



Phospholipid composition of bile and blood in rats under correction of experimental fatty hepatosis

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Abstract. To date, the aetiology and molecular mechanisms of the development of fatty hepatitis, which is quite common in mammals, have not yet been sufficiently explained. This pathology requires detailed study not only because of functional disorders of the liver and biliary system, but also because of the high probability of dangerous complications – fibrosis, cirrhosis, or hepatocellular carcinoma. The purpose of this study was to determine marker changes in the phospholipid composition of bile and blood in rats with experimental fatty hepatitis and with the use of corrective therapy. Hepatopathology was modelled in *Wistar* laboratory rats by intragastric administration of a 4% solution of tetracycline hydrochloride at the rate of 0.25 g/kg body weight for seven days. Using the method of thin-layer chromatography, the phospholipid components of animal bile and blood were studied. It was found that during experimental fatty hepatitis in rats, there was a decrease in the total phospholipid content in bile, mainly due to a decrease in the level of phosphatidylcholine (by 22.4-27.0%), the total fraction of inositol phosphatide and phosphatidylinositol (by 20.0-27.3%), and phosphatidylethanolamine (by 17.5-25.2%). Conversely, the introduction of milk phospholipids in the form of a dietary supplement “FLP-MD” in sick animals contributed to an increase in bile levels of phosphatidylserine by 67.1-99.8%, inositol phosphatide and phosphatidylinositol by 48.6-57.6%, phosphatidylcholine by 38.8-60.2%, phosphatidylethanolamine by 45.6-57.4%, and sphingomyelin by 30.4-46.3%. In the blood of such rats, a significant decrease in the content of phosphatidylcholine, phosphatidylserine, and sphingomyelin was found, which was not observed after administration of the “FLP-MD” dietary supplement to sick animals. In the case of using the supplement in healthy animals, only a 29.3% increase in the blood content of inositol phosphatide and phosphatidylinositol was noted. The determination of the most sensitive indicators in the phospholipid spectrum of blood and bile reveals the features of changes in molecular processes for the development of fatty hepatitis in animals, and also contributes to preclinical tests of the corrective effectiveness of newly created drugs according to established markers

Keywords: tetracycline hydrochloride; thin-layer chromatography; corrective therapy; dietary supplement “FLP-MD”

Introduction

Fatty liver (fatty hepatitis, steatohepatosis) is a series of liver abnormalities that often begin as simple steatosis and progresses to steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma. Due to the growing prevalence of fatty degeneration of liver, it is becoming a serious scientific and clinical problem (Pydyn *et al.*, 2020; Huh *et al.*, 2022). In veterinary practice, animal liver damage, in particular fatty degeneration of liver is noted as often occurring (up to 25%) and has a different aetiology, requires the development of appropriate non-invasive diagnostic methods and effective complex treatment

(Imbery *et al.*, 2022; Korolova *et al.*, 2023). Development of fatty hepatitis according to experts (Ota, 2021) is associated with impaired glucose and lipid metabolism in the liver, with hydrophobic bile acid toxicity, macrophage dysfunction and liver stellate cell activation, progressive fibrogenesis, and due to genetic, epigenetic, and environmental factors that contribute to the progression of fibrosis and the risk of hepatocellular carcinoma.

The leading factor of deepening pathological changes in the liver in its fatty hepatitis and the aetiological factor of hepatocellular

carcinoma is the accumulation of lipids in liver cells. S. Kartsoli *et al.* (2020) point to the fact that liver tissue samples with fatty degeneration show noticeable changes in the composition of fatty acids and phospholipids, indicating a violation of lipid metabolism as a key factor in the pathogenesis and progression of this disease. Current studies on lipotoxicity and progress in the analysis of the lipid profile, as a leading indicator of pathophysiological mechanisms in fatty hepatitis, show a link between lipid metabolism disorders and chronic inflammation and cell damage. In their studies of liver and serum lipids in patients with this hepatopathy, S. Kartsoli *et al.* (2020) found that as a result of metabolic disorders, several complex lipids, including sphingolipids and other phospholipids, are involved in the lipotoxicity and pathogenesis of steatohepatitis. Among the mechanisms that prevent the transition of fatty hepatitis to hepatocellular carcinoma, D.E. Berardi *et al.* (2022) consider both coordinated autophagic degradation of lipid droplets and stimulation of lipid excretion from hepatocytes.

B. Zhang *et al.* (2021) note that the liver has a pathway for removing various lipid constituents from the body as components of bile. Relatedly, cholesterol and phospholipids synthesised in hepatocytes, together with free fatty acids and triacylglycerols, are secreted to the bile tubules in combination with bile acids and determine the physical and chemical properties of bile. As noted by J.L. Boyer & C.J. Soroka (2021), fatty acids, triacylglycerols, cholesterol and its esters, phospholipids are mandatory lipid components of mammalian bile, but a violation of their ratio can provoke the development of gallstones and damage to the epithelial cells of the bile ducts and the mucous membrane of the gallbladder.

S. Kartsoli *et al.* (2020) note that in the case of fatty hepatitis, the qualitative and quantitative composition of lipids in blood plasma

and liver cells changes. First of all, there is a significant accumulation of fatty acids, triacylglycerol, and even phospholipids in the internal environment of the body and a clear correlation between the increase in lipids in the liver parenchyma and in the blood. The search for therapeutic measures that will reduce the intensity of lipid accumulation in the liver and prevent the pathological processes provoked by this accumulation, in particular, increased lipid peroxide oxidation, and inflammation, is an urgent scientific task.

Modern scientific studies using various models of fatty degeneration of liver and statistical analysis of patients' condition indicators in this hepatopathy indicate positive effects in the case of phospholipid-containing drugs and the introduction of phospholipid-containing supplements into the diet of patients with pathology of the hepatobiliary system (Dajani & Popovic, 2020). Essential phospholipids have a hepatoprotective effect, which has been demonstrated in studies on hepatocytes *in vitro* by D. Wupperfeld *et al.* (2022), and in animal models by D. Korolova *et al.* (2023). Phospholipid-containing drugs are actively used in the treatment of patients with fatty liver disease (Yin *et al.*, 2021). Numerous laboratory and clinical studies have investigated the hepatoprotective and therapeutic effects of phospholipids, mainly of plant origin and made from seafood, which are supplemented with omega-3 polyunsaturated fatty acids, food phospholipid-containing egg yolk preparations in combination with fat-soluble vitamins, etc. (Lüchtenborg *et al.*, 2020; Mitrovic *et al.*, 2022).

Affordable and promising in their unique composition and properties are preparations based on milk phospholipids (Gryshchenko *et al.*, 2019). Given the urgency of the problem of finding effective drugs in the treatment of patients with fatty liver degeneration and based on previous studies, the purpose of this

study was to investigate the effects of milk phospholipids in the form of dietary supplements “FLP-MD” on the indicators of the total content of phospholipids and five of their individual fractions in the bile and blood of animals with experimental fatty hepatosis.

Literature Review

Fatty liver disease has a number of histological and biochemical features. Among them is the accumulation of lipids in the cells of the liver parenchyma, which is a common feature of pathological conditions of all levels of severity in this disease (Younossi *et al.*, 2016; Gryshchenko, 2017). This liver disease is considered the result of a metabolic syndrome, which, in addition to the accumulation of lipids in the liver tissue, is characterised by abdominal obesity, hyperglycemia, dyslipidemia (Chalasan *et al.*, 2018). It is extremely important to diagnose fatty hepatosis before it develops into steatohepatitis, which can progress to cirrhosis of the liver with complications such as ascites, portal hypertension, and encephalopathy (McGlinchey *et al.*, 2022). The next threatening complication of fatty liver dystrophy and cirrhosis is hepatocellular carcinoma with an unfavourable prognosis.

Non-invasive and minimally invasive tests, including serum biomarkers, are cost-effective and convenient. However, these tests currently have several limitations, such as variability, insufficient accuracy, and risk factors for errors. The limitations and variability of these tests encourage researchers to combine them into diagnostic algorithms to produce more accurate tools (Gryshchenko, 2017; Bassal *et al.*, 2022). Identifying patients with significant fibrosis is important for targeted therapy to prevent disease progression. Effective screening using non-invasive tests can be crucial for patient risk stratification and early diagnosis. Given the need to accumulate information about

various biochemical markers of blood in fatty liver degeneration, it is important to conduct a comprehensive analysis of blood composition (Zhou *et al.*, 2019).

The pathogenesis of fatty liver disease is still unclear. It is believed that a number of different damaging factors induce the development of this pathological condition in individuals with a corresponding genetic predisposition (Borrelli *et al.*, 2018). There is also sexual dimorphism of this hepatic pathology, differences in the course and prognosis in people of different sexes (Martin-Grau & Monleon, 2023). Fat disease is one of the main liver diseases, which determines the relevance of its study. The pathological profile includes fatty liver disease and steatohepatitis. However, in clinical practice, there is no reliable non-invasive parameter for detecting characteristic differences between them. With the help of lipidomic analysis, it is possible to distinguish between these two pathological conditions. Moreover, it is in the content of various blood phospholipids that the most striking differences between fatty liver disease and steatohepatitis are revealed (Wang *et al.*, 2021).

Studies of the mechanisms of development of fatty liver disease are carried out simultaneously with the search for effective treatment methods. The course of pathological processes is associated with the state and functioning of cell membranes, and therefore membrane lipids are key structural, signalling, and regulatory components involved in the course of fatty hepatosis in mammals. Therefore, a violation of the ratio of membrane lipids is one of the mechanisms of the pathogenesis of fat degeneration in this organ. In steatohepatosis and steatohepatitis, noticeable changes were found not only in the composition of fatty acids, but also in the total content of phospholipids in the liver parenchyma (Puri *et al.*, 2007). Since these lipids affect many aspects of the pathophysiology of fatty liver disease, they can potentially be

prescribed for the treatment of patients (Welch *et al.*, 2022). At the same time, it was found that the effectiveness of drugs in this pathology is also associated with their effect on lipid metabolism and lipid homeostasis (Park *et al.*, 2020).

Later studies have demonstrated changes in the content of various phospholipid fractions in liver biopsies of patients with steatohepatitis, which indicates a violation of their synthesis and is associated with disease progression (Chiappini *et al.*, 2017). The most pronounced statistically significant changes were observed when comparing the content of phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylcholine, and sphingomyelin in the blood plasma of healthy subjects and patients with fatty hepatitis and steatohepatitis (Tiwari-Heckler *et al.*, 2018). In non-toxic concentrations, essential phospholipids (0.1 and 0.25 mg/mL), polyenylphosphatidylcholine and phosphatidylinositol (0.1 and 1 mg/mL) increase the fluidity of the hepatocyte cell membrane, reduce the percentage of apoptosis, and stimulate hepatocellular transport processes that ensure the elimination of substances from liver cells. In combination, this can significantly improve the functional state of the liver (Wupperfeld *et al.*, 2022).

The results of the analysis of the use of phospholipids in the composition of drugs for the treatment of fatty liver disease confirm the improvement in the condition of patients and indicate the need for further study of all aspects of the effectiveness of phospholipid-containing drugs, to clarify the mechanisms of their action (Dajani & Popovic, 2020).

Materials and Methods

The experimental research was conducted during 2021-2022 at the scientific laboratories of the Faculty of Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine (NUBIP of Ukraine) and

the educational and scientific centre Institute of High Technologies of Taras Shevchenko National University of Kyiv. In the study of the effect of dietary supplements “FLP-MD” based on milk phospholipids on the total content of phospholipids in bile and their five individual representatives: the total fraction of inositol phosphatide and phosphatidylinositol, phosphatidylserine, sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, male *Wistar* laboratory rats with a body weight of 200±50 g (n=13) were used. Electronic scales ORION OS-0K22 (ORION ELECTRONICS LTD, Europe) were used for weighing animals.

Laboratory rats were kept in standard vivarium conditions at a temperature 22-24°C, with a 14-hour light period of the day, on a standardised complete diet and with free access to water. The following methods were observed during the experiment: European Convention “For the Protection of Vertebrate Animals Used for Research and other Scientific Purposes” (1986), and Law of Ukraine No. 3447-IV “On the Protection of Animals from Cruelty” (2006, February). All surgical interventions during acute experiments were also performed in compliance with ARRIVE recommendations and were performed without violating the guidelines of Council Directive 2010/63/EU “On the Protection of Animals Used for Scientific Purposes” (2010).

Fatty hepatitis was reproduced according to the author’s own method (Gryshchenko *et al.*, 2019). During the modulation of fatty hepatitis, rats were administered a 4% solution of tetracycline hydrochloride using a soft silicone probe at the rate of 0.25 g/kg body weight daily intragastrically for seven days. The rats developed tetracycline-induced fatty hepatitis. The “Self-rehabilitation” group (n=5) was formed from animals that did not receive any therapy under the conditions of antibiotic administration. To determine the corrective efficacy of the liposomal form of a 1% solution of

the dietary supplement “FLP-MD”, whose main active ingredient is milk phospholipids, on the phospholipid content in bile, rats were orally administered the aforementioned dietary supplement one hour before the administration of tetracycline hydrochloride and additionally for the following two days after the completion of antibiotic administration. The “Correction” group (n=4) was formed from animals treated with tetracycline hydrochloride and dietary supplements “FLP-MD”. For animals of this group, the daily dose of dietary supplement “FLP-MD” was 13.5 mg/kg of body weight (Pat. 86516 UA). Rats in the control group (“Control”, n=4) were intragastrically injected with an equivalent volume of distilled water. During the study, the animals’ body weight was regularly monitored, and drug doses were recalculated considering changes in body weight during the experiment. Bile samples were collected in acute experiments on rats with a cannulated bile duct and their phospholipid content was determined. Bile samples were collected under certain conditions. Namely, the day before the acute experiment, the rats were weighed and then kept on a starvation diet with free access to drinking water. Animals were restricted in feeding during the day before surgery during an acute experiment to eliminate possible effects of feed consumption on the formation and secretion of bile.

Immediately before surgery, the animals were intraperitoneally injected with sodium thiopental at a dose of 7 µg/100 g of body weight as anaesthesia. After the animal was put under anaesthesia, the abdominal wall was dissected along a white line and the bile duct was cannulated using a plastic cannula connected to a micropipette. Next, the opening of the abdominal wall was covered with a dressing moistened with saline solution to prevent moisture loss from the dissected abdominal cavity. In the future, bile samples were collected from the immobilised animal in separate plastic containers

every 30 minutes for three hours of acute experiment. Thus, at the end of the three-hour acute experiment, six individual half-hour bile samples were obtained. At the third hour of the acute experiment, blood samples were also taken from animals to determine the content of phospholipids in it: total and fractional composition. In samples of bile and native blood stabilised with heparin, after appropriate extraction, a complex of phospholipids characteristic of these biolids was examined, which were divided by thin-layer chromatography on standard “Silufol” plates (Czech Republic) into individual fractions: total inositol phosphatide and phosphatidylinositol, phosphatidylserine, sphingomyelin, phosphatidylcholine, phosphatidylethanolamine (Pat. 99031324 UA). The following mixture was used as a solvent for determining five individual phospholipid fractions in prepared lipid-containing bile samples: chloroform:methanol:water:acetic acid in a ratio of 63:25:4:2.

The results obtained were statistically processed using the “Statistica 5.0” software suite (“StatSoft Inc.”, USA). As an indicator of the statistical significance of the differences found between the indicators in different experimental groups, the Student’s t-test was calculated in the case of normal distribution of data. The Shapiro-Wilk test was used to determine the nature of the distribution (normal or abnormal). The differences between the two indicators of the compared samples at $P < 0.05$, $P < 0.01$, and $P < 0.001$ were considered statistically significant (Filimonova *et al.*, 2005).

Results and Discussion

A study of the concentration of phospholipids in bile obtained from rats with experimental fatty hepatitis showed that both the total content of phospholipids and their individual fractions – mandatory components of liver secretions – decreased compared to the control

(Fig. 1, Tables 1-5). Thus, the total concentration of phospholipids in the bile of animals with experimental pathology (“Self-rehabilitation” group) was 20.1-25.4% less than the control indicators (50.8-58.7 mg%). The use of dietary supplements “FLP-MD” based on milk phospholipids led to a significant increase in the concentration of total phospholipids in the bile of experimental rats compared to the control. In particular, the content of phospholipids in the bile of animals of the “Correction” group

was 79.2-86.2 mg%, which is 40.4-56.1% more than the control indicators. The differences between the indicators of total phospholipids in the bile of laboratory rats from the “Self-rehabilitation” and “Correction” groups are even more significant. Thus, in sick animals that were orally administered dietary supplements “FLP-MD” based on milk phospholipids, the concentration of phospholipids in bile samples increased by 75.8-109.3% compared to rats of the “Self-rehabilitation” group (Fig. 1).

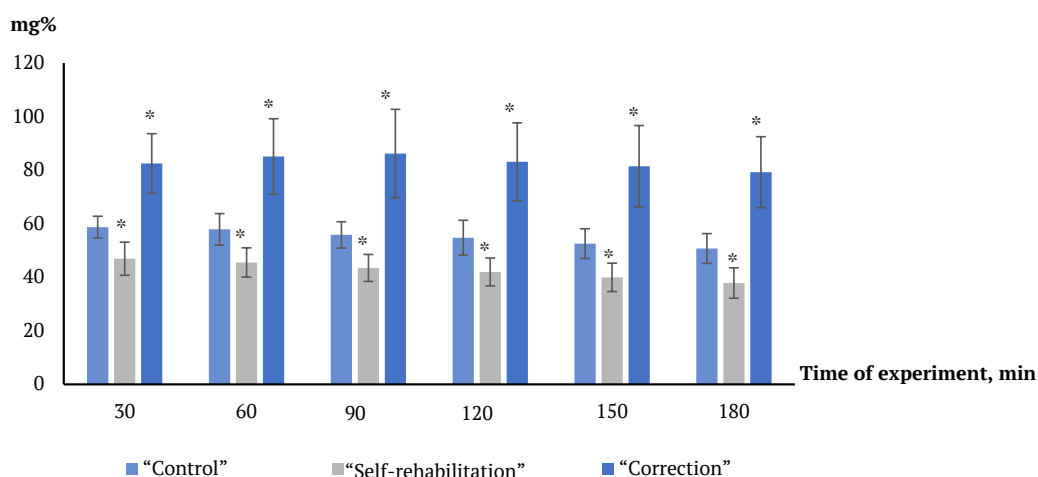


Figure 1. Concentration (mg%) of phospholipids in the bile of rats of the “Control”, “Self-rehabilitation”, and “Correction” groups

Notes: * $P < 0.05$, a statistically significant difference compared to the control;

“Control” – $n=4$; rats with experimental fatty hepatitis “Self-rehabilitation” – $n=5$; sick rats that received the dietary supplements “FLP-MD” based on milk phospholipids “Correction” – $n=4$

Source: developed by the author

Consequently, the use of a 1% solution of the liposomal form of dietary supplement “FLP-MD” based on milk phospholipids in an experimentally determined therapeutic dose caused significant changes in the processes of phospholipid secretion in the bile ducts. This was accompanied by a significant increase in the concentration of phospholipids in hepatic secretions. But the question arises whether

phospholipids of the dietary supplement “FLP-MD” affect the metabolic and transport processes in hepatocytes, or to a large extent, absorbed by liver cells, transit them and are released to the bile ducts. One of the ways to investigate the molecular mechanism of the effect of milk phospholipids in the form of dietary supplements “FLP-MD” on the phospholipid composition of bile was to determine the

spectrum of phospholipids of hepatic secretions in rats of all three experimental groups.

The author's modification of the components of the solvent mixture using the thin-layer chromatography method allowed detecting five phospholipid fractions in the collected rat bile samples. They were evaluated in conjunction with the results of a study of individual phospholipids in rat blood samples from three

experimental groups. It was established that in the conditions of simulated fatty hepatosis, the concentration of the total fraction of inositol phosphatide and phosphatidylinositol in the blood of experimental animals was statistically significantly different from the control values only in blood samples from the group of rats receiving only dietary supplement "FLP-MD" based on milk phospholipids (Fig. 2).

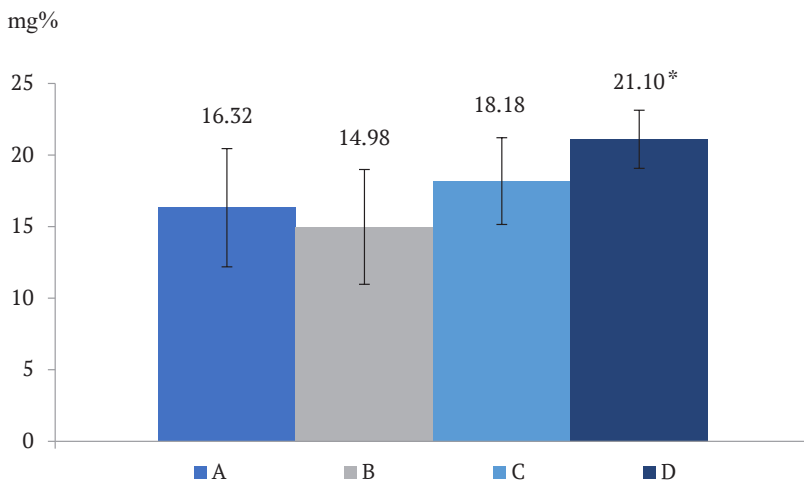


Figure 2. Concentration (mg%) of the total fraction of inositol phosphatide and phosphatidylinositol in the blood of rats of the "Control", "Self-rehabilitation", and "Correction" groups and in clinically healthy animals that received dietary supplement "FLP-MD" based on milk phospholipids

Notes: * $P < 0.05$, a statistically significant difference compared to the control; A – "Control" group ($n=4$); B – modelling fatty hepatosis ("Self-rehabilitation" group, $n=5$); C – rats with modelling fatty hepatosis, which were obtained the dietary supplement ("Correction" group, $n=4$); D – rats, which were obtained only the dietary supplement ("FLP-MD" group, $n=4$)

Source: developed by the author

In the bile of rats under the conditions of simulated pathology ("Self-rehabilitation" group), a decrease in the concentration of the total fraction of inositol phosphatide and phosphatidylinositol was observed by 20.0-27.3% compared to the control group (Table 1). The applied phospholipids of milk in the form of dietary supplements caused an increase in the

concentration of the total fraction of inositol phosphatide and phosphatidylinositol in the bile of rats by 48.6-57.6% in the first two bile samples from six collected in an acute experiment compared to the control indicators and in all the studied samples by 85.8-100.7% compared to the indicators of animals of the "Self-rehabilitation" group (Table 1).

Table 1. Concentration (mg%) of inositol phosphatide and phosphatidylinositol in the bile of rats of the “Control”, “Self-rehabilitation”, and “Correction” groups

Group of rats Time of experiment, min	“Control”	“Self-rehabilitation”	“Correction”
30	3.70±0.45	2.96±0.45**	5.50±0.76*###
60	3.60±0.45	2.90±0.44**	5.68±1.10*##
90	3.42±0.40	2.68±0.41**	5.20±1.65 ^a
120	3.28±0.41	2.54±0.43**	4.93±1.55 ^a
150	3.10±0.39	2.38±0.41**	4.63±1.35 [#]
180	3.00±0.39	2.18±0.41**	4.38±1.36 [#]

Notes: * $P < 0.05$, ** $P < 0.01$ statistically significant difference compared to the control; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ statistically significant difference in the indicators of the “Correction” group compared to the indicators of the “Self-rehabilitation” group. “Control” group – (n=4); for modelling fatty hepatitis – “Self-rehabilitation” group (n=5); sick rats of the “Correction” group (n=4), which were injected with the dietary supplement “FLP-MD”

Source: developed by the author

The results obtained suggest that the applied correction method promotes the elimination of inositol phosphatide and phosphatidylinositol with bile. At the same time, the concentration of these lipids was characterised by a tendency to increase in the blood of animals with fatty hepatitis when using dietary supplements.

As noted by C.N. Feriod *et al.* (2017), inositol triphosphatide plays a key role in the formation of lipid droplets in hepatocytes and is also involved in the development of fatty liver disease. Together with diacylglycerol, it is produced from membrane phosphatidylinositol in response to regulatory physiological and pathophysiological signals. In the future, this secondary mediator reveals its effects on the course of various intracellular processes. Considering the involvement of inositol triphosphate in the development of fatty liver disease, namely, stimulating the synthesis and accumulation of fat droplets in hepatocytes, the resulting effect of dietary supplements “FLP-MD” prevents the development of fatty degeneration of the liver parenchyma.

Phosphatidylinositol belongs to the minor phospholipid fractions in terms of its content in both blood and bile, which coincides with its own results of chromatographic examination of the corresponding samples of biological material. The authors suggest that the study of the content of this phospholipid fraction in biologicals will provide a better understanding of the possible links of its metabolic transformations in normal and pathological conditions. In particular, F. de Oliveira Lemos *et al.* (2019) noted that additional studies of the intracellular regulatory pathway inositol triphosphate-calcium may open up prospects for developing pharmacological strategies for the treatment of liver diseases specifically targeting each intracellular receptor isoform of this secondary mediator.

Under the conditions of experimental fatty hepatitis, a significant decrease in the content of phosphatidylserine in the blood of rats by 25.7% compared with its concentration in control samples was noted (Fig. 3). In animals of other experimental groups, this indicator did not differ statistically significantly from the range of control values.

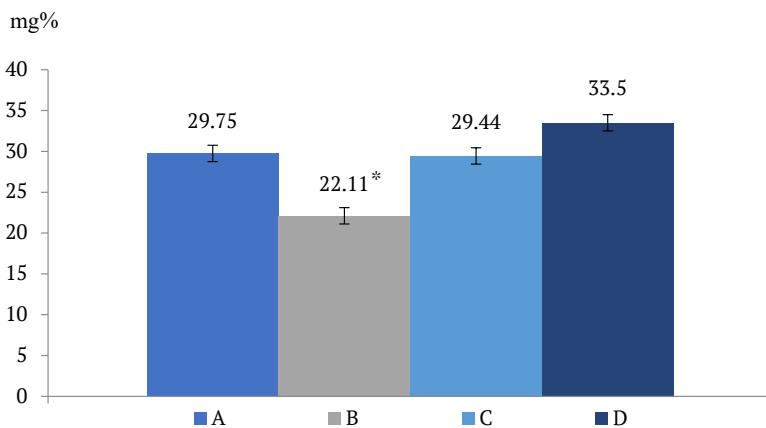


Figure 3. Concentration (mg%) of phosphatidylserine in the blood of rats of the “Control”, “Self-rehabilitation”, and “Correction” groups and in clinically healthy animals that received dietary supplement “FLP-MD”

Notes: * $P < 0.05$, a statistically significant difference compared to the control; A – “Control” group ($n=4$); B – modelling fatty hepatitis (“Self-rehabilitation” group, $n=5$); C – rats with modelling fatty hepatitis, which were obtained the dietary supplement (“Correction” group, $n=4$); D – rats, which were obtained only the dietary supplement (“FLP-MD” group, $n=4$)

Source: developed by the author

Under the conditions of simulated pathology (“Self-rehabilitation” group), there was a tendency to reduce the concentration of phosphatidylserine in the bile of rats compared to the control indicators. The introduction of a dietary supplement based on milk phospholipids to rats of the “Correction” group provokes an increase in the concentration of phosphatidylserine in rat bile by 42.0-62.1% compared to the control group. At the same time, in the bile of rats with

fatty hepatitis treated with dietary supplements “FLP-MD”, the content of phosphatidylserine increased by 67.1-99.8% compared to the indicators in animals of the “Self-rehabilitation” group. Thus, the increase in the content of phosphatidylserine in the bile of animals that were administered dietary supplements “FLP-MD” against the background of experimental pathology exceeded the stimulating effect of the dietary supplement compared to the control group (Table 2).

Table 2. Concentration (mg%) of phosphatidylserine in the bile of rats of the groups “Control”, “Self-rehabilitation”, “Correction”

Group of rats Time of experiment, min	“Control”	“Self-rehabilitation”	“Correction”
30	4.40±0.67	3.74±0.49	6.25±0.70*##
60	4.27±0.70	3.56±0.46	6.42±1.12*#
90	4.08±0.68	3.44±0.50	6.53±1.36#
120	3.90±0.72	3.26±0.52	6.13±1.11#
150	3.70±0.72	3.02±0.48	5.85±1.12#

Table 2. Continued

Group of rats	“Control”	“Self-rehabilitation”	“Correction”
Time of experiment, min			
180	3.50 ± 0.67	2.84 ± 0.50	5.68 ± 1.28 [#]

Notes: * $P < 0.05$, a statistically significant difference compared to the control; # $P < 0.05$, ## $P < 0.01$, a statistically significant difference in the indicators of the “Correction” group compared to the indicators of the “Self-rehabilitation” group. “Control” group – (n=4); for modelling fatty hepatosis – “Self-rehabilitation” group (n=5); sick rats of the “Correction” group (n=4), which were injected with the dietary supplement “FLP-MD”

Source: developed by the author

Y. Zhou et al. (2021) reported that phosphatidylserine enriched with docosahexaenoic acid has the ability to reduce the likelihood of negative consequences for fatty liver disease and effectively reduces serum levels of total cholesterol, triacylglycerols, non-esterified fatty acids and low-density lipoprotein cholesterol, as well as significantly increases the level of high-density lipoprotein cholesterol. According to the results obtained to determine the effectiveness of dietary supplements “FLP-MD” against the

background of experimental liver pathology, an increased intake of phosphatidylserine to bile was noted, which coincided with maintaining its content in the blood at the level of control values. The study of other lipid fractions in the blood and bile is considered for the future. In the conditions of experimental hepatopathy (“Self-rehabilitation” group), the concentration of sphingomyelin in the blood of laboratory animals was characterised by a significant decrease of 1.5 times compared to the control (Fig. 4).

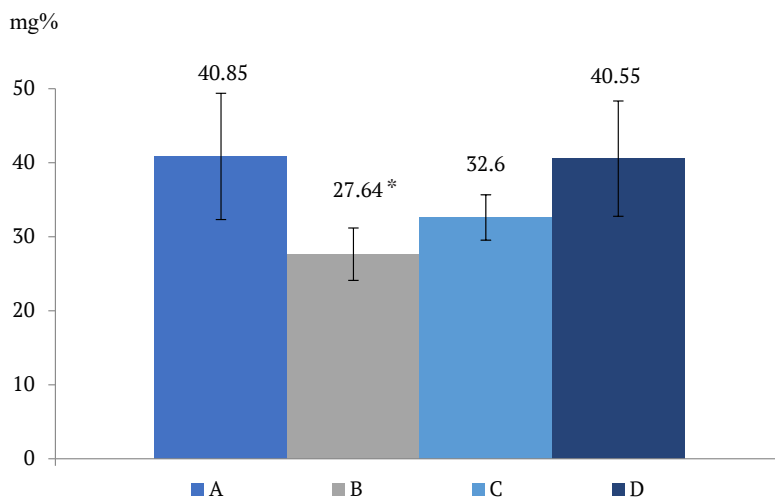


Figure 4. Concentration (mg%) of sphingomyelin in the blood of rats of the “Control”, “Self-rehabilitation”, and “Correction” groups and in clinically healthy animals that received dietary supplement “FLP-MD”

Notes: * $P < 0.05$, a statistically significant difference compared to the control; A – “Control” group (n=4); B – modelling fatty hepatosis (“Self-rehabilitation” group, n=5); C – rats with modelling fatty hepatosis, which were obtained the dietary supplement (“Correction” group, n=4); D – rats, which were obtained only the dietary supplement (“FLP-MD” group, n=4)

Source: developed by the author

The introduction of the “FLP-MD” dietary supplement (“Correction” group) to sick animals restrained from sharp quantitative changes in this indicator in the blood, which corresponded to the range of concentration values of the control group. And the introduction of dietary supplements to clinically healthy animals did not show a statistically significant effect. S. Tiwari-Heckler *et al.* (2018) reported a significant increase in serum sphingomyelin in patients with fatty liver dystrophy and steatohepatitis compared to the control against the background of metabolic disorders caused by diabetes mellitus, unfavourable PNPLA3 genotype, and other malformations. The inconsistency of the data obtained may be related to the different background clinical conditions of patients, lipid examination of serum rather than

whole blood, and differences in methodological approaches (the researchers used liquid chromatography mass spectrometry (LC-MS/MS)).

In the conditions of simulated pathology (“Self-rehabilitation” group), there is a tendency to reduce the concentration of sphingomyelin in the bile of rats compared to the control indicators. However, oral administration of a milk phospholipid supplement to rats (“Correction” group) caused a significant increase in the concentration of sphingomyelin in the bile of rats by 30.4-46.3% compared to its value in animals of the control group and by 50.5-76.6% compared to the indicators in rats from the “Self-rehabilitation” group. Consequently, dietary supplement “FLP-MD” based on milk phospholipids effectively stimulated the flow of sphingomyelin to bile in animals with experimental fatty hepatosis (Table 3).

Table 3. Concentration (mg%) of sphingomyelin in the bile of rats of the “Control”, “Self-rehabilitation”, and “Correction” groups

Group of rats Time of experiment, min	“Control”	“Self-rehabilitation”	“Correction”
30	5.43±0.56	4.70±0.50	7.08±0.90*##
60	5.30±0.76	4.50±0.50	7.27±1.15*##
90	5.13±0.77	4.36±0.47	7.50±1.36*##
120	4.95±0.75	4.20±0.47	7.15±1.28*##
150	4.70±0.79	3.96±0.44	6.80±1.43*##
180	4.50±0.75	3.68±0.46	6.50±1.23*##

Notes: * $P < 0.05$, a statistically significant difference compared to the control; ## $P < 0.01$, a statistically significant difference in the indicators of the “Correction” group compared to the indicators of the “Self-rehabilitation” group. “Control” group – (n=4); for modelling fatty hepatosis – “Self-rehabilitation” group (n=5); sick rats of the “Correction” group (n=4), which were injected with the dietary supplement “FLP-MD”

Source: developed by the author

In experimental fatty hepatosis, the concentration of phosphatidylcholine in the blood of experimental rats in the “Self-rehabilitation” group experienced a significant decrease of 1.1 times compared to animals in the “Control” group (Fig. 5). Administration of dietary supplements “FLP-MD” based on milk phospholipids (“Correction” group) to animals contributed to the

tendency to increase the content of phosphatidylcholine in the blood of sick rats, compared with the control. In addition, the dietary supplement administered to clinically healthy animals (“Dietary supplement” group) also caused a similar trend with the previous group of animals regarding quantitative changes in the blood concentration of phosphatidylcholine (Fig. 5).

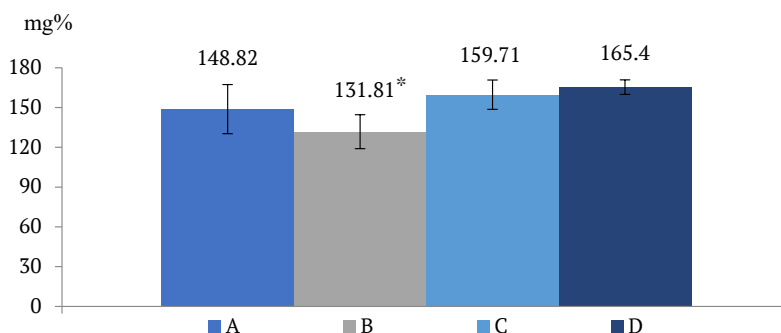


Figure 5. Concentration (mg%) of phosphatidylcholine in the blood of rats of the “Control”, “Self-rehabilitation”, and “Correction” groups and in animals that received dietary supplement “FLP-MD”
Notes: * $P < 0.001$, a statistically significant difference compared to control; A – “Control” group ($n=4$); B – modelling fatty hepatosis (“Self-rehabilitation” group, $n=5$); C – rats with modelling fatty hepatosis, which were obtained the dietary supplement (“Correction” group, $n=4$); D – rats, which were obtained only the dietary supplement (“FLP-MD” group, $n=4$)
Source: developed by the author

In the conditions of experimental hepatopathy, a significant decrease in the concentration of phosphatidylcholine in the bile of sick rats (“Self-rehabilitation” group) was observed by 22.4-27.0% compared to its indicators in the control (Table 4). Oral administration of milk phospholipids in the form of the “FLP-MD” dietary supplement to experimental animals caused a significant increase in the concentration of phosphatidylcholine in the

bile by 38.8-60.2% compared to the control and by 78.9-119.5% compared to the corresponding indicators in rats of the “Self-rehabilitation” group (Table 4). Consequently, the decrease in phosphatidylcholine content in rat bile caused by tetracycline hydrochloride in the simulation of fatty hepatosis was not only eliminated by the use of dietary supplements “FLP-MD”, but also led to an increase in the content of this important phospholipid component in it.

Table 4. Concentration (mg%) of phosphatidylcholine in the bile of rats of the “Control”, “Self-rehabilitation”, and “Correction” groups

Experimental group	“Control”	“Self-rehabilitation”	“Correction”
Time of experiment, min			
30	35.98±1.54	27.92±3.89**	49.95±7.14****
60	35.75±3.33	27.16±3.21**	51.95±8.10****
90	34.40±2.44	25.86±2.87**	53.10±8.90****
120	33.98±3.97	25.16±2.90**	51.98±7.85****
150	32.60±3.03	24.08±3.17**	51.88±8.47****
180	31.60±3.16	23.06±3.34**	50.63±7.34****

Notes: ** $P < 0.01$, a statistically significant difference compared to the control; **** $P < 0.001$, a statistically significant difference in the indicators of the “Correction” group compared to the indicators of the “Self-rehabilitation” group. “Control” group – ($n=4$); for modelling fatty hepatosis – “Self-rehabilitation” group ($n=5$); sick rats of the “Correction” group ($n=4$), which were injected with the dietary supplement “FLP-MD”

Source: developed by the author

The study of phosphatidylethanolamine content in the blood and bile of animals with experimental fatty hepatitis is relevant, since the literature notes that this phospholipid is one of the possible pathogenetic factors that leads to the progression of fatty liver disease in existing obesity (Shama *et al.*, 2023). Thus, the researchers noted an increase in the concentration of phosphatidylethanolamine against the background of a clinically established diagnosis of obesity. It is assumed that this effect is associated with a causal cascade of influence on the functional ability of various liver cells. In contrast to the described fact, according to the data presented in the paper by S. Tiwari-Heckler *et al.* (2018), on the contrary, there was a significant decrease in serum phosphatidylethanolamine in patients

with fatty liver dystrophy and steatohepatitis compared to the control, which occurred against the background of metabolic disorders and other clinical diagnoses. According to the results of the analysis of blood samples taken from experimental rats with a drug-induced form of fatty hepatitis against the background of tetracycline hydrochloride administration, a significant decrease in the content of phosphatidylethanolamine in the experimental group “Self-rehabilitation” was revealed, which coincides with the data of S. Tiwari-Heckler *et al.* (2018) and is shown in Figure 6. The use of dietary supplements “FLP-MD” (“Correction” group) allowed normalising the level of phosphatidylethanolamine in the blood of experimental animals to values close to the control (Fig. 6).

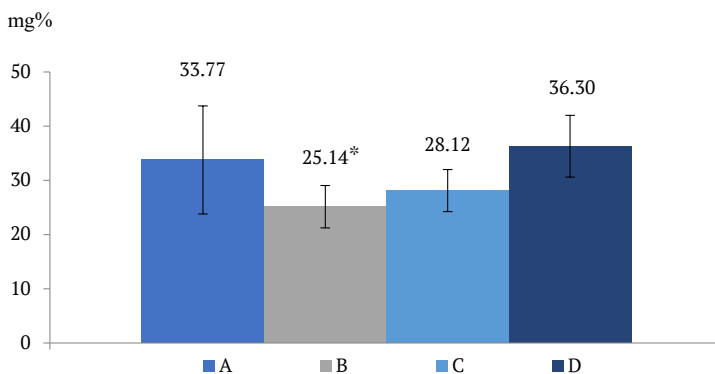


Figure 6. Concentration (mg%) of phosphatidylethanolamine in the blood of rats of the “Control”, “Self-rehabilitation”, and “Correction” groups and in animals that received dietary supplement “FLP-MD”

Notes: * $P < 0.05$, a statistically significant difference compared to the control. A – “Control” group (n=4); B – modelling fatty hepatitis (“Self-rehabilitation” group, n=5); C – rats with modelling fatty hepatitis, which were obtained the dietary supplement (“Correction” group, n=4); D – rats, which were obtained only the dietary supplement (“FLP-MD” group, n=4)

Source: developed by the author

This contradictory nature of the results of the study of the content of phosphatidylethanolamine in the blood, first of all, is explained by the specifics of experiments and the features of clinical diagnoses in the examined individuals.

Under the conditions of tetracycline-induced hepatitis, a significant decrease in the concentration of phosphatidylethanolamine in rat bile was observed by 17.5-25.2% compared to the control group. The use of milk phospholipids in the

form of dietary supplements caused a significant increase in the concentration of phosphatidylethanolamine in the bile of rats compared to the

control by 45.6-57.4%, and in comparison with similar data in animals of the “Self-rehabilitation” group, it increased by 80.3-97.5% (Table 5).

Table 5. Concentration (mg%) of phosphatidylethanolamine in the bile of rats of the “Control”, “Self-rehabilitation”, and “Correction” groups

Experimental group Time of experiment, min	“Control”	“Self-rehabilitation”	“Correction”
30	9.23±0.85	7.60±0.84*	13.70±1.64*####
60	8.98±0.64	7.40±0.84*	13.77±2.62*####
90	8.80±0.63	7.14±0.80*	13.85±3.24*####
120	8.65±0.68	6.82±0.88*	12.93±2.77*####
150	8.45±0.63	6.52±0.81**	12.30±2.83*####
180	8.15±0.58	6.10±0.97**	12.05±2.04*####

Notes: * $P < 0.05$, ** $P < 0.01$, statistically significant difference compared to the control; #### $P < 0.01$, ### $P < 0.001$, statistically significant difference in the indicators of the “Correction” group compared to the indicators of the “Self-rehabilitation” group. “Control” group – (n=4); for modelling fatty hepatosis – “Self-rehabilitation” group (n=5); sick rats of the “Correction” group (n=4), which were injected with the dietary supplement “FLP-MD”

Source: developed by the author

According to M. Masoodi *et al.* (2021), changes in blood phospholipid levels are one of the biomarkers of fatty liver disease. According to the results described in this paper, the content of various individual phospholipids in the bile of rats undergoes significantly greater changes than in the blood of animals with experimental fatty hepatosis. This indicates the need for a comprehensive lipid study, considering changes in both mammalian blood and bile. The multidirectional nature of quantitative changes in individual phospholipid fractions can be explained by various mechanisms of influence of these phospholipids on liver function and their participation in the pathogenesis of fatty liver. The use of dietary supplements “FLP-MD” based on milk phospholipids in the experiment statistically significantly changed the content of phosphatidylserine, sphingomyelin, and phosphatidylcholine in the blood of sick animals, which was also noted by other researchers (Gundermann *et al.*, 2016; Dajani &

Popovic, 2020; Lüchtenborg *et al.*, 2020). Instead, a significant increase in the concentration of all five phospholipid fractions was observed in the bile of these rats compared not only with animals with experimental fatty hepatosis, but also with controls. The intake of phospholipids in the bile reflects the course of transmembrane transport processes that ensure the movement of these mandatory components of bile to the bile tubules. In conditions of pathology, transport processes are disrupted, which leads to changes in the ratio of lipids in the blood, bile, and liver cells. Scientific sources also emphasise other mechanisms of effects of phospholipid use in the case of fatty liver disease (Ota, 2021; Bassal *et al.*, 2022; Mitrovic *et al.*, 2022). These include a reduction of the intensity of oxidative stress by regulating the activity of enzymes that produce reactive oxygen species, a controlled decrease in the intensity of inflammation and fibrogenesis in the liver (Dajani & Popovic, 2020), an increase in

the fluidity of hepatocyte membranes along with a decrease in apoptosis and intensification of hepatocellular transport (Wupperfeld *et al.*, 2022). Both according to the results of the conducted studies and according to other researchers, the effectiveness and systemic effect of phospholipid-containing drugs in fatty liver disease was confirmed. However, it is the milk phospholipids used in the creation of the dietary supplement “FLP-MD” that demonstrate a significant positive effect on the bile secretory function of the liver and show a corrective effect on the concentration of individual phospholipids in the blood of animals during artificial reproduction of fatty hepatosis.

Conclusions

The processes of phospholipid entry into hepatocytes and their secretion into bile are significantly disrupted in the experimental tetracycline-induced form of fatty hepatosis in laboratory rats. Phospholipid components of dietary supplements “FLP-MD” are of animal origin (from milk), and therefore, differ in the most pronounced bioavailability, as evidenced by a significant increase in the content of all certain fractions of phospholipids in the blood of experimental rats. In the future, phospholipids of dietary supplements “FLP-MD”, entering the liver, accelerate the restoration of metabolic and transport processes in its cells, contributing to the establishment of a complete composition of bile according to the main biochemical parameters. Considering pathological disorders of lipid homeostasis during experimental fatty liver degeneration, the high effectiveness of dietary supplements “FLP-MD” in normalising the content of phospholipids both in the blood and in the bile of sick animals was established. This gives

reason to consider promising the use of dietary supplements “FLP-MD” to correct the structural and functional state of the liver and improve the metabolism of phospholipids for its damage, in particular, with tetracycline antibiotics. When using the supplement on clinically healthy rats, a neutral effect on the studied indicators was observed, with the exception of an increase in the concentration of the total fraction of inositol phosphatide and phosphatidylinositol in the blood by 29.3% compared to the control.

In accordance with the results obtained, it is reasonable to recommend the dietary supplement “FLP-MD” as a drug effective for the development of fatty hepatosis, including for preventing the occurrence of complications in the form of intrahepatic and extrahepatic cholestasis. The latter is explained by the fact that an experimentally determined increase in the content of phosphatidylserine, sphingomyelin, phosphatidylcholine, phosphatidylethanolamine in the bile of sick animals is a favourable sign, since it improves its physical and chemical properties and allows removing phosphatidylinositol from liver cells as a risk factor for increased fatty degeneration of liver tissue.

In the future, it is planned to investigate the features of the lipid and bile acid spectrum of blood and bile in laboratory rats in experimental hepatopathology and to determine the corrective effectiveness of dietary supplements “FLP-MD” based on milk phospholipids in a model experiment.

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None.

Conflict of Interest

None.

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Фосфоліпідний склад жовчі та крові в щурів за корекції експериментального жирового гепатозу

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Анотація. На сьогодні ще недостатньо з'ясовано етіологію і молекулярні механізми розвитку досить поширеного в свавців жирового гепатозу. Ця патологія потребує детального вивчення не лише через функціональні порушення печінки та біліарної системи, але й високу ймовірність виникнення небезпечних ускладнень – фіброзу, цирозу або гепатоцелюлярної карциноми. Мета цього дослідження полягала у визначенні маркерних змін фосфоліпідного складу жовчі та крові в щурів за експериментального жирового гепатозу та при застосуванні коригувальної терапії. Моделювання гепатопатології в лабораторних щурів лінії *Wistar* здійснювали шляхом внутрішньошлункового введення 4 %-го розчину тетрацикліну гідрохлориду з розрахунку 0,25 г/кг маси тіла тварини, впродовж семи діб. Завдяки використанню методу тонкошарової хроматографії проведено дослідження фосфоліпідних компонентів жовчі та крові тварин. Встановлено, що за експериментального жирового гепатозу в щурів відбувалося зменшення в жовчі загального вмісту фосфоліпідів, переважно за рахунок зниження рівня фосфатидилхоліну (на 22,4-27,0 %), сумарної фракції інозитолфосфатиду і фосфатидилінозитолу (на 20,0-27,3 %) та фосфатидилетаноламіну

(на 17,5-25,2 %). І, навпаки, введення хворим тваринам фосфоліпідів молока у вигляді біодобавки «FLP-MD» сприяло зростанню в жовчі рівня фосфатидилсерину на 67,1-99,8 %, інозитолфосфатиду і фосфатидилінозиту на 48,6-57,6 %, фосфатидилхоліну на 38,8-60,2 %, фосфатидилетаноламіну на 45,6-57,4 % та сфінгомієліну на 30,4-46,3 %. У крові таких щурів виявлялося вірогідне зменшення вмісту фосфатидилхоліну, фосфатидилсерину і сфінгомієліну, що не відмічалось за введення хворим тваринам біодобавки «FLP-MD». У випадку застосування біодобавки здоровим тваринам відзначали лише збільшення в крові вмісту інозитолфосфатиду і фосфатидилінозиту на 29,3 %. Визначення найчутливіших показників у фосфоліпідному спектрі крові та жовчі розкриває особливості змін молекулярних процесів за розвитку в тварин жирового гепатозу, а також сприяє проведенню доклінічних випробувань корегувальної ефективності новостворених препаратів за встановленими маркерами

Ключові слова: тетрацикліну гідрохлорид; тонкошарова хроматографія; корегувальна терапія; біодобавка «FLP-MD»