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PHARMACOLOGICAL ACTION OF THICK EXTRACT OF COMMON TANSY (*Tanacetum vulgare* L.) FLOWERS IN AN EXPERIMENTAL MODEL OF ESTROGEN-INDUCED CHOLESTASIS

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The aim. *To study the protective effect of thick extract of Tanacetum vulgare L. flowers (TETVF) in an experimental model of estrogen-induced cholestasis (EIC).*
Materials and methods. *EIC was reproduced by subcutaneous administration of 7α-ethinylestradiol (E) (5 mg/kg) to rats. TETVF and the reference preparation (RP) cholelesan (ChL) were administered to animals intragastri-cally once a day for 3 days before and 5 days of the modeling period. One day after the last E administration, the animals were anesthetized (thiopental sodium, 50 mg/kg, intraperitoneally), the volume of bile secreted in 60 min was determined, and the bile secretion rate (BSR) was calculated. The activity of alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GTT), the content of TBA-active products (TBA-AP) and reduced glutathione (RG) was determined in the blood serum. For histological examination, prepared liver sections were stained with hematoxylin, eosin and sudan IV to identify lipids. A semi-quantitative assessment of EIC signs was performed.*
Results of the study. *In animals with EIC TETVF contributed to the normalization of bile volume and BSR to the level of intact control (IC), a significant ($p < 0.05$) decrease in the studied parameters: ALT, LF and GTT, a decrease in lipid peroxidation (LPO) processes and an increase in liver antioxidant defense, as evidenced by a significant decrease in the level of TBA-AP (30.98 ± 0.90 μmol/g vs. 44.87 ± 4.26, $p < 0.05$) and a 1.9-fold increase in RG (4.11 ± 0.36 μmol/g vs. 2.19 ± 0.16, $p < 0.05$) compared to the control pathology (CP). In general, TETVF showed a normalizing effect like RP, but was inferior to it in terms of its effect on BER and on ALT activity and TBA-AP level. After the introduction of TETVF the severity of ductal proliferation significantly decreased by 1.6 times ($p < 0.05$), periductal inflammation by 3.8 times ($p < 0.05$), and fatty degeneration of hepatocytes of the periductal zones by 2.3 times ($p < 0.05$) compared to CP.*
Conclusion. *The ability of TETVF to improve impaired liver function under conditions of EEIS and reduce patho-logical manifestations was established*
Keywords: *estrogen-induced cholestasis (EIC), rats, thick extract of Tanacetum vulgare L. flowers (TETVF), an-tioxidant properties, protective effect*

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1. Introduction

Intrahepatic cholestasis, which is caused by drugs and is accompanied by impaired bile formation and secretion, is a common phenomenon [1, 2]. Intrahepatic accumulation of cytotoxic bile acids (BA) provokes cholestatic liver damage, which is manifested by impaired hepatocyte integrity, inflammation, fibrosis, cirrhosis, and an increased risk of cancer [3, 4]. Agents that can cause intrahepatic cholestasis are estrogens, sex steroid hormones that play a crucial role in regulating the female reproductive system, bone density, cholesterol mobilization, electrolyte balance, brain function, cardiovascular system, and central nervous system [5]. However, estrogens and their metabolites can cause cholestasis in pregnant women and postmenopausal women receiving oral contraceptives or hormone replacement therapy [5]. Estrogen-induced cholestasis (EIC) is a specific condition in the second or third trimester of pregnancy in women. The incidence of EIC ranges from 0.2 to 5.6% [6]. EIC often presents as generalized pruritus in association with elevated serum bile acids and/or transaminases in the late second or third trimester [7]. Despite a favorable maternal prognosis, the disease is associated with an increased risk of adverse perinatal outcomes, including meconium contamination of amniotic fluid, preterm labor, and stillbirth [8]. Currently, therapeutic options for estrogen-induced cholestasis are limited, and treatment strategies are primarily aimed at maintaining liver function and alleviating symptoms. In this context, natural products have attracted increasing attention due to their multifactorial mechanisms of action. Bioactive compounds such as flavonoids, phenolics, alkaloids, and saponins have demonstrated antioxidant, anti-inflammatory, and hepatoprotective properties, including modulation of bile acid homeostasis and hepatic transporter activity [9]. Recent systematic reviews and meta-analyses suggest that herbal medicines

may improve clinical symptoms and certain biochemical parameters in patients with intrahepatic cholestasis; however, the available evidence remains insufficient, highlighting the need for further high-quality experimental and clinical studies [10].

Therefore, given the limited availability of effective targeted therapies and the promising pharmacological potential of plant-derived compounds, the present study aims to investigate the pharmacological activity of a newly developed thick extract of *Tanacetum vulgare* L. flowers (TETVF) in an experimental model of estrogen-induced cholestasis.

The aim of the work is to investigate the pharmacological activity of the newly created thick extract of *Tanacetum vulgare* L. flowers (TETVF) in an experimental model of estrogen-induced cholestasis.

2. Planning (methodology) of research

Step 1. Analysis of publications on the prevalence of estrogen-induced cholestasis (EIC), possible pharmacocorrection approaches and justification of the feasibility of studying the effectiveness of the newly created thick extract of *Tanacetum vulgare* L. flowers (TETVF) on experimental EIC.

Step 2. Modeling EIC in female rats and administration of tested agents.

Step 3. Determination of indicators of bile secretion, the level of hepatocyte cytolysis indicators, inflammatory processes, lipid peroxidation and activity of the liver antioxidant system and preparation of liver micropreparations for histological examination.

Step 4. Identification of signs of damage to hepatocytes, bile ducts and severity of steatosis after staining sections with hematoxylin and eosin, sudan IV, respectively.

Step 5. Histological analysis of the liver and bile ducts and semi-quantitative assessment of EIC signs: ductular proliferation, inflammatory periductular reaction, steatosis.

Step 6. Processing and analysis of the results.

Step 7. Identification of promising directions for further research.

3. Materials and methods

The studied TETVF was obtained at the Department of Botany at the National university of Pharmacy. Tansy flowers were crushed to a particle size of 2–3 mm and extracted three times with 70% water-ethanol solution. The ratio of the mass of the raw material to the total volume of the extractant was 1 : 5. The resulting extracts were combined and allowed to stand for 24 hours at a temperature of 2–4°C, after that they were filtered and evaporated on a rotary vacuum evaporator until a thick mass with humidity of not more than 25% was obtained. The dense extract obtained was a viscous mass of a dark brown color with a specific odor; it stretched into threads and again mixed into a solid mass [10].

TETVF was analyzed by thin-layer chromatography, which allowed the identification of three dominant phenolic compounds: luteolin, luteolin-7-glycoside, and chlorogenic acid. The quantitative content of phenolic substances in the TETVF was determined by spectrophotometry. The obtained data allowed for the standardization of TETVF for its total flavonoid content of 3.69% (calculated as luteolin) and total hydroxycinnamic acids of 16.88% (calculated as chlorogenic acid) [11, 12].

This study was conducted at the Educational and Scientific Institute of Applied Pharmacy of the National University of Pharmacy, certified by the State Expert Center of the Ministry of Health of Ukraine. Preliminary safety tests of pure TETVF were conducted, and its toxicological properties were studied. It was determined that intragastric administration of TETVF to mature rats of both sexes at a dose of 5000 mg/kg did not result in animal mortality and had no adverse effects on the condition of the animal's organs and systems. This allows the TETVF to be classified as practically non-toxic. Also, during preliminary screening studies on healthy intact rats, a conditionally effective dose of extract was selected – 100 mg/kg [13].

The EIC model most closely reproduces the pathogenesis and morphofunctional changes in the liver and biliary tract during estrogen administration [14]. Given this, this experimental model was used in the study. Intrahepatic cholestasis was reproduced in sexually mature female rats (body weight 150–180 g) by subcutaneous administration of 7α-ethinylestradiol (E) (5 mg/kg) dissolved in an equal volume of propylene glycol, once daily for 5 days [14]. E was obtained from Sigma-Aldrich (St. Louis, MO, USA). The studied extract and the reference preparation (RP) were administered to animals in a therapeutic and prophylactic regimen intragastrically once daily for three days before the start of pathology modeling and 1.5 hours before E administration throughout the modeling period.

The herbal medicine cholelesan (ChL) (produced by the Arterium Corporation, Ukraine) was chosen as RP as an analogue of the study agent in terms of indications for use and therapeutic effect. Cholelesan contains the following components: extract of dense wild carrot fruits and calendula flowers ((7.75–13.4):1) – 60 mg; extract of dry immortelle flower (40:1) – 50 mg; curcumin C3 ((64–66):1) – 20 mg; turmerone oil – 5 mg; peppermint essential oil – 7.5 mg [15].

Animals were divided into groups: 1 – intact control (IC), animals of which received distilled water; 2 – control pathology group (CP), animals of which received E (5 mg/kg, s. c.); 3 – animals that received E and TETVF at a dose of 100 mg/kg, which was found to be effective in terms of bile secretion [13]; 4 – animals that received E and RP ChL at a dose of 35 mg/kg as an analogue of pharmacological activity. The dose of RP was calculated considering the species sensitivity coefficients [16].

During the experiment, the animals were kept in standard vivarium conditions with a natural light regime "day-night" and free access to water and food. All manipulations were carried out in accordance with the provisions of the "European convention for the protection of vertebrate animals used for experimental and other scientific purposes" [17] and the methodological recommendations of the Ministry of Health of Ukraine [16]. The distribution of animals into groups was carried out according to the principle of randomization.

One day after the last administration of E (9th day of the experiment), the animals were anesthetized with intraperitoneal administration of thiopental sodium at a dose of 50 mg/kg, fixed on the operating table, and the abdominal cavity was opened with an incision in the epigastric region 2 cm long. In the anesthetized animals, the place of entry of the bile duct into the duodenum was found and a polyethylene tube-cannula was fixed in the duct, through which bile entered the test tube. The volume of bile secreted in 60 min was recorded within one hour after the administration of TETVF and RP by collecting bile from the test tube with a graduated syringe. Bile was collected for 1 hour and the bile secretion rate (BSR) was calculated (mg/min/100 g of animal body weight).

In blood serum, the activity of alanine aminotransferase (ALT) (Reitman-Frenkel method, by reaction with 2,4-diphenylhydrazine), alkaline phosphatase (ALP) (photometrically, with 4-aminophenazone) and gamma-glutamyl transpeptidase (GGT) (photometrically, with γ-L-(+)-glutamyl-4-nitroanilide) was determined using diagnostic kits PLIVA-Lachema Diagnostika (Czech Republic) and Filisit-Diagnostika (Ukraine). The content of thiobarbituric acid-active products (TBA-AP) was determined by the method [16], the level of reduced glutathione (RG) by the method of Beutler E.D. et al. [18].

All actual material was processed by methods of variational statistics (mean value and its standard error, $M \pm m$) using parametric (analysis of variance, Newman-Keuls test) and nonparametric analysis methods (the Kruskal-Wallis test followed by Dunn's multiple comparisons test with Bonferroni correction). The significance level was accepted as $p < 0.05$. To obtain statistical conclusions, the standard STATISTICA program package (version 6) was used [19].

For histological examination, liver slices were fixed in 10% formalin solution, dehydrated in alcohols of increasing strength, and embedded in paraffin. Sections 5-6 μm thick were obtained using a sledge microtome, mounted on a glass slide, and stained with hematoxylin and eosin. Additionally, formalin-fixed animal liver samples were cut on a freezing microtome. Sections for identification of total lipids were stained with sudan IV. In addition to the qualitative analysis of the state of the liver parenchyma, a semi-quantitative assessment of the severity of simulated intrahepatic cholestasis was performed: ductular proliferation, inflammatory periductular reaction, and steatosis according to the following score: 0 points – no sign; 1 point – proliferation of bile ducts in individual triads of single lobules on a micropreparation (MP) and/or vague cellular infiltration periductally in triads of individual lobules on MP and/or dust-like or small droplet lipid infiltration of the cytoplasm of individual periportal hepatocytes in MP; 2 points – proliferation of bile ducts in ¼ triads of lobules on a MP with slight spread towards adjacent portal tracts and/or weak cellular infiltration periductally in approximately ¼ triads of lobules on MP and/or small droplet lipid infiltration of the cytoplasm of periportal hepatocytes in approximately ¼ triads of lobules on MP; 3 points – proliferation of bile ducts in ½ triads of lobules on the MP with noticeable spread towards adjacent portal tracts and/or pronounced cellular infiltration periductally in approximately ½ triads of lobules on the MP and/or small droplet lipid infiltration of the cytoplasm of periportal hepatocytes in approximately ½ triads of lobules on the MP; 4 points – proliferation of bile ducts in approximately ⅔ triads of lobules on the MP with noticeable spread towards adjacent portal tracts, germination of newly formed ducts into the depth of the lobules and/or pronounced cellular infiltration periductally in approximately ⅔ triads of lobules on the MP and/or small droplet lipid infiltration of the cytoplasm of periportal hepatocytes in approximately ⅔ triads of lobules on the MP.

4. Result

The study results (Table 1) showed that the administration of E (CP group) caused a decrease in bile volume and BSR by 2 (0.22 ml vs. 0.44 ml, $p < 0.05$) and 1.8 times (1.42 mg/min/100 g vs. 2.54 mg/min/100 g, $p < 0.05$), respectively, which indicates the development of intrahepatic cholestasis.

Table 1

Effect of TETVF and RP ChL on the volume and bile secretion rate (BSR) in rats with EIC, $M \pm m$, $n = 6$

Research groups	IC	CP	Pathology + TETVF, 100 mg/kg	Pathology + RP ChL, 35 mg/kg
Bile volume, ml	0.44 ± 0.03	0.22 ± 0.04*	0.44 ± 0.06	0.55 ± 0.03
Bile secretion rate (BSR), mg/min/100 g	2.54 ± 0.19	1.42 ± 0.13*	3.16 ± 0.27**	4.25 ± 0.32 */**/#

*Note: * – relative to IC (Newman-Keuls test), $p < 0.05$; ** – relative to CP (Newman-Keuls test), $p < 0.05$; # – relative to RP ChL (Newman-Keuls test), $p < 0.05$; n – number of animals in the group.*

In the CP group, a significant increase in the hepatocyte cytolysis marker ALT enzyme by 4.4 times (1.39 ± 0.04 mmol/(hxl) vs 0.33 ± 0.02, $p < 0.05$), the cholestasis marker ALP by 2 times (475.13 ± ± 27.09 IU d/l vs 231.92 ± 22.13, $p < 0.05$) and GGT by 1.85 times (16.71 ± 1.67 IU d/l vs 9.00 ± 0.11, $p < 0.05$) was also found in relation to the IC, which indicates impaired liver function. The study of lipid peroxidation and antioxidant protection indicators showed a 2.4-fold increase in the content of TBA-AP (44.87 ± 4.26 μmol/g versus 18.38 ± 1.22, $p < 0.05$) and a 2.7-fold decrease in RG (2.19 ± 0.16 μmol/g versus 5.82 ± 0.51, $p < 0.05$) (Table 2).

Prophylactic and therapeutic administration of TETVF to rats with EIC contributed to the normalization of the volume and BSR indicators almost to the level of IC. In terms of the normalizing effect on the rate of bile secretion, TETVF was inferior to RP ChL (3.16 ± 0.27 mg/min/100 g vs 4.25 ± 0.32, $p < 0.05$). After administration of TETVF to rats with EIC, a significant ($p < 0.05$) decrease in the studied indicators was observed relative to CP: ALT, ALP and GTT. TETVF contributed to the reduction of LPO processes and the enhancement of antioxidant protection of the liver, as evidenced by a significant decrease in the level

of TBA-AP compared to CP (30.98 ± 0.90 μmol/g vs. 44.87 ± 4.26, $p < 0.05$) and a 1.9-fold increase in RG (4.11 ± 0.36 μmol/g vs. 2.19 ± 0.16, $p < 0.05$). TETVF exhibited a normalizing effect similar to RP but was inferior in its effect on ALT activity and TBA-AP level.

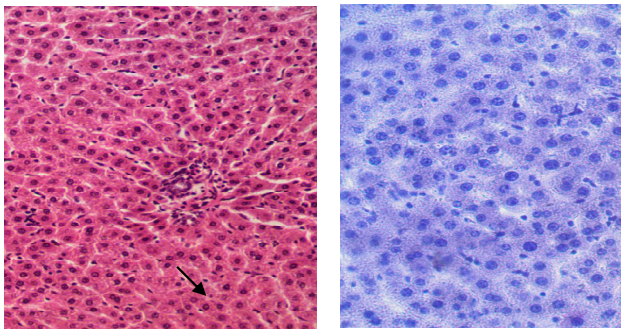
Table 2

Effect of TETVF and RP ChL on biochemical parameters in blood serum and liver homogenate of rats with EIC, $M \pm m$, $n = 6$

Research groups	IC	CP	Pathology + TETVF, 100 mg/kg	Pathology + RP ChL, 35 mg/kg
blood serum				
ALT, mmol/(hxl)	0.33 ± 0.02	1.39 ± 0.04*	1.06 ± 0.04*/**/#	0.93 ± 0.04*/**
ALP, IU d/l	231.92 ± 22.13	475.13 ± 27.09*	317.65 ± 16.84*/**	271.54 ± 24.43**
GGT, IU d/l	9.00 ± 0.11	16.71 ± 1.67*	11.83 ± 0.70*/**	10.46 ± 0.95**
liver homogenate				
TBA-AP, μmol/g	18.38 ± 1.22	44.87 ± 4.26*	30.98 ± 0.90*/**/#	26.07 ± 1.27*/**
RG, μmol/g	5.82 ± 0.51	2.19 ± 0.16*	4.11 ± 0.36*/**	4.77 ± 0.32**

*Note: * – relative to IC (Newman-Keuls test), $p < 0.05$; ** – relative to CP (Newman-Keuls test), $p < 0.05$; # – relative to RP ChL (Newman-Keuls test), $p < 0.05$; n – number of animals in the group.*

Results of histological studies. On MP the liver parenchyma of intact rats had a typical structure for this species of animals. The condition of the endothelium of the bile ducts and the epithelium of the terminal branches of blood vessels (veins, arteries) in triads, as well as the endothelium of other blood vessels, was normal. Hepatocytes had a characteristic polygonal shape; their border was quite clear. The nuclei of the cells were of regular round shape, centrally located. The heterogeneity of the size of the nuclei was within the physiological norm. The cytoplasm of hepatocytes was evenly stained, optically dense, small accumulations of fine-grained basophilic material were visible in the perinuclear zone. Mitoses were not visible in the cells. The lumen of the intralobular sinusoidal hemocapillaries was normal, as a rule, it did not contain blood, a moderate number of lymphoid cells were observed. Stellate reticuloendotheliocytes (Kupffer cells) were without features. Lipid staining was negative (Fig. 1).

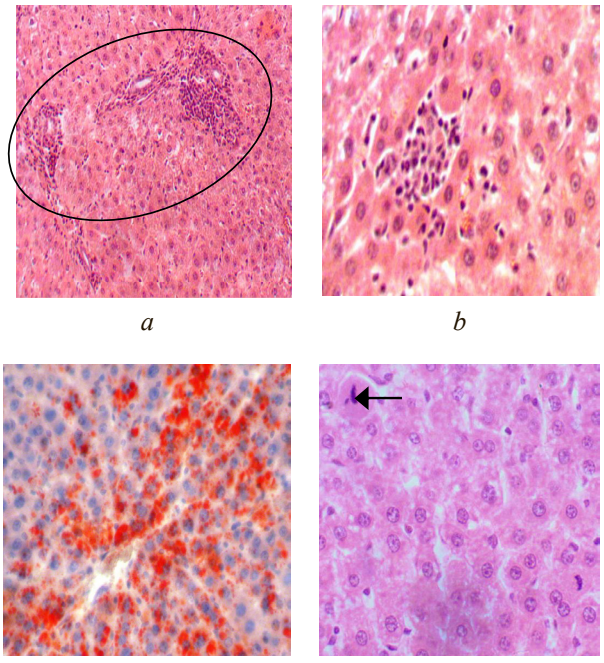


a *b*

Fig. 1. MP of the liver of an intact rat: *a* – the radial orientation of the hepatic lobules is not changed, the transverse profiles of the artery, bile duct, and vein are clearly visible in the triad (hematoxylin-eosin, × 250); *b* – the absence of fat in the cytoplasm of hepatocytes (sudan IV, × 400)

In all rats from the CP group, a pronounced round cell infiltration was observed in several triads around the bile duct (Fig. 2, *a*), small foci of necrosis of individual hepatocytes and groups of hepatocytes with infiltration by lympho-macrophage cells replacing dead cells were visible (Fig. 2, *c*). In the periportal zones, lipid accumulation was detected in the cytoplasm of hepatocytes (Fig. 2, *c*). In all rats from the CP group, the number of hepatocytes in a state of division was significantly increased, with changes indicating certain disorders (Fig. 2, *d*).

The results of semi-quantitative assessment of the severity of ductular proliferation, the intensity of periductal inflammation, and fatty degeneration of periportal hepatocytes in rats of the CP group and others are presented in Table 3.



a *b* *c* *d*

Fig. 2. MP of the liver of a rat that was administered E (5 mg/kg, subcutaneously, 5 days): *a* – round cell infiltration around the proliferating bile ducts; *b* – necrosis of a group of hepatocytes (hematoxylin-eosin, × 200); *c* – accumulation of lipids in the cytoplasm of hepatocytes (sudan IV, × 250); *d* – hepatocytes in a state of division (hematoxylin-eosin, × 200)

After therapeutic and prophylactic administration of TETVF at a dose of 100 mg/kg, no noticeable proliferation of bile ducts was detected in one rat (16.66%) (Fig. 3, *a*), the structure of the liver parenchyma corresponded to that of intact animals, and fatty dystrophy of hepatocytes was not detected (Fig. 3, *d*). In the remaining 83.33% of rats, moderate proliferation of ductules in triads occurred, as well as

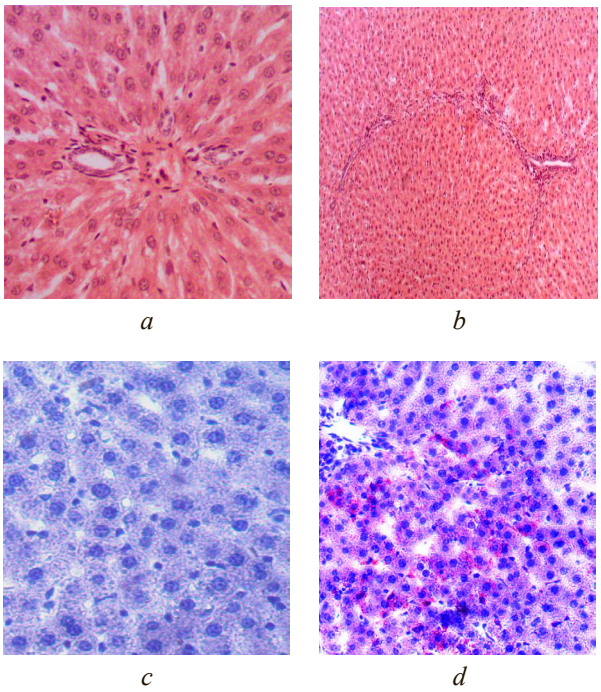
pronounced spread of bile ducts in the direction of other portal tracts (Fig. 3, *b*). Newly formed small ductules in the depth of the lobules were practically not found. The cellular inflammatory reaction was significantly reduced or absent (Fig. 3, *c*). In periportal hepatocytes, the severity of fatty dystrophy, although noticeably varied in different rats, was generally less than in CP (Fig. 3, *d*). Isolated small foci of necrosis were observed in only 33% of rats (with more prominent signs of ductular proliferation), as was an increase in hepatocytes in the division phases.

Table 3

Effect of TETVF and RP ChL on the severity of some histological signs of EIC in rats, $n = 6$

Group of animals	Signs of cholestasis (points)		
	Ductular proliferation	Periductular inflammation	Steatosis
Intact control (IC)	0 (0; 0)	0 (0; 0)	0 (0; 0)
Control pathology (CP)	3.0 (2; 3) *	2.5 (0; 3)	3.0 (2; 3) *
Pathology + TETVF	1.5 (0; 2) */**	0.66 (0; 1) **	1.16 (0; 2) **
Pathology + ChL	1.66 (0; 2) */**	1.0 (1; 1) **	0 (0; 0) **/#

*Note: Data are presented as median (Q1–Q3); statistical analysis was performed using the Kruskal–Wallis test followed by Dunn's multiple comparisons test with Bonferroni correction; * – $p < 0.05$ vs intact control (IC); ** – $p < 0.05$ vs control pathology (CP); n – number of animals in the group.*

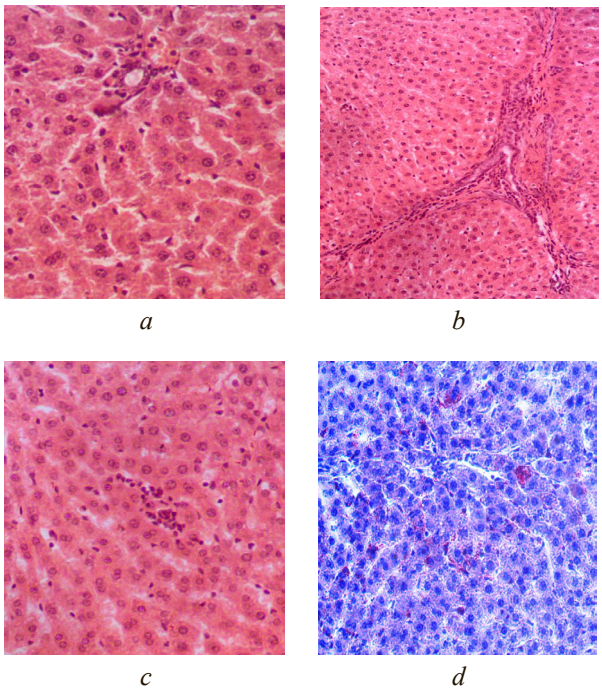


a *b* *c* *d*

Fig. 3. MP of rat liver upon administration of E (5 mg/kg, subcutaneously) and TETVF (100 mg/kg intragastrically): *a, b* – absence of proliferation of ductules and ductular epithelium in the triad (hematoxylin-eosin, × 250); *c, d* – absence of lipid droplets in the cytoplasm of hepatocytes (sudan IV, ×400)

Analysis of semi-quantitative assessment of the liver condition of rats after administration of TETVF showed that the severity of ductal proliferation significantly decreased by 1.6 times, periductal inflammation by 3.78 times, and fatty degeneration of hepatocytes of periductal zones by 2.3 times compared to animals from the CP group (Table 3).

The administration of the RP ChL generally significantly reduced both bile duct proliferation and periductal inflammatory reaction in all rats. At the same time, in some animals, against the background of a general decrease in the severity of ductular proliferation, quite noticeable growths of newly formed bile ducts in the direction of neighboring triads were observed in individual lobules (Fig. 4). Small foci of necrosis with cellular infiltration, replacing dead hepatocytes, were visualized, and the level of mitoses was reduced. When stained with sudan, lipid droplets were detected in the cytoplasm of single hepatocytes of the periportal zones, and a weak dust-like presence of fatty substances was visible in the intercellular spaces (Fig. 4, *d*).



a *b* *c* *d*

Fig. 4. MP of the rat liver upon administration of E (5 mg/kg, subcutaneously) and RP ChL (35 mg/kg intragastrically) cholelesan: *a* – absence of ductule proliferation and cellular reaction in the triad zone (hematoxylin-eosin, × 250), *b* – growth of bile ducts towards neighboring triads, moderate increase in inflammatory reaction (× 200); *c* – small foci of necrosis of several hepatocytes with cellular infiltration replacing them (hematoxylin-eosin, × 250), *d* – lipid droplets in the cytoplasm of individual hepatocytes, dust-like presence of fatty substances in the intercellular spaces (sudan IV, × 400)

In animals receiving RP ChL, the severity of ductular proliferation was significantly reduced by 1.6 times compared to CP, the inflammatory reaction was reduced by 2.5 times, and fatty infiltration of hepatocytes in problem areas was not recorded (Table 3).

5. Discussion

It is well known that E, a synthetic estrogen, induces intrahepatic cholestasis and is widely used to establish an experimental model in rodents [20]. In the liver, estrogen penetrates the plasma membrane of hepatocytes to interact with estrogen receptors (ERα and ERβ), which can suppress farnesoid X receptor expression and then regulate the expression of other genes. Estrogens increase the activity of bile acid synthase (CYP7A1 and CYP8B1) and inhibit their metabolic enzymes (CYP3A1, CYP3A11 and Sult2a1) and also contribute to the increase in bile acid content in hepatocytes. Estrogens also reduce the levels of bile transporters bile salt export pump (BSEP) and multidrug-resistance-associated protein 2 (MRP2) and inhibit Na^+-taurocholate co-transport polypeptide (NTCP) and organic anion transporter polypeptides (OATP), resulting in reduced bile flow. In addition, EIC is associated with increased levels of reactive oxygen species (ROS) and inflammatory response, which may also aggravate liver injury [21].

Elevated serum ALT, ALP, GTT, and bile acids in the presence of decreased bile volume and rate are generally considered markers of cholestasis. In this study, rats exposed to E (5 mg/kg p.c.) showed significant increases in serum ALT, ALP, GTT, and TBA-AP, which is consistent with the findings of other investigators [14]. Treatment with TETVF significantly improved the elevated biochemical parameters induced by E, suggesting that the biologically active substances (BAS) of TETVF exert a protective effect on the liver in E injury.

Many studies [9, 22] have suggested that free radicals, oxidative stress, and lipid peroxidation are involved in cholestatic liver injury. E induces changes in the balance between antioxidant and prooxidant activity and ultimately increases hepatic malondialdehyde production and decreases the activity of the hepatic antioxidant system [23]. TBA-AP are the main products of lipid peroxidation, which is considered an effective marker of oxidative stress. RG is a potent endogenous intracellular antioxidant that protects cells from oxidative stress, and its deficiency leads to the accumulation of highly reactive free radicals and subsequent degenerative changes in the organ [24]. This study showed that E administration significantly increased the content of hepatic TBA-AP and decreased the activity of hepatic RG compared with intact controls, while TETVF significantly decreased the level of hepatic TBA-AP and increased the level of RG. This suggests the potential protective effects of TETVF BAS against EIC partly due to antioxidant effects.

Recent studies on biological activity and mechanisms of plant substances flavonoids, phenols, acids, quinones, saponins, alkaloids, glycosides have shown that they function as complex regulators by improving oxidative stress, inflammation and apoptosis, restoring the balance of bile acids with hepatic transporters and correcting immune disorders. Moreover, the main targets for the activity of natural products are believed to be nuclear factor erythroid-related factor 2, reactive oxygen species production, heme oxygenase-1, NF-κB, cholesterol-7 alpha-hydroxylase, and farnesoid X receptors [9].

As is known, the hepatoprotective effect of flavonoids, in particular luteolin [24], and chlorogenic acid [25] contained in TETVF, is realized due to their antioxidant activity by inhibiting the processes of lipid peroxidation of cell membranes, preventing cytolysis and normalizing energy synthesis and energy metabolism in liver cells. Also, flavonoids of tansy flowers prevent fatty degeneration and prevent the development of inflammation, proliferation and fibrosis in liver tissues in general [9, 24].

Cholestasis is usually accompanied by rapid damage to hepatocytes, inflammation, bile duct proliferation and fibrosis. Despite oxidative stress, inflammation plays a central role in the development of cholestasis [21].

Histological study confirmed that subcutaneous administration of E (5 mg/kg) to female rats for 5 days induces a state of intrahepatic cholestasis with characteristic changes: ductular proliferation, pronounced periductal inflammation, and fatty dystrophy of periportal hepatocytes. TETVF at a dose of 100 mg/kg, administered in a prophylactic and therapeutic regimen, reduces the severity of bile duct proliferation, inflammatory infiltration, and fatty dystrophy of periductal hepatocytes. In terms of its positive effect on the state of the liver parenchyma, TETVF was not inferior to RP ChL (35 mg/kg intragastrically).

Thus, the ability of TETVF at a dose of 100 mg/kg to improve impaired liver function under EIC conditions and reduce pathological manifestations of developed intrahepatic cholestasis has been established.

Practical significance. The results obtained are a scientific justification for the possible use of drugs based on *Tanacetum vulgare* L. in estrogen-induced cholestasis.

Study limitations. The study was conducted in a single experimental model, which does not allow assessing the effectiveness of the extract in other types of cholestasis.

Prospects for further research. Considering the established protective effect of TETVF on the liver with intrahepatic cholestasis, it is advisable to further study the deeper mechanisms of this effect.

6. Conclusions

In the estrogen-induced cholestasis (EIC) model in rats, thick extract of *Tanacetum vulgare* L. flowers (TETVF) administered in a prophylactic and therapeutic regimen at a dose of 100 mg/kg contributes to a decrease in the severity of cytolytic and inflammatory processes, lipid peroxidation, an increase in the activity of the organ's antioxidant system, normalization of bile formation and the bile secretion rate.

Histologically, it has been proven that TETVF reduces the severity of bile duct proliferation, inflammatory infiltration, and fatty degeneration of periductal hepatocytes.

In terms of its positive effect on the liver condition under EIC conditions, TETVF at a dose of 100 mg/kg is generally not inferior to reference preparation (RP) cholelesan (35 mg/kg).

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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Data availability

Data will be made available on reasonable request

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies in creating the submitted work.

Authors' contributions

Oksana Mishchenko: Conceptualization, Methodology, Management, Project Administration; **Yaroslava Butko:** Writing – Review and Editing; **Oksana Tkachova:** Research, Writing; **Olena Khalieieva:** Research, Resources; **Andrii Berezniakov:** Writing – Initial Draft; **Oleksii Andriianenkov:** Formal Analysis, Visualization.

References

1. Yu, L., Liu, Y., Wang, S., Zhang, Q., Zhao, J., Zhang, H. et al. (2023). Cholestasis: exploring the triangular relationship of gut microbiota-bile acid-cholestasis and the potential probiotic strategies. Gut Microbes, 15 (1). https://doi.org/10.1080/19490976.2023.2181930

2. Kolarić, T. O., Ninčević, V., Smolić, R., Smolić, M., Wu, G. Y. (2019). Mechanisms of Hepatic Cholestatic Drug Injury. Journal of Clinical and Translational Hepatology, 7 (1), 86–92. https://doi.org/10.14218/jcth.2018.00042

3. Onofrio, F. Q., Hirschfield, G. M. (2020). The Pathophysiology of Cholestasis and Its Relevance to Clinical Practice. Clinical Liver Disease, 15 (3), 110–114. https://doi.org/10.1002/cld.894

4. Yu, L., Liu, X., Li, X., Yuan, Z., Yang, H., Zhang, L., Jiang, Z. (2016). Protective effects of SRT1720 via the HNF1α/FXR signalling pathway and anti-inflammatory mechanisms in mice with estrogen-induced cholestatic liver injury. Toxicology Letters, 264, 1–11. https://doi.org/10.1016/j.toxlet.2016.10.016

5. Chen, C., Gong, X., Yang, X., Shang, X., Du, Q., Liao, Q. et al. (2019). The roles of estrogen and estrogen receptors in gastrointestinal disease (Review). Oncology Letters, 18 (6), 5673–5680. https://doi.org/10.3892/ol.2019.10983

6. Smith, D. D., Rood, K. M. (2020). Intrahepatic Cholestasis of Pregnancy. Clinical Obstetrics & Gynecology, 63 (1), 134–151. https://doi.org/10.1097/grf.0000000000000495

7. Mutlu, M. F., Aslan, K., Guler, I., Mutlu, I., Erdem, M., Bozkurt, N., Erdem, A. (2017). Two cases of first onset intrahepatic cholestasis of pregnancy associated with moderate ovarian hyperstimulation syndrome after IVF treatment and review of the literature. Journal of Obstetrics and Gynaecology, 37 (5), 547–549. https://doi.org/10.1080/01443615.2017.1286302

8. Mor, M., Shmueli, A., Krispin, E., Bardin, R., Sneh-Arbib, O., Braun, M. et al. (2020). Intrahepatic cholestasis of pregnancy as a risk factor for preeclampsia. Archives of Gynecology and Obstetrics, 301 (3), 655–664. https://doi.org/10.1007/s00404-020-05456-y

9. Ma, X., Jiang, Y., Zhang, W., Wang, J., Wang, R., Wang, L. et al. (2020). Natural products for the prevention and treatment of cholestasis: A review. Phytotherapy Research, 34 (6), 1291–1309. https://doi.org/10.1002/ptr.6621

10. Wang, R., Cheng, N., Peng, R., Yu, Z., Nan, M., Cao, H. (2020). Oral herbal medicine for women with intrahepatic cholestasis in pregnancy: a systematic review of randomized controlled trials. BMC Complementary Medicine and Therapies, 20 (1). https://doi.org/10.1186/s12906-020-03097-x

11. Mishchenko, O. Ya., Hontova, T. M., Yurchenko, K. Yu., Zolotaikina, M. Yu., Herbina, N. A., Ruban, O. A., Kalko, K. O. (2020). Pat. 139684 UA. Analhetychnyi Zasib. MKP (2006) A61K 36/00, A61K 9/02 (2006.01) A61R 29/00. No. u201907609; declareted: 08.07.2019; published:10.01.2020, Bul. No. 1/2020.

12. Herbina, N. A., Ruban, O. A., Gontova, T. M., Yaremenko, M. S., Yurchenko, C. Yu. (2021). The study of the qualitative and quantitative content of the amount of flavonoids and hydroxycinnamic acids in a dense extract of common tansy flowers. News of Pharmacy, 2 (102), 8–13. https://doi.org/10.24959/nphj.21.55

13. Yurchenko, C., Mishchenko, O., Herbina, N., Kapustyanskiy, I. (2021). Screening study of the choleretic and cholekinetic effects of the the common tansy (*Tanacetum vulgare* L.) flowers thick extract. Pharmacology and Drug Toxicology, 15 (3), 197–203. https://doi.org/10.33250/15.03.197

14. Xu, Y.-J., Yu, Z.-Q., Zhang, C.-L., Li, X.-P., Feng, C.-Y., Lei, K. et al. (2025). CORRIGENDUM: Protective Effects of Ginsenosides on 17α-Ethynyelstradiol-Induced Intrahepatic Cholestasis via Anti-Oxidative and Anti-Inflammatory Mechanisms in Rats. The American Journal of Chinese Medicine, 53 (8), 2603–2604. https://doi.org/10.1142/s0192415x2592003x

15. Kholelesan® kapsuly blister No. 30. Compendium. Available at: https://compendium.com.ua/dec/338632/446070/

16. Drohovoz, S. M., Hubskyi, Yu. I., Skakun, M. P., Kovalenko, V. M., Derymedvid, L. V. (2001). Eksperymentalne vyvchennia zhovchohinnoi, kholespazmolitychnoi, kholelitiaznoi ta hepatoprotektornoi aktyvnosti novykh likarskykh zasobiv. Doklinichne doslidzhennia likarskykh zasobiv. Kyiv, 334–351.

17. European convention for the protection of vertebrate animals used for the experimental and other scientific purposes: European Treaty Series No. 123: Text amended according to the provisions of the Protocol (ETS No. 170), as of its entry into force, on 2 December 2005 (1986). Strasbourg, 48.

18. Beutler, E., Duron, O., Kelly, B. M. (1963). Improved method for the determination of blood glutathione. Journal of Laboratory and Clinical Medicine, 61, 882–888.

19. Golovanova, I. A., Belikova, I. V., Lyakhova, N. O. (2017). Osnovy medychnoi statystyky. Poltava, 113.

20. Crocenzi, F. A., Sánchez Pozzi, E. J., Pellegrino, J. M., Favre, C. O., Rodríguez Garay, E. A., Mottino, A. D. et al. (2001). Beneficial Effects of Silymarin on Estrogen–Induced Cholestasis in the Rat: A Study In Vivo And in Isolated Hepatocyte Couplets. Hepatology, 34 (2), 329–339. https://doi.org/10.1053/jhep.2001.26520

21. Zu, Y., Yang, J., Zhang, C., Liu, D. (2021). The Pathological Mechanisms of Estrogen-Induced Cholestasis: Current Perspectives. Frontiers in Pharmacology, 12. https://doi.org/10.3389/fphar.2021.761255

22. Kawamura, K., Kobayashi, Y., Kageyama, F., Kawasaki, T., Nagasawa, M., Toyokuni, S. et al. (2000). Enhanced Hepatic Lipid Peroxidation in Patients With Primary Biliary Cirrhosis. American Journal of Gastroenterology, 95 (12), 3596–3601. https://doi.org/10.1111/j.1572-0241.2000.03300.x

23. Hussein, M. A. (2013). Prophylactic Effect of Resveratrol Against Ethinylestradiol-Induced Liver Cholestasis. Journal of Medicinal Food, 16 (3), 246–254. https://doi.org/10.1089/jmf.2012.0183

24. Mureşana, M., Benedeca, D., Vlasea, L., Opreana, R., Toiua, A., Onigaa, I. (2015). Screening of polyphenolic compounds, antioxidant and antimicrobial properties of *Tanacetum vulgare* from Transylvania. Studia Universitatis Babes-Bolyai Chemia, 60 (1), 127–138.

25. Mishchenko, O., Kyrychenko, I., Gontova, T., Kalko, K., Hordiei, K. (2022). Research on the phenolic profile, antiradical and anti-inflammatory activity of a thick hydroalcoholic feverfew (Tanacetum parthenium L.) herb extract. ScienceRise: Pharmaceutical Science, 5 (39), 91–99. https://doi.org/10.15587/2519-4852.2022.266400

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