Original Article

Thai Journal of Pharmaceutical Sciences

Hepatoprotective and choleretic activity of dried extract of *Tanacetum vulgare* flowers

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Received: Jul 11, 2018
Accepted: Oct 30, 2018
Published: Dec 10, 2018

ABSTRACT

Objectives: The article presents the study of hepatoprotective and choleretic properties of *Tanacetum vulgare* flowers dried extract *(TVFDE) in vivo*. **Materials and Methods**: The research was carried out using the model of subchronic toxic hepatitis in rats, caused by intragastric administration of 50% tetrachloromethane oil solution (0.4 ml/100 g) and subcutaneous administration of 40% ethyl alcohol (1.3 ml/100 g) within 4 days. An evaluation of the effect of TVFDE on the treatment-prophylactic regimen in doses of 25, 50, 75, 100, and 150 mg/kg was performed in 72 h after the last administration of toxins. **Results:** It has been established that TVFDE has dose-dependent hepatoprotective and choleretic effects. TVFDE in doses of 75 and 100 mg/kg decreased thiobarbituric acid active products on 25% and 29% ($P < 0.05$), increased the concentration of reduced glutathione on 40% and 34% ($P < 0.05$), decreased the activity of alanine aminotransferase in 1.7 times ($P < 0.05$) and 1.3 times ($P < 0.05$), and the activity of aspartate aminotransferase in 1.3 ($P < 0.05$) and 1.2 ($P > 0.05$) times, respectively, versus control hepatitis. The use of TVFDE in doses of 50, 75, 100, and 150 mg/kg (excluding 25 mg/kg) contributed to the normalization of the bile volume and rate of bile secretion. Among all the studied doses, TVFDE only in a dose of 75 mg/kg showed the significant improvement in bile composition: The bile acids content was significantly increased in 1.5 ($P < 0.05$), cholesterol in 1.7 times ($P < 0.05$), and influenced the total volume of allocated bile, which was significantly higher in 2.3 times ($P < 0.05$) and contributed to alkaline phosphatase activity decrease in 1.4 times ($P < 0.05$). **Conclusions:** TVFDE makes dose-dependent hepatoprotective and choleretic effects. Two most effective doses of TVFDE 75 and 100 mg/kg were determined. Based on the total assessment of the extract effect on the prooxidant-antioxidant imbalance, the activity of cytolysis and cholestasis, bile-synthetic and bile-secretory liver function TVFDE in a dose of 75 mg/kg was found to be most effective; therefore, it was selected for further research.

Keywords: Antioxidant activity, choleretic activity, hepatoprotective activity, subchronic toxic hepatitis, *Tanacetum vulgare* flowers

INTRODUCTION

Among the most common non-infectious liver diseases are fatty hepatosis, non-alcoholic steatohepatitis and in its outcome liver cirrhosis, as well as medicine and alcohol damage of the liver.[1-3] Without any connection to the etiological factor causes liver damage, oxidative stress, which leads to the activation of lipid peroxidation of the cell and subcellular membrane lipids, is played key role.[4-6] The products of lipid peroxidation play an important role in fibrogenesis, activating stellate cells of

the liver. In its turn, fibrosis leads to the cirrhosis formation, which is the main cause of patients death.[7-9] The damage of the liver is often accompanied by a disruption of the biliary and bile excretory functions of the liver. It is generally known that bile components (hydrophobic bile acids [BA], bilirubin, and cholesterol) have a toxic effect both on the tubular epithelium of the bile ducts and on the hepatocytes, in particular on its mitochondria, directly or indirectly blocking the respiratory cycle and oxidation of fatty acids. The result is not only further disruption of hepatocyte function but also the secondary stimulation of lipid peroxidation processes, which leads to cell damage.[10,11] Hepatocellular cholestasis predominantly takes place under the influence of metabolic disorders, toxic substances, medicines, and alcohol. Hepatoprotectors are widely used in the complex treatment of liver diseases. The ability of the hepatoprotector to positively influence on the cholestasis state is an important characteristic of its pharmacological effect.[12] Considering the above, it is advisable to evaluate the hepatoprotective effect together with choleretic, when searching for a new hepatoprotector. It is known from the experience of traditional medicine that *Tanacetum vulgare* flowers, which is common in Ukraine, has a choleretic effect,[13] and research data[14] confirm a similar effect of *T. vulgare* liquid extract. However, phytomedicines from *T. vulgare* flowers are lacking in the pharmaceutical market of Ukraine. That is why the development of a new medicine from *T. vulgare* flowers is efficient.

At the Department of Botany of the National University of Pharmacy, under the guidance of professor T. N. Gontova, *T. vulgare* flowers dried extract (TVFDE) was purposely received, and the presence of hepatoprotective effect along with the ability to positively influence the processes of bile formation and secretion was predicted. Study of the pharmacological properties of TVFDE was provided at doses of 25, 50, 75, 100, and 150 mg of extract per kg rat body weight. The choice of such a range of TVFDE doses is due to the necessity of the effective dose determination.

MATERIALS AND METHODS

Phytochemical Part

Plant materials and extraction procedures

T. vulgare flowers were collected during flowering in 2014 on the territory of Ukraine in the Kharkiv region, Kolomatsiy district. The raw material, according to the rules of purchasing, was exposed to the air-shadow method of drying, crushed into powder, stored in well-sealed containers and used for phytochemical analysis.

TVFDE was prepared by fractional maceration. *T. vulgare* flowers were infused with 70% ethyl alcohol at room temperature 3 times when the ratio of raw material to extractant was 1:5. The first extraction was carried out within 12 h, the second and third within 1 h each. Received filtered extracts were combined, purified and evaporated to dryness under vacuum. The technology of obtaining was tested in manufacturing conditions of the pharmaceutical manufacturer "Chervona zirka" (Ukraine). The novelty of the research is confirmed by patent number 113431, January 25, 2017.[15]

Preliminary phytochemical screening

The determination of phenolic compounds of TVFDE was carried out by a thin-layer chromatography identification method using Merk Silica gel 60 F254 as a mobile phase. Standard solutions of luteolin, luteolin-7-glycoside, caffeine and chlorogenic acids were used. The content of phenolic compounds was studied using high-performance liquid chromatography (HPLC) method with a Shimadzu HPLC - system, ser. 20 with a diode array detector. The spectrophotometry method has been used for the determination of quantitative content of flavonoids (wavelength – 410 nm, per luteolin) and sum of hydroxycinnamic acids (wavelength - 525 nm, per chlorogenic acid).[13]

Pharmacological Part

Animals

Male rats weighing 190–220 g were used. The animals were obtained from the vivarium of the Central Research Laboratory (National University of Pharmacy, Kharkiv, Ukraine). All experimental protocols were approved by the Bioethics Commission of the National University of Pharmacy (animal use protocol number 12, December 20, 2017). Experiments were conducted in accordance with the "Directive 2010/63/EU of the European Parliament of the Council of 22 September 2010 on the protection of animals used for scientific purposes." The rats were housed in standard polypropylene cages and kept at 20–26°C and 50% humidity in a well-ventilated room with a 12 h light/dark cycle with free access to food and water.[16]

Preparation of the extract and the reference medicine for biological research

A freshly prepared aqueous suspension of TVFDE was stabilized with Tween-80, and a freshly prepared aqueous silymarin suspension (comparison agent) was also stabilized with Tween-80.

Influence on subchronic toxic hepatitis

Laboratory animals (male rats weighing 190–220 g) were divided into eight groups according to the experimental conditions: Group I - intact control (normal control); Group II- control hepatitis; Groups III–VII- animals with hepatitis, which were administered TVFDE in doses of 25, 50, 75, 100, and 150 mg/kg, respectively; Group VIII - animals with hepatitis, which were treated with reference medicine silymarin (Karsil, Sopharma, Bulgaria) at a dose of 100 mg/kg (silymarin content per tablet is 22.5 mg).[17]

To obtain the representative results and provide the correct statistical analysis of experimental data, the eight rats were used in each group. Less number of experimental animals can lead to distortion of experimental results due to the variability of biological test systems.[18]

A model of subchronic toxic lesion of the liver was reproduced by a single subcutaneous administration of tetrachloromethane 50% oil solution in a dose of 0.4 ml/100 g of rat weight with subsequent intragastric 40% ethyl alcohol introduction in a dose of 1.3 ml/100 g for 4 days.[18] TVFDE was administered in chosen doses to rats 7 days before hepatitis modeling 1 time/day (prophylactic mode) and during hepatitis 1 h before tetrachloromethane administration and 2 h after (treatment regimen). After the last injection of toxins (on the 4 days), TVFDE was administered once a day, the last time in an hour before the procedure of bile collecting.

Influence on bile formation and bile secretion

On the 4th day of hepatitis modeling, 1 h after the introduction of toxins, animals were anesthetized with barbamil 1% aqueous solution and laparotomy of the abdominal cavity was conducted. The duodenum and the place where the bile duct was introduced into was found, where the incision was made. Into the incision of the bile duct, a catheter was injected through which bile was collected in a test tube. The allocated total volume (V_{total}) of bile was recorded. The bile activity of TVFDE and silymarin at the rate of bile secretion (RBS) within an hour (mg/min/100 g of rat weight) and its quantitative components: The content of BA and cholesterol was evaluated.[19] A method based on the ability of a cooled iron chloride solution to give colored complexes with BA and cholesterol with absorption peaks at different wavelengths was used.[18] Photometric measurements of the studied parameters were performed on a spectrophotometer SPh-46. Furthermore, the influence of TVFDE on the activity of alkaline phosphatase (APh), a marker of cholestasis, was studied by the kinetic method for the rate of para-nitrophenol formation, which was directly proportional to the activity of the enzyme.[20]

Influence on the state of cytolytic processes and antioxidant-prooxidative imbalance

After studying the state of bile secretion processes, the rats were decapitated with subsequent serum obtaining according to generally accepted methods. In serum, the concentration of marker enzymes of cytolysis alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was determined using the unified Reitman-Frankel dinitrophenylhydrazine method based on the reaction of transamination involving these enzymes to form a colored hydrazone in an alkaline medium, having a characteristic absorption peak at a wavelength of 500–560 nm.[21] The manufacturer sets of the Scientific-Research Enterprise "Filisit-Diagnostika" (Ukraine) were used.

The content of thiobarbituric acid-active products (TBA-AP) and reduced glutathione (RG) in liver homogenate made the ability to evaluate the effect of TVFDE on the prooxidant-antioxidant imbalance. To obtain a homogenate of the liver, 0.5 M Tris HCl buffer with a pH of 7.8 was used. The content of TBA-AP in the liver homogenate by colorimetric method, based on the ability of these compounds to form in the acidic medium trimethine complexes with TBA, having a maximum absorption at a wavelength of 532 nm,[22] was determined. The determination of the content of RG is based on the formation of a colored compound in the destruction of the bisulfide bond in the glutathione molecule under the influence of sulfosalicylic acid.[20] The optical density of the colored compound at a wavelength of 412 nm was measured.

Statistical Analysis

Statistical processing of the obtained results using the program "Statistica 8.0" was carried out. The non-parametric Mann–Whitney U-test was used, the level of significance $P < 0.05$ was adopted.[23]

It was considered that TVFDE provides pharmacological activity in case of significant differences ($P < 0.05$) compared with the model pathology (control hepatitis). For the determination of the benefits of TVFDE, the comparison with standard treatment with silymarin ($P < 0.05$) was carried out.

RESULTS AND DISCUSSION

Previous Phytochemical Screening

Previously, it was detected that TVFDE contains the following phenolic compounds: Flavonoids (luteolin 0.34%, routine 0.37%, hyperoside 0.16%, acacetin 7-glycoside 1.02%, apigenin 0.06%, and kaempferol 0.05%), hydroxycinnamic acids (chlorogenic acid 2.40%, neochlorogenic acid 0.13%, 3,5-dicaffeoylquinic acid 7.32%, and 4,5-dicaffeoylquinic acid 1.83% of acid). Trace amounts of coumarins (umbelliferone), flavonoids (catechin, apigenin-7-glycoside), hydroxycinnamic acids (caffeic acid, ferulic acid), and phenolcarboxylic acids (gallic acid)[24] have been found.

Pharmacological Activity

Influence on subchronic hepatitis

Influence on antioxidant-prooxidant imbalance and activity of cytolytic processes

The composition of the biologically active compounds of TVFDE, which has antioxidant properties, in particular routine, apigenin, luteolin, hydroxycinnamic acids,[25-27] allowed to predict the possible hepatoprotective effect. This is very important, as noted above, regardless of the etiological factor causing hepatocytes damage, the key role is given to oxidative stress.[4-6]

Experimental hepatitis was accompanied by an increase in the processes of lipid peroxidation: The TBA-AP content increased in 2.5 times while the antioxidant content of RG decreased in 1.9 compared to normal control [Table 1].

This indicates the development of oxidative stress, which resulted in the destruction of cellular and subcellular membranes, which is confirmed by the growth of cytolysis activity markers: ALT in 2.5 times and AST in 2.4 times [Table 2].

The most beneficial effect on the normalization of the prooxidant-antioxidant imbalance was observed in TVFDE in doses of 75 and 100 mg/kg: TBA-AP decreased on 25% and 29% ($P < 0.05$), while the concentration of RG increased on 40 % and 34 % ($P < 0.05$), respectively.

Table 1: TVFDE influence on the prooxidant-antioxidant imbalance under the conditions of subchronic toxic hepatitis

Group of animals	RG, μkmol/g	TBA-AP, μkmol/g
Normal control	1.76±0.16	33.33±3.53
Control hepatitis	0.94±0.27*	82.05±22.34*
TVFDE, 25 mg/kg	0.97±0.27#	77.77±21.84#
TVFDE, 50 mg/kg	0.87±0.41#	73.93±30.41
TVFDE, 75 mg/kg	1.32±0.33**	61.54±5.06**/#
TVFDE, 100 mg/kg	1.26±0.26**	58.12±8.53**
TVFDE, 150 mg/kg	1.19±0.25#	65.60±22.59
Silymarin, 100 mg/kg	1.96±0.39**	49.14±9.98**

*Significantly different from the normal control ($P<0.05$). **Significantly different from the control hepatitis ($P<0.05$). #Significantly different from the standard treatment with silymarin ($P<0.05$). $n=8$ rats per group. Values are expressed as means±SD. TBA-AP: Thiobarbituric acid-active products, RG: Reduced glutathione, TVFDE: *Tanacetum vulgare* flowers dried extract

TVFDE in doses of 25, 50, and 150 mg/kg did not show a pronounced effect on the prooxidant-antioxidant imbalance. The reference medicine silymarin showed a more pronounced effect than TVFDE: In doses of 25, 50, and 150 mg kg, respectively, the content of RG was in 2.02, 2.25, and 1.65 times higher ($P < 0.05$) and the, respectively, content of TBA-AP was in 1.58 and 1.3 times lower ($P < 0.05$) than under the influence of the extract in doses of 25 and 75 mg/kg [Table 1].

TVFDE in doses of 75 and 100 mg/kg decreased the activity of ALT in 1.7 times ($P < 0.05$) and 1.3 times ($P < 0.05$) and the activity of AST in 1.3 ($P < 0.05$) and 1.2 ($P > 0.05$) times comparing with control hepatitis [Table 2]. Administration of TVFDE in doses of 25 and 50 mg/kg did not lead to significant changes in transaminases activity. TVFDE in doses of 150 mg/kg caused a significant decrease only in ALT activity. Silymarin reduced the markers of cytolysis in 1.5 times ($P < 0.05$). There was no significant difference between the effect of silymarin 100 mg/kg and TVFDE in doses of 75 and 100 mg/kg. Anti-cytolytic activity of TVFDE in a dose of 75 mg/kg was practically the same as in silymarin and significantly higher than TVFDE in dose of 100 mg/kg (ALT activity 2.20 ± 0.14 vs. 2.70 ± 0.50 μkmol/h × ml, $P < 0.05$).

Thus, the expressed anti-cytolytic activity of TVFDE in doses of 75 and 100 mg/kg is established, which is believed to be realized under the antioxidant mechanism, since the flavonoids (kaempferol, apigenin, apigenin-7-glycoside, luteolin, hyperoside, routine, acacetin-7-glycoside, and catechin) and hydroxycinnamic acids, which according to the literature[25,28-30] is "scavengers" of free radicals. The more pronounced anti-cytolytic effect of TVFDE at a dose of 75 mg/kg compared with the doses of 100 and 150 mg/kg is probably due to the ability of flavonoids and hydroxycinnamic acids, which are presenting in the composition of dried extract, at higher doses to provide prooxidant properties.[31] The absence of the antioxidant effect of TVFDE at a dose of 150 mg/kg can also be explained by this fact.

Influence on the processes of bile formation and bile secretion

Against the background of aggressive pathology induced by the introduction of tetrachloromethane and alcohol, there was a suppression of biliary excretion: V_{total} and RBS decreased in 2.0 times ($P < 0.05$) [Table 3]. BA and cholesterol synthesis was suppressed: BA decreased in 1.6 times ($P < 0.05$), cholesterol - in 1.9 times ($P < 0.05$), respectively, relatively to normal control [Table 4].

Furthermore, in the blood serum APh activity was increased (in 1.5 times, $P < 0.05$), which is a marker of cholestasis, indicating on the development of inflammation in the bile ducts. The above data indicate the development of cholestasis syndrome.[32]

TVFDE dose-dependently effected on bile-forming and bile-secretion functions, probably due to hepatoprotective properties described in the previous section. The manifestation of cholestasis is a consequence of cytodestruction of hepatocytes and cholangiocytes, and the root cause of this process is oxidative stress.[33] TVFDE in doses of 50, 75, 100, and 150 mg/kg (excluding 25 mg/kg) significantly affect on the V_{total} of bile and RBS. TVFDE in doses of 75 and 100 mg/kg had the most potent normalizing effect on the functional parameters of the bile-synthetic and bile-secretory function of the liver, which is confirmed by a greater volume of secreted bile and its secretion rate, compared to other studied doses of the extract and to standard treatment with silymarin ($P < 0.05$) [Table 3]. Effect of TVFDE in doses of 75 and 100 mg/kg on bile-synthetic and bile-secretory liver function was superior to the effect of standard treatment with silymarin ($P < 0.05$).

Among all the studied doses, TVFDE only in a dose of 75 mg/kg showed the significantly improvement in bile composition: The BA content was significantly increased in 1.5 ($P < 0.05$), cholesterol in 1.7 times ($P < 0.05$), and influenced the total volume of allocated bile, which was significantly higher in 2.3 times ($P < 0.05$) and contributed to APh activity decrease in 1.4 times ($P < 0.05$) comparing with control hepatitis [Table 4]. The influence of TVFDE in a dose of 75 mg/kg on the BAs and cholesterol content in bile and blood serum Aph activity was the same as standard treatment with silymarin. The decrease in activity of serum APh under the influence of TVFDE in a dose of 75 mg/kg and silymarin indicated a normalizing effect on cholestasis syndrome.

Table 2: TVFDE influence on cytolysis markers activity under the conditions of subchronic toxic hepatitis

Group of animals	ALT, μkmol/h×ml	AST, μkmol/h×ml
Normal control	1.49±0.28	1.45±0.16
Control hepatitis	3.66±0.23*	3.43±0.61*
TVFDE, 25 mg/kg	3.29±0.75$^{\#}$	3.48±0.48$^{\#}$
TVFDE, 50 mg/kg	3.42±0.42$^{\#}$	2.81±0.95$^{\#}$
TVFDE, 75 mg/kg	2.20±0.14**	2.75±0.92**
TVFDE, 100 mg/kg	2.70±0.50**/$^{\$}$	2.79±0.83
TVFDE, 150 mg/kg	2.70±0.30**/$^{\$}$	3.25±0.60$^{\#}$
Silymarin, 100 mg/kg	2.48±0.44**	2.29±0.36**

*Significantly different from the normal control ($P<0.05$). **Significantly different from the control hepatitis ($P<0.05$). $^{\$}$Significantly different from the TVFDE in dose 75 mg/kg; $n=8$ rats per group. Values are expressed as means±SD. TVFDE: *Tanacetum vulgare* flowers dried extract, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase

Table 3: TVFDE influence on bile-synthetic and bile-secretory liver function under the conditions of subchronic toxic hepatitis

Group of animals	Vtotal, ml	RBS, mg/min/100
Normal control	7.86±0.88	4.66±0.66
Control hepatitis	3.91±1.24*	2.35±0.25*
TVFDE, 25 mg/kg	5.10±0.60$^{\#}$	3.00±0.12$^{@}$/$^{\#}$
TVFDE, 50 mg/kg	6.26±0.55**	3.79±0.13$^{@}$
TVFDE, 75 mg/kg	8.94±0.61**/$^{\#}$	5.12±0.20**/$^{\#}$
TVFDE, 100 mg/kg	8.60±0.68**/$^{\#}$	5.07±0.22**/$^{\#}$
TVFDE, 150 mg/kg	7.71±1.54**	4.51±0.37**
Silymarin, 100 mg/kg	6.84±0.70**	4.01±0.14**

*Significantly different from the normal control ($P<0.05$). **Significantly different from the control hepatitis ($P<0.05$). $^{\#}$Significantly different from the silymarin group ($P<0.05$). $^{@}$Significantly different from the group of animals administered TVFDE in doses of 75 and 100 mg/kg ($P<0.05$). $n=8$ rats per group. Values are expressed as means±SD. TVFDE: *Tanacetum vulgare* flowers dried extract, V_{total}: Total volume, RBS: Rate of bile secretion

Table 4: TVFDE influence on the bile acids and cholesterol content in bile and blood serum Aph activity under the conditions of subchronic toxic hepatitis

Group of animals	Bile acids, mg %	Cholesterol, mg %	APh, U/l
Normal control	828.78±203.11	33.08±12.38	141.62±24.20
Control hepatitis	529.24±121.76*	17.60±10.45*	216.97±27.41*
TVFDE, 25 mg/kg	567.15±108.62#	19.55±15.56	200.47±33.65#
TVFDE, 50 mg/kg	667.75±171.39	31.16±19.39	179.02±36.58
TVFDE, 75 mg/kg	796.57±137.32**	29.15±5.92**	156.2±17.29**
TVFDE, 100 mg/kg	724.47±285.78	26.42±13.09	168.85±35.26
TVFDE, 150 mg/kg	604.77±312.62	26.54±39.46	181.64±35.26
Silymarin, 100 mg/kg	707.65±141.40**	25.58±14.22**	160.05±20.80**

*Significantly different from the normal control ($P<0.05$). **Significantly different from the control hepatitis ($P<0.05$). #Significantly different from the silymarin group ($P<0.05$). $n=8$ rats per group. Values are expressed as means±SD. TVFDE: *Tanacetum vulgare* flowers dried extract, Aph: Alkaline phosphatase

According to the results of the study, two most effective doses of TVFDE 75 and 100 mg/kg were determined. Taking into account that TVFDE in a dose of 75 mg/kg significantly exceeded the extract in a dose of 100 mg/kg for inhibitory effect on cytolytic processes (ALT activity 2.20 ± 0.14 vs. 2.70 ± 0.50 μkmol/h × ml, $P < 0.05$) and had a significant effect on the cholesterol and bile secretion function of the liver, a dose of 75 mg/kg was chosen for further studies.

CONCLUSIONS

Thus, the TVFDE makes dose-dependent hepatoprotective and choleretic effects. Two most effective doses of TVFDE 75 and 100 mg/kg were determined. Based on the total assessment of the extract effect on the prooxidant-antioxidant imbalance, the activity of cytolysis and cholestasis, bile-synthetic and bile-secretory liver function TVFDE in a dose of 75 mg/kg was found to be most effective: Significantly exceeded the extract in a dose of 100 mg/kg for inhibitory effect on cytolytic processes and had a significant effect on the cholesterol and bile secretion function of the liver; therefore, it was selected for further study. TVFDE at a dose of 75 mg/kg has shown less antioxidant activity and the same anti-cytolytic effect as standard treatment with silymarin but inferior to silymarin for normalization of bile formation and secretion.

REFERENCES

1. Williams CD, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, *et al.* Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: A prospective study. Gastroenterology 2011;140:124-31.
2. Takahashi Y, Sugimoto K, Inui H, Fukusato T. Current pharmacological therapies for nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. World J Gastroenterol 2015;21:3777-85.
3. Kazyulin AN. Place of ursodeoxycholic acid in the treatment of non-alcoholic fatty liver disease at different stages of the disease: Steatosis, steatohepatitis, fibrosis/cirrhosis. Riga Med J 2017;17:1248-57.
4. Cichoz-Lach H, Michalak A. Oxidative stress as a crucial factor in liver diseases. World J Gastroenterol 2014;20:8082-91.
5. Lavine JE. Vitamin E treatment of nonalcoholic steatohepatitis in children: A pilot study. J Pediatr 2000;136:734-8.
6. de Andrade KQ, Moura FA, dos Santos JM, de Araújo OR, de Farias Santos JC, Goulart MO, *et al.* Oxidative stress and inflammation in hepatic diseases: Therapeutic possibilities of N-acetylcysteine. Int J Mol Sci 2015;16:30269-308.
7. Kumar P, Smith T, Raeman R, Chopyk DM, Brink H, Liu Y, *et al*. Periostin promotes liver fibrogenesis by activating lysyl oxidase in hepatic stellate cells. J Biol Chem 2018;2108:RA117.
8. Wang L, Yue Z, Guo M, Fang L, Bai L, Li X, *et al*. Dietary flavonoid hyperoside induces apoptosis of activated human LX-2 hepatic stellate cell by suppressing canonical NF-kB signaling. Biomed Res Int 2016;2016:1068528.
9. Lee UE, Friedman SL. Mechanisms of hepatic fibrogenesis. Best Pract Res Clin Gastroenterol 2011;25:195-206.
10. Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: The multiple parallel hits hypothesis. Hepatology 2010;52:1836-46.
11. Zvyagintseva TD, Chernobay AI. Intrahepatic cholestasis: From pathogenesis to treatment. Ukr Med J 2012;3:79-83.
12. Yakovenko EP. Modern approaches to the choice of hepatoprotectors use in chronic liver disease. Ukr Med J 2008;6:16-9.
13. Zolotaykina MY, Syra LM, Gontova TM. Morphological and anatomical signs of the above-ground bodies of *Tanacetum vulgare*. Pharm J 2016;1:23-9.
14. Kalko KO, Mishchenko OY, Pozdniakova AY, Derymedvid LV, Zolotaikina MY, Gontova TM, *et al*. Screening studies of the common tansy grass (*Tanacetum vulgare*) liquid extract choleretic activity under subchronic hepatitis in rats. Pharm Drug Toxicol 2018;1:39-44.
15. Zolotaykina MY, Gontova TM. Method of Peppermint Common Flowers Dry Extract Obtaining: Pat. 113431 Ukraine: IPC A 61 K 133/00. Number u 201608022; Stated. 07.19.2016; 2017. p. 4.
16. Deacon RM. Housing, husbandry and handling of rodents for behavioral experiments. Nat Protoc 2006;1:936-46.
17. Mironov AN, Bunatyan ND, Vasilyeva AN, Verstakova OL, Zhuravlev MV, Lepakhin VK, *et al*. Guidelines for Preclinical Research on Medicines. part One. Moscow, Russian Federation: Grif and K; 2012. p. 944.
18. Stefanov OV. Pre-clinical Research of Medicinal Products (Methodical Recommendations). Kyiv, Ukraine: Avicenna Publishing House; 2018. p. 528.
19. Skakun NP, Oleinik AN. Comparative effect of atropine and methacin on the extra-secretory function of the liver. Pharm Toxicol 1967;3:334-7.
20. Kishkun AA. Manual on Laboratory Methods of Diagnostics. Moscow, Russian Federation: GEOTAR-Media; 2007.
21. Kamyshnikov VS. A Reference Book on Clinical and Biochemical Laboratory Diagnostics. Vol. 1. Minsk: Belarus; 2000.
22. Stalnaya ID, Garishvili TG. Method of Malonic Dialdehyde

Determination with the Use of Thiobarbituric Acid. Modern Methods in Biochemistry. Moscow, Russian Federation: Medicine; 1977.
23. Truhacheva NV. Mathematical Statistics in Medical-biological Researches Using the Package Statistica. Moscow, Russian Federation: GEOTAR-Media; 2012.
24. Zolotaikina MY, Gontova TM, Kotov AH, Ilyina TV, Kryvoruchko OV, *et al*. Study of dry extract of tansy (*Tanacetum vulgare*) using the method of high-performance liquid chromatography. Der Pharm Chem 2017;9:1-4.
25. Shashank K, Abhay KP. Chemistry and biological activities of flavonoids: An overview. Sci World J 2013;2013. Doi. org/10.1155/2013/162750.
26. Rašković A, Gigov S, Čapo I, Paut Kusturica M, Milijašević B, Kojić-Damjanov S, *et al.* Antioxidative and protective actions of apigenin in a paracetamol-induced hepatotoxicity rat model. Eur J Drug Metab Pharmacokinet 2017;42:849-56.
27. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radic Res 1995;22:375-83.
28. Damasceno SS, Dantas BB, Ribeiro-Filho J, Antônio M Araújo D, Galberto M da Costa J. Chemical properties of caffeic and ferulic acids in biological system: Implications in cancer therapy. A Review. Curr Pharm Des 2017;23:3015-23.
29. Patel SB, Attar UA, Ghane SG. Antioxidant potential of wild *Lagenaria siceraria* (Molina) Standl. TJPS 2018;42:90-6.
30. Razzaghi-Asl N, Garrido J, Khazraei H, Borges F, Firuzi O. Antioxidant properties of hydroxycinnamic acids: A review of structure- activity relationships. Curr Med Chem 2013;20:4436-50.
31. Procházková D, Boušová I, Wilhelmová N. Antioxidant and prooxidant properties of flavonoids. Fitoterapia 2011;82:513-23.
32. Uchaikin VF, Cherednichenko TV, Smirnov AV. Infectious Hepatology: A Manual for a Doctor. Moscow: Top Doctors; 2002. p. 640.
33. Alaca N, Özbeyli D, Uslu S, Şahin HH, Yiğittürk G, Kurtel H, *et al.* Treatment with milk thistle extract (*Silybum marianum*), ursodeoxycholic acid, or their combination attenuates cholestatic liver injury in rats: Role of the hepatic stem cells. Turk J Gastroenterol 2017;28:476-84