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Blood lipid profile in rats with tetracycline-induced liver damage

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Abstract. Drug-induced liver injury leads to significant morbidity and mortality due to the development of severe complications, making it a leading cause of acute liver failure in animals. Among medications with direct cytotoxic effects on liver cells, tetracycline antibiotics are notable, as improper use can induce fatty liver disease. This study aimed to examine changes in the blood lipid profile of laboratory rats with tetracycline-induced liver damage, alongside assessing the corrective potential of milk phospholipids. The lipid composition of native blood was analysed using thin-layer chromatography. The findings indicate that, in cases of artificially induced tetracycline liver damage in rats, there was a deficit in all five indicators of the native blood lipid profile, including a 30.8% reduction in total lipids, a 45.3% decrease in triacylglycerols, a 37.0% decline in phospholipids, a 46.2% drop in free fatty acids, a 23.1% decrease in free cholesterol, and a 32.0% reduction in esterified cholesterol, compared to the control. This may be attributed to diminished appetite in affected animals, insufficient lipid absorption in the digestive tract, and the inhibition of endogenous lipid synthesis in the livers of affected rats. The administration of milk phospholipids as the dietary supplement "FLP-MD" to diseased animals demonstrated a pronounced corrective effect, manifested by the restoration of quantitative parameters across all studied lipid fractions and their overall content. Oral administration of this supplement to clinically healthy rats showed no toxic impact on liver cells or the organism as a whole. Additionally, a tendency towards an increase in the absolute content of most examined blood lipid fractions was observed. Thus, this experimental study revealed

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marker changes in the quantitative parameters of the native blood of the laboratory rats, lipid profile, which may be valuable in understanding the pathogenesis of tetracycline-induced injury in mammals and testing newly developed hepatoprotective agents

Keywords: corrective therapy; milk phospholipids; fatty liver disease; thin-layer chromatography; lipid fractions

Introduction

The liver plays a crucial role in regulating lipid metabolism, and any impairment of its functions leads to significant alterations in the lipid profile, which may serve as an early marker of toxicity. Studying the blood lipid profile in cases of tetracycline-induced liver damage provides a means to assess the extent of metabolic disturbances and aids in understanding the mechanisms underlying the development of fatty liver disease. This is vital not only for the diagnosis and monitoring of liver health but also for developing therapeutic approaches aimed at preventing and treating liver failure induced by toxic drug doses.

N. Stefan *et al.* (2019) emphasised that metabolic disorders in mammals lead to fatty liver disease, triggering numerous structural and functional disturbances, which subsequently progress to fibrosis, cirrhosis, and liver cancer. As well, K. Mohammadhasani *et al.* (2024) suggested that the pathogenic mechanisms underlying fatty liver degeneration are associated with various pathological factors, including drug-induced damage, cardiovascular conditions, extrahepatic complications, and diabetes. Implementing a regimen of active physical exercise, along with hypolipidaemic agents and insulin sensitisers, is considered, as noted by S. Pouwels *et al.* (2022), to be a potential therapeutic strategy for patients with fatty liver disease. However, these treatments demonstrate limited efficacy and are often accompanied by adverse effects. Consequently, there is a pressing need to develop new therapeutic approaches that minimise toxic effects on living organisms.

F. Zhang *et al.* (2022) identified that liver metabolites of a lipid nature, such as sphingomyelin and glycerophospholipid, exert a corrective effect in cases of toxic liver injury by regulating fatty acid synthesis, correcting lipid metabolism disorders, and reducing inflammatory responses. Furthermore, it was found that structural lipids within cell membranes help maintain stability in lipid metabolism, disruptions of which can lead to the development of fatty liver disease. The authors highlighted various types of membrane lipids, including their metabolites – phospholipids, cholesterol, and sphingolipids – whose dysregulation was implicated in the onset of fatty liver disease. Through the use of new drug-based artificial models involving laboratory animals, the role of certain lipid classes in the pathogenic mechanisms underlying hepatopathology, particularly fatty liver degeneration, has been demonstrated, confirming their potential use in treating fatty liver disease. As noted by X. Gao *et al.* (2024), most conventional therapies target pathways of lipid accumulation within liver cells, as well as inflammation and hepatocyte damage, which are typically consequences of metabolic stress in the context of steatosis. This highlights that fundamental research on pharmaceutical support for patients with fatty liver disease predominantly focuses on cellular mechanisms responsible for these pathophysiological outcomes in mammals.

T. Harayama & N. Riezman (2018) found that a primary disturbance in the development of fatty liver disease is the dysregulation of lipid metabolism, leading to intense deposition of cholesterol and fatty acids within hepatocytes, a phenomenon known as hepatic lipotoxicity. Lipid-based substances play a central role in maintaining numerous cellular functions and are considered essential structural components

of biomembranes. The researchers highlighted key lipid classes found in the cell membranes of eukaryotes: phospholipids, sterol lipids, sphingolipids, and cholesterol. In animals, phospholipid molecules contain one hydrophilic group and two hydrophobic acyl chains, with ceramides acting as primary precursors in sphingolipid formation, while cholesterol possesses a steroid backbone and serves as an important sterol component of cell membranes. H. Duan *et al.* (2024) noted that carbohydrates form complex compounds with proteins and lipids, resulting in glycoproteins and glycolipids, respectively. Membrane lipids are also critical in maintaining lipid homeostasis, energy storage, cell signalling, and providing a supportive environment for protein function. Additionally, L.Y. Swindale *et al.* (2023) emphasised the unique structural and functional characteristics of biomembranes, stemming from their distinct lipid organisation. Further research is needed to clarify the responses of various structural lipids during the progression of pathological processes, including fatty liver disease.

V.A. Gryshchenko *et al.* (2019) indicated that the liver plays a central role in lipid metabolism within mammalian organisms. Alongside hepatocytes, the primary functional cells, other liver cells, including hepatic stellate cells, cholangiocytes, and Kupffer cells, also contribute to maintaining homeostasis in animals, especially under conditions of liver pathology. These cells all eventually sustain damage due to fatty infiltration and lipotoxicity in the liver. Numerous experimental and clinical studies have shown that fatty liver degeneration arises in response to alterations in the intracellular lipid composition of the liver. Additionally, most studies, including those in pharmacology and genetics, underscore the importance of triacylglycerol accumulation as a key factor in the development of fatty liver disease. Given the complexity of its pathogenesis, the accumulation of triacylglycerols alone is insufficient for the development of this hepatopathy. Therefore, the authors highlighted the need for a novel research approach in lipid studies to determine the roles of specific lipid classes, including cholesterol, phospholipids, and sphingolipids, in the progression of fatty liver disease. For example, research by M. Jiang *et al.* (2019) demonstrates that obesity affects ceramide levels in the liver parenchyma, resulting in insulin resistance, steatosis, and hepatocyte necrosis.

The study by J.N. van der Veen *et al.* (2017) indicated that phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are among the most prevalent phospholipids in mammalian cells, primarily localised within membrane structures. The stability of the cell plasma membrane is maintained by preserving the balance between PC and PE. Consequently, any disturbance in membrane composition increases the susceptibility of hepatocyte plasma membranes to various pathological damages, including toxic lipid radicals, activators, and immune messengers, which can lead to inflammation, fatty liver disease, and cell necrosis. As noted by R.G. Joergensen (2022), substantial evidence supports the critical role of phospholipids in preventing and potentially reversing fatty liver disease. Thus, phospholipid-based therapies with hepatoprotective and reparative properties have become increasingly relevant in treating fatty liver degeneration, showing high efficacy in clinical applications. The aim of this study was, therefore, to assess the effect of milk phospholipids on the lipid composition of native blood in rats while simultaneously artificially inducing fatty liver disease using tetracycline hydrochloride as a provoking factor.

Materials and Methods

Laboratory studies were conducted in 2023 using the scientific facilities of the Faculty of Veterinary Medicine at the National University of Life and Environmental Sciences of Ukraine (Kyiv) and the Educational and Scientific Institute of High Technologies at Taras Shevchenko National University (Kyiv). The experimental work was carried out at the vivarium of the Educational and Scientific Institute of High Technologies at Taras Shevchenko National University (Kyiv), which is equipped with plumbing, drainage, ventilation, and air

conditioning. Regular cleaning with disinfectants was performed in the vivarium. Laboratory rats were housed in cages with wood shavings as bedding, which were replaced every two days.

Laboratory white rats weighing 200-250 g were used for the experiment. Consequently, four groups were formed (three experimental groups and one control group), each consisting of eight individuals. Each group of animals was housed in separate cages with free access to water and food. Prior to the commencement of the experiment, the animals were quarantined, during which their clinical condition was monitored. Throughout the experiment, observations were made regarding the amount of water consumed, appetite, and changes in body weight among the animals in all groups. The experimental studies were conducted over a period of nine days.

The conduct of scientific research involving laboratory animals adhered to the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (1986) and Law of Ukraine No. 3447-IV (2006). All planned procedures on the laboratory rats were performed following the ARRIVE guidelines and the fundamental principles of Council Directive 2010/63/EU (2010).

In the two experimental groups of rats ("Self-rehabilitation" and "Correction"), hepatosis was experimentally modelled using the antibacterial agent tetracycline hydrochloride, administered orally at a dose of 250 mg/kg of body weight as a solution once daily for 7 days (Gryshchenko *et al*., 2019). Notably, the rats in the first experimental group ("Self-rehabilitation") did not receive any treatment, whereas the laboratory animals in the second experimental group ("Correction") were given a 1% solution of milk phospholipids in the form of liposomes (the dietary supplement (DS) "FLP-MD") at a dosage of 13.5 mg/kg of body weight for 9 days. The third experimental group ("Healthy animals + DS "FLP-MD") consisted of clinically healthy rats that were administered the DS "FLP-MD" at the same dosage for 9 days. The control group comprised intact rats that were provided with an equivalent volume of distilled water. Each group included 8 animals.

On the 10th day, blood samples were obtained from rats in all groups for subsequent analysis of their lipid composition. Blood sampling was conducted under ether anaesthesia from the abdominal aorta. The lipid profile of the native blood was determined using thin-layer chromatography according to the methodology (Kostenko *et al*., 2001).

Lipid extraction from the plasma was performed using a one-phase system of organic solvents in the following ratio: chloroform:acetone:ethanol (7:2:1). The amount of extracting solvent was taken in a ratio of 20:1 to the sample. In a test tube, 1 mL of the specified solvent was added to the crushed chromatographic paper containing the blood samples, which was then left for 15 minutes in a shuttle apparatus. The resulting liquid was collected into a beaker, and an additional 0.5 cm^3 of the extracting liquid was added twice, with a 5-minute interval, in the shuttle apparatus. The obtained extract, containing the blood lipids, was subjected to analysis after the solvent had been evaporated.

The chromatographic separation of total lipids from native blood was conducted on thin-layer plates "Silufol" (15×15 cm), which were pre-activated in a thermostat at 110°C for one hour. During this time, filter paper was placed in the chromatographic chamber to enhance saturation, and a mixture of solvents – hexane:diethyl ether:acetic acid (7:23:1) – was added. The dry lipid residue was dissolved in chloroform:benzene:acetone mixture (1:2:1) in volumes ranging from 20 to 100 µL and then applied to the premarked chromatogram.

Over a period of 10-15 minutes, the blood lipids were separated on the plate into fractions: cholesterol esters, free cholesterol, triacylglycerols, free fatty acids, and phospholipids. The solvent was removed from the chromatogram in a fume hood. Subsequently, the chromatogram was stained with a 10% solution of phosphomolybdic acid in ethanol using a glass laboratory sprayer.

To visualise the lipid fractions, the stained chromatogram was placed in a thermostat set to 110°C. The quantitative assessment of blood lipids was performed using a densitometer model DO-1M (Ukraine).

Statistical analysis of the experimental data was conducted using the computer programme Statistica 5.0 (StatSoft Inc., USA), taking into account the Student's t-test under normal distribution of results. Differences were considered statistically significant at $P < 0.05$.

Results and Discussion

The chromatographic analysis of the lipid spectrum in the native blood samples from laboratory rats undergoing artificially induced tetracycline-induced hepatosis (first experimental group, "Self-rehabilitation") revealed that their qualitative composition included five lipid fractions: phospholipids, free fatty acids, free cholesterol, esterified cholesterol, and triacylglycerols. The total content of these fractions corresponded to the parameter for total lipids (Table 1).

Table 1. *Lipid spectrum of native blood in experimental rats (M ± m, n = 8, mg%)*

Parameter	Group "Control"	First experimental group "Self-rehabilitation"
Total lipids	639.43 ± 49.56	402.56 ± 31.80*
Phospholipids	374.21 ± 29.12	221.43 ± 20.94*
Free fatty acids	41.42 ± 2.61	22.30 ± 1.84*
Free cholesterol	96.11 ± 7.32	73.88 ± 3.21*
Esterified cholesterol	99.34 ± 8.13	67.54 ± 4.64*
Triacylglycerols	26.35 ± 2.38	14.41 ± 1.17*

Note: * – P<0.05 compared to the control group
Source: developed by the authors

During the development of tetracycline-induced hepatosis (first experimental group, "Self-rehabilitation"), a significant decrease in the total lipid content was observed in the native blood of affected rats, with a reduction of 37.0% compared to intact animals (control group) (Table 1). This trend may be attributed to a decreased appetite in the affected animals due to the overall intoxication resulting from the toxic effects of tetracycline hydrochloride. Additionally, it could be related to disorders in the absorption of dietary lipids in the intestine caused by the onset of cholestasis, which is characteristic of this clinical situation, and to disruptions in the molecular mechanisms of their endogenous synthesis in the liver cells, potentially stemming from a reduced energy potential in hepatocytes. As noted by P. Rada *et al.* (2020) and Y. Ni *et al.* (2023), most researchers' findings indicated that hepatic steatosis is primarily associated with alterations in the lipid composition within hepatocytes. Furthermore, according to the findings of numerous studies, including pharmacological and genetic research summarised in the scientific study of V.F. Williams *et al.* (2019), there is a prevailing consensus regarding the primary importance of the accumulation of triacylglycerols as a key etiological factor in the development of fatty hepatosis. However, as indicated by S.H. Park *et al.* (2020) and G. Targher *et al.* (2021), the pathophysiology of the drug-induced form of fatty hepatosis is exceedingly complex, and thus the accumulation of triacylglycerols alone is insufficient for the onset of this hepatic pathology. M. Welch *et al.* (2022) recommended a more in-depth investigation into the roles of various classes of lipids, particularly sphingolipids, phospholipids, and cholesterol, in the pathogenesis of fatty hepatosis.

In the case of artificially modelling drug-induced liver damage, disturbances were observed in the lipid profile of the native blood of affected rats (first experimental group, "Self-rehabilitation"), similar to the noted decrease in total lipid levels. In the rats undergoing self-rehabilitation (first experimental group), a significant reduction in the level of phospholipids in the native blood

was noted, amounting to 40.8% (Table 1), indicating the development of a deficient level of these lipids within the organisms of these animals. According to Y. Zhou *et al.* (2023), the liver serves as the primary site for the endogenous formation of phospholipids in mammals. Consequently, the established quantitative changes in phospholipids may be attributed to insufficient synthesis resulting from pathological alterations in liver function, as well as a concomitant reduction in the intensity of fatty acid utilisation for the formation of phospholipid molecules, along with shifts in the activity of relevant enzymes. Thus, as reported by M. Welch *et al.* (2022), the incorporation of fatty acids into phospholipid synthesis is hindered. At the same time, as noted by E. Calzada *et al.* (2016) and O. Ali & A. Szabó (2023), among all known phospholipids characteristic of animal organisms, the highest proportion is comprised of phosphatidylcholine and phosphatidylethanolamine, which dominate the structure of cellular membranes. Consequently, the stability of the structural organisation of the plasmalemma is maintained by the balance of these lipids, and any disruption of this balance can provoke the development of fatty liver disease. For instance, when the gene Pcyt1a, which encodes the enzyme limiting the intensity of phosphatidylcholine formation (CTP: phosphocholine cytidylyltransferase-α, or CTα), is blocked in the livers of mice, a sex- and diet-dependent effect on lipid transformation in the liver is observed, which can lead to hepatic steatosis. Overall, according to R.L. Jacobs *et al.* (2004), phosphatidylcholine plays a crucial role in the processes of triglyceride formation and secretion (very low-density lipoproteins, VLDLs), and an increase in the extracellular triglyceride content correlates with the development of liver steatosis and its fatty degeneration. Similar effects were noted by J. Li *et al.* (2023) in mice when the activity of phosphatidylethanolamine N-methyltransferase (PEMT−/−), which catalyses the unique hepatic pathway for phosphatidylcholine production, was inhibited. This inhibition resulted in a decreased molar ratio of phosphatidylcholine/phosphatidylethanolamine and an increase in triglyceride content in hepatocytes. Thus, abnormal alterations in the phospholipid composition of membrane structural organisation heighten the susceptibility of cellular membranes to various damages, including the effects of toxic lipids and activators, which initiate hepatic fat infiltration, the development of inflammatory responses, and cellular destruction.

In the native blood of the affected rats (first experimental group, "Self-rehabilitation"), deviations in the level of free fatty acids were also observed (Table 1). Specifically, a significant reduction in free fatty acid levels by 46.2% was noted compared to that in intact animals (the "Control" group), which was likewise reflected in the overall lipid levels in the blood. Furthermore, in line with findings from previous research (Gryshchenko *et al.,* 2019) on the fatty acid composition of hepatic lipids under drug-induced liver damage from toxic doses of this antibiotic, polyunsaturated fatty acids (PUFAs) were primarily affected. Consequently, the diseased animals exhibited a deficient level of eicosapentaenoic (20:5ω3), docosapentaenoic (22:5ω3), and docosahexaenoic (22:6ω3) acids, along with a decrease in the total saturated fatty acids (SFA) and an alteration in the ratio of saturated to unsaturated fatty acids by a factor of 1.3, as well as the ω3/ω6 ratio by 1.3.

The molecular mechanisms underlying deviations in the quantitative parameters of fatty acid composition in response to tetracycline-induced liver pathology remain insufficiently understood. The specifics of fatty acid intermediary metabolism in living organisms depend on the structural organisation of the tissue. Additionally, gene activity is influenced not only by fatty acids but also by other endocrine, paracrine, and autocrine factors within the internal environment, exemplifying the broader integration of metabolic processes.

Furthermore, as highlighted in the research by V.F. Williams *et al.* (2019), the liver is also the primary site for endogenous cholesterol synthesis in mammalian organisms. Consequently, liver

pathology is likely to lead to deviations in this sterol's concentration in the bloodstream. It is well-established that cholesterol metabolism involves over 300 proteins with enzymatic activity dependent on liver function. As noted by A.S. Mutlu *et al.* (2021), cholesterol, like phospholipids, is an essential structural component of biological membranes in mammals, playing a crucial role in transmembrane processes and signal transmission. Cholesterol synthesis occurs in the cytosol and endoplasmic reticulum of cells through the mevalonate pathway, using active acetate. 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) is the rate-limiting enzyme for steroid biosynthesis. In addition, cholesterol esters are formed through the catalytic action of acyl-CoA acyl-transferase (ACAT), which limits the accumulation of free cholesterol in blood plasma and cellular membranes. Maintaining cholesterol homeostasis across the organism and within cells is a regulated process that prevents excessive accumulation or deficiency. The liver plays a central role in regulating blood plasma cholesterol levels via a complex metabolic pathway involving sterol transporters, nuclear and lipoprotein receptors, and transcriptional gene expression.

In rats with tetracycline-induced hepatosis (first experimental group, "Self-rehabilitation"), a significant reduction was observed in native blood levels of both free cholesterol (by 23.1%) and esterified cholesterol (by 32.0%) compared to the control group (Table 1). This finding effectively demonstrates disruptions in cholesterol biosynthesis within hepatocytes and other tissues, as well as certain impairments in the formation of esterified cholesterol and the corresponding lipoprotein complexes. According to P. Malhotra *et al.* (2019), disturbances in cholesterol metabolic pathways that govern its transport, synthesis, and conversion play a critical role in the development of hepatopathologies. Low-density and very-low-density lipoproteins, which are particularly rich in cholesterol, are primarily synthesised in the liver. Z. Li *et al.* (2023) suggested that the non-inflammatory intracellular accumulation of fat may result from an imbalance between lipid distribution and clearance from the liver. In turn, as noted by J. Aron-Wisnewsky *et al.* (2020), this imbalance can eventually lead to fibrosis, cirrhosis, and hepatocellular carcinoma.

Furthermore, in the native blood of rats with tetracycline-induced hepatosis (first experimental group, "Self-rehabilitation"), the triacylglycerol content decreased by 45.3% compared to the control group (Table 1). While these lipids hold limited diagnostic value, researchers such as P. Rada *et al.* (2020) and S. Lang & B. Schnabl (2020) have highlighted their importance as a metabolic fuel reserve for the organism. Consequently, analysis of triacylglycerol levels is commonly applied in laboratory diagnostics to study related animal diseases.

Therefore, as a result of a detailed analysis of the lipid profile of native blood in rats with experimental tetracycline-induced hepatitis (first experimental group, "Self-rehabilitation"), the formation of a deficient level of both total lipids and individual lipid fractions was established. The identified patterns of the native blood lipid profile in diseased animals may be the result of a decrease in appetite, insufficient digestion and absorption of lipids in the gastrointestinal tract, intensive energy expenditure by the organism, rapid depletion of lipid depots, impaired synthesis and utilisation of lipid molecules in hepatocytes, etc.

When rats were orally administered milk phospholipids in the form of the DS "FLP-MD" in a liposomal form (second experimental group, "Correction"), a restoration of total lipid content in native blood was observed, reaching the levels of intact animals (Table 2).

Table 2. *Lipid spectrum of native blood in experimental rats (M ± m, n = 8, mg%)*

Parameter	Group "Control"	Second experimental group "Correction"	Third experimental group "Healthy animals + DS "FLP-MD"
Total lipids	639.43 ± 49.56	607.90 ± 43.13	685.53 ± 51.82

Table 2. *Continued*

Parameter	Group "Control"	Second experimental group "Correction"	Third experimental group "Healthy animals + DS "FLP-MD"
Phospholipids	374.21 ± 29.12	355.31 ± 30.10	401.03 ± 27.23
Free fatty acids	41.42 ± 2.61	40.83 ± 2.32	46.93 ± 4.55
Free cholesterol	96.11 ± 7.32	90.12 ± 3.86	102.13 ± 9.12
Esterified cholesterol	99.34 ± 8.13	93.81 ± 4.33	107.58 ± 8.43
Triacylglycerols	26.35 ± 2.38	27.83 ± 2.52	27.86 ± 2.49

Note: P < 0.05 compared to the control group
Source: developed by the authors

This finding can be attributed to improved appetite in the experimental animals, which clinically distinguished them from the diseased rats of the first experimental group ("Self-rehabilitation"), as a result of the gradual restoration of exogenous lipid absorption processes in the intestine and their endogenous synthesis in hepatocytes. At the same time, the administration of milk phospholipids as a dietary supplement to clinically healthy animals led to a tendency towards an increase in total lipid content compared to the control. This research result is explained by the additional introduction of exogenous lipids (primarily phospholipids) contained in the dietary supplement into the organism of the experimental animals.

It should be noted that the phospholipid content in the native blood of rats in the second experimental group "Correction" and the third experimental group "Healthy animals + DS "FLP MD" (Table 2), which additionally received the dietary supplement, was characterised by normalisation of their parameters, even in animals with modelled tetracycline-induced hepatitis. In clinically healthy rats of the third experimental group "Healthy animals + DS "FLP MD", this indicator even showed a tendency to increase, which may indicate the effective absorption of exogenous phospholipids in the gastrointestinal tract. Similarly to the previously described indicators in the native blood of rats in both experimental groups, a lack of significant quantitative changes in free fatty acids was established. This confirms the corrective effect of the supplement on the intermediate stages of fatty acid metabolism in the experimental animals.

When determining the patterns of quantitative changes in individual cholesterol fractions in the native blood of the experimental rats, a normalisation of their content was established in animals of the second experimental group ("Correction") and a tendency towards an increase in the values of these indicators was observed in animals of the third experimental group ("Healthy animals + DS "FLP-MD") (Table 2) compared to the control. The established pattern may be the result of their additional intake into the body as part of the dietary supplement and activation of the intermediate metabolism of this steroid in the internal organs and tissues of clinically healthy rats. The established fact also indirectly indicates the detection of hepatoprotective effects of the phospholipid-containing dietary supplement "FLP-MD" and the absence of its toxic effect on liver cells. In the study of D.O. Melnychuk *et al.* (2014), the membrane-tropic and hepatoprotective properties of milk phospholipids were also described, which have a stabilising effect on the indicators of pigment metabolism during artificial irradiation of rats and the introduction of cadmium compounds into their organism, which was confirmed by a significant improvement in the functional state of the hepatobiliary system.

As with the previously discussed lipid profile parameters, a similar pattern was observed regarding the triacylglycerol content in the native blood of rats in the second experimental group "Correction" and the third experimental group "Healthy animals + DS "FLP-MD" (Table 2). Triacylglycerols are present in a small amount in the

dietary supplement, which may also contribute to the restoration of the pool of these substances, which are important for energy-supplying synthetic processes in the organism of diseased animals. The established results of the blood lipid profile correspond to the parameters in the animals of the control group, which, in combination with the results of other studies, suggests that the absence of a hepatotoxic effect of the components of the phospholipid-containing dietary supplement on liver cells and the organism as a whole, as well as its pronounced corrective effectiveness.

Therefore, as a result of an in-depth analysis of the lipid spectrum of native blood in laboratory rats with experimentally modelled tetracycline-induced hepatitis and the administration of the DS "FLP-MD" to both diseased and clinically healthy animals, an effective restoration of the content of total lipids and their individual fractions was established. This indicates the ability of milk phospholipids within the dietary supplement to exert a corrective and hepatoprotective effect in the event of drug-induced hepatopathology in animals.

Conclusions

This study presents a laboratory evaluation of the lipid composition of native blood in laboratory rats with experimentally modelled tetracycline-induced hepatitis and oral administration of milk phospholipids in the form of the DS "FLP-MD". The use of thin-layer chromatography allowed for the separation of total lipids in native blood into individual fractions in laboratory rats with modelled tetracycline-induced hepatitis. It was established that their qualitative composition remained unchanged and contained five classical fractions: phospholipids, free fatty acids, triacylglycerols, free and esterified forms of cholesterol. At the same time, the lipid composition of native blood in laboratory rats with experimental tetracycline-induced hepatitis (first experimental group "Self-rehabilitation") was characterised by a decrease in the content of both total lipids and individual components of the lipid profile. In particular, there was a deficit in total lipids – by 30.8%, phospholipids – by 37.0%, free fatty acids – by 46.2%, triacylglycerols – by 45.3%, free cholesterol – by 23.1% and esterified cholesterol by 32.0% compared to the corresponding control. The established disturbances in the lipid profile of native blood in diseased animals may be caused by appetite disorders in these patients, as well as insufficient absorption of dietary lipids at the level of the intestine, inhibition of the activity of specific enzymatic systems and the associated endogenous synthesis of them in liver cells.

Administration of the DS "FLP-MD" to diseased rats resulted in the restoration of both total lipid levels and individual fractions, demonstrating the pronounced corrective and hepatoprotective effects of the components of the milk phospholipid-based dietary supplement. Concurrently, the administration of the phospholipid-containing dietary supplement to clinically healthy laboratory rats was accompanied by the effective restoration of all lipid fractions and their total content. This effectively indicates the absence of a toxic effect of the dietary supplement components on the organism of laboratory rats, as well as a pronounced corrective and hepatoprotective effect, which allows recommending specified DS for use in diseased animals in cases of drug-induced hepatopathology. In addition, the use of thin-layer chromatography can be recommended for effective laboratory diagnosis of lipid metabolism disorders in the organism of diseased animals during the development of drug-induced hepatopathy and the determination of the pathogenesis of internal diseases, in particular, the liver at the molecular level.

In the future, it is planned to conduct studies of the fractional composition of blood proteins during the artificial modelling of drug-induced hepatopathology against the background of simultaneous determination of the effectiveness of corrective therapy using laboratory animals.

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None.

Conflict of Interest

None.

References

[1] Ali, O., & Szabó, A. (2023). Review of eukaryote cellular membrane lipid composition, with special attention to the fatty acids. *International Journal of Molecular Sciences*, 24(21), article number 15693. doi: 10.3390/ijms242115693.

[2] Aron-Wisnewsky, J., Warmbrunn, M.V., Nieuwdorp, M., & Clément, K. (2020). Nonalcoholic fatty liver disease: Modulating gut microbiota to improve severity? *Gastroenterology*, 158(7), 1881-1898. doi: 10.1053/j.gastro.2020.01.049.

[3] Calzada, E., Onguka, O., & Claypool, S.M. (2016). Phosphatidylethanolamine metabolism in health and disease. *International Review of Cell and Molecular Biology*, 321, 29-88. doi: 10.1016/bs.ircmb.2015.10.001.

[4] Council Directive 2010/63/EU "On the Protection of Animals Used for Scientific Purposes". (2010, September). Retrieved from https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF.

[5] Duan, H., Song, S., Li, R., Hu, S., Zhuang, S., Liu, S., Li, X., & Gao, W. (2024). Strategy for treating MAFLD: Electroacupuncture alleviates hepatic steatosis and fibrosis by enhancing AMPK mediated glycolipid metabolism and autophagy in T2DM rats. *Diabetology & Metabolic Syndrome*, 16(1), article number 218. doi: 10.1186/s13098-024-01432-7.

[6] European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes. (1986, March). Retrieved from https://zakon.rada.gov.ua/go/994_137.

[7] Gao, X., Zhu, Z., Bao, Y., Li, Y., Zhu, W., He, X., Ge, X., Huang, W., Wang, H., Wei, W., Du, J., Chen, L., Li, H., & Sheng, L. (2024). *Chrysanthemum morifolium* Ramat extract and probiotics combination ameliorates metabolic disorders through regulating gut microbiota and PPARα subcellular localization. *Chinese Medicine*, 19(1), article number 76. doi: 10.1186/s13020-024-00950-w.

[8] Gryshchenko, V., Danchenko, O., & Musiychuk, V. (2019). Modification of modeling method of toxic dystrophy of liver in rats. In V. Nadykto (Ed.), *Modern development paths of agricultural production* (pp. 689-697). Cham: Springer. doi: 10.1007/978-3-030-14918-5_67.

[9] Harayama, T., & Riezman, H. (2018). Understanding the diversity of membrane lipid composition. *Nature Reviews. Molecular Cell Biology*, 19(5), 281-296. doi: 10.1038/nrm.2017.138.

[10] Jacobs, R.L., Devlin, C., Tabas, I., & Vance, D.E. (2004). Targeted deletion of hepatic CTP:phosphocholine cytidylyltransferase alpha in mice decreases plasma high density and very low density lipoproteins. *The Journal of Biological Chemistry*, 279(45), 47402-47410. doi: 10.1074/jbc.M404027200.

[11] Jiang, M., Li, C., Liu, Q., Wang, A., & Lei, M. (2019). Inhibiting ceramide synthesis attenuates hepatic steatosis and fibrosis in rats with non-alcoholic fatty liver disease. *Frontiers in Endocrinology*, 10, article number 665. doi: 10.3389/fendo.2019.00665.

[12] Joergensen, R. G. (2022). Phospholipid fatty acids in soil-drawbacks and future prospects. *Biology and Fertility of Soils,* 58, 1-6. doi: 10.1007/s00374-021-01613-w.

[13] Kostenko, S.I., Liashchenko, P.S., Gorenko, Z.A., Kurovska, L.F., & Veselskyi, S.P. (2001). *Patent No. 33564 "The Method of Preparing Samples of Biofluids for Determining the Content of Substances of a Lipid Nature"*. Retrieved from http://surl.li/tfkmdq.

[14] Lang, S., & Schnabl, B. (2020). Microbiota and fatty liver disease-the known, the unknown, and the future. *Cell Host & Microbe*, 28(2), 233-244. doi: 10.1016/j.chom.2020.07.007.

[15] Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty". (2006, February). Retrieved from https://zakon.rada.gov.ua/go/3447-15.

[16] Li, J., Xin, Y., Li, J., Chen, H., & Li, H. (2023). Phosphatidylethanolamine N-methyltransferase: From functions to diseases. *Aging and Disease*, 14(3), 879-891. doi: 10.14336/AD.2022.1025.

[17] Li, Z., Zheng, D., Zhang, T., Ruan, S., Li, N., Yu, Y., Peng, Y., & Wang, D. (2023). The roles of nuclear receptors in cholesterol metabolism and reverse cholesterol transport in nonalcoholic fatty liver disease. *Hepatology Communications*, 8(1), article number e0343. doi: 10.1097/HC9.0000000000000343.

[18] Malhotra, P., Gill, R.K., Saksena, S., & Alrefai, W. A. (2020). Disturbances in cholesterol homeostasis and non-alcoholic fatty liver diseases. *Frontiers in Medicine*, 7, article number 467. doi: 10.3389/fmed.2020.00467.

[19] Melnychuk, D.O., Hryshchenko, V.A., & Veselskyi, S.P. (2014). Indicators of the metabolism of bile pigments under the conditions of the effect of ecopathogenic factors on the body and after correction with liposomes. *The Ukrainian Biochemical Journal*, 86(3), 125-132. doi: 10.15407/ubj86.03.125.

[20] Mohammadhasani, K., Vahedi Fard, M., Mottaghi Moghaddam Shahri, A., & Khorasanchi, Z. (2024). Polyphenols improve non-alcoholic fatty liver disease via gut microbiota: A comprehensive review. *Food Science & Nutrition*, 12(8), 5341-5356. doi: 10.1002/fsn3.4178.

[21] Mutlu, A.S., Duffy, J., & Wang, M.C. (2021). Lipid metabolism and lipid signals in aging and longevity. *Developmental Cell*, 56(10), 1394-1407. doi: 10.1016/j.devcel.2021.03.034.

[22] Ni, Y., Wang, X., Wu, Q., Yao, Y., Xu, Y., Li, Y., Feng, Q., Zhou, M., & Gou, X. (2023). Qushi Huayu decoction ameliorates non-alcoholic fatty liver disease in rats by modulating gut microbiota and serum lipids. *Frontiers in Endocrinology*, 14, article number 1272214. doi: 10.3389/fendo.2023.1272214.

[23] Park, S.H., Lee, J.E., Lee, S.M., Lee, J., Seo, C.S., Hwang, G.S., & Jung, J. (2020). An unbiased lipidomics approach identifies key lipid molecules as potential therapeutic targets of Dohongsamul-tang against non-alcoholic fatty liver diseases in a mouse model of obesity. *Journal of Ethnopharmacology*, 260, article number 112999. doi: 10.1016/j.jep.2020.112999.

[24] Pouwels, S., Sakran, N., Graham, Y., Leal, A., Pintar, T., Yang, W., Kassir, R., Singhal, R., Mahawar, K., & Ramnarain, D. (2022). Non-alcoholic fatty liver disease (NAFLD): A review of pathophysiology, clinical management and effects of weight loss. *BMC Endocrine Disorders*, 22(1), article number 63. doi: 10.1186/s12902-022-00980-1.

[25] Rada, P., González-Rodríguez, Á., García-Monzón, C., & Valverde, Á.M. (2020). Understanding lipotoxicity in NAFLD pathogenesis: Is CD36 a key driver? *Cell Death & Disease*, 11(9), article number 802. doi: 10.1038/s41419-020-03003-w.

[26] Stefan, N., Häring, H.U., & Cusi, K. (2019). Non-alcoholic fatty liver disease: Causes, diagnosis, cardiometabolic consequences, and treatment strategies. *The Lancet. Diabetes & Endocrinology*, 7(4), 313-324. doi: 10.1016/S2213-8587(18)30154-2.

[27] Swindale, L.Y., Stevens, A.J., Whalley, S.J., Currie, R.A., Jessop, K.M., Lai, F., Lim, P.P., McInnes, E.F., Cowie, D., & Kende, A. (2023). Predictive liver lipid biomarker signature of Acetyl-coenzyme A carboxylase inhibitor related developmental toxicity in non-pregnant female Han Wistar rats – lipidomics biomarker discovery and validation. *Toxicology Letters*, 380, 62-68. doi: 10.1016/j.toxlet.2023.03.009.

[28] Targher, G., Tilg, H., & Byrne, C.D. (2021). Non-alcoholic fatty liver disease: A multisystem disease

requiring a multidisciplinary and holistic approach. *The Lancet. Gastroenterology & Hepatology*, 6(7), 578-588. doi: 10.1016/S2468-1253(21)00020-0.

[29] van der Veen, J.N., Kennelly, J.P., Wan, S., Vance, J.E., Vance, D.E., & Jacobs, R.L. (2017). The critical role of phosphatidylcholine and phosphatidylethanolamine metabolism in health and disease. *Biochimica et Biophysica Acta. Biomembranes*, 1859(9(B)), 1558-1572. doi: 10.1016/j.bbamem.2017.04.006.

[30] Welch, M., Secunda, C., Ghimire, N., Martinez, I., Mathus, A., Patel, U., Bhogoju, S., Al-Mutairi, M., Min, K., & Lawan, A. (2022). Characterization and roles of membrane lipids in fatty liver disease. *Membranes*, 12(4), article number 410. doi: 10.3390/membranes12040410.

[31] Williams, V.F., Taubman, S.B., & Stahlman, S. (2019). Non-alcoholic fatty liver disease (NAFLD), active component, U.S. Armed Forces, 2000-2017. *Medical Surveillance Monthly Report*, 26(1), 2-11.

[32] Zhang, F., Wu, J., Ruan, H., Xia, J., Xue, H., Wang, J., Li, Q., & Shi, Y. (2022). ZeXie decoction alleviates non-alcoholic fatty liver disease in rats: The study of genes, lipids, and gut microbiotas. *Biochemical and Biophysical Research Communications*, 632, 129-138. doi: 10.1016/j.bbrc.2022.09.097.

[33] Zhou, Y., Dai, Z., Deng, K., Wang, Y., Ying, J., Chu, D., Zhou, J., & Tang, C. (2023). Eight Zhes Decoction ameliorates the lipid dysfunction of nonalcoholic fatty liver disease using integrated lipidomics, network pharmacology and pharmacokinetics. *Journal of Pharmaceutical Analysis*, 13(9), 1058-1069. doi: 10.1016/j.jpha.2023.05.012.

Ліпідограма крові в щурів за тетрациклінового ураження печінки

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Анотація. Медикаментозно зумовлене ураження печінки спричинює значну кількість захворювань та смертності внаслідок розвитку небезпечних ускладнень, залишаючи його провідною причиною гострої печінкової недостатності в тварин. Серед препаратів з прямим цитотоксичним ефектом впливу на клітини печінки виділяють антибіотики тетрациклінової групи, некоректне застосування яких здатне викликати жировий гепатоз. Тому метою роботи було дослідити закономірності щодо змін у ліпідограмі нативної крові в лабораторних щурів за тетрациклінового ураження печінки з одночасним визначенням коригувальної здатності фосфоліпідів молока. Ліпідний склад нативної крові досліджували з використанням методу тонкошарової хроматографії. Встановлено, що у разі штучного моделювання в щурів тетрациклінового ураження печінки відмічали формування дефіцитного рівня усіх п'яти показників ліпідограми нативної крові, зокрема, зменшення вмісту загальних ліпідів – на 30,8 %, триацилгліцеролів – на 45,3 %, фосфоліпідів – на 37,0 %, вільних жирних кислот – на 46,2 %, вільного холестеролу – на 23,1 % та естерифікованого холестеролу на 32,0 % порівняно з відповідним контролем. Це можна пояснити погіршенням у хворих тварин апетиту, недостатнім засвоєнням ліпідів у травному каналі та інгібуванням їх ендогенного синтезу в печінці хворих щурів. Застосування хворим тваринам фосфоліпідів молока у вигляді біодобавки «FLP-MD» виявляло виражений коригувальний ефект, що проявлялося у відновленні кількісних параметрів усіх досліджуваних ліпідних фракцій та їх загального вмісту. У разі перорального введення цієї біодобавки клінічно здоровим щурам відмічали відсутність токсичного впливу її компонентів як на клітини печінки, так і на весь організм. При цьому відмічено тенденцією до зростання абсолютного вмісту більшості з досліджуваних фракцій загальних ліпідів крові. Таким чином, в результаті експериментального дослідження розкрито маркерні зміни ліпідограми нативної крові в лабораторних щурів, що може бути корисним у визначенні патогенезу тетрациклінового ураження в ссавців та при випробуванні новостворених лікарських засобів гепатопротекторної дії

Ключові слова: коригувальна терапія; фосфоліпіди молока; жировий гепатоз; тонкошарова хроматографія; фракції ліпідів