dysbiosis and disorders of the hepatobiliary system. Despite the fact that scientific developments in the field of the creation of new laxatives and hepatoprotective agents are ongoing, the arsenal of these agents is still relatively limited. That is why the creation of modern effective and safe medicines for the pharmacocorrection of constipation of different etiology, which are accompanied by disorders of the functional state of the liver, is quite relevant [4, 5, 6].

A promising area of pharmacotherapy for gastrointestinal tract and liver diseases is the use of herbal remedies. Advantages of pharmacotherapy by herbal remedies are: absence of toxic action, possibility of long-term use, considerable range and «softness» of influence, optimum pharmacoeconomic component, possibility of application to patients of all age categories. Therefore, Plum was selected for research due to unique chemical composition of the fruits and long experience of its use in folk medicine.

Plum (Prunus domestica L., fam. Rosaceae) is widespread horticultural crops in Ukraine, it has a lot zoned and local varieties and the number of plantations is inferior to only apples, pears and cherries [7]. According to the research, the presence and content of organic acids (malic, citric, chlorogenic, neochlorogenic, caffeic), anthocyanins, rutin, gallic acid, homo- and heteropolysaccharides was determined in the plum fruits [8, 9, 10, 11, 12].

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According to the available literature data Plum fruits are a source of polyphenolic compounds with a wide spectrum of action and are widely used in the treatment of diseases of the gastrointestinal tract and hepatobiliary system (constipation and atony of the intestine, bile stagnation, liver disease) [13, 14, 15, 16].

Therefore, the study of pharmacological properties and experimental substantiation of the possibility of application of Plum fruits extracts in the treatment of gastrointestinal tract and hepatobiliary system diseases is of great relevance.

Previous experimental studies have shown that of the four Plum extracts, the plum extract containing fibers (PEF) and extract containing plum polysaccharide complex (PEPC) exhibit a clear laxative effect and moderate hepatoprotective activity, which is probably related to their chemical content homo- and heteropolysaccharides, as well as sums of phenolic compounds [17, 18].

The aim of the present work was an experimental study of anti free radical activity and antioxidant properties by indicators of lipid peroxidation and the antioxidant system (AOS) of two Plum extracts (PEF and PEPC), detecting the most effective extract and determining its effective dose.

Materials and methods

The object of the study were two dried extracts from the Plum (Prunus domestica L., fam. Rosaceae) fruits of the Ugorka variety (PEF and PEPC), which were selected as a result of previous screening studies of laxative and hepatoprotective activities of four extracts.

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. According to the phytochemical analysis, the 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 [17, 18].

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 ED₃₀ of this hepatoprotective agent [19].

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 ml/kg for 7 days [20]. The dry extracts PEF and PEPC were administered intragastrically at doses of 100 mg/kg and 200 mg/kg.

All extracts were dissolved or suspended in 4 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 [21]. 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 [21].

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

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 minutes 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 SF-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 [22] and was calculated by the formula:

\[ A = E \cdot K \cdot 0X, \]

where \( E \) – extinction of the test sample;

\( K \) – coefficient of molar extinction \( 2,2 \cdot 10^{-5} \text{ M}^{-1} \cdot \text{cm}^{-1} \);

\( 0X \) – dilution of the sample.

The content of LPO was determined by a standard biochemical method using a redox
reaction with Fe³⁺ ions using the kit «Lipid Hydroperoxide (LPO) 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 Uchiyama M. & Michara M.
in the modification of I. A. Volchevorsky by the test with TBA-AP. During the reaction, 3 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 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 [23] and was
calculated by the formula:

\[ A = (E_{535} - E_{580}) \cdot K \cdot 0X, \]

where \( E_{535} \) и \( E_{580} \) – extinction at an appropriate wavelength;

\( K \) – coefficient of molar extinction \( 1,88 \cdot 10^{-5} \text{ M}^{-1} \cdot \text{cm}^{-1} \);

\( 0X \) – dilution of the sample.

The content of RG in the liver homogenate was determined by spectrophotometric method
with Ellman’s reagent [24]. The principle of the method is based on the use of a specific thiol
reagent – 5,5 diithiobisnitrobenzoic 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% trichloracetic acid, stirred and centrifuged for 10 min at 1500 rpm. To
0,5 ml of centrifuge was added 2 ml of Ellman’s solution. Incubated for 10 min at t 18–22 °C. The
extinction was determined with an SF-46 spectrophotometer 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:

\[ C = E \cdot 1094, \]

where \( E \) – extinction of the test sample;

1094 – estimated coefficient.
Determination of α-tocopherol was performed by color reaction with Fe³⁺ (indicator α, α-bipyridyl); the results of the determination were corrected for the presence of cholesterol [25].

Statistical processing of the obtained experimental data was performed using Statistica (StatSoftInc., USA, version 6.0) [26].

Animals were kept in the same conditions, on a standard diet in accordance with the sanitary and hygiene requirements [27] 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). The experiments 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, 1986, as amended in 1998) and the Law of Ukraine No. 3447-IV dated February 21, 2006, as amended, «On the Protection of Animals from Cruelty», Order of the Ministry of Education and Science, Youth and Sports of Ukraine No. 249 dated March 1, 2012, «Procedure for conducting scientific experiments, experiments on animals». The Committee on Bioethics of National University of Pharmacy (Protocol No. 01 of 02.10.2019) found no violations of moral and ethical standards during the experiment.

Results and discussion

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 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 1).

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 1).

<table>
<thead>
<tr>
<th>Animals group</th>
<th>DCs, µmol/g</th>
<th>TBA-AP, µmol/g</th>
<th>LPO, nmol/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact control (IC)</td>
<td>7,63 ± 0,65</td>
<td>35,28 ± 2,62</td>
<td>79,53 ± 4,52</td>
</tr>
<tr>
<td>Control pathology (CP)</td>
<td>13,92 ± 0,96*</td>
<td>63,15 ± 3,19*</td>
<td>160,44 ± 8,98*</td>
</tr>
<tr>
<td>Silibor, 25 mg/kg</td>
<td>10,92 ± 0,53***</td>
<td>57,82 ± 2,79*</td>
<td>148,00 ± 6,62*</td>
</tr>
<tr>
<td>PEF, 100 mg/kg</td>
<td>12,07 ± 1,04*</td>
<td>60,37 ± 2,44*</td>
<td>152,27 ± 4,11*</td>
</tr>
<tr>
<td>PEF, 200 mg/kg</td>
<td>9,84 ± 0,78***</td>
<td>49,71 ± 3,51***</td>
<td>124,25 ± 7,85***</td>
</tr>
<tr>
<td>PEPC, 100 mg/kg</td>
<td>13,57 ± 0,86*</td>
<td>64,27 ± 1,41*</td>
<td>153,58 ± 9,42*</td>
</tr>
<tr>
<td>PEPC, 200 mg/kg</td>
<td>12,28 ± 0,60***</td>
<td>59,75 ± 3,54*</td>
<td>151,91 ± 4,73*</td>
</tr>
</tbody>
</table>

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.
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 2).

**Table 2**

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

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».

**Conclusions**

1. 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 use 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.

2. 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.

3. 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).

4. 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.
References


References

Надійшла до редакції 2 листопада 2019 р. Прийнято до друку 20 листопада 2019 р.
The study of pharmacological properties and experimental substantiation of application possibilities of the plum fruits extracts in the treatment of the gastrointestinal tract and hepatobiliary system diseases is relevant, because plum is a source of phenolic compounds with a wide range of action and is used in folk medicine for treating diseases of the digestive system.

The aim of the present work was an experimental study of anti free radical activity by lipid peroxidation indicators and antioxidant properties by the antioxidant system (AOS) indicators of two Plum extracts, detecting the most effective extract and determining its effective dose.

The objects of research were extracts obtained from the fresh plum fruits: plum extract containing fibers (PEF) and extract containing plum polysaccharide complex (PEPC).

Research methods: pharmacological (modeling of alcoholic damage to rat liver), biochemical (determination of the level of diene conjugates, TBA-AP, lipid hydroxyperoxides, reduced glutathione and α-tocopherol in rat liver homogenate) and statistical (using the program «Statistica 6.0», Student’s t-test).

Experimental data on the study of the anti free radical properties of the plum extracts showed inhibitory effects on lipid peroxidation, reducing the content of diene conjugates, TBA and lipid hydroxyperoxides under conditions of alcoholic liver damage. Regarding the effect of the investigated extracts on the markers of the antioxidant system of hepatocytes, there was an increase in the content of reducing glutathione and α-tocopherol on the alcoholic hepatitis model. The fiber-containing extract (PEF) was the most effective in normalizing the functional state of the liver at a dose of 200 mg/kg. Thus, it was determined that an effective dose of extract with fibers (PEF) was 200 mg/kg. The investigated effects of PEF in 200 mg/kg exceeded the effects of PEPS at the tested doses and were at the level of activity of reference drug «Silibor» in the dose of 25 mg/kg. Anti free radical and antioxidant properties of the PEF are probably related to its chemical composition namely phenolic compounds (anthocyanins and hydroxycinnamic acids). Considering the results of screening, the most promising subject for further in-depth pharmacological study is plum extract PEF at dose 200 mg/kg.
to be expected, the effectiveness of a functional test, which is expected to be effective at a dose of 200 mg/kg. Thus, the effective dose of CEW extract was determined, which was 200 mg/kg. The effects of the CEW extract at a dose of 200 mg/kg exceeded the action of the CEPK extract at the studied doses and were at the level of activity of the control agent "Silibor" at a dose of 25 mg/kg. Antioxidant and antioxidant properties of the CEW extract, likely, are associated with the presence in its chemical composition of a sum of phenolic compounds (anthocyanins and oxycorice acids). Considering the results of screening, the most promising object for further in-depth pharmacological study is the CEW extract obtained from the plums of domestic plum, containing fibers (CEW), at a dose of 200 mg/kg.

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(Senin I. V.)