Searching of drugs that normalize the function of the digestive glands are important because disruption of digestion underlie the pathogenesis of many diseases such as gastroesophageal reflux disease, hepatitis, gastric ulcer, irritable bowel syndrome, cancer etc. [1‒5]. The use of herbal objects containing fibres is a promising direction for solving of the mentioned problem. We were attracted by fruits of *Prunus domestica* which are rich in fibres (homo- and heteropolysaccharides) [6] and are used in folk medicine as a laxative and hepatoprotective agent. In previous studies of the pharmacological properties of extracts obtained from the fruits of *Prunus domestica*, their expressive laxative, moderate hepatoprotective, antioxidant, anti-exudative and prebiotic activities were confirmed. The data of screening studies of four new extracts from *Prunus domestica* fruits for laxative and hepatoprotective activity revealed the most active extract. Fibre-containing extract (FCE) was selected as the most active and named «Prunofit». The dose 200 mg/kg was determined as its effective dose.

The aim of this experimental study was to investigate the lipotropic properties of «Prunofit» extract, as its normalizing effect on the functional state of the liver can be used to prevent fatty infiltration of the liver. In addition, one of the consequences of chronic liver damage with alcohol is the development of liver steatosis. According to modern notions, steatosis, or fatty degeneration of the liver, is a condition characterized by excessive accumulation of fat in hepatocytes. At the same time, it is marked by changes in the content of lipids and lipoproteins in the blood plasma.

Materials and methods

The study of lipotropic properties of the extract from *Prunus domestica* fruits «Prunofit» was carried out in the conditions of subacute toxic liver damage caused by the introduction of ethanol.

Lipotropic drug – «Methionine» (JSC «Kyiv Vitamin Plant»), which is a proteinogenic essential amino acid involved in transmethylation processes, was used as the reference drug.
It has lipotropic action, promotes the synthesis of choline, phospholipids; participates in the synthesis of adrenaline, creatine; activates the action of hormones, vitamins, enzymes. It has detoxifying properties due to its ability to methylate toxic products, reduces blood cholesterol and increases phospholipid content.

Twenty four albino rats both sexes weighing between 180 to 200 g were used in the experiment. The animals were randomly divided into four groups (n = 6). The first group of animals (intact control – IC) was without affected liver and was treated with the equal volume of water. The second group of animals (control pathology – CP) had induced alcoholic hepatitis and was treated with the equal volume of water. The third and fourth groups of animals with alcoholic hepatitis were treated with the reference drug «Methionine» at a dose of 155 mg/kg [7] and «Prunofit» extract at a dose of 200 mg/kg, respectively.

Alcoholic hepatitis was induced by intragastric administration of 40% ethanol at a dose of 7 ml/kg for 7 days [8]. All investigated agents dissolved or suspended in 4 ml of purified water and administered intragastrically 1 hour after the introduction of ethanol solution.

Seventy-two hours after the last introduction of hepatotoxin, animals were decapitated under chloralose-urethane anesthesia, blood was collected and serum was obtained by centrifugation at 3 000 rpm. The liver was perfused with cold 0.1 M Tris-HCl buffer (pH 7.4). The tissue of the liver was crushed, a mixture of ethanol:diethyl ether (3:1, by volume) was added in a ratio of 15 ml of the mixture to 300 mg of tissue [9].

The content of cholesterol was determined in obtained tissue extract by the colorimetric method named Liebermann-Burchard on photocolorimeter KFK-2 at a wavelength of 630‒690 nm [10]. The calculation was made according to the standard; the results were expressed in mmol/g of tissue. Triacylglycerols (TG) content was determined using a standard set of reagents «Lachema» (Czech Republic). The calculation was made according to the standard; the results were expressed in mg/g of tissue. The obtained tissue extract was used to determine the content of unsaturated fatty acids (UFA) by the colorimetric method named Laurel–Tibbling at a wavelength of 550 nm; the calculation was performed according to the standard; the results were expressed in mmol/g of tissue [10]. The content of total phospholipids (TPL) was determined by the Blur method using KFK-2 at a wavelength of 605‒730 nm; the calculation was performed according to the standard and the results were expressed in mmol/g of tissue [10]. The total lipids (TL) content was determined using Felitis–Diagnostics reagent kits (Dnipro, Ukraine).

The content of total lipids (TL), total cholesterol (TCh), triacylglycerols (TGs), unsaturated fatty acids (UFA) and total phospholipids (TPL) were also determined in blood serum by appropriate methods.

**Results and discussion**

It has been found that subacute alcoholic liver damage leads to a violation of lipid metabolism in the liver tissue and in the blood serum. Thus, TL content was significantly increased in the liver tissue by 41%, due to an increase in the content of TCh, TGs and UFA by 47%, 42.84% and 43%, respectively, in comparison with IC. There was a significant decrease of TPL content in hepatocytes by 56.6% compared with IC. There was also a tendency to increase the content of TL, TCh, TGs and UFA by 39.27%, 34.2%, 46.7% and 50%, respectively, in the blood serum of rats with alcoholic liver damage. The content of TPL in rats with alcoholic liver damage decreased by 50.2% (Table). Phosphatidylcholine is one of the main phospholipids of liver cells and it is a part of the plasmatic and intracellular membranes of cells. Thus, a decrease in the phospholipids content may indicate the membranes damage that can lead to impaired cell integrity and the development of necrotic processes. These assumptions confirmed by some biochemical parameters that were determined in serum after ethanol administration [11]. The cause of the phospholipids...
decrease may be inhibition of their formation by enhancing the synthesis of triacylglycerols and enhancing their hydrolysis with the participation of phospholipases, as evidenced by the increase in UFA content. Unsaturated fatty acids are the main substrate of lipid peroxidation (LPO), which confirmed by the data of the enhancement of LPO under these experimental conditions. Changes in lipid spectrum in the blood serum reflect the deviation of lipid metabolism in the liver of animals with experimental alcoholic hepatitis: decreased TPL, increased TGs, TCh, and UFA.

Pharmacological correction of liver toxic damage is directed at normalization of lipid metabolism and TCh, stimulation the mobilization of lipids from the liver and oxidizing them, enhancing the synthesis of phospholipids in liver cells.

The introduction of the investigated extract from Prunus domestica fruits «Prunofit» to rats with subacute alcoholic liver damage led to normalization of lipid metabolism in the liver tissue and blood serum of experimental animals.

**Table Study of lipotropic effect of extract from Prunus domestica fruits «Prunofit» on a model of subacute alcoholic hepatitis (n = 6)**

| Groups of animals       | TL, mg/g | TCh, mmol/g | TGs, mg/g | UFA, mmol/g | TPL, mmol/g |
|-------------------------|----------|-------------|-----------|-------------|-------------|
| **In liver tissue**     |          |             |           |             |             |
| Intact control          | 161.52 ± 3.5 | 15.65 ± 3.12 | 5.63 ± 0.21 | 3.27 ± 0.72 | 45.67 ± 1.85 |
| Control pathology       | 273.64 ± 6.28* | 29.48 ± 1.83* | 9.85 ± 0.65* | 5.74 ± 0.81* | 19.82 ± 1.94* |
| Prunofit, 200 mg/kg     | 197.36 ± 2.45 | 27.35 ± 1.96*** | 7.14 ± 0.83** | 3.56 ± 0.54** | 31.63 ± 2.16*** |
| Methionine, 155 mg/kg   | 198.47 ± 2.46*** | 27.86 ± 1.79*** | 7.32 ± 0.86*** | 4.46 ± 0.63*** | 31.75 ± 1.89*** |
| **In the serum**        |          |             |           |             |             |
| Intact control          | 2.35 ± 0.13 | 6.47 ± 0.51 | 0.72 ± 0.16 | 0.87 ± 0.84 | 0.68 ± 1.26 |
| Control pathology       | 3.87 ± 0.56* | 9.83 ± 0.95* | 1.35 ± 0.18 | 1.74 ± 0.96* | 5.32 ± 1.12* |
| Prunofit, 200 mg/kg     | 2.79 ± 0.48*** | 5.68 ± 0.87* | 1.24 ± 0.13* | 0.92 ± 0.75 | 7.62 ± 1.25*** |
| Methionine, 155 mg/kg   | 1.98 ± 0.34*** | 6.21 ± 0.53* | 0.97 ± 0.85* | 1.15 ± 0.39 | 6.95 ± 1.12*** |

**Notes:** * – p < 0,05 versus intact control group; ** – p < 0,05 versus control pathology group.

The analysis of the experimental data showed that administration of the «Prunofit» extract led to a significant decrease the content TL, TCh, TGs and UFA in liver homogenate by 27.9%, 7.2%, 27.5% and 38%, respectively, compared with CP, and significant increase of TPL level by 37.34%. The slight stabilization of lipid metabolism in serum of animals treated with the investigated extract was also observed. It was shown a decrease of TL by 28%, TCh by 42.2%, TGs by 8.15% and UFA by 47.1%, and increase in TPL content by 30.2% compared with CP (Table 1). An increase in TPL level correlates with a decrease in UFA level may be due to the antioxidant effect of the extract. It is known that the development of oxidative stress in the liver leads to activation of phospholipase A2, destruction of phospholipids and an increase in UFA level [12].

The reference drug «Methionine» (155 mg/kg) also had a pronounced effect on lipid metabolism in the liver and blood serum. The introduction of «Methionine» decreased the content of TL, TCh, TGs and UFA in the liver by 27.47%, 5.5%, 25.7%, and 22.3%
compared with CP. The TPL content increased by 37.57%. The administration of the reference drug had a normalizing effect on serum lipid metabolism, reducing TL by 48.8%, TCh by 36.8%, TGs by 28.14%, UFA by 34% and increasing TPL by 23.45 % (Table 1).

Thus, the obtained experimental data indicate that the extract from the Prunus domestica fruits «Prunofit» has a lipotropic effect in subacute alcoholic hepatitis. It is manifested by reducing the intensity of lipolysis, fatty hepatosis and signs of hyperlipidemia. Obtained data indicates the ability of «Prunofit» extract to improve of metabolic processes in the liver in case of the long-term use.

**Conclusions**

1. The introduction of «Prunofit» extract at a dose of 200 mg/kg on the background of alcoholic liver damage led to a decrease in the intensity of the lipolysis processes, fatty hepatosis, manifestations of hyperlipidemia, reducing the content of total lipids, cholesterol, triacylglycerols and free fatty acids in rats.

2. «Prunofit» extract was at the level of the reference drug «Methionine» at a dose 155 mg/kg by its ability to inhibit fatty liver infiltration.

3. The lipotropic action of «Prunofit» extract is probably mediated by its antioxidant properties and by the presence of phenolic compounds (anticyanins and oxycoric acids) in its chemical composition.

**References**

1. Wu T., Rayner C. K., Young R. L., Horowitz M. Gut motility and enteroendocrine secretion // Curr. Opin. Pharmacol. – 2013. – V. 13, N 6. – P. 928–934.

2. Drinnan M., Powell J., Nikkar-Esfahani A., Heading R. C., Doyle J. Gastroesophageal and extraesophageal reflux symptoms: Similarities and differences // Laryngoscope. – 2014. – N 30.

3. Dressman D. A., Hasler W. L. Rome IV – Functional GI Disorders: Disordersof Gut-Brain Interaction // Gastroenterol. – 2016. – V. 150, N 6. – P. 1257–1261.

4. American Gastroenterological Association Medical Position Statement on Constipation // Gastroenterol. – 2013. – N 144. – P. 211–217. https://doi.org/10.1053/j.gastro.2012.10.029

5. Quigley E. M. Prucalopride: safety, efficacy and potential applications. Ther. Adv. // Gastroenterol. – 2012. – V. 5, N 1. – P. 23–30.

6. Patent на винахід № C2 118602. Спосіб одержання водорозчинного полісахаридного комплексу з послаблюючою активністю з плодів сливи домашньої / Комісаренко А. М., Упир Т. В., Сенюк І. В. та ін. – Заявл. 06. 03. 2017; Опубл. 11. 02. 2019, Бюл. № 3.

7. Anroop B. Nair, Shery Jacob. A simple practice guide for dose conversion between animals and human // J. Basic. Clin. Pharm. – 2016. – V. 7, N 2. – P. 27–31. https://doi.org/10.4103/0976-0105.177703

8. Doklinicheskie issledovaniya lekarstvennykh sredstv: metod. rek. / Pod red. A. V. Stefanova. – K: Avitsenna, 2002. – 528 s.

9. Orel N. M. Biokhimiya membran: metod. posobie k lab.zanyatiyam dlya stud. biol. f-ta. – Minsk: BGU, 2010. – 28 s.

10. Prokhorova M. I. Metody biokhimicheskih issledovaniy (lipidnyy i energeticheskiy obmen): uch. posobie. – L.: Izd-vо Leningr. un-tа, 1982. – 272 s.

11. Evans U. G., Morre D. D. Biologicheskie membrany. Metody / Pod red. Findleya Dzh., Evansa U. G. – M: Mir, 1990. – 424 s.

12. Ježek J., Jabůrek M., Zelenka J., Ježek P. Mitochondrial phospholipase A2 activated by reactive oxygen species in heart mitochondria induces mild uncoupling // Physiol. Res. – 2010. – V. 59, N 5. – P. 737–747.

**Список використаної літератури**

1. Wu T., Rayner C. K., Young R. L., Horowitz M. Gut motility and enteroendocrine secretion // Curr. Opin. Pharmacol. – 2013. – V. 13, N 6. – P. 928–934.

2. Drinnan M., Powell J., Nikkar-Esfahani A., Heading R. C., Doyle J. Gastroesophageal and extraesophageal reflux symptoms: Similarities and differences // Laryngoscope. – 2014. – N 30.

3. Dressman D. A., Hasler W. L. Rome IV – Functional GI Disorders: Disorders of Gut-Brain Interaction // Gastroenterol. – 2016. – V. 150, N 6. – P. 1257–1261.

4. American Gastroenterological Association Medical Position Statement on Constipation // Gastroenterol. – 2013. – N 144. – P. 211–217. https://doi.org/10.1053/j.gastro.2012.10.029

5. Quigley E. M. Prucalopride: safety, efficacy and potential applications. Ther. Adv. // Gastroenterol. – 2012. – V. 5, N 1. – P. 23–30.

6. Патент на винахід № C2 118602. Спосіб одержання водорозчинного полісахаридного комплексу з послаблюючою активністю з плодів сливи домашньої / Комісаренко А. М., Упир Т. В., Сенюк І. В. та ін. – Заявл. 06. 03. 2017; Опубл. 11. 02. 2019, Бюл. № 3.
THE STUDY OF LIPOTROPIC ACTION OF EXTRACT FROM FRUIT PRUNUS DOMESTICA «PRUNOFIT» BY ANIMAL MODELS OF ALCOHOLIC LIVER DISEASES

Key words: lipotropic action, methionine, total lipids, cholesterol, triacylglycerols, unsaturated fatty acids, total phospholipids, alcoholic hepatitis, Prunus domestica fruits

Abstract

Searching of drugs that normalize the function of the digestive glands are important because disruption of digestion underlie the pathogenesis of many diseases such as gastroesophageal reflux disease, hepatitis, gastric ulcer, irritable bowel syndrome, cancer etc. The use of herbal objects containing fibres is a promising direction for solving of the mentioned problem. We were attracted by fruits of Prunus domestica which are rich in fibres (homo- and heteropolysaccharides) and are used in folk medicine as a laxative and hepatoprotective agent.

The aim of this experimental study was to investigate the lipotropic properties of «Prunofit» extract. The study of lipotropic properties of the «Prunofit» was carried out in the conditions of subacute toxic liver damage caused by the introduction of ethanol. The content of total lipids (TL), total cholesterol (TCh), triacylglycerols (TGs), unsaturated fatty acids (UFA) and total phospholipids (TPL) were determined in liver and blood serum.

Obtained results of the lipotropic properties study of the «Prunofit» extract at a dose of 200 mg/kg against the background of alcoholic liver damage showed a decrease in the intensity of lipolysis, fatty hepatosis, and manifestations of hyperlipidemia. It has occurred due to a decrease in the content of total lipids, cholesterol, triglycerides and free fatty acids in the homogenate rat liver by 27.9%, 7.2%, 27.5% and 38%, respectively, and rat serum by 28%, 42.2%, 8.15%, 47.1%, respectively, compared with the control pathology. Against the background of model pathology, «Prunofit» extract tended to increase the content of total phospholipids in the liver homogenate by 37.34% and in serum by 30.2% compared with the control pathology. According to its ability to inhibit fatty liver infiltration, the «Prunofit» extract was at the level of the reference drug «Methionine» at a dose of 155 mg/kg.
Метою цього експериментального дослідження було вивчення ліпотропних властивостей екстракту «Прунофіт». Вивчення ліпотропних властивостей «Прунофіта» проводили в умовах підострого токсичного ураження печінки, спричиненого введенням етанолу. У печінці і сироватці крові визначали вміст загальних ліпідів, загального холестеролу, триацилгліцеролів, ненасичених жирних кислот і загальних фосфоліпідів.

У результаті вивчення ліпотропних властивостей екстракту «Прунофіт» у дозі 200 мг/кг на тлі алкогольного ураження печінки спостерігалося зменшення виразності процесів ліполізу, жирового гепатозу, провів гіперліпідемії, яке відбувається за рахунок зниження вмісту загальних ліпідів, холестеролу, триацилгліцеролів та вільних жирних кислот у гомогенаті печінки шурів на 27,9%, 7,2%, 27,5% та 38% відповідно, в сироватці крові шурів на 28%, 42,2%, 8,15%, 47,1% відповідно порівняно з контрольною патологією. На тлі модельної патології екстракт «Прунофіт» виявив тенденцію до збільшення вмісту загальних фосфоліпідів у гомогенаті печінки на 37,34% та у сироватці крові на 30,2% відносно контрольної патології. За здатністю гальмувати жирову інфільтрацію печінки екстракт «Прунофіт» був на рівні препарату порівняння «Метіонін» у дозі 155 мг/кг.

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ІЗУЧЕНИЕ ЛИПОТРОПНОЙ АКТИВНОСТИ ЭКСТРАКТА ИЗ ПЛОДОВ СЛИВЫ ДОМАШНЕЙ "ПРУНОФИТ" НА МОДЕЛИ АЛКОГОЛЬНОГО ГЕПАТИТА

Ключевые слова: липотропное действие, метионин, общие липиды, алкогольный гепатит, плоды сливы домашней.

АННОТАЦИЯ
Поиск лекарств, нормализующих функцию пищеварительных желез, важен, поскольку нарушение процессов пищеварения лежит в основе патогенеза многих заболеваний, таких как гастроэзофагеальная рефлюксная болезнь, гепатит, язва желудка, синдром раздраженного кишечника, рак и др. Перспективным направлением решения указанной проблемы является применение растительных объектов, содержащих волокна. Нас привлекли плоды Prunus domestica, богатые клетчаткой (гомо- и гетерополисахариды), которые используются в народной медицине как слабительное и гепатопротекторное средство.

Целью этого экспериментального исследования было изучение липотропных свойств экстракта «Прунофит». Изучение липотропных свойств «Прунофита» осуществляли в условиях подострого токсического поражения печени, вызванного введением этанола. В печени и сыворотке крови определяли содержание общих липидов, общего холестерина, триацилглицеролов, ненасыщенных жирных кислот и общих фосфолипидов.

В результате изучения липотропных свойств экстракта «Прунофит» в дозе 200 мг/кг на фоне алкогольного поражения печени наблюдалось уменьшение выраженности процессов липолиза, жирового гепатоза, проявленной гиперлипидемии, которое происходило за счет снижения содержания общих липидов, холестерола, триацилглицеролов и свободных жирных кислот в гомогенате печени крыс на 27,9%, 7,2%, 27,5% и 38% соответственно, и сыворотке крови крыс на 28%, 42,2%, 8,15%, 47,1% соответственно по сравнению с контрольной патологией. На фоне модельной патологии экстракт «Прунофит» проявлял тенденцию к увеличению содержания общих фосфолипидов в гомогенате печени на 37,34% и в сыворотке крови на 30,2% по сравнению с контрольной патологией. По способности ингибировать жировую инфилтрацию печени экстракт «Прунофит» был на уровне препарата сравнения «Метионин» в дозе 155 мг/кг.

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