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Correction of Triacylglycerols and Free Fatty Acids in Rat Bile in Experimental Hepatic Steatosis

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Abstract. The relevance of the scientific study is associated with a substantial spread of hepatic steatosis in domestic animals (up to 40%) and the development of health-threatening complications in the form of cirrhosis of the liver, liver failure, and cancer. The purpose of this study was to determine the corrective effectiveness of the “FLP-MD” dietary supplement based on milk phospholipids in relation to the content of triacylglycerols and free fatty acids in the bile of rats with tetracycline-induced hepatic steatosis. Modelling of the drug form of hepatic steatosis was conducted by intragastric administration of a 4% solution of tetracycline hydrochloride at the rate of 0.5 g/kg of animal body weight for seven days. As a corrective therapy, for nine days the animals were intragastrically administered a dietary supplement “FLP-MD” based on milk phospholipids at a dose of 15.5 mg/kg of body weight. At the end of the experiment, bile samples were taken from rats for three hours every 30 minutes, in which the content of triacylglycerols and free fatty acids was determined by thin-layer chromatography. It was determined that the concentration of triacylglycerols in the bile of sick rats at the third hour of its selection is 63.0% lower than the control indicators. In laboratory rats that received a phospholipid-containing supplement against the background of modelling drug-induced hepatitis, this indicator in bile corresponded to the values of the control group. Therewith, the concentration of free fatty acids in bile samples at the third hour of its selection in sick rats was marked by a decrease of 47.2% compared to the control. The use of the dietary supplement under study in sick animals caused an increase in the concentration of free fatty acids in bile by 2.85 times compared to the control, which reduces the intensity of their use for the synthesis of triacylglycerols and prevents the development of fatty liver infiltration. Therefore, the phospholipid-containing dietary supplement is a highly effective corrective agent for impaired metabolism of triacylglycerols and free fatty acids in rats with drug-induced hepatic steatosis. This gives grounds to recommend it as a corrective therapy and for the prevention of the development of hepatic steatosis, especially in the case of the use of tetracycline antibiotics in animals

Keywords: milk phospholipids, biologically active supplement, tetracycline hydrochloride, corrective therapy

Suggested Citation:

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Introduction

Fatty liver disease or Hepatic steatosis is a hepatopathy, common in domestic animals (up to 40%), which is characterised by a disruption of the balance between the synthesis and utilisation of triacylglycerols, their excessive accumulation in hepatocytes, dyslipidemia, activation of fatty acid peroxide oxidation, and disorders of the bile secretory function of the liver [1; 2]. A particular threat to their health is the consequences of this pathology in the form of cirrhosis of the liver (in 25% of sick animals), further development of liver failure and hepatocellular carcinoma [1; 3].

The liver plays a leading role in lipid metabolism in the body of mammals. The development of hepatic steatosis primarily consists in the intrahepatic accumulation of triacylglycerols [1]. Their excessive deposition in hepatocytes can be reversed or vice versa. Therewith, this disease can occur secondary to various pathological conditions and be a consequence of viral infection, alcohol consumption, incorrect use of medications, etc. [3; 4]. In particular, excessive accumulation of lipids in the liver parenchyma is observed in obesity, metabolic syndrome, diabetes mellitus with insulin resistance [5; 6]. The most common type of fatty liver degeneration is non-alcoholic steatohepatitis (NASH). In addition, there is a tendency to increase cases of hepatic steatosis caused by drugs [5; 7]. Both non-alcoholic hepatitis and hepatic steatosis are caused by various groups of drugs are characterised by damage to hepatocytes, inflammatory processes in the liver parenchyma, and fibrosis, which substantially increases the risk of other complications [1; 3].

The molecular mechanisms of hepatic steatosis development are still poorly understood. It is believed [1] that the accumulation of triacylglycerols in hepatocytes in hepatic steatosis is not the leading factor of lipidotoxicity. Along with this, abnormal accumulation of triacylglycerols in liver cells is accompanied by an increase in the intrahepatocytic content of other lipids, which in turn, are able to trigger numerous intracellular mechanisms of lipidotoxicity, namely: activation of death receptors, endoplasmic reticulum stress, modification of mitochondrial function, and oxidative stress. In particular, with fatty degeneration of the liver, there is an accumulation of cholesterol in its cells, which, with its excessive intracellular amount, exhibits a number of toxic effects leading to liver damage. Maintenance of intrahepatic cholesterol homeostasis depends on the smooth flow of a number of complex processes: the synthesis of cholesterol in hepatocytes, the transport of cholesterol through the sinusoidal (basal) and, especially, canalicular (apical) domains of the plasma membrane of hepatocytes (this mechanism allows removing cholesterol in the bile), the conversion of cholesterol into primary bile acids involving specific intrahepatocytic enzyme, and the absorption of cholesterol in the intestine [6]. Along with this, it was determined that in laboratory rats with an experimental drug form of hepatic steatosis, the excretion of cholesterol and, especially, cholesterol esters to the bile tubules is suppressed and their ratio in bile is substantially disrupted, which is likely due to their accumulation in liver cells [8].

Notably, such a common disease in dogs as chronic hepatitis is accompanied by hypercholesterolemia and hypertriacylglycerolemia [9]. Therewith, dyslipidemia in dogs with chronic hepatitis, which is considered a disease

similar to non-alcoholic fatty degeneration of the liver in humans, has several causes: increased synthesis of cholesterol and triacylglycerols in the liver, inhibition of triacylglycerol catabolism, and cholestatic phenomena with an increase in the concentration of bile acids in the blood of sick animals. In this case, a violation of the excretion of both excess cholesterol and excess triacylglycerols in the bile is one of the possible causes of an increase in the level of these lipid compounds in the blood.

However, it is still an open question which of the mechanisms – increased synthesis of cholesterol and triacylglycerols in hepatocytes, inhibition of their catabolism, or disruption of the transport of these lipids from hepatocytes is leading in the pathogenesis of hepatic steatosis [9]. In addition, it was also determined that when triacylglycerols are stimulated to enter the primary bile tubules in case of disruption of the further outflow of bile by the biliary system, for example, as noted in pancreatobiliary reflux, their excessive accumulation in the bile ducts and gallbladder is observed. This, in turn, leads to the fact that triacylglycerols become one of the main factors in the formation of gallstones in the gallbladder [10].

It should be noted the important role of free fatty acids in liver damage due to its fatty degeneration, which occurs due to increased oxidative stress and the subsequent development of inflammatory processes in the parenchyma. There is a hepatoprotective effect of measures that help reduce the content of free fatty acids in hepatocytes, which prevents the progression of fatty degeneration of this organ [11]. Drugs that can correct lipid metabolism in the liver include bile acids (in particular, ursodeoxycholic acid preparations), omega-3 of unsaturated fatty acids [12; 13]. Phospholipids play an important role in the treatment of patients with fatty liver degeneration [14; 15]. Largely the content of various fractions of lipid components in bile reflects the course of lipid metabolism in liver cells. Considering the close relationship between the metabolism of free fatty acids and triacylglycerols and their role in the course of pathologies with fatty liver degeneration, the purpose of the study was to examine the corrective effect of phospholipid-containing biologically active supplement (BAS) “FLP-MD” on the dynamics of the content of free fatty acids and triacylglycerols in rat bile under the conditions of modelling the drug form of hepatic steatosis.

Literature Review

The liver is the central organ of the metabolism of fatty acids, which both enter liver cells from the blood and are synthesised in hepatocytes *de novo*. Free fatty acids are either oxidised in cells as an energy resource or used for the synthesis of triacylglycerols, which, as part of low-density lipoprotein complexes, enter the blood plasma and then enter all organs and tissues of the body with its flow. According to the physiological state, both free fatty acids and triacylglycerols (up to 5% of the organ mass) do not accumulate in hepatocytes in substantial amounts [16]. It is noted that the intracellular accumulation of triacylglycerols, which is characteristic of hepatic steatosis, is primarily associated with a violation of their excretion from hepatocytes, in particular, as part of lipoprotein complexes, and not with an increase in the intake of fatty acids to liver cells from the blood [17].

Steatosis of the liver due to its fatty degeneration caused by metabolic disorders is associated with the excessive synthesis of triacylglycerols in the liver using fatty acids obtained from various sources: from white adipose tissue, as a product of lipogenesis *de novo*, and from endocytic residues rich in triacylglycerol lipoproteins [17]. Due to the high content of lipids in the liver, the elimination of very low-density lipoproteins into the bloodstream should be expected to increase, which becomes the main cause of complex dyslipidemia characteristic of patients with hepatic steatosis. Measures that reduce the secretion of very low-density lipoproteins into the blood can prevent the development of dyslipidemia, but simultaneously cause exacerbation of hepatic steatosis [17; 18]. This means that the balance between intracellular accumulation of lipids and their secretion from hepatocytes into the blood is a critical parameter that determines the course of diseases associated with lipid metabolism disorders and fatty degeneration of the liver.

To date, it has already been confirmed [18] that increasing energy processes using fatty acids as an energy resource reduces the lipid load on the liver. In addition, hepatocellular synthesis of triacylglycerols from fatty acid derivatives can positively change the course of fatty liver damage. However, more studies are needed to determine the effect of individual transporter proteins, enzymes, and their isoforms on the development of steatosis and dyslipidemia *in vivo*. Attention should also be paid to the factor of insufficient enzymatic transformations of cholesterol lipoproteins, which leads to their substantial accumulation in the cytosol of hepatocytes. Although it appears to be less important compared to other reasons. However, “cholesterol load” affects the course of inflammatory processes and leads to the progression of fatty liver degeneration associated with metabolic disorders [18].

Transport systems of the plasma membrane of liver cells play a substantial role in maintaining a certain intracellular content of free fatty acids and triacylglycerols: translocase of fatty acids ((FAT)/CD36), a plasma membrane protein that binds fatty acids (FABPpm) and Caveolin-1. It is noted that the activity of these transport systems and their significance in the development of fatty liver degeneration require in-depth investigation [16]. Notably, hypertriacylglycerolemia is defined as one of the risk factors for developing cholelithiasis [10; 19; 20]. Both metabolic and genetic factors affect the content of triacylglycerols in liver cells, identifying their effects by various mechanisms. Therefore, as noted in the review papers [21-23], further investigation is needed to determine the effects of metabolic and genetic interaction, which will allow the development of personalised and potentially effective therapeutic measures for correcting lipid metabolism disorders in fatty liver degeneration.

Materials and Methods

Experimental animal studies and biochemical analysis of bile were conducted based on the scientific laboratory of the educational and Scientific Centre “Institute of High Technologies” of Taras Shevchenko National University of Kyiv in the second half of 2021. In the study of the effect of BAS “FLP-MD” based on milk phospholipids on the content of triacylglycerols and free fatty acids in bile, male laboratory rats of the line were used Wistar, whose body weight

was 200 ± 50 g ($n = 13$). ORION OS-0k22 electronic scales (ORION ELECTRONICS LTD, Europe) were used for weighing animals. Laboratory rats were kept in standard vivarium conditions at a temperature of 22-24 C with a 14-hour light period of the day, on a standardised full-fledged diet and with free access to water. During the experiment, they adhered to the “European Convention for the Protection of Vertebrates Used for Experimental and Scientific Purposes” (Strasbourg, 1986) [24], and the Law of Ukraine No. 692 “On the Protection of Animals from Cruelty” (3447-IV) of 02/21/2006 [25]. All surgical interventions were performed with the use of painkillers (thiopental sodium at a dose of $7 \mu\text{g}/100$ g of body weight) and compliance with the rules of humane treatment of laboratory animals.

Reproduction of hepatic steatosis was conducted according to the author’s own technique [26]. Using a soft silicone probe, rats were given a 4% Solution of tetracycline hydrochloride daily intragastric administration at the rate of 0.5 g/kg of body weight for seven days. The animals developed tetracycline-induced hepatic steatosis, and since these rats did not receive therapy, a “Self-rehabilitation” group was formed from them ($n = 5$). Rats were intragastrically administered with the specified dietary supplement for seven days an hour before the use of tetracycline and additionally the next two days after the completion of the seed, (group “Correction”, $n = 4$) to determine the corrective properties of BAS “FLP-MD” (the main active substance – phospholipids of milk) in relation to the content of triacylglycerols and free fatty acids in bile samples. The daily dose of BAS “FLP-MD” was 13.5 mg/kg of body weight [27]. Animals of the control group (“Control”, $n = 4$) were injected intragastrically with an equivalent volume of distilled water. During the study, the animals’ body weight was monitored, and the doses of drugs were determined individually, considering changes in body weight during the entire experiment period.

Bile samples were taken to determine the content of triacylglycerols and free fatty acids in it in acute experiments with bile duct cannulation. The day before the acute experiment, the rats were weighed and kept on a starvation diet with free consumption of drinking water. Animals’ access to feed during the day before the end of the experiment was restricted to eliminate the influence of feed on the processes of bile formation and on the composition and secretion of bile. Before surgery, rats were drugged by intraperitoneal administration of thiopental sodium at the dose indicated above. After that, laparotomy and cannulation of the bile duct were performed using a plastic cannula, which was connected to a micropipette. Bile samples were taken every 30 minutes for three hours of acute experiment. Thus, at the end of the three-hour acute experiment, six half-an-hour bile samples (No. 1-6) were obtained from each animal. These samples were analysed for the content of triacylglycerols and free fatty acids by thin-layer chromatography in the author’s modification [28] on standard plates of the company “Silufol” (Czech Republic) in the solvent system petroleum, ether-diethyl, ether-acetic acid (90 : 70 : 1).

Statistical processing of the obtained results was conducted using the software “Statistica 5.0” (“StatSoft Inc.”, USA). As an indicator of the statistical significance of the identified differences in the values of the studied indicators in the experimental groups, the Student’s t-test was

considered for the normal distribution of data. The normality of the distribution was evaluated using the Shapiro-Wilk test. The indicators obtained during the experiment had a normal distribution. The differences between the two indicators of the compared samples at $P < 0.05$, $P < 0.01$, and $P < 0.001$ were considered statistically substantial [29].

Results and Discussion

As a result of the study of the concentration of triacylglycerols in rat bile, it was determined that in animals of the control group it averages 3.01 ± 0.28 mg%. Therewith, their concentration in bile samples from animals with tetracycline-induced liver damage on average corresponded to values of 1.39 ± 0.28 mg%, which is 53.8% less than in the control. But in laboratory rats that received BAS “FLP-MD” against the background of experimental tetracycline liver damage, the content of triacylglycerols in bile samples averaged 2.83 ± 0.69 mg%, that is, it did not differ from the values of this indicator in bile samples of animals of the control group.

During the three-hour acute experiment, bile samples were taken every half an hour. This allowed examining the dynamics of changes in the concentration of triacylglycerols in rat bile during the entire acute experiment.

During the first hour of the experiment in two half-an-hour bile samples (No. 1 and No. 2) obtained from animals with tetracycline-induced liver damage (“Self-rehabilitation” group), the concentration of triacylglycerols was 1.60 ± 0.25 mg% (samples No. 1) and 1.62 ± 0.31 mg% (samples No. 2), which is 49.5% ($P < 0.001$) and 46.0% ($P < 0.001$) less than their value in the control – 3.18 ± 0.26 (samples No. 1) and 3.00 ± 0.12 (samples No. 2). Similar differences in the concentration of triacylglycerols in bile samples were also observed in rats between the “Self-rehabilitation” and “Correction” groups. Namely, in bile samples No. 1 in sick animals (“Self-rehabilitation” group), the concentration of triacylglycerols was 44.8% ($P < 0.01$) less than in the bile of rats that, in addition to tetracycline load, received a course of BAS “FLP-MD” (group “Correction”), which corresponded to the values of 2.90 ± 0.61 ($P < 0.01$, samples No. 1). In addition, in bile samples No. 2 obtained from animals of the “Self-rehabilitation” group, the concentration of triacylglycerols was 45.5% ($P < 0.01$) less than in rats of the “Correction” group (2.98 ± 0.77 , $P < 0.01$, samples No. 2). There were no statistically substantial differences between the concentration of triacylglycerols in bile samples No. 1 and No. 2 in animals of the control group and the “Correction” group (Fig. 1).

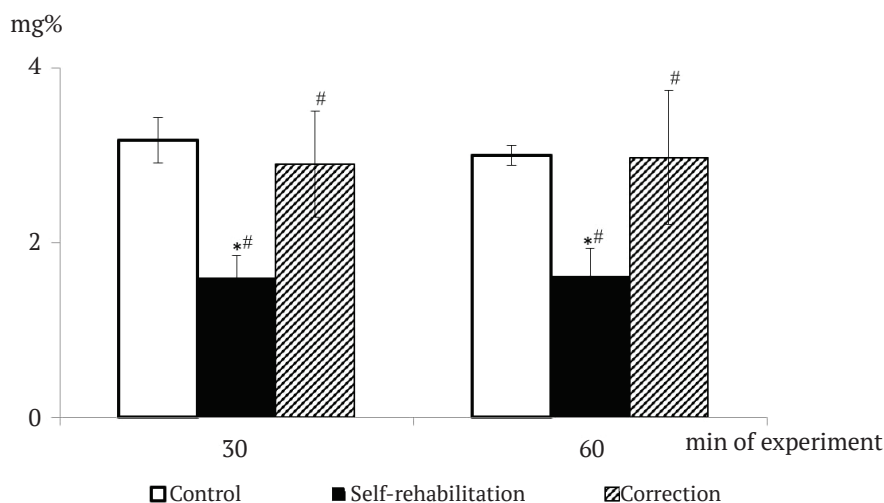


Figure 1. The concentration of triacylglycerols in bile samples of rats (“Control” group), with experimental hepatic steatosis (“Self-rehabilitation” group) and in sick animals that were additionally administered BAS “FLP-MD” (“Correction” group) obtained during the first hour of the acute experiment

Notes: * $P < 0.001$, a statistically substantial difference compared to the control; # $P < 0.01$, a statistically substantial difference between the indicators of the “Self-rehabilitation” and “Correction” groups

During the second hour of the acute experiment, a substantially lower concentration of triacylglycerols was recorded in bile samples (No. 3 and No. 4) in animals with experimental hepatic steatosis compared to the control indicators. Thus, in rats of the “Self-rehabilitation” group, the concentration of triacylglycerols in bile samples No. 3 and No. 4 was 1.46 ± 0.31 and 1.30 ± 0.25 mg%, respectively, which is 51.3% ($P < 0.001$) and 55.2% ($P < 0.001$)

lower than their values in the control (3.00 ± 0.28 mg% and 2.90 ± 0.22 mg%, respectively). The concentration of triacylglycerols in similar bile samples in animals of the “Self-rehabilitation” group (1.46 ± 0.31 mg% and 1.30 ± 0.25 mg%) and the “Correction” group (3.03 ± 0.89 mg% and 2.75 ± 0.70 mg%) differed upwards in the latter, respectively, by 51.8% ($P < 0.01$) and 55.7% ($P < 0.01$) (Fig. 2).

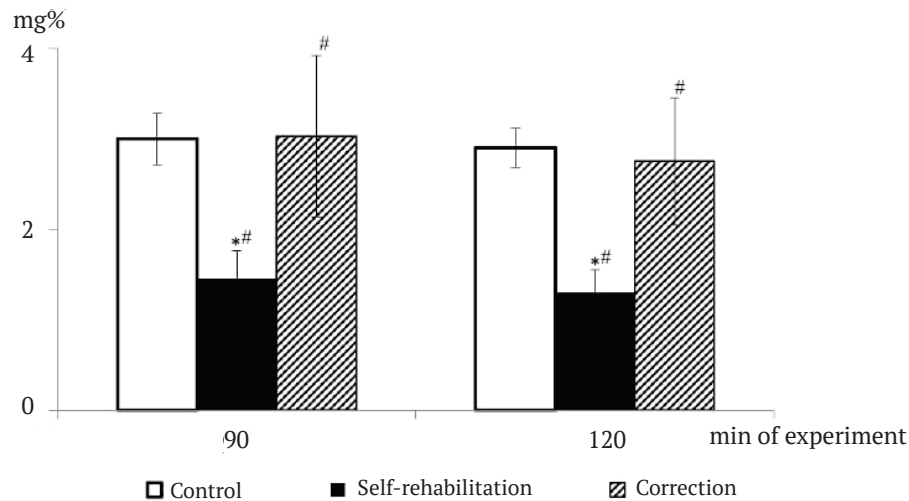


Figure 2. Concentration of triacylglycerols in bile samples of rats (“Control” group), with experimental hepatic steatosis (“Self-rehabilitation” group) and in sick animals that were additionally administered BAS “FLP-MD” (“Correction” group) obtained during the second hour of the acute experiment

Notes: * $P < 0.001$, a statistically substantial difference compared to the control; # $P < 0.01$, a statistically substantial difference between the indicators of the “Self-rehabilitation” and “Correction” groups

Notably, in comparison with the content of triacylglycerols in bile samples collected during the first and second hours of the acute experiment, further inhibition of their entry into the bile composition was observed. This may be due to insufficient activity of the corresponding transport systems of the canalicular domain of the plasma membrane of hepatocytes.

Thus, in bile samples taken during the third hour of acute experiment in rats with hepatic steatosis, the concentration of triacylglycerols remained substantially reduced compared to the corresponding indicators of the control group. In animals with hepatic steatosis (“Self-rehabilitation” group), it was 1.22 ± 0.29 mg% (samples No. 5) and

1.12 ± 0.26 mg% (samples No. 6) and was respectively lower by 58.6% ($P < 0.001$) and 63.0% ($P < 0.001$) than in the control, which corresponded to the values of 2.95 ± 0.33 mg% (samples No. 5) and 3.03 ± 0.50 mg% (samples No. 6). In animals that received a course of BAS “FLP-MD” under the conditions of modelling hepatic steatosis, the concentration of triacylglycerols was close to that in the control: 2.65 ± 0.53 mg% (samples No. 5) and 2.68 ± 0.61 mg% (samples No. 6). Therewith, the content of triacylglycerols in the bile of rats of the “Self-rehabilitation” group was characterised by values lower than those of the “Correction” group, respectively, by 54.0% ($P < 0.01$) in half-an-hour samples No. 5 and by 58.2% ($P < 0.01$) in half-an-hour samples No. 6 (Fig. 3).

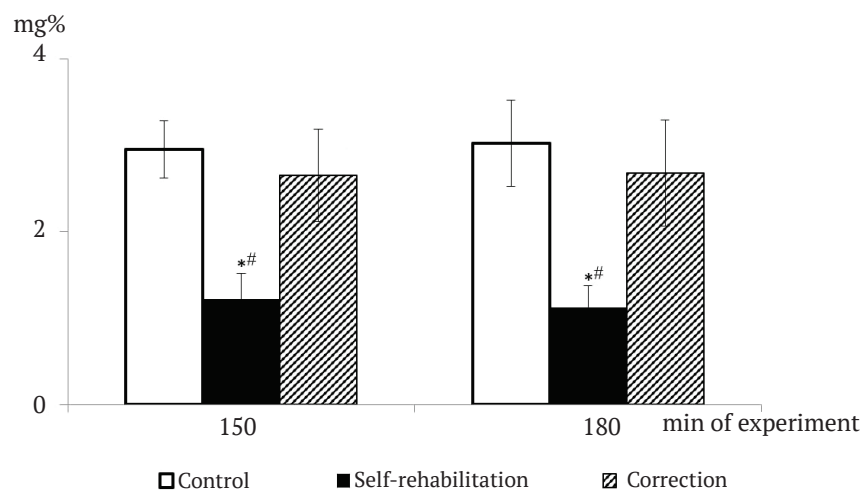


Figure 3. Concentration of triacylglycerols in bile samples of rats (control group), with experimental hepatic steatosis (“Self-rehabilitation” group) and in sick animals that were additionally administered BAS “FLP-MD” (“Correction” group), obtained during the third hour of an acute experiment

Notes: * $P < 0.001$, a statistically substantial difference compared to the control; # $P < 0.01$, a statistically substantial difference between the indicators of the “Self-rehabilitation” and “Correction” groups

Thus, under the conditions of experimental hepatic steatosis in experimental rats, a substantial decrease in the content of triacylglycerols in bile is observed. This indirectly indicates a disruption of the mechanisms of entry of triacylglycerols into the primary bile tubules with the accumulation of these lipids in liver cells and is one of the factors in the progression of fatty liver dystrophy. The use of the dietary supplement “FLP-MD” in animals under the conditions of modelling tetracycline-induced liver damage completely eliminates the negative effect of the applied toxic dose of the antibiotic on the structures of hepatocytes, which ensures the restoration of the processes of triacylglycerol intake to bile. Mainly, the corrective effect of phospholipid-containing dietary supplements is due to its membranotropic and membranoprotective properties [27]. It is known [30] that tetracycline in high doses has a cytotoxic effect on hepatocytes. Therewith, the result of this action is the development of fatty liver dystrophy, accompanied by the accumulation of triacylglycerols in liver cells [31]. This is the result of a disruption of the balance between lipid formation and catabolism, a decrease in the activity of mitochondrial beta-oxidation of fatty acids, an increase in the synthesis of endogenous fatty acids, and insufficient incorporation of triacylglycerols into low-density lipoproteins [4; 32]. In addition, due to the toxic effect of tetracycline on the body, activation of lipid peroxide oxidation reactions is also observed. This, in turn, leads to excessive formation of free radicals, covalent binding of electrophilic metabolites to proteins, a decrease in the content and oxidation of free glutathione [3].

Modelling of hepatic steatosis in rats also substantially

reduces the concentration of free fatty acids in bile. The average concentration of free fatty acids in the bile of animals with toxic liver damage (“Self-rehabilitation” group) was 14.70 ± 1.89 mg%. While the average concentration of free fatty acids in the bile of animals in the control group was 21.88 ± 3.47 mg%. Therewith, the average concentration of free fatty acids in the bile of animals that received BAS “FLP-MD” (“Correction” group) against the background of modelling tetracycline-induced liver damage corresponded to the values of 39.69 ± 2.90 mg%.

Considering these changes in stages, in the bile samples obtained from animals of the “Self-rehabilitation” group during the first hour of the acute experiment, the concentration of free fatty acids was 16.50 ± 1.82 mg% (samples No. 1) and 16.26 ± 1.29 mg% (samples No. 2), which is lower than in animals of the “Control” group by 23.3% (21.53 ± 3.85 , $P < 0.05$) and 21.4% (20.68 ± 3.55 , $P < 0.05$), respectively. The concentration of free fatty acids in the bile of rats of the “Correction” group corresponded to the values of 41.15 ± 3.64 mg% (samples No. 1) and 42.95 ± 3.64 mg% (samples No. 2), which is 91.2% ($P < 0.001$) and 107.7% ($P < 0.001$), respectively, more than in the control. Notably, the concentration of free fatty acids in the first two half-an-hour samples of bile of rats that received a dietary supplement under the conditions of modelling hepatic steatosis exceeded the concentration of these components in the bile of animals with hepatic steatosis without correction by 2.5 and 2.6 times, respectively (Fig. 4). This result indicates a pronounced corrective and choleric effect of the dietary supplement components on the hepatobiliary system of sick animals.

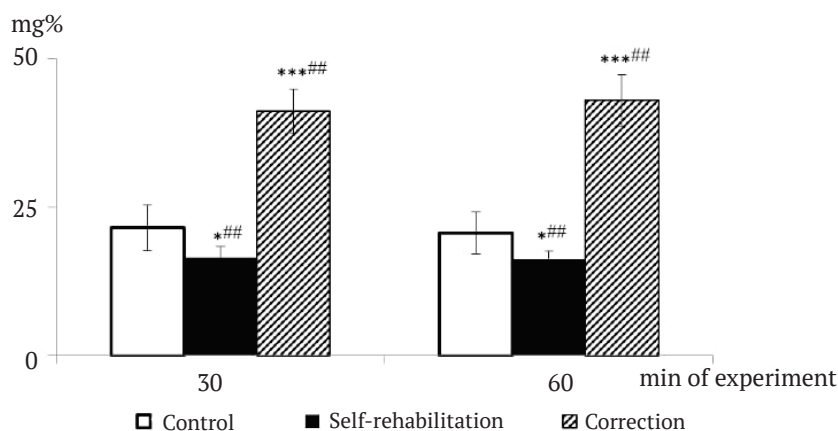


Figure 4. Concentration of free fatty acids in bile samples of rats (“Control” group), with experimental hepatic steatosis (“Self-rehabilitation” group) and in sick animals that were additionally administered BAS “FLP-MD” (“Correction” group) obtained during the first hour of the acute experiment

Notes: * $P < 0.05$ and *** $P < 0.001$ statistically substantial difference compared to the control; ## $P < 0.001$ statistically substantial difference between the indicators of the “Self-rehabilitation” and “Correction” groups

At the second hour of the acute experiment, animals with hepatic steatosis (“Self-rehabilitation” group) demonstrated a further decrease in the concentration of free fatty acids, respectively, by 26.9% ($P < 0.05$) and 34.6% ($P < 0.01$), compared with the control – 21.15 ± 3.51 mg% (samples No. 3) and 21.88 ± 3.16 mg% (samples No. 4), which corresponded to the values of 15.46 ± 1.77 mg% (in samples No. 3) and 14.30 ± 2.22 mg% (in samples No. 4). Whereas

in animals of the “Correction” group, the concentration of free fatty acids in bile was 42.28 ± 0.86 mg% (samples No. 3) and 39.13 ± 2.48 mg% (samples No. 4), which is 99.9% ($P < 0.001$) and 78.9% ($P < 0.001$) higher than in animals of the “Control” group, respectively. The difference in the content of free fatty acids in the bile of rats with hepatopathology (“Self-rehabilitation” group) and in rats who were treated with BAS “FLP-MD” (“Correction” group)

is even greater than during the first hour of the acute experiment. In particular, in both half-an-hour bile samples from animals of the “Self-rehabilitation” group, the

concentration of free fatty acids was 2.7 times ($P < 0.001$) lower than in similar samples from rats of the “Correction” group (Fig. 5).

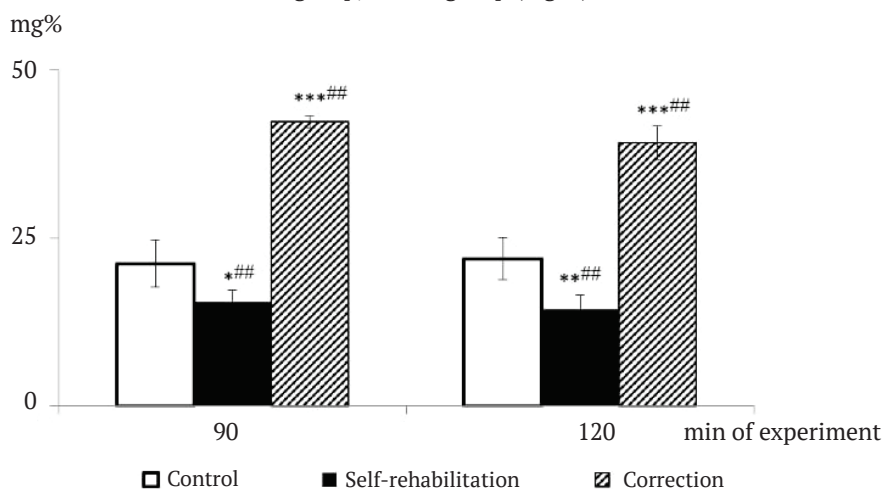


Figure 5. Concentration of free fatty acids in bile samples of rats (“Control” group), with experimental hepatic steatosis (“Self-rehabilitation” group) and in sick animals that were additionally administered BAS “FLP-MD” (“Correction group”) obtained during the second hour of the acute experiment

Notes: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ statistically substantial difference compared to the control; ## $P < 0.001$ statistically substantial difference between the indicators of the “Self-rehabilitation” and “Correction” groups

In the bile samples during the final third hour of the acute experiment, the most pronounced differences in the concentration of free fatty acids were identified between animals of the “Control” group and rats with experimental hepatic steatosis (“Self-rehabilitation” group), and between animals that received BAS “FLP-MD” (“Correction” group) during the simulation of hepatic steatosis, and rats with tetracycline-induced hepatic steatosis without the specified correction (“Self-rehabilitation” group). Thus, the concentration of free fatty acids in the bile of animals with experimental hepatic steatosis was 13.24 ± 2.17 mg% (samples No. 5) and 12.46 ± 2.08 mg% (samples No. 6), which, respectively, was 41.1% ($P < 0.01$) and 47.2% ($P < 0.001$) lower than their concentration in the control (22.48 ± 3.40 mg% (samples No. 5) and 23.60 ± 3.37 mg% (samples # 6). In animals of the “Correction” group, the concentration of free fatty acids in bile samples was

37.10 ± 2.85 mg% (samples No. 5) and 35.55 ± 3.28 mg% (samples No. 6), i.e. it was 65.1% ($P < 0.001$) and 50.6% ($P < 0.001$) higher than in rats in the “Control” group. It was at the end of the acute experiment that the most substantial differences between the concentration of free fatty acids in the “Self-rehabilitation” and “Correction” groups were observed in bile samples No. 5 and No. 6. In particular, in animals that, in addition to tetracycline for modelling hepatic steatosis, were administered BAS “FLP-MD” (“Correction” group), the concentration of free fatty acids in bile samples No. 5 was 2.80 times ($P < 0.001$) higher than in similar bile samples obtained from animals of the “Self-rehabilitation” group. In bile samples No. 6 from animals of the “Correction” group, the concentration of free fatty acids was 2.85 times ($P < 0.001$) higher than in the corresponding half-an-hour bile samples of rats of the “Self-rehabilitation” group (Fig. 6).

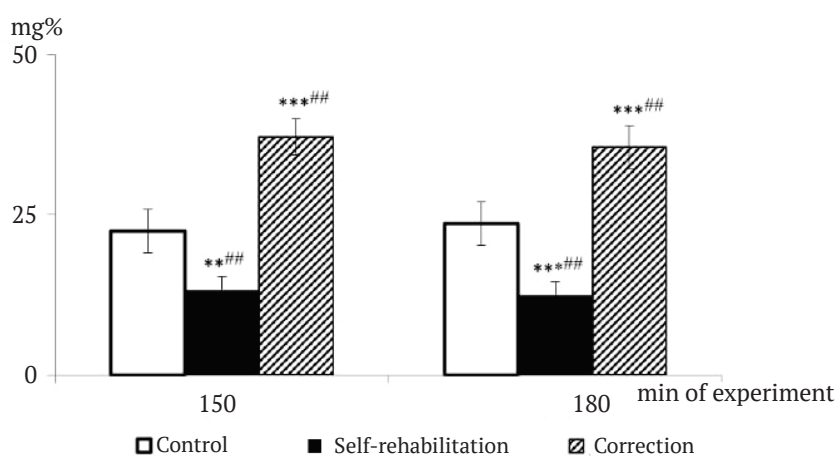


Figure 6. The concentration of free fatty acids in bile samples of rats (“Control” group), with experimental hepatic steatosis (“Self-rehabilitation” group) and in sick animals that were additionally administered BAS “FLP-MD” (“Correction” group), obtained during the second hour of the acute experiment

Notes: ** $P < 0.01$ and *** $P < 0.001$ statistically substantial difference compared to the control; ## $P < 0.001$ statistically substantial difference between the indicators of the “Self-rehabilitation” and “Correction” groups

The described changes in the quantitative characteristics of free fatty acids in bile samples obtained from animals of different groups are marked by trends similar to the dynamics of the content of triacylglycerols and also indicate a pronounced choleric effect of phospholipid-containing dietary supplement. This fact is especially important if the pathogenesis of hepatopathy indicates the development of intrahepatic cholestasis, which is also diagnosed with tetracycline-induced hepatosis, as described in the previous study [15].

A similar effect of phospholipid-containing drugs, regardless of the nature of the origin of the active substance, is noted in their works by other authors [7; 33]. Thus, according to the results obtained, the introduction of the dietary supplement "FLP-MD" (the main active substance – phospholipids of milk) to laboratory rats under experimental conditions allows not only successfully correcting the metabolic processes of the described lipid fractions disrupted by hepatic steatosis, but also substantially stimulating the mechanisms of their entry into bile.

Conclusions

Under the conditions of a drug-induced (tetracycline) form of hepatic steatosis in laboratory rats, inhibition of the processes that ensure the intake of triacylglycerols and free fatty acids in the bile is observed. The use of BAS "FLP-MD" (the main active substance is milk phospholipids) in rats simultaneously with the simulation of tetracycline-induced liver

damage identifies a pronounced corrective effect on their content in bile samples during the entire 3-hour study period. Therewith, the concentration of triacylglycerols in the bile of animals that received BAS "FLP-MD" against the background of the development of hepatic steatosis was restored to values characteristic of animals of the "Control" group. The use of dietary supplements in rats in parallel with the simulation of tetracycline-induced hepatic steatosis stimulates the excretion of free fatty acids in the bile and leads to an increase in their concentration in hepatic secretions compared to the control and group of sick animals for Self-rehabilitation.

Considering the substantial disruptions of lipid homeostasis in hepatic steatosis and the identified effects of BAS "FLP-MD" on the content of triacylglycerols and free fatty acids in the bile of sick animals, it is possible to assert the prospects of using this supplement to correct the metabolism of the described individual lipid fractions in conditions of drug-induced liver damage, primarily with tetracycline group antibiotics. Especially relevant is the use of phospholipid-containing drugs in hepatopathy, accompanied by the development of intrahepatic cholestasis. Since bile secretory processes and physico-chemical properties of bile largely depend on the secretion of phospholipids and cholates by hepatocytes to the primary bile tubules, an important aspect of further investigation will be the experimental determination of the effects of BAS "FLP-MD" on the phospholipid and bile-acid composition of bile in the normal state and hepatopathy.

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Корекція вмісту триацилгліцеролів і вільних жирних кислот у жовчі щурів за експериментального жирового гепатозу

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Анотація. Актуальність наукового дослідження пов'язана із значним поширенням жирового гепатозу в свійських тварин (до 40 %) та розвитком небезпечних для здоров'я ускладнень у вигляді цирозу печінки, печінкової недостатності та онкології. Метою цієї роботи було визначити коригувальну ефективність біодобавки «FLP-MD» на основі фосфоліпідів молока щодо вмісту триацилгліцеролів і вільних жирних кислот у жовчі щурів із тетрациклініндукованим жировим гепатозом. Моделювання медикаментозної форми жирового гепатозу здійснювали шляхом внутрішньошлункового введення 4 %-го розчину препарату тетрацикліну гідрохлориду з розрахунку 0,5 г/кг маси тіла тварини протягом семи діб. В якості коригувальної терапії впродовж дев'яти діб тваринам внутрішньошлунково вводили біодобавку «FLP-MD» на основі фосфоліпідів молока у дозі 13,5 мг/кг маси тіла. На завершенні експерименту впродовж трьох годин через кожні 30 хв у щурів відбирали зразки жовчі, в яких визначали вміст триацилгліцеролів і вільних жирних кислот методом тонкошарової хроматографії. Встановлено, що концентрація триацилгліцеролів у жовчі хворих щурів на третю годину її відбору на 63,0 % нижча за контрольні показники. У лабораторних щурів, які на тлі моделювання медикаментозного гепатозу отримували фосфоліпідовмісну біодобавку, цей показник у жовчі відповідав значенням контрольної групи. Водночас концентрація вільних жирних кислот у зразках жовчі на третю годину її відбору в хворих щурів відзначалася зниженням на 47,2 % порівняно з контролем. Застосування досліджуваної біодобавки у хворих тварин викликало зростання концентрації вільних жирних кислот у жовчі в 2,85 рази порівняно з контролем, що зменшує інтенсивність їх використання на синтез триацилгліцеролів та запобігає розвитку жирової інфільтрації печінки. Отже, фосфоліпідовмісна біодобавка є високоефективним коригувальним засобом за порушення обміну триацилгліцеролів і вільних жирних кислот у щурів із медикаментозним жировим гепатозом. Це дає підстави рекомендувати її в якості коригувальної терапії та для профілактики розвитку жирового гепатозу, особливо у випадку застосування тваринам антибіотиків тетрациклінової групи

Ключові слова: фосфоліпіди молока, біологічно активна добавка, тетрацикліну гідрохлорид, коригувальна терапія