



Milk phospholipids for correcting bile acid composition in rats with experimental fatty hepatitis

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Abstract. The unique functions of the liver require detailed study, since hepatopathology is a serious veterinary problem, which also negatively affects the productivity of farm animals. It is possible to clarify the pathogenetic mechanisms and its development using artificial modeling of hepatopathology and determine the therapeutic efficacy of hepatoprotective drugs, especially based on raw materials of animal origin. The aim of the study was to determine the effect of the use of milk phospholipids in the composition of the bioadditive “FLP-MD” on the secretion of bile acids by the liver in artificial modeling of fatty hepatitis in rats. To reproduce hepatopathology, laboratory rats were administered intragastrically with a 4% solution of tetracycline hydrochloride at a dose of 0.25 g/kg of body weight for seven days and the bioadditive was used at a dose of 13.5 mg/kg of body weight for nine days. Bile samples were collected from rats by conducting acute experiments. Six fractions of conjugated bile acids were determined in bile samples by thin-layer chromatography. It was found that in laboratory rats with experimental fatty hepatitis, the processes of biotransformation of primary and secondary cholates by conjugation with taurine were inhibited. In particular, a decrease in the concentration of taurocholic acid in the bile of sick animals by 20.5%-38.1% ($P < 0.01$), and of the complex of taurochenodeoxycholic and taurodeoxycholic acids by 21.8%-25.7% ($P < 0.05$) was recorded. In the case of using the bioadditive “FLP-MD” in rats with experimental fatty hepatitis, the concentration of taurocholic, taurochenodeoxycholic and taurodeoxycholic acids in bile significantly increased. The concentration of glycoconjugated bile acids and free cholates corresponded to their level in the control. The use of the bioadditive “FLP-MD” based on milk phospholipids in experimental fatty hepatitis eliminated the negative impact of the antibiotic in a toxic dose on the processes of biotransformation and the formation of cholates. This allows to recommend the bioadditive “FLP-MD” based on milk phospholipids as a hepatoprotective agent in the case of the use of antimicrobial drugs in animals

Keywords: bile acids; cholesterol; bioadditive “FLP-MD”; tetracycline hydrochloride; hepatocyte; hepatopathology

Introduction

One of the unique metabolic functions of hepatocytes is the conversion of cholesterol to the bile acids. Concentration changes of various cholates in the bile reflect the path of synthesis and biotransformation reactions and transport processes in the liver parenchyma. Bile acids are synthesised from cholesterol with the participation of enzymes specific exclusively for hepatocytes. Considering the importance of cholates in the liver as a unique site of their synthesis and biotransformation, it is important to investigate the effects of various factors, including pathological ones, on the functional

state of the liver and on the transformation and transport of bile acids.

A. Allameh *et al.* (2023) and T. Nakamura *et al.* (2025) emphasised that the liver is damaged by many factors and, at the same time, numerous processes in hepatocytes are disrupted. I. Lupaina *et al.* (2021) and C. Silva *et al.* (2023) demonstrated that under the influence of various endogenous and exogenous substances, the cholate synthesising function of hepatocytes can be both stimulated and inhibited. In particular, the known property of antibiotics to cause serious disorders

of liver function can lead to changes in the synthesis, biotransformation and transport of bile acids. For example, doxycycline in excessive doses exhibits prooxidant properties and promotes the accumulation in tissues of toxic metabolites: aldehydes, ketones, hydroperoxides, resulting in increased lipid peroxidation, changes in the structure and permeability of cell membranes, impaired metabolic processes and developed cholestasis and inflammation of the liver (Varma *et al.*, 2021; Nikolajevic *et al.*, 2024). A.M. Liashevych *et al.* (2021) showed that during modeling of doxycycline-induced hypercholesterolemia and stress-induced hypercholesterolemia, the bile flow of conjugated bile acids is inhibited. C. Wei *et al.* (2022) indicated that the antibiotic tetracycline effects on the liver are hepatocellular injury, cytolysis, cholestatic injury, liver failure. D. Wupperfeld *et al.* (2022) showed that the antibiotic tetracycline inhibits succinate dehydrogenase, cytochrome oxidase, alkaline phosphatase, arginase, reduces the level of aerobic respiration and associated oxidative phosphorylation, disrupts the metabolism of proteins, fats, carbohydrates, pigments, and causes an increase in peroxide oxidation of lipids and causes destructive changes in hepatocyte membranes. As K. Taylor *et al.* (2025) in particular pointed out, the “targets” of the damaging effect of antibiotics on liver cells have been largely identified, and the use of antibiotics to model experimental liver damage has become widespread in research practice. Artificial pathology modeling is used to experimentally study the effectiveness of various drugs. V. Tiwari *et al.* (2025) noted that the study of the effects of tetracycline and its derivatives is due to their widespread use in medical practice and animal husbandry. Taking into account possibility of side effects, it is important to develop and study drugs with hepatoprotective properties. It is known that

phospholipids have hepatoprotective effects across many different liver diseases. In particular, essential phospholipids are used for the supportive treatment of non-alcoholic fatty liver disease as biologically active substances that improve the fluidity of hepatocyte membranes and transmembrane transport (Wupperfeld *et al.*, 2024).

The liver's unique ability to synthesise bile acids from cholesterol, which consists of many energy-dependent enzymatic reactions in hepatocytes, can be significantly impaired under the influence of tetracycline. Disturbances of lipid metabolism and changes in the regulation of liver cell function by bile acids in conditions of simulated fatty hepatitis remain insufficiently studied. Given the need for long-term use of tetracycline in medical practice and possible side effects of the antibiotic on the liver, the search for effective hepatoprotectors is urgent. The aim of the study was to study the effect of the dietary supplement “FLP-MD”, which contains milk phospholipids, unsaturated fatty acids and antioxidants, on the synthesis and biotransformation of cholates and their concentration in the bile of rats with fatty hepatitis, modeled using tetracycline hydrochloride.

Literature Review

The synthesis of bile acids from cholesterol and the transmembrane transport of cholates to the primary bile tubules are energy-consuming processes (Boyer & Soroka, 2021). As noted by V.M.P. de Bruijn *et al.* (2022) the body does not lose a significant amount of bile acids after its participating in digestion in the intestine. Bile acids biotransformed by the intestinal microbiota return to the liver with blood via the portal vein. This phenomenon is named bile acid enterohepatic circulation. Cholates, processed by the enzymes of the intestinal microbiome, are called secondary bile

acids. Enterohepatic circulation of bile acids occurs due to the coordinated functioning of the transport systems of the membranes of enterocytes and hepatocytes. In liver cells, secondary bile acids are biotransformed into primary ones, which most effectively perform the digestive function in the intestine.

Bile acids are essential components of bile necessary for digestion. They emulsify dietary fats, activate digestive enzymes, and affect the intestinal microbiota. It should be noted that bile acids are not only a necessary component of bile as a digestive secretion. They perform the functions of regulatory compounds, realising their effects through the corresponding intracellular nuclear receptors – farnesoid X receptor (FXR) (Qi *et al.*, 2024) and G-protein bound membrane receptors (Fleishman & Kumar, 2024; Jia *et al.*, 2024). The intestinal and hepatic transport systems regulated by farnesoid X receptors ensure the efficiency of enterohepatic circulation of cholates and lead to a negative feedback loop between the return of secondary bile acids to hepatocytes and the intensity of de novo bile acid synthesis. Activation of hepatocyte FXR by bile acids leads to the suppression of the expression of enzymes for primary cholate synthesis. F. Stellaard & D. Lütjohann (2021) found that when the flow of bile acids from the intestine decreases, the inhibition of synthesis is abolished and the processes of synthesis of primary bile acids from cholesterol are intensified in hepatocytes.

Changes in the content of bile acids in the internal environment of the body are primarily caused by changes in their synthesis, bile formation, and bile excretion. The synthesis of bile acids from cholesterol is a multi-step enzymatic process. Bile contains various bile acids that have different physicochemical properties, exert different detergent effects on biomembranes, and change the properties of bile as a digestive secret. S. Choudhuri & C.D. Klaassen (2022)

noted that hydrophobic bile acids more effectively increase cholesterol absorption compared to more hydrophilic bile acids in the digestive tract. C.D. Fuchs *et al.* (2025) emphasised that trihydroxycholanic bile acids, such as cholate and its tauroconjugates and glycoconjugates, effectively regulate lipid metabolism in the body, as well as regulate the enterohepatic circulation of cholates. One of the important stages of the metabolic transformations of bile acids in hepatocytes is their conjugation with taurine or glycine. Y. Fu *et al.* (2025) found that conjugation of bile acids with taurine or glycine and changes in the ratio of different conjugated and free bile acids significantly affect the course of inflammatory processes in the gastrointestinal tract. At the same time, disorders of metabolic transformations of cholate cause cholestatic lesions of the liver and its fatty degeneration and significantly affect the metabolism in the body as a whole (Nimer *et al.*, 2021; Farooqui *et al.*, 2022).

The conversion of cholesterol to bile acids, which occurs in hepatocytes, requires the activity of many hepatospecific enzymes. The conversion of cholesterol into cholate is the most important mechanism for maintaining cholesterol homeostasis, and thus the prevention of many diseases associated with metabolic disorders and the accumulation of cholesterol in the body. J. Guo *et al.* (2024) indicated that bile acids, as well as intermediates of their biosynthesis, regulate the expression of genes whose products provide cholesterol homeostasis. In summary, bile acids play a central role in the digestion and absorption of dietary lipids in animals, while also participating in regulatory mechanisms that influence hepatic metabolism, intestinal immunity, and cholesterol balance. Alterations in bile acid synthesis or enterohepatic circulation may contribute to metabolic disturbances and hepatobiliary pathologies in veterinary species.

Materials and Methods

Research was carried out in scientific laboratories of National University of Life and Environmental Sciences of Ukraine and Taras Shevchenko National University of Kyiv. Experiments and biochemical analysis of the obtained biological fluids were carried out during 2023-2024. Studies were performed on white laboratory male rats with body weight 200 ± 50 g ($n = 13$), kept in the vivarium at room temperature $22-24^{\circ}\text{C}$ with 14-hour light period and standard diet and free water access. All work with animals was carried out based on the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and Scientific Purposes (1986), the Law of Ukraine No. 3447 (2006), which was confirmed by the conclusion of the Bioethics Commission of the National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine (Protocol No. 002/2023 of October 26, 2023).

The experiment included the following stages:

- 1) a control group of animals received water intragastrically for five days (control group);
- 2) the first experimental group of animals received intragastrically a 4% solution of tetracycline hydrochloride at a dose of 0.25 g/kg of body weight for seven days (self-rehabilitation group);
- 3) the second experimental group of animals received the milk phospholipid-containing bioadditive (Ukraine, author's development) daily intragastrically one hour before the introduction of tetracycline hydrochloride and for the next two days after the antibiotic administration (treatment group);
- 4) a day after the completion of intragastric administration of substances, an acute experiment was performed with cannulation of the bile duct to obtain bile;
- 5) biochemical analysis: in bile, the content of conjugated and free bile acids was determined by thin-layer chromatography;

6) a statistical analysis of the obtained results of measurements of the content of bile acids in animals of all experimental groups.

Liver damage in the form of fatty hepatitis was simulated by administering to rats a 4% solution of tetracycline hydrochloride intragastrically at a dose of 0.25 g/kg of body weight of the animal for seven days (Korolova *et al.*, 2023). The bioadditive made from milk phospholipids was administered daily to experimental animals one hour before tetracycline hydrochloride and for the next two days after the discontinuation of the antibiotic to observe cholate productive function of the liver. The daily oral dose of the bioadditive "FLP-MD" was 13.5 mg/kg of body weight. The bioadditive "FLP-MD" is an authors' development (Melnychuk *et al.*, 2009). The composition of this bioadditive includes the following components: milk phospholipids with a set of fatty acids typical of cell membranes, a mixture of unsaturated fatty acids (oleic, linoleic, linolenic) extracted from linseed oil, and vitamins A and E as antioxidants.

The day before the start of the acute experiment with cannulation of the common bile duct, all animals were weighed and placed in a separate cage without access to food and with free consumption of water. The rats were then anesthetised with sodium thiopental ($7 \mu\text{g}/100$ g body weight, intraperitoneally). Immobilised animals underwent laparotomy and cannulation of the bile duct using a plastic cannula connected to a micropipette to bile collecting. Bile samples were collected every 30 min for 3 h in an acute experiment. Each bile sample was analysed for the content of bile acids of six different fractions.

Determination of bile cholates was performed by a modified method of thin layer chromatography (Melnychuk *et al.*, 2009). Before extraction of cholate from the collected samples of liver secretion, to 1.9 cm^3 of a cold mixture of ethanol-acetone (1 : 3) was added

0.1 cm³ of bile. Subsequently, the resulting mixture was kept at a temperature from -10°C to 0°C in a chamber for 30 min and then centrifuged for 10 min at 3,000-4,000 rpm. The obtained extracts were transferred into conical glass tubes and dried. The dry residue was dissolved in 50-100 µL of a mixture of ethanol water (6:4). Each sample in a volume of 5-10 µL was applied to chromatographic plates coated with Silicagel (Kavalier, Czech Republic). Free and conjugated bile acids were separated in a system containing amyl ester of acetic acid, toluene, butanol, acetic acid and water (3:1:1:3:1, respectively) in glass chromatographic chambers. Staining of the chromatograms was performed with a dye of the following composition: 15 cm³ of glacial acetic acid, 1 g of phosphomolybdic acid, 1 cm³ of sulfuric acid and 5 cm³ of a 50% trichloroacetic acid solution. After staining, the chromatogram was dried at 60-70°C for 5 minutes. Quantitative determination of bile acid content was performed using a GP-920 densitometer (Shimadzu, Japan) at λ 620 nm. Modification of the chromatographic method contributed to the determination of six fractions of conjugated bile acids, in particular: taurocholic, taurochenodeoxycholic and taurodeoxycholic (mixture), glycocholic, glycochenodeoxycholic and glycodeoxycholic (mixed) and free bile acids: cholic, chenodeoxycholic and deoxycholic (mixture) acids (Veselsky *et al.*, 2001).

The experimental data were processed by variation statistics methods using OriginPro 2018 (OriginLab Corporation, USA) and STATISTICA 7.0 (Stat Soft, USA) software packages. The obtained and digitised values of the research results indicators were checked for normality of the distribution of the general populations using the Shapiro-Wilk test. Significant differences between sample means were examined by parametric analysis of variance (ANOVA). Levels of variance were assessed using Levene's test. Post-test comparisons were performed using

the Bonferroni test. The Kruskal-Wallis nonparametric ANOVA was used to determine reliable differences between the parameters of the distributions of the samplings, if non-equal variances were estimated with Levene's test. In all cases the results were considered reliable on condition of the probability value P under 5% ($P < 0.05$) or 1% ($P < 0.01$) (Deo & Ranganathan, 2024). The results were presented as the arithmetic mean \pm standard deviation, n – number of experiments.

Results and Discussion

Effect of the milk phospholipid-containing bioadditive on the secretion of bile acid tauroconjugates under the conditions of experimental fatty hepatitis in rats

The influence of phospholipid-containing bioadditive components on the secretion of tauroconjugates of bile acids in the conditions of experimental fatty hepatitis in rats was investigated. It was found that, in the conditions of simulated tetracycline hydrochloride liver damage, the concentration of taurocholic, and taurochenodeoxycholic and taurodeoxycholic acids in the bile of rats is significantly reduced. The content of taurocholate in the hepatic secretion was lower by 20.5%-38.1% ($P < 0.01$) than the control values, and that of taurochenodeoxycholic and taurodeoxycholic acids was lower by 21.8%-25.7% ($P < 0.05$) (Table 1).

Thus, under conditions of tetracycline hydrochloride usage, the processes of biotransformation of bile acids (hydroxylation and conjugation of bile acids with taurine) are suppressed. When rats were administered the phospholipid-containing dietary supplement "FLP-MD" while taking tetracycline hydrochloride, the effect of the antibiotic on the processes of biotransformation and the formation of bile acids was significantly reduced. This is evidenced by the absence of statistically significant differences in the concentration of taurocholate in the first bile sample from the rats treated with

tetracycline hydrochloride and the bioadditive (treatment group) compared to the control, and a statistically significant increasing of the taurocholate concentration in other samples of bile obtained from rats in the treatment group (tetracycline hydrochloride + bioadditive “FLP-MD”). The concentration of taurocholic acid in bile samples from the rats in the

treatment group was even more significantly higher compared to the self-rehabilitation group. The use of the phospholipid-containing dietary supplement in animals with simulated fatty hepatitis led to an increase in the concentration of taurocholic acid in bile by 28.7%-86.3% compared to the indicators of animals in the self-rehabilitation group.

Table 1. Concentration (mg/100 mL) of bile acid tauroconjugates in the bile of rats during self-rehabilitation and administration of the phospholipid-containing bioadditive “FLP-MD” against the background of tetracycline-induced fatty hepatitis (n = 13), M ± SD

No. samples	Experiment time, min	Group of animals	Bile acid	
			taurocholic	taurochenodeoxycholic and taurodeoxycholic
1	30	Control	219.5 ± 15.9	125.4 ± 18.3
		Self-rehabilitation	174.1 ± 8.1**	90.7 ± 9.2*
		Treatment	224.78 ± 15.3	121.7 ± 6.1
2	60	Control	212.2 ± 1.3	113.4 ± 19.03
		Self-rehabilitation	168.75 ± 9.8*	88.7 ± 9.9
		Treatment	257.6 ± 4.8*	136.7 ± 6.6
3	90	Control	207.2 ± 23.2	108.4 ± 19.1
		Self-rehabilitation	160.2 ± 12.05*	83.1 ± 8.2
		Treatment	250.4 ± 12.0**	115.2 ± 14.3
4	120	Control	201.6 ± 23.0	103.5 ± 18.1
		Self-rehabilitation	152.8 ± 11.0**	78.1 ± 6.9*
		Treatment	258.5 ± 12.7*	126.9 ± 10.6
5	150	Control	197.1 ± 24.3	98.1 ± 16.7
		Self-rehabilitation	138.4 ± 6.4**	74.1 ± 5.7*
		Treatment	238.5 ± 7.4**	112.1 ± 9.9
6	180	Control	189.0 ± 21.7	91.8 ± 13.2
		Self-rehabilitation	128.4 ± 6.8**	68.2 ± 3.7*
		Treatment	239.2 ± 3.4**	112.7 ± 4.2*

Note: * $P < 0.05$; ** $P < 0.01$, statistically significant differences compared to control

Source: authors' development

The concentration of taurochenodeoxycholate and taurodeoxycholate in the bile of the rats with fatty hepatitis treated with the phospholipid-containing bioadditive was similar to control concentration value in the first – fifth samples. The concentration of taurochenodeoxycholate and taurodeoxycholate in the sixth sample of the bile of the treated rats was higher by 22.8% ($P < 0.05$) than the control values. The concentration of taurochenodeoxycholate

and taurodeoxycholate in bile samples from the rats in the treatment group was significantly higher compared to the self-rehabilitation group. The use of the phospholipid-containing dietary supplement in animals with simulated fatty hepatitis provided an increase in the concentration of taurochenodeoxycholate and taurodeoxycholate in bile samples by 37.5%-65.2% compared to the values in bile samples from the rats in the self-rehabilitation group (Table 1).

The described multidirectional changes in the content of bile acid tauroconjugates in the bile of rats during self-rehabilitation and administration of the phospholipid-containing bioadditive “FLP-MD” against the background of tetracycline-induced fatty hepatitis led to differences in their ratio in the hepatic secret obtained during the first thirty minutes of the acute experiment (Fig. 1). In the first 30-minute sample of bile obtained from animals of the control group, the proportion of taurocholic acid is 39%, taurochenodeoxycholic and taurodeoxycholic acids – 22%. During the tetracycline hydrochloride usage, the proportion of taurocholate decreased to 37%, and dihydroxycholane tauroconjugates decreased to 19% (Fig. 2).

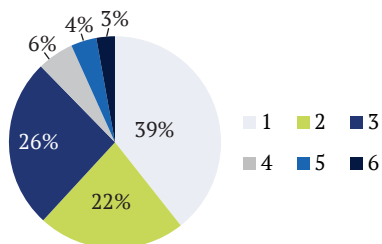


Figure 1. The ratio of 6 different fractions of bile acids in the first 30-minute sample of bile from the rats of the control group

Note: 1 – taurocholic, 2 – taurochenodeoxycholic and taurodeoxycholic (mixture), 3 – glycocholic, 4 – glycochenodeoxycholic and glycodeoxycholic (mixture), 5 – cholic, 6 – chenodeoxycholic and deoxycholic (mixture) acids

Source: authors’ development

In the first 30-minute bile sample from the rats treated with the phospholipid-containing bioadditive “FLP-MD” in addition to tetracycline hydrochloride, the ratio of tauroconjugates of bile acids was changed significantly. Namely, the part of taurocholate tauroconjugates was 42%, while that of dihydroxycholane tauroconjugates was 23%. Note that in the control, the proportion of all bile acids conjugated with taurine was 61%. In the self-rehabilitation

group of samples the percentage of bile acids conjugated with taurine was 56%. The use of the dietary supplement caused an increase in the proportion of taurocholates to 65% (Fig. 3).

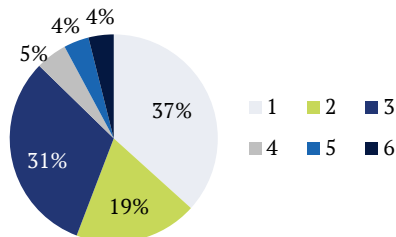


Figure 2. The ratio of 6 different fractions of bile acids in the first 30-minute sample of rat bile under the action of tetracycline hydrochloride

Note: 1 – taurocholic, 2 – taurochenodeoxycholic and taurodeoxycholic (mixture), 3 – glycocholic, 4 – glycochenodeoxycholic and glycodeoxycholic (mixture), 5 – cholic, 6 – chenodeoxycholic and deoxycholic (mixture) acids

Source: authors’ development

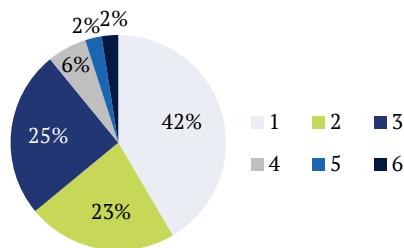


Figure 3. The ratio of 6 different fractions of bile acids in the first 30-minute sample of bile from the rats with the introduction of the phospholipid-containing bioadditive “FLP-MD” against the background of tetracycline-induced hepatitis

Note: 1 – taurocholic, 2 – taurochenodeoxycholic and taurodeoxycholic (mixture), 3 – glycocholic, 4 – glycochenodeoxycholic and glycodeoxycholic (mixture), 5 – cholic, 6 – chenodeoxycholic and deoxycholic (mixture) acids

Source: authors’ development

Thus, the phospholipid-containing dietary supplement under conditions of experimental

tetracycline-induced fatty hepatitis contributed to the enhancement of the processes of conjugation of free bile acids with taurine. T. Miyazaki *et al.* (2023) in their studies on mice confirmed that both the synthesis of taurine and the pathways of amidation of free bile acids with taurine are regulated by the activation of intracellular nuclear receptors (FXR) sensitive to bile acids. In view of this, it could be assumed that factors that change the metabolism of bile acids in hepatocytes affect the further transformations of bile acids through their interaction with cholates. The described differences in the ratio of different fractions of tauroconjugated cholates persist throughout the experiment. Thus, in the control group in the last sample of bile, taurocholic acid was 42%, taurochenodeoxycholic and taurodeoxycholic acids were 21%. That is, in the sixth bile sample from rats in the control group, the total proportion of bile acids conjugated with taurine was 63%. Thus, in control, tauroconjugates were the predominant fraction of bile acids in rat bile (Fig. 4).

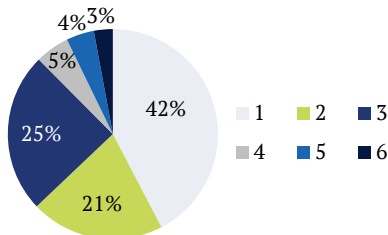


Figure 4. The ratio of 6 different fractions of bile acids in the last (180-minute) sample of bile from the rats of the control group

Note: 1 – taurocholic, 2 – taurochenodeoxycholic and taurodeoxycholic (mixture), 3 – glycocholic, 4 – glycochenodeoxycholic and glycodeoxycholic (mixture), 5 – cholic, 6 – chenodeoxycholic and deoxycholic (mixture) acids

Source: authors' development

In the last sixth sample of bile from the rats with tetracycline-induced hepatitis, the proportion of taurocholate was 34%, while that of

taurochenodeoxycholate and taurodeoxycholate was 18%. As was observed, the proportion of taurocholic, and taurochenodeoxycholic and taurodeoxycholic acids in the last bile sample collected in the acute experiment from rats in the self-rehabilitation group was lower compared to the control. During the acute experiment, due to the interruption of the enterohepatic circulation of bile acids, the concentration of bile acids in bile was expected to decrease. In the conditions of the acute experiment with cannulation of the bile duct, hepatocytes needed to intensify the synthesis of bile acids to maintain their proper concentration in bile. It is noteworthy that in rats with tetracycline-induced hepatitis, the processes of synthesis of bile acid tauroconjugates were suppressed. The percentage of bile acid tauroconjugates in the sixth bile sample from the rats with tetracycline-induced hepatitis was 52% (Fig. 5).

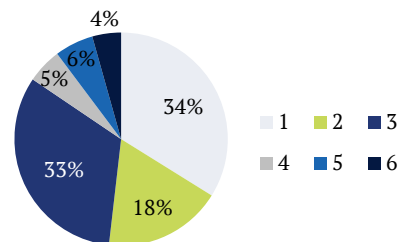


Figure 5. The ratio of 6 different fractions of bile acids in the last 180-minute sample of rat bile under the action of tetracycline hydrochloride

Note: 1 – taurocholic, 2 – taurochenodeoxycholic and taurodeoxycholic (mixture), 3 – glycocholic, 4 – glycochenodeoxycholic and glycodeoxycholic (mixture), 5 – cholic, 6 – chenodeoxycholic and deoxycholic (mixture) acids

Source: authors' development

Administration of the bioadditive based on milk phospholipids to animals with tetracycline-induced fatty hepatitis showed the effect of “drug protection” against the background of antibiotic usage, which was manifested in

maintaining the ratio of different fractions of free and conjugated bile acids. As shown in Figure 6, the proportion of taurocholate was 45%, while that of taurochenodeoxycholate and taurodeoxycholate was 21%. Thus, the percentage of taurocholate even exceeded the control figure, which was 42%. The total proportion of taurine-conjugated bile acids in the bile of the animals with fatty hepatitis that received the bioadditive was 66%.

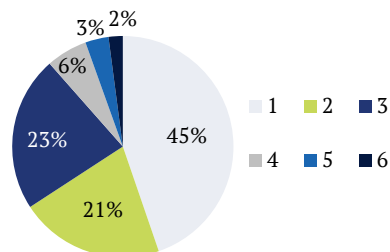


Figure 6. The ratio of 6 different fractions of bile acids in the last 180-minute sample of bile from the rats with the introduction of the phospholipid-containing bioadditive “FLP-MD” against the background of tetracycline-induced hepatitis

Note: 1 – taurocholic, 2 – taurochenodeoxycholic and taurodeoxycholic (mixture), 3 – glycocholic, 4 – glycochenodeoxycholic and glycodeoxycholic (mixture), 5 – cholic, 6 – chenodeoxycholic and deoxycholic (mixture) acids

Source: authors’ development

As was found in the study, in conditions of simulated fatty hepatitis, the mechanisms

of synthesis of bile acid tauroconjugates were suppressed. A particularly significant decrease in the fraction of tauroconjugates was detected in the last sixth sample of bile from animals in the self-rehabilitation group. The use of the dietary supplement led to an increase in the percentage of tauroconjugates in the bile of the rats in the treatment group. The percentage of taurocholic acid in the bile of the rats in the treatment group even exceeded the proportion of taurocholate in control samples. Thus, the effect of the phospholipid-containing bioadditive was to stimulate the secretion of tauroconjugates of bile acids into bile in animals with experimental fatty hepatitis.

Effect of the milk phospholipid-containing bioadditive on the secretion of bile acid glycoconjugates under the conditions of experimental fatty hepatitis in rats

Bioadditive influenced the secretion of glycoconjugates of chenodeoxycholic and deoxycholic bile acids in the conditions of experimental fatty hepatitis in rats. At the same time, the content of glycocholic acid in the bile of the animals with tetracycline-induced fatty hepatitis did not show significant changes compared to the control. Note that in the bile samples from the rats, which were administered the phospholipid-containing bioadditive “FLP-MD” and tetracycline hydrochloride, the concentration of glycocholates exceeded that in the control (Table 2).

Table 2. Concentration (mg/100 mL) of bile acid glycoconjugates in the bile of rats during self-rehabilitation and administration of the phospholipid-containing bioadditive against the background of tetracycline-induced fatty hepatitis (n = 13), M ± SD

No. samples	Experiment time, min	Group of animals	Bile acid	
			glycocholic	glycochenodeoxycholic and glycodeoxycholic
1	30	Control	144.2 ± 7.1	31.1 ± 3.4
		Self-rehabilitation	149.4 ± 13.1	23.1 ± 3.3*
		Treatment	135.7 ± 12.4	32.2 ± 3.7
2	60	Control	135.65 ± 11.3	27.4 ± 3.2
		Self-rehabilitation	146.7 ± 16.9	22.4 ± 3.1
		Treatment	140.8 ± 16.3	33.8 ± 2.8

Table 2. Continued

No. samples	Experiment time, min	Group of animals	Bile acid	
			glycocholic	glycochenodeoxycholic and glycodeoxycholic
3	90	Control	134.3±3.9	27.4±3.0
		Self-rehabilitation	142.6±16.6	21.8±0.7
		Treatment	140.6±12.2	34.8±5.2*
4	120	Control	128.0±4.4	25.7±3.04
		Self-rehabilitation	137.5±16.4	21.1±1.9
		Treatment	134.1±13.2	30.4±2.7
5	150	Control	121.3±3.5	24.9±2.4
		Self-rehabilitation	133.4±15.1	20.3±1.6
		Treatment	127.7±13.9	34.0±6.1*
6	180	Control	110.0±5.3	23.4±2.0
		Self-rehabilitation	123.7±15.9	20.1±1.9
		Treatment	121.3±9.2	32.2±6.6*

Note: * $P < 0.05$; ** $P < 0.01$, statistically significant differences compared to control

Source: authors' development

In the first 30-minute sample of bile obtained from animals of the control group, the proportion of glycocholic acid was 26% (Fig. 1) and during tetracycline hydrochloride usage the part of glycocholate increased to 31% (Fig. 2). In the first 30-minute bile sample from the rats treated with the phospholipid-containing bioadditive "FLP-MD" in addition to tetracycline hydrochloride, the ratio of glycocholic acids was close to that in the control. Namely, the proportion of glycocholate was 25% (Fig. 3).

A decrease in the concentration of glycochenodeoxycholic and glycodeoxycholic acids in the first sample of bile by 25.7% ($P < 0.05$) was observed in animals with self-rehabilitation under the conditions of simulated tetracycline hydrochloride liver damage (Table 2). In the bile of the rats, that were administered the phospholipid-containing bioadditive and tetracycline hydrochloride, the concentration of glycochenodeoxycholic and glycodeoxycholic acids was higher than control in the third, fifth and sixth samples (Table 2). Namely, the use of the phospholipid-containing bioadditive stimulated the processes that ensure the formation and secretion of glycoconjugates of

dihydroxycholan bile acids – glycochenodeoxycholic and glycodeoxycholic bile acids. As a result, the content of this fraction of bile acids was 27.0%-37.6% higher than the control.

Effect of the milk phospholipid-containing bioadditive on the secretion of free bile acids under the conditions of experimental fatty hepatitis in rats

The milk phospholipid-containing bioadditive influenced the secretion of free bile acids under the conditions of experimental fatty hepatitis in rats. The synthesis of free bile acids under the influence of tetracycline hydrochloride in the dose used for modeling fatty hepatitis did not change statistically significantly. Therefore, in conditions of simulated fatty hepatitis, the mechanisms of synthesis of conjugated bile acids were primarily damaged, in particular the mechanisms of tauroconjugation. It is important to note that the concentration of free cholic acid, chenodeoxycholic acid and deoxycholic acid in the bile samples from the animals with tetracycline hydrochloride liver disease did not differ statistically significantly from the control (Table 3).

Table 3. The concentration (mg/100 mL) of free bile acids in the bile of rats during self-rehabilitation and the introduction of the phospholipid-containing bioadditive against the background of tetracycline-induced fatty hepatitis (n = 13), M ± SD

No. samples	Experiment time, min	Group of animals	Bile acid	
			cholic	chenodeoxycholic deoxycholic
1	30	Control	21.8 ± 3.6	15.8 ± 1.4
		Self-rehabilitation	18.6 ± 1.9	18.6 ± 1.9
		Treatment	13.4 ± 3.7	13.4 ± 3.7
2	60	Control	21.7 ± 3.3	15.4 ± 2.6
		Self-rehabilitation	25.3 ± 3.9	19.3 ± 1.6
		Treatment	20.0 ± 2.4	13.2 ± 4.6
3	90	Control	20.9 ± 2.9	14.9 ± 1.8
		Self-rehabilitation	24.2 ± 4.3	18.2 ± 1.8
		Treatment	20.8 ± 4.0	13.8 ± 5.4
4	120	Control	20.6 ± 2.5	14.1 ± 2.0
		Self-rehabilitation	24.5 ± 4.3	17.5 ± 1.8
		Treatment	18.0 ± 2.1#	11.8 ± 3.1#
5	150	Control	19.2 ± 2.5	13.3 ± 2.1
		Self-rehabilitation	23.0 ± 3.8	17.4 ± 2.0
		Treatment	18.6 ± 3.7	11.5 ± 3.5#
6	180	Control	19.1 ± 2.3	13.0 ± 2.8
		Self-rehabilitation	22.3 ± 3.6	16.5 ± 1.4
		Treatment	18.2 ± 3.4	11.1 ± 3.5

Note: # $P < 0.05$, statistically significant differences compared to self-rehabilitation group

Source: authors' development

It was discovered that the use of the phospholipid-containing bioadditive in rats with tetracycline-induced fatty hepatitis prevents the growth of the content of dihydroxycholan bile acids in the bile – chenodeoxycholic and deoxycholic acids – under the influence of an antibiotic at the dose used to model fatty hepatitis (Table 3). Note that the percentage of chenodeoxycholic and deoxycholic acids out of all bile acids in the bile of the first sample of the control group animals was 3% (Fig. 1). In the first and last samples of the bile of the self-rehabilitation group of rats, the percentage of chenodeoxycholic and deoxycholic acids out of all bile acids was 4% (Figs. 2 and 5). The smallest percentage (2%) of chenodeoxycholic and deoxycholic acids out of all bile acids was found in the first and last (sixth) samples of the bile of the treatment group (Figs. 3 and 6).

The category of primary bile acids includes cholic acid. This acid is synthesised by the so-called classical route, which begins with 7 α -hydroxylation of cholesterol by CYP7A1 of liver. Because of the absence of notable variation in the concentration of cholic acid in the bile samples from the animals in experimental fatty hepatitis, it can be assumed that this link in the transformation of cholesterol during cholic acid synthesis does not undergo critical changes in the modelling pathology. It is known that dihydroxycholanic bile acids, in particular deoxycholic acid, have a more significant detergent effect on the biomembranes of liver parenchymal cells and bile duct cells. Thus, such damaging factors as antibiotics cause an increase in the content of dihydroxycholanic bile acids in the hepatobiliary system. In turn, dihydroxycholanic bile acids enhance the

damaging effect on liver cells. The phospholipid-containing bioadditive decreased the concentration of free dihydroxycholane bile acids in the bile of the rats with tetracycline-induced fatty hepatosis. The formation of more dihydroxycholane chenodeoxycholic acid in hepatocytes is the result of the enhanced activation of the so-called “acid” route of cholate synthesis, which starts with 27-hydroxylation by CYP27A1. It is important to note that the classical route of synthesis of bile acids from cholesterol usually takes place under physiological conditions. K. Nemeth *et al.* (2024) in particular noted that bile acid salts accumulate in the bile ducts and penetrate into cholangiocytes to a significant extent at low pH levels, at which the increased acidity of the bile ducts is a factor in accelerated apoptosis of bile duct cells. The cytoprotective mechanism of action of ursodeoxycholic acid explains its clinical use in the prevention of cholestasis and the treatment of cholangiopathies. It is known that endogenous conjugated trihydroxycholanic bile acids in physiological concentrations do not exhibit a significant damaging effect on liver cells. The increase in the content of conjugated trihydroxycholanic bile acids under the influence of the dietary supplement, determined in the conducted studies, reveals one of the possible mechanisms of the hepatoprotective effect of the dietary supplement “FLP-MD”, which was also described in the work of D.O. Melnychuk & V.A. Hryshchenko (2014).

Free bile acids are conjugated with taurine and glycine in hepatocytes. Conjugated bile acids are critical for lipid absorption in the gut. In liver cells, the enzyme bile acid-CoA amino acid N-acyltransferase ensures the formation of bile acid conjugates. Violation of this enzyme leads to significant losses of phospholipids and fat-soluble vitamins. A significant violation of the conjugation of compounds with taurine is observed in liver pathologies. According to

D. Zhang *et al.* (2022), this may be related to the inhibition of taurine synthesis and transport in hepatocytes, and the inhibition of enzyme functioning. Disorders in the formation of bile acid conjugates with taurine were detected in the artificial modeling of fatty hepatosis in rats. The milk phospholipid-containing bioadditive eliminated the inhibition of the formation and secretion of tauroconjugates of bile acids in experimental fatty hepatosis. Decreased levels of bile acid tauroconjugates indicate significant changes in detoxification reactions during tetracycline hydrochloride usage. Namely, the conjugation of free cholates with taurine was inhibited. Instead, conjugation with glycine (an evolutionarily older metabolic pathway) in experimental fatty hepatosis was more stable, in particular the concentration of glycocholate did not change compared to control. The usage of the bioadditive based on milk phospholipids during tetracycline hydrochloride administration to the animals significantly eliminated the effects of antibiotics on the processes of biotransformation and formation of bile acids. This was confirmed by the absence of statistically significant differences with the control of the concentration of glycocholic acid, cholic acid and free dihydroxycholane bile acids in the bile of the rats that received the phospholipid-containing bioadditive for tetracycline-induced hepatosis. A special effect of the milk phospholipid-containing bioadditive was the increasing of taurocholate, taurochenodeoxycholate and taurodeoxycholate secretion into the bile of the rats in the conditions of experimental fatty hepatosis. This can be explained by the enhanced formation of glycine and taurine conjugates with free bile acids under the influence of the dietary supplement containing milk phospholipids. Accordingly, the secretion of free dihydroxycholane bile acids under the influence of the bioadditive in rats with fatty hepatosis was reduced compared to the animals that did not receive the bioadditive but had fatty hepatosis.

The established patterns regarding the quantitative changes of six fractions of cholates in the bile of animals with fatty hepatitis indicated the manifestation of the effect of “medical protection” when using the milk phospholipid-containing bioadditive against the background of the introduction of antibiotics. Y. Ikeda (2020) noted that the specific interaction of bile acids with the transport protein ABCB4 in hepatocytes is necessary for the release of phospholipid molecules by ABCB4 into bile. The researcher suggested that understanding the mechanisms of bile acid-phospholipid interaction will help in the development of new therapeutic agents for cholestatic liver diseases.

As stated by X.Y. Yeo *et al.* (2023), it should be taken into account that changes in the composition of bile acids due to impaired metabolic transformations in the liver affect inflammatory reactions, permeability of the blood-brain barrier and synaptic functions of neurons. Thus, pathological changes in the synthesis, biotransformation, secretion into the primary bile tubules, detergent action of unconjugated dihydroxycholic acids lead not only to damage to liver cells and progression of its damage. Changes in the physiological ratio of the different types of bile acids in bile, disorders in the biotransformation of cholates lead to violations of the mechanisms of nervous and humoral regulation of both liver functions and the organism as a whole. The determination of bile acid concentrations in biofluid samples is not yet standard practice in veterinary medicine. A special effect of the milk phospholipid-containing bioadditive on the functional state of the liver and the organism presented in this work and described by other researchers indicates the need for further thorough studies of the synthesis and biotransformation of cholates in normal and pathological conditions. The role of bile acids of various types in the pathogenesis of fatty hepatitis may be particularly significant.

In view of this, it is relevant to study the possible involvement of bile acids and enzymes that regulate their synthesis and biotransformation, as well as transport proteins that ensure the translocation of cholates through various domains of the plasma membrane of hepatocytes and enterocytes. Therefore, it is especially important to normalise the biotransformation of bile acids in hepatocytes. This allows to recommend the bioadditive “FLP-MD” as a hepatoprotective agent when administering potent drugs to animals that negatively affect liver cells and the processes of bile acid biotransformation.

Conclusions

The milk phospholipid-containing bioadditive eliminates the decrease in the concentration of bile acid tauroconjugates in the bile of the rats with experimental fatty hepatitis, which was detected during the study. This bioadditive stimulates the processes of conjugation of bile acids with taurine in hepatocytes. Under its influence, the concentration of bile acid glycoconjugates in the bile of the rats with fatty hepatitis approaches the control values. Therefore, the use of the milk phospholipid-containing bioadditive in animals stabilises the processes of conjugation of free bile acids with glycine in hepatocytes. The mechanisms of its effective action on the processes of conjugation of trihydroxycholic and dihydroxycholic bile acids are associated with the effect on the transmembrane transport of bile acids, taurine and glycine in hepatocytes, as well as with changes in the activity of the enzyme systems of cholate biotransformation. Under conditions of experimental fatty hepatitis, the milk phospholipid-containing bioadditive causes a decrease in the concentration of free bile acids in bile. The decrease in the secretion of cholic acid, chenodeoxycholic and deoxycholic acids with bile is due

to the stimulating effect of the components of the bioadditive on the conjugation of taurine with free bile acids in hepatocytes. An increase in the content of tauroconjugates of bile acids in bile significantly changes its physicochemical properties, efficiency in the processes of digestion, and the course of enterohepatic circulation of bile acids. The conjugation of substances with taurine and glycine is one of the effective detoxification processes in liver cells. The milk phospholipid-containing bioadditive acts on the mechanisms of conjugation in hepatocytes, stimulates the synthesis of tauroconjugates, which contributes to more effective detoxification of toxic compounds in the liver of animals with artificially modeled fatty hepatosis. Thus, the milk phospholipid-containing bioadditive reduces the content of toxic compounds in the internal environment of the body and, in particular, in the liver and promotes their effective removal from the body. Due to this, it is expected to slow down destructive changes in liver tissue in its fatty hepatosis.

The analysis of the ratio of conjugated and free bile acids in the bile of mammals with ar-

tificially modeled fatty hepatosis when using the milk phospholipid-containing bioadditive indicates prospects for studying the bioadditive as an effective means for improving the detoxification function of the liver. Further studies will be aimed at determining the features of changes in cholate secretion by the liver of rats during the development of experimental diclofenac-induced toxic hepatitis. It is planned to investigate the effectiveness of the influence of the components of the milk phospholipid-containing bioadditive “FLP-MD” on the molecular mechanisms of cholate synthesis and secretion in this hepatopathology and to conduct a comparative assessment with the patterns already described in this publication.

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Conflict of Interest

None.

References

- [1] Allameh, A., Niayesh-Mehr, R., Aliarab, A., Sebastiani, G., & Pantopoulos, K. (2023). Oxidative stress in liver pathophysiology and disease. *Antioxidants (Basel)*, 12(9), article number 1653. doi: [10.3390/antiox12091653](https://doi.org/10.3390/antiox12091653).
- [2] Boyer, J.L., & Soroka, C.J. (2021). Bile formation and secretion: An update. *Journal of Hepatology*, 75(1), 190-201. doi: [10.1016/j.jhep.2021.02.011](https://doi.org/10.1016/j.jhep.2021.02.011).
- [3] Choudhuri, S., & Klaassen, C.D. (2022). Molecular regulation of bile acid homeostasis. *Drug Metabolism and Disposition*, 50(4), 425-455. doi: [10.1124/dmd.121.000643](https://doi.org/10.1124/dmd.121.000643).
- [4] de Bruijn, V.M.P., Wang, Z., Bakker, W., Zheng, W., Spee, B., & Bouwmeester, H. (2022). Hepatic bile acid synthesis and secretion: Comparison of *in vitro* methods. *Toxicology Letters*, 365, 46-60. doi: [10.1016/j.toxlet.2022.06.004](https://doi.org/10.1016/j.toxlet.2022.06.004).
- [5] Deo, V., & Ranganathan, P. (2024). Statistical tools and packages for data collection, management, and analysis – a brief guide for health and biomedical researchers. *Perspectives in Clinical Research*, 15(4), 209-212. doi: [10.4103/picr.picr_160_24](https://doi.org/10.4103/picr.picr_160_24).
- [6] European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. (1986, March). Retrieved from <https://rm.coe.int/168007a67b>.

- [7] Farooqui, N., Elhence, A., & Shalimar. (2022). A current understanding of bile acids in chronic liver disease. *Journal of Clinical and Experimental Hepatology*, 12(1), 155-173. doi: [10.1016/j.jceh.2021.08.017](https://doi.org/10.1016/j.jceh.2021.08.017).
- [8] Fleishman, J.S., & Kumar, S. (2024). Bile acid metabolism and signaling in health and disease: Molecular mechanisms and therapeutic targets. *Signal Transduction and Targeted Therapy*, 9(1), article number 97. doi: [10.1038/s41392-024-01811-6](https://doi.org/10.1038/s41392-024-01811-6).
- [9] Fu, Y., Guzior, D.V., Okros, M., Bridges, C., Rosset, S.L., González, C.T., Martin, C., Karunarathne, H., Watson, V.E., & Quinn, R.A. (2025). Balance between bile acid conjugation and hydrolysis activity can alter outcomes of gut inflammation. *Nature Communications*, 16(1), article number 3434. doi: [10.1038/s41467-025-58649-x](https://doi.org/10.1038/s41467-025-58649-x).
- [10] Fuchs, C.D., Simbrunner, B., Baumgartner, M., Campbell, C., Reiberger, T., & Trauner, M. (2025). Bile acid metabolism and signalling in liver disease. *Journal of Hepatology*, 82(1), 134-153. doi: [10.1016/j.jhep.2024.09.032](https://doi.org/10.1016/j.jhep.2024.09.032).
- [11] Guo, J., Chen, S., Zhang, Y., Liu, J., Jiang, L., Hu, L., Yao, K., Yu, Y., & Chen, X. (2024). Cholesterol metabolism: Physiological regulation and diseases. *MedComm*, 5(2), article number e476. doi: [10.1002/mco2.476](https://doi.org/10.1002/mco2.476).
- [12] Ikeda, Y. (2020). Mechanism of taurohyodeoxycholate-induced biliary phospholipid efflux –understanding the function of the ABCB4 enhancer for developing therapeutic agents against bile salt-induced liver injury. *Yakugaku Zasshi*, 140(11), 1329-1334. doi: [10.1248/yakushi.20-00156](https://doi.org/10.1248/yakushi.20-00156).
- [13] Jia, W., Li, Y., Cheung, K.C.P., & Zheng, X. (2024). Bile acid signaling in the regulation of whole body metabolic and immunological homeostasis. *Science China. Life Sciences*, 67(5), 865-878. doi: [10.1007/s11427-023-2353-0](https://doi.org/10.1007/s11427-023-2353-0).
- [14] Korolova, D., Gryshchenko, V., Chernyshenko, T., Platonov, O., Hornytska, O., Chernyshenko, V., Klymenko, P., Reshetnik, Y., & Platonova, T. (2023). Blood coagulation factors and platelet response to drug-induced hepatitis and hepatosis in rats. *Animal Models and Experimental Medicine*, 6(1), 66-73. doi: [10.1002/ame2.12301](https://doi.org/10.1002/ame2.12301).
- [15] Law of Ukraine No. 3447 “On the Protection of Animals from Cruelty”. (2006, February). Retrieved from <https://zakon.rada.gov.ua/laws/show/3447-15#Text>.
- [16] Liashevych, A.M., Lupaina, I.S., Davydovska, T.L., Tsybalyuk, O.V., Oksentiuk, Y.R., & Makarchuk, M.Y. (2021). The effect of Corvitin on the content of bile acids in the liver of rats under conditions of chronic social stress. *Regulatory Mechanisms in Biosystems*, 12(3), 419-424. doi: [10.15421/022157](https://doi.org/10.15421/022157).
- [17] Lupaina, I., Liashevych, A., Reshetnik, Y., Veselsky, S., & Makarchuk, M. (2021). The effect of testosterone on the bile acid and bile lipid composition in rats. *Scientific Reports of the National University of Life and Environmental Sciences of Ukraine*, 17(5), 28-38. doi: [10.31548/dopovidi2021.05.003](https://doi.org/10.31548/dopovidi2021.05.003).
- [18] Melnychuk, D., Hryshchenko, V., & Litvinenko, O. (2009). *Veterinary bioactive addition of liposomal form and method of reparative therapy in hepatology*. (Patent of Ukraine No. 86516). Retrieved from <https://sis.nipo.gov.ua/uk/search/detail/422804/>.
- [19] Melnychuk, D.O., & Hryshchenko, V.A. (2014). [Exchange of bile pigments under the action of ecopathogenic factors on organism](#). *The Ukrainian Biochemical Journal*, 86(5), article number 156.

- [20] Miyazaki, T., Ueda, H., Ikegami, T., & Honda, A. (2023). Upregulation of taurine biosynthesis and bile acid conjugation with taurine through FXR in a mouse model with human-like bile acid composition. *Metabolites*, 13(7), article number 824. doi: [10.3390/metabo13070824](https://doi.org/10.3390/metabo13070824).
- [21] Nakamura, T., Masuda, A., Nakano, D., Amano, K., Sano, T., Nakano, M., & Kawaguchi, T. (2025). Pathogenic mechanisms of metabolic dysfunction-associated steatotic liver disease (MASLD)-associated hepatocellular carcinoma. *Cells*, 14(6), article number 428. doi: [10.3390/cells14060428](https://doi.org/10.3390/cells14060428).
- [22] Nemeth, K., Sterczar, Á., Kiss, D.S., Lányi, R.K., Hemző, V., Vámos, K., Bartha, T., Buzás, A., & Lányi, K. (2024). Determination of bile acids in canine biological samples: Diagnostic significance. *Metabolites*, 14(4), article number 178. doi: [10.3390/metabo14040178](https://doi.org/10.3390/metabo14040178).
- [23] Nikolajevic, N., Nikolajevic, M., Pantic, I., Korica, B., Kotseva, M., Alempijevic, T., Jevtic, D., Madrid, C.I., & Dumic, I. (2024). Drug-induced liver injury due to doxycycline: A case report and review of literature. *Cureus*, 16(5), article number e59687. doi: [10.7759/cureus.59687](https://doi.org/10.7759/cureus.59687).
- [24] Nimer, N., et al. (2021). Bile acids profile, histopathological indices and genetic variants for non-alcoholic fatty liver disease progression. *Metabolism: Clinical and Experimental*, 116, article number 154457. doi: [10.1016/j.metabol.2020.154457](https://doi.org/10.1016/j.metabol.2020.154457).
- [25] Qi, Y., Ma, Y., & Duan, G. (2024) Pharmacological mechanisms of bile acids targeting the farnesoid X receptor. *International Journal of Molecular Sciences*, 25(24), article number 13656. doi: [10.3390/ijms252413656](https://doi.org/10.3390/ijms252413656).
- [26] Silva, C., Merim, S., Sevivas, R., Mota, J., & Leitão, A. (2023). Bismuth subcitrate, metronidazole and tetracycline – a rare cause of drug-induced liver injury. *European Journal of Case Reports in Internal Medicine*, 10(12), article number 004119. doi: [10.12890/2023_004119](https://doi.org/10.12890/2023_004119).
- [27] Stellaard, F., & Lütjohann, D. (2021). Dynamics of the enterohepatic circulation of bile acids in healthy humans. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, 321(1), G55-G66. doi: [10.1152/ajpgi.00476.2020](https://doi.org/10.1152/ajpgi.00476.2020).
- [28] Taylor, K., et al. (2025) Perspective: How complex *in vitro* models are addressing the challenges of predicting drug-induced liver injury. *Frontiers in Drug Discovery*, 5, article number 1536756. doi: [10.3389/fddsv.2025.1536756](https://doi.org/10.3389/fddsv.2025.1536756).
- [29] Tiwari, V., Shandily, S., Albert, J., Mishra, V., Dikhatwar, M., Singh, R., Sah, S.K., & Chand, S. (2025). Insights into medication-induced liver injury: Understanding and management strategies. *Toxicology Reports*, 14, article number 101976. doi: [10.1016/j.toxrep.2025.101976](https://doi.org/10.1016/j.toxrep.2025.101976).
- [30] Varma, S., Nathanson, J., Dowlatshahi, M., Del Portillo, A., Ramirez, I., & Garcia-Carrasquillo, R. (2021). Doxycycline-induced cholestatic liver injury. *Clinical Journal of Gastroenterology*, 14(5), 1503-1510. doi: [10.1007/s12328-021-01475-7](https://doi.org/10.1007/s12328-021-01475-7).
- [31] Veselskyi, S., Liaschenko, P., Kostenko, S., Horenko, Z., & Kurovska, L. (2001). *The method of preparing samples of biofluids for determining the content of substances of a lipid nature*. (Patent of Ukraine No. 33564). Retrieved from <https://sis.nipo.gov.ua/uk/search/detail/342563/>.
- [32] Wei, C., Liu, Y., Jiang, A., & Wu, B. (2022). A pharmacovigilance study of the association between tetracyclines and hepatotoxicity based on Food and Drug Administration adverse event reporting system data. *International Journal of Clinical Pharmacy*, 44(3), 709-716. doi: [10.1007/s11096-022-01397-5](https://doi.org/10.1007/s11096-022-01397-5).
- [33] Wupperfeld, D., Fricker, G., Bois De Fer, B., & Popovic, B. (2024). Essential phospholipids impact cytokine secretion and alter lipid-metabolizing enzymes in human hepatocyte cell

- lines. *Pharmacological Reports: PR*, 76(3), 572-584. [doi: 10.1007/s43440-024-00595-4](https://doi.org/10.1007/s43440-024-00595-4).
- [34] Wupperfeld, D., Fricker, G., Bois De Fer, B., Frank, L., Wehrle, A., & Popovic, B. (2022). Essential phospholipids decrease apoptosis and increase membrane transport in human hepatocyte cell lines. *Lipids in Health and Disease*, 21(1), article number 91. [doi: 10.1186/s12944-022-01698-8](https://doi.org/10.1186/s12944-022-01698-8).
- [35] Yeo, X.Y., Tan, L.Y., Chae, W.R., Lee, D.Y., Lee, Y.A., Wuestefeld, T., & Jung, S. (2023). Liver's influence on the brain through the action of bile acids. *Frontiers in Neuroscience*, 17, article number 1123967. [doi: 10.3389/fnins.2023.1123967](https://doi.org/10.3389/fnins.2023.1123967).
- [36] Zhang, D., Zheng, J., Qiu, G., Niu, T., Gong, Y., & Cui, S. (2022). CCl₄ inhibits the expressions of hepatic taurine biosynthetic enzymes and taurine synthesis in the progression of mouse liver fibrosis. *Human & Experimental Toxicology*, 41, article number 9603271221135033. [doi: 10.1177/09603271221135033](https://doi.org/10.1177/09603271221135033).

Фосфоліпіди молока в корекції жовчнокислотного складу жовчі в щурів за експериментального жирового гепатозу

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Анотація. Унікальні функції печінки вимагають детального вивчення, оскільки гепатопатологія є серйозною ветеринарною проблемою, яка також негативно впливає на продуктивність сільськогосподарських тварин. Використовуючи штучне моделювання гепатопатології, можливо з'ясувати патогенетичні механізми її розвитку та визначати терапевтичну ефективність гепатопротекторних препаратів, особливо на основі сировини тваринного походження. Мета дослідження – визначити ефект від застосування фосфоліпідів молока у складі біодобавки «FLP-MD» на секрецію печінкою жовчних кислот за штучного моделювання в щурів жирового гепатозу. Для відтворення гепатопатології лабораторним щурам впродовж семи діб внутрішньошлунково вводили 4 % розчин тетрацикліну гідрохлориду в дозі 0,25 г/кг маси тіла та впродовж дев'яти діб використовували біодобавку в дозі 13,5 мг/кг маси тіла. Зразки жовчі в щурів відбирали шляхом проведення гострих експериментів. У зразках жовчі методом тонкошарової хроматографії було визначено шість фракцій кон'югованих жовчних кислот. Встановлено,

що в лабораторних щурів за експериментального жирового гепатозу гальмувалися процеси біотрансформації первинних і вторинних холатів шляхом кон'югації з таурином. Зокрема, фіксували зменшення у жовчі хворих тварин концентрації таурохолевої кислоти на 20,5–38,1 % ($P < 0,01$), а комплексу з таурохенодезоксихолевої та тауродезоксихолевої кислот – на 21,8–25,7 % ($P < 0,05$). У разі застосування біодобавки щурам за експериментального жирового гепатозу концентрація в жовчі таурохолевої, таурохенодезоксихолевої та тауродезоксихолевої кислот достовірно підвищувалася. Концентрація глікокон'югованих жовчних кислот і вільних холатів відповідала їх рівню в контролі. Біодобавка на основі фосфоліпідів молока за експериментального жирового гепатозу усувала негативний вплив антибіотика в токсичній дозі на процеси біотрансформації та утворення холатів. Це дозволяє рекомендувати біодобавку «FLP-MD» на основі фосфоліпідів молока в якості засобу гепатопротекторного профілю у разі застосування тваринам антимікробних препаратів

Ключові слова: жовчні кислоти; холестерол; біодобавка «FLP-MD»; тетрацикліну гідрохлорид; гепатоцит; гепатопатологія