PharmacologyOnLine

Archives • 2021 • vol.3 • 1705-1716

THE EFFECT OF A COMPLEX PHARMACEUTICAL COMPOSITION AT LIPID METABOLISM AND LIVER STATUS OF RATS UNDER CONDITIONS OF EXPERIMENTAL METABOLIC SYNDROME

Mishchenko O. Ya., Dukhnich N. Yu., Larianovska Yu. B., *Kalko K. O., Berezniakov A. V.

National University of Pharmacy, Kharkiv, Ukraine
*ketrin27kalko@gmail.com

Abstract

Among the population of the world the number of patients with non-alcoholic fatty liver disease (NAFLD) is growing along with the epidemic of obesity and metabolic syndrome. Hepatic steatosis is a consequence of the accumulation of lipids in the liver tissue.

The experimental model of metabolic syndrome in rats was caused by a high content of carbohydrates and fats in the diet by enriching the diet with fructose (adding fructose to feed and replacing drink with 10.0% fructose solution - a total of up to 20.0% of daily calories) and animal fats (lard and fat in total in the diet up to 20.0% of daily calories) for 18 weeks.

Thus, it was found that CPC has a positive effect on lipid profile (decrease in TG, VLDL cholesterol, LDL cholesterol and increase in HDL cholesterol) and in terms of its effect on TG and HDL cholesterol, it is superior to vitamin E and metformin. CPC also has some protective effect on the manifestations of hepatic steatosis: there was a decrease in the prevalence and severity of fatty degeneration of hepatocytes. It was found that CPC in terms of the severity of "antisteatotic" activity is superior to the comparison drug vitamin E and inferior to the comparison drug metformin.

Keywords: *complex pharmaceutical composition, lipid metabolism, liver status, experimental metabolic syndrome*

Introduction

Among the population of the world the number of patients with non-alcoholic fatty liver disease (NAFLD) is growing along with the epidemic of obesity and metabolic syndrome [1, 33, 16, 2, 14]. Hepatic steatosis is a consequence of the accumulation of lipids in the liver tissue. Pathological progression of NAFLD is a consequence of «three strokes», namely steatosis, lipotoxicity and inflammation [36, 30, 32]. NAFLD is diagnosed in the presence of steatosis in more than 5% of hepatocytes [19, 40]. NAFLD is considered as a manifestation of metabolic syndrome in the liver, and its development and progression is influenced by the complex interaction of environmental and genetic factors [8, 28, 31, 29].

At the same time, the question of the use of vitamins, vitamin-like substances and microelements by patients with NAFLD and other non-infectious liver diseases is debatable today [27, 21, 6]. Given that previous studies have already established a positive effect of a complex pharmaceutical composition (CPC) (Aevit Premium production Joint-Stock Company «Kyiv Vitamin Factory») of next composition: ethyl esters of Omega-3 acids - 280 mg; vitamin E - 65 mg; coenzyme Q10 - 30 mg; zinc (as a part of zinc oxide) (15 mg; vitamin A - 1765 mcg; biotin - 150 mcg; selenium (as part of sodium selenite) - 100 mg and excipients) [3] at the histological structure of pancreatic tissue [9].

The aim of this work was to study the effect of CPC at lipid metabolism and liver status in experimental metabolic syndrome in rats induced by a diet high in carbohydrates and fats in the diet.

Materials and Methods

The experimental model of metabolic syndrome in rats was caused by a high content of carbohydrates and fats in the diet by enriching the diet with fructose (adding fructose to feed and replacing drink with 10.0% fructose solution - a total of up to 20.0% of daily calories) and animal fats (lard and fat in total in the diet up to 20.0% of daily calories) for 18 weeks [13].

Experimental rats were randomized to 5 experimental groups with 6 animals in each: I – intact control (IC) rats, which received a standard balanced diet of proteins, fats, carbohydrates, vitamins, macro- and micronutrients; II – control pathology (CP) rats, which reproduced the experimental metabolic syndrome (EMS) (described above); III – rats with EMS, which received CPC at a dose of 25.8 mg/kg intragastrically [26]; IV – animals with EMS, which received vitamin E at a dose of 100 mg/kg [11]; V – animals with EMS, which received metformin at a dose of 60 mg/kg [34]. The studied CPC and comparison drugs were used starting from 15 weeks of EMS modeling for 4 weeks, ie in the treatment regimen.

Euthanasia of female rats of all groups was performed by decapitation under light chloroform anesthesia. The indicators of lipid metabolism and the structure of the liver of rats were studied.

The content of total cholesterol and high-density lipoprotein (HDL) cholesterol was determined in two stages: enzymatic hydrolysis followed by oxidation according to Trinder [20]. The content of triglycerides (TG), low-density lipoprotein cholesterol (LDL cholesterol) and very low-density lipoprotein cholesterol (VLDL cholesterol) was determined using a set of reagents from Philisit-Diagnostics (Dnipro, Ukraine) [17].

These samples of rat liver were fixed in 10% formalin solution, dehydrated in alcohols of increasing strength, poured into paraffin. Paraffin blocks with liver samples were cut on a sled microtome MC-1, the sections were placed on a glass slide, stained with hematoxylin and eosin. To verify the neutral fats in the liver, the samples, after fixation in formalin, were cut on a freezing microtome and stained with Sudan IV [23, 25]. Examination of micropreparations was performed under a Granum light microscope, photographing microscopic images was performed with a digital video camera Granum DCM 310. Photographs were processed on a Pentium 2.4GHz computer using a program Toup View.

All obtained data were processed by methods of variation statistics [38].

Results and Discussion

Violation of the lipid profile: a decrease in HDL cholesterol by 1.37 times ($p < 0.05$) and an increase in TG, VLDL cholesterol and LDL cholesterol by 2.0; 2.0 and 1.19 times ($p < 0.05$) (Table 1) were found in rats with EMS.

The use of CPC contributed to significant reduction of TG content by 1.26 times ($p < 0.05$); VLDL cholesterol by 31% ($p<0.05$) and LDL cholesterol by 13% ($p < 0.05$) relative to animals in control pathology ($p<0,05$). Due to the activity of CPC there was an increase in the content of antiatherogenic fraction of lipids - HDL cholesterol by 1.5 times ($p<0,05$) relative to the same indicator in animals with EMS (table. 1). In terms of the decrease in the content of TG in the blood serum, CPC significantly outweighed the drugs comparing metformin (1.21 vs 1.34 mm/l) and vitamin E (1.21 vs 1.69 mm/l).). Similarly, the effect of CPC was more significant at the increase of HDL content by 23 and 16% relative to this indicator in the group of animals treated with vitamin E and metformin (Table 1).

Violation of the lipid profile on the background of EMS was reflected at changes in liver tissue. The histological structure of the liver was normal in animals of the intact control group. The particle pattern of the fabric is not expressive. Liver lobes consisted of strands of hepatocytes, which had a fairly clear radial orientation. The boundaries of the particles were determined by triads. The triad zones were narrow. The condition of the epithelium of blood vessels in the triads and other vessels was within normal limits. Intraparticle sinusoidal hemocapillaries were moderately dilated, containing a moderate number of lymphoid cells. Kupffer cells (stellar reticuloendotheliocytes) were normal. Between the parenchyma of the liver and the connective tissue surrounding the branches of the portal vein, small gaps were visible – space of Mall, components of the lymphatic outflow system in the liver [24], they contain a small number of lymphocytes and macrophages. Hepatocytes in the beams had a characteristic shape and size, the cytoplasm was stained evenly, optically dense, did not contain inclusions. The nuclei of hepatocytes were normochromic, centrally located, contained 1, sometimes 2 nucleoli. The pool of dinuclear cells was sufficient. Sudan staining do not reveal the accumulation of fat in the cells (Fig. 1).

Diffuse vacuolar hepatocyte dystrophy was detected in the liver of EMS rats. The cell volume was visually enlarged, the cells were swollen. The nuclei of some cells are hypertrophied, others are pale, with signs of karyolysis. Around the nuclei, along the cell membranes there are visible remnants of weakly eosinophilic granular cytoplasm, cell boundaries are quite clear. In most areas, hepatocytes are tightly adjacent to each other, which leads to a pronounced narrowing of the lumens of the sinusoidal blood capillaries, which are visible due to Kupffer cells (which are in the activated state). The beam pattern of such areas is «smeared». In some areas (without clear signs of vacuolar dystrophy) the lumen of hemocapillaries was expanded, in some sinusoids observed pictures of blood stasis, erythrocyte sludge (microthrombosis). Manifestations of lymph flow disorders were quite often observed - expansion of Mall's lymphatic spaces, infiltration by lymphoid cells and macrophages, migration of lymphocytes into the parenchyma, formation of lymphoid nodules. Vacuol cell dystrophy was combined with fat dystrophy (Sudan staining), the symptoms of which varied in severity at different rats. Fat inclusions were small droplet or dusty in nature, evenly filling the entire cytoplasm of cells, with little effect on the localization of the nuclei (Fig. 2).

The use of CPC on the background of EMS had a positive effect on the condition of the liver. In the vast majority of animals receiving CPK, there was no vacuolar and fatty degeneration of hepatocytes in this organ, the beam pattern was preserved, binuclear hepatocytes were present and not visually altered. Only some rats in the areas adjacent to the triad area of the lobes we observed less pronounced than in the control pathology signs of vacuolar dystrophy (with much less pronounced cell hypertrophy and, accordingly, narrowing of the lumen of hemocapillaries). Mall's lymphatic spaces were visually expanded, migration of lymphocytes and macrophages, formation of temporary lymphoid nodules was traced to a lesser extent and not in all triads. The was an accumulation of small drops of lipids in the cytoplasm of certain groups of hepatocytes in some animals (Fig. 3).

In the liver of rats treated with vitamin E, was found varying in severity vacuolar dystrophy of hepatocytes. Such cells (depending on their size) to some extent preserved beam pattern, hepatocytes often had hypertrophied nuclei. Mall spaces were often enlarged, filled with lymphocytes and macrophages, and temporary formed lymph nodes . Fat accumulated in the cytoplasm of hepatocytes, the

severity of which correlated with the severity of vacuolar dystrophy (Fig. 5).

The introduction of the comparison drug "Metformin" at the background of EMS contributed to the fact that in the liver of these animals there was almost no violation of the pattern of liver beams, vacuolar cell dystrophy was observed in isolated cases and had a small focus. At the same time, there was still an expansion of part of the lymphatic spaces of Mall, with the migration of lymphocytes and macrophages, the formation of temporary lymph nodes. Sudan staining did not show signs of fatty degeneration of hepatocytes in most of the studied animals (Fig. 4). Impaired lipid metabolism was found at rats with EMS induced by a diet high in carbohydrates and fats. The microscopic picture of the hepatic parenchyma of animals with EMS can be identified as steatosis (diffuse), which occurs at the background of local hypoxia and lymphatic flow [22, 15]. Circulatory disorders were expressed in uneven narrowing of the lumen of sinusoidal blood capillaries, erythrocyte stasis. Disorders of lymph flow were in the form of dilatation of the lymphatic spaces of Mall, their infiltration by cells of the lymphoid series, the migration of lymphocytes into the parenchyma, the formation of lymphoid nodules. According to the literature, the expansion of the lymphatic spaces of Mall is evidence of a tense state of the tissues of nonvascular vascular microcirculation, difficulty in advancing the fluid components discharged from the hepatic lobe. The formation of lymphoid nodules is a temporary accumulation of lymphoid tissue, which is created in response to damage - excessive intake of animal fats [35].

Thus, it was found that CPC has a positive effect on lipid profile (decrease in TG, VLDL cholesterol, LDL cholesterol and increase in HDL cholesterol) and in terms of its effect on TG and HDL cholesterol, it is superior to vitamin E and metformin. CPC also has some protective effect on the manifestations of hepatic steatosis: there was a decrease in the prevalence and severity of fatty degeneration of hepatocytes. It was found that CPC in terms of the severity of "antisteatotic" activity is superior to the comparison drug vitamin E and inferior to the comparison drug metformin.

The positive effect of CPC on the regulation of lipid profile is probably due to the composition of CPC: ethyl esters of Omega-3 acids, vitamin E, coenzyme Q10, zinc, vitamin A, biotin, selenium. In particular, the ability of omega 3 acid ethyl esters to be included in the modulation of lipid metabolism, regulation of adipokines (adiponectin and leptin), to facilitate inflammation of adipose tissue and to promote adipogenesis with changes in epigenetic mechanisms [5, 37, 18]. Vitamin E inhibits the activity of an enzyme involved in the synthesis of cholesterol [12]. Coenzyme Q10, zinc, vitamin A, biotin and selenium can indirectly affect the regulation of lipid metabolism through the implementation of direct and indirect antioxidant action, the ability to inhibit subchronic inflammation in the body [10, 39, 41, 4].

Obtained results substantiate the feasibility of using CPC to correct lipid metabolism in metabolic syndrome.

References

1. Abdelmalek MF. Nonalcoholic fatty liver disease: another leap forward. Nat Rev Gastroenterol Hepatol. 2021 Feb;18(2):85-86. doi: 10.1038/s41575-020-00406-0. PMID: 33420415; PMCID: PMC7791336.
2. Administration of «CORVITIN®» as an off-label agent for pharmacological correction of metabolic syndrome/Kalko K. O., Drogovoz S. M., Mishchenko O. Ya., Komisarenko M. A., Komissarenko A. M., Bondariev Y. V., Moeen Dababneh. Проблеми ендокринної патології. №1. 2021. С. 97-102. https://www.jpep.endocrinology.org.ua/index.php/1/article/view/124
3. AEVIT PREMIUM: instructions for medical use. [Electronic resource] - Access mode: https://compendium.com.ua/info/350158/aevit-premium/
4. Aguilera-Mendez A, Hernández-Equihua MG, Rueda-Rocha AC, Guajardo-López C, Nieto-Aguilar R, Serrato-Ochoa D, Ruíz Herrera LF, Guzmán-Nateras JA. Protective effect of supplementation with biotin against high-fructose-induced metabolic syndrome in rats. Nutr Res. 2018 Sep;57:86-96. doi: 10.1016/j.nutres.2018.06.007. Epub 2018 Jun 30. PMID: 30122199.

5. Albracht-Schulte K, Kalupahana NS, Ramalingam L, Wang S, Rahman SM, Robert-McComb J, Moustaid-Moussa N. Omega-3 fatty acids in obesity and metabolic syndrome: a mechanistic update. J Nutr Biochem. 2018 Aug;58:1-16. doi: 10.1016/j.jnutbio.2018.02.012. Epub 2018 Feb 27. PMID: 29621669; PMCID: PMC7561009.
6. Bjelakovic G, Nikolova D, Bjelakovic M, Gluud C. Vitamin D supplementation for chronic liver diseases in adults. Cochrane Database Syst Rev. 2017 Nov 3;11(11):CD011564. doi: 10.1002/14651858.CD011564.pub2. Update in: Cochrane Database Syst Rev. 2021 Aug 25;8:CD011564. PMID: 29099543; PMCID: PMC6485973.
7. Borodin YI, Trufakin VA, Michurina SV. Structural organization of the liver, lymphatic, immune, endocrine systems in violation of the light regime and the introduction of melatonin. Novosibirsk: Manuscript, 2012. - p 208
8. Chen YY, Yeh MM. Non-alcoholic fatty liver disease: A review with clinical and pathological correlation. J Formos Med Assoc. 2021 Jan;120(1 Pt 1):68-77. doi: 10.1016/j.jfma.2020.07.006. Epub 2020 Jul 9. PMID: 32654868.
9. Effect of complex pharmaceutical composition at the histostructure of the pancreas under the conditions of experimental metabolic syndrome in rats/Dukhnich N. Yu., Mishchenko O. Ya., Larianovska Yu. B., Kalko K. O. Pharmacologyonline. 2021. Vol. 2. P. 1192-1202. https://pharmacologyonline.silae.it/files/archives/2021/vol2/PhOL_2021_2_A134_Dukhich.pdf.
10. El-Khadragy M, Al-Megrin WA, AlSadhan NA, Metwally DM, El-Hennamy RE, Salem FEH, Kassab RB, Abdel Moneim AE. Impact of Coenzyme Q10 Administration on Lead Acetate-Induced Testicular Damage in Rats. Oxid Med Cell Longev. 2020 Jun 2;2020:4981386. doi: 10.1155/2020/4981386. PMID: 32566085; PMCID: PMC7290879.
11. Geddawy A, Hussian M, Kamel MY, Kamal R, Ibrahim MA. Effects of Liraglutide and Vitamin E in Fructose-Induced Metabolic Syndrome in Rats. Pharmacology. 2017;99(1-2):48-56. doi: 10.1159/000449429. Epub 2016 Oct 1. PMID: 27694749.
12. Geddawy A, Hussian M, Kamel MY, Kamal R, Ibrahim MA. Effects of Liraglutide and Vitamin E in Fructose-Induced Metabolic Syndrome in Rats. Pharmacology. 2017;99(1-2):48-56. doi: 10.1159/000449429. Epub 2016 Oct 1. PMID: 27694749
13. Gorbenko NI, Borikov OY, Ivanova OV. Modeling of metabolic syndrome of different genesis in experimental animals (guidelines) – Kharkiv, 2019 – 39 p.
14. Hepatoprotective and choleretic activity of dried extract of Tanacetum vulgare flowers/Mishchenko O. Ya., Kalko K. O., Zolotaikina M. Yu. et al. Thai Journal of Pharmaceutical Sciences. 2019. № 43 (1). P. 30-35.
15. Investigation of the hepatoprotective effect of the common cat's foot herb dry extract/Slobodianiuk, L., Budniak, L., Marchyshyn, S., Basaraba, R. Pharmacologyonline. 2020. Vol. 3. P. 310-318.
16. Kalko K. O. «Korvitin®» - new therapeutic opportunities Pharmacologyonline. 2021. Vol. 2. P. 1310-1316. https://pharmacologyonline.silae.it/files/archives/2021/vol2/PhOL_2021_2_A144_Kalko.pdf
17. Kamyshnikov VS Handbook of clinical and biochemical research and laboratory diagnostics/VS Kamyshnikov. - M .: MEDpressinform, 2009. 896 p.
18. Kawada T. Omega-3 polyunsaturated fatty acids and metabolic syndrome. Clin Nutr. 2020 Jul;39(7):2319. doi: 10.1016/j.clnu.2020.05.033. Epub 2020 Jun 1. PMID: 32540183.
19. Khneizer G, Rizvi S, Gawrieh S. Non-alcoholic Fatty Liver Disease and Diabetes Mellitus. Adv Exp Med Biol. 2021;1307:417-440. doi: 10.1007/5584_2020_532. PMID: 32424494.
20. Klimov AN Exchange of lipids and liproteins and its disturbances / A. N. Klimov, NG Nikulcheva. - 1999. - p. 47-87.

21. Kozeniecki M, Ludke R, Kerner J, Patterson B. Micronutrients in Liver Disease: Roles, Risk Factors for Deficiency, and Recommendations for Supplementation. Nutr Clin Pract. 2020 Feb;35(1):50-62. doi: 10.1002/ncp.10451. Epub 2019 Dec 16. PMID: 31840874.
22. Loginov AS, Aruin LI. Clinical morphology of the liver. M .: Medicine. - 1985. - p. 240.
23. Merkulov GA. Course of pathological and histological technique. - M .: Медицина, Ленингр. otd-nie, 1969.– p. 424.
24. Michurina SV, Vasendin DV, Ishchenko I.Yu. Structural and functional changes in the liver of Wistar rats with experimental obesity and melatonin correction. –2014. - V. 1., Part 1. - p. 291–299.
25. Pierce E. Histochemistry. M .: Inostr. Literature, 1962. - p 967.
26. Rybolovlev Yu. R., Sidlyarov DP, Afonin NI. Prognostic assessment of safety of substances for the person on constants of their biological activity. Moscow, December 16-18, 1981 -p. 9–10.
27. Saeed A, Dullaart RPF, Schreuder TCMA, Blokzijl H, Faber KN. Disturbed Vitamin A Metabolism in Non-Alcoholic Fatty Liver Disease (NAFLD). Nutrients. 2017 Dec 29;10(1):29. doi: 10.3390/nu10010029. PMID: 29286303; PMCID: PMC5793257.
28. Savych, A., & Mazur, O. (2021). Antioxidant activity in vitro of antidiabetic herbal mixtures. PharmacologyOnLine, 2, 17-24.
29. Savych, A., & Polonets, O. (2021). Study of hypoglycemic activity of antidiabetic herbal mixture on streptozotocin-nicotinamide-induced rat model of type 2 diabetes. PharmacologyOnLine, 2, 62-67.
30. Savych, A., Basaraba, R., & Gerush, O. (2021). Comparative analysis of hypoglycemic activity of herbal mixtures by glucose tolerance tests (message 2). PharmacologyOnLine, 2, 1118-1127.
31. Savych, A., Duchenko, M., Shepeta, Y., Davidenko, A. & Polonets, O. (2021). Analysis of carbohydrates content in the plant components of antidiabetic herbal mixture by GC-MS. Pharmacia, 68(4), 721–730. DOI: 10.3897/pharmacia.68.e69107
32. Savych, A., Gerush, O., & Basaraba, R. (2021). Determination of hypoglycemic activity of the herbal mixtures by means of glucose loading tests (message 3). PharmacologyOnLine, 2, 1128-1137.
33. Sicras-Mainar A, Aller R, Crespo J, Calleja JL, Turnes J, Romero Gómez M, Augustín S. Overall clinical and economic impact of non-alcoholic fatty liver disease. Rev Esp Enferm Dig. 2021 Jun;113(6):396-403. doi: 10.17235/reed.2020.7238/2020. PMID: 33222473.
34. Siofor: instructions for medical use. [Electronic resource] - Access mode: https://mozdocs.kiev.ua/likiview.php?id=38861.
35. Slobodianiuk, L., Budniak, L., Marchyshyn, S., Berdey, I., Slobodianiuk, O. Study of the hypoglycemic effect of the extract from the tubers of Stachys sieboldii MIQ. Pharmacologyonline. 2021. Vol. 2. P. 167-178.
36. Svegliati-Baroni G, Pierantonelli I, Torquato P, Marinelli R, Ferreri C, Chatgilialoglu C, Bartolini D, Galli F. Lipidomic biomarkers and mechanisms of lipotoxicity in non-alcoholic fatty liver disease. Free Radic Biol Med. 2019 Nov 20;144:293-309. doi: 10.1016/j.freeradbiomed.2019.05.029. Epub 2019 May 29. PMID: 31152791.
37. Tortosa-Caparrós E, Navas-Carrillo D, Marín F, Orenes-Piñero E. Anti-inflammatory effects of omega 3 and omega 6 polyunsaturated fatty acids in cardiovascular disease and metabolic syndrome. Crit Rev Food Sci Nutr. 2017 Nov 2;57(16):3421-3429. doi: 10.1080/10408398.2015.1126549. PMID: 26745681.
38. Trukhacheva NV Mathematical statistics in biomedical research using the Statistica package. M .: GEOTAR-Media, 2012. 379 p
39. Vijayaraghavan K, Iyyampillai S, Subramanian SP. Antioxidant potential of zinc-flavonol complex studied in streptozotocin-diabetic rats. J Diabetes. 2013 Jun;5(2):149-56. doi: 10.1111/j.1753-0407.2012.00226.x. PMID: 22834931.

40. Yamamura S, Nakano D, Hashida R, Tsutsumi T, Kawaguchi T, Okada M, Isoda H, Takahashi H, Matsuse H, Eguchi Y, Sumida Y, Nakajima A, Gerber L, Younossi ZM, Torimura T. Patient-reported outcomes in patients with non-alcoholic fatty liver disease: A narrative review of Chronic Liver Disease Questionnaire-non-alcoholic fatty liver disease/non-alcoholic steatohepatitis. J Gastroenterol Hepatol. 2021 Mar;36(3):629-636. doi: 10.1111/jgh.15172. Epub 2020 Jul 16. PMID: 32627871.
41. Zou C, Qiu Q, Chen H, Dou L, Liang J. Hepatoprotective effects of selenium during diabetes in rats. Hum Exp Toxicol. 2016 Feb;35(2):114-23. doi: 10.1177/0960327115579207. Epub 2015 Mar 27. PMID: 25820154.

Table 1. Influence of CPC and comparison drugs at blood lipid profile of rats with experimental metabolic syndrome

	Cholesterol, mm/l	TG, mm/l	Cholesterol VLDL, mm/l	Cholesterol LDL, mm/l	Cholesterol HDL, mmol/l
IC	3,96	0,765	0,355	1,56	2,05
EMS (CP)	3,78	1,53*	0,68*	1,86*	1,5*
EMS + CPC, 25,8 mg/kg	4	1,21*/**	0,47*/**	1,62*/**	2,3*/**/@
EMS + Vit E, 100 mg/kg	3,93	1,69*/**	0,46*/**	1,3*/**	1,86*/**
EMS + Metformin, 60 mg/kg	3,64	1,34*/**	0,4*/**	1,51*/**	1,98*/**

Notes:
* - reliable relative to intact control, p<0,05;
** - reliable for animals of control pathology, p<0,05;
@ - reliable for animals treated with metformin, p<0,05;
n - umber of animals in the group.

Figure 1. Rat liver: normal state of hepatocytes, sinusoidal capillaries and Kupffer cells (a), Mall space (arrows) with a small number of lymphocytes and macrophages (b) – hematoxylin-eosin; lack of lipids in the cytoplasm of hepatocytes (c) – Sudan IV-hematoxylin. x250.

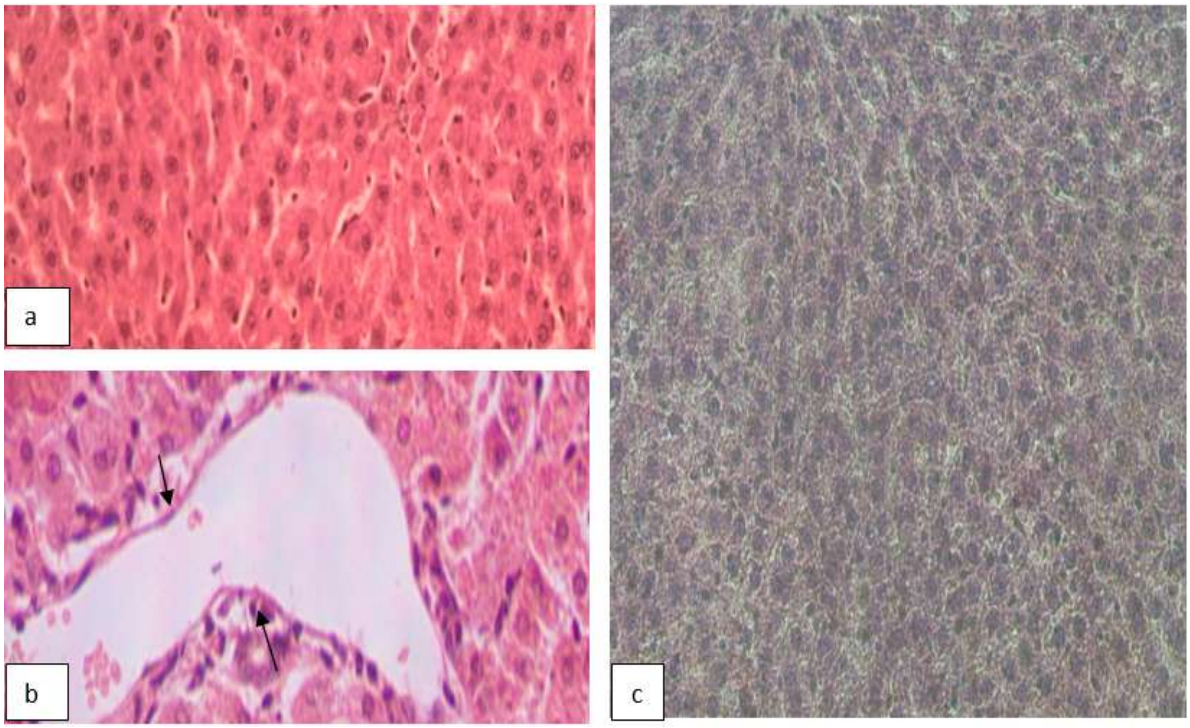

Figure 2. Rat liver at the background of EMS: large focal vacuolar dystrophy of hepatocytes, narrowing of sinusoidal hemocapillaries, beam pattern smeared (a); hemocapillary lumen dilated, stasis, erythrocyte sludge (b); accumulation of lymphocytes and macrophages in the expanded Malla space (arrows), migration of lymphocytes into the liver parenchyma with the formation of a lymphoid nodule (c); fine-grained fatty degeneration of hepatocytes (d). a, b, c - hematoxylin-eosin, d - Sudan IV-hematoxylin. x250.

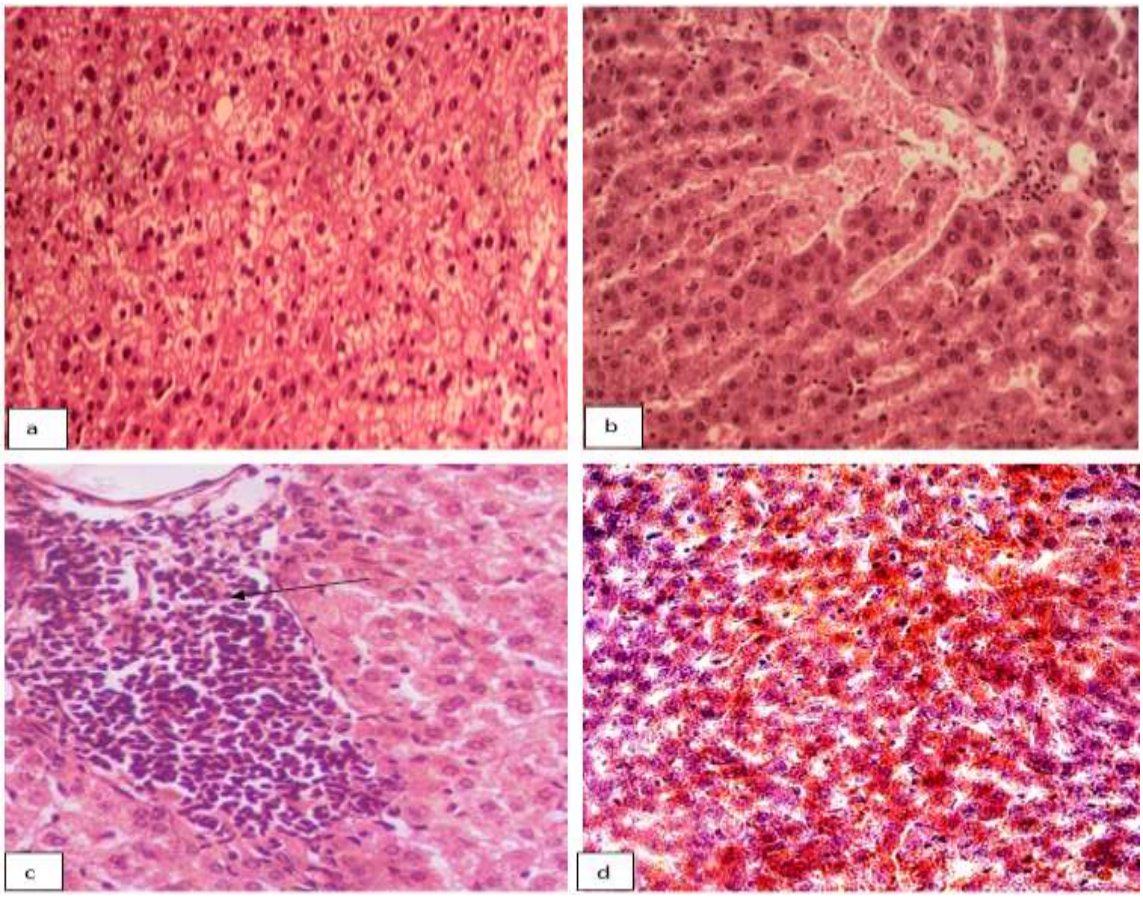

Figure 3. Rat liver at the background of EMS, which was injected with CPC: fluctuations in the severity of vacuolar (a-b) and fatty (c-d) hepatocyte dystrophy; migration of lymphocytes into the expanded Malla space (e). a, b, e – hematoxylin-eosin, c-d – Sudan IV-hematoxylin. a - x250; b, e – x400, c-d – x200.

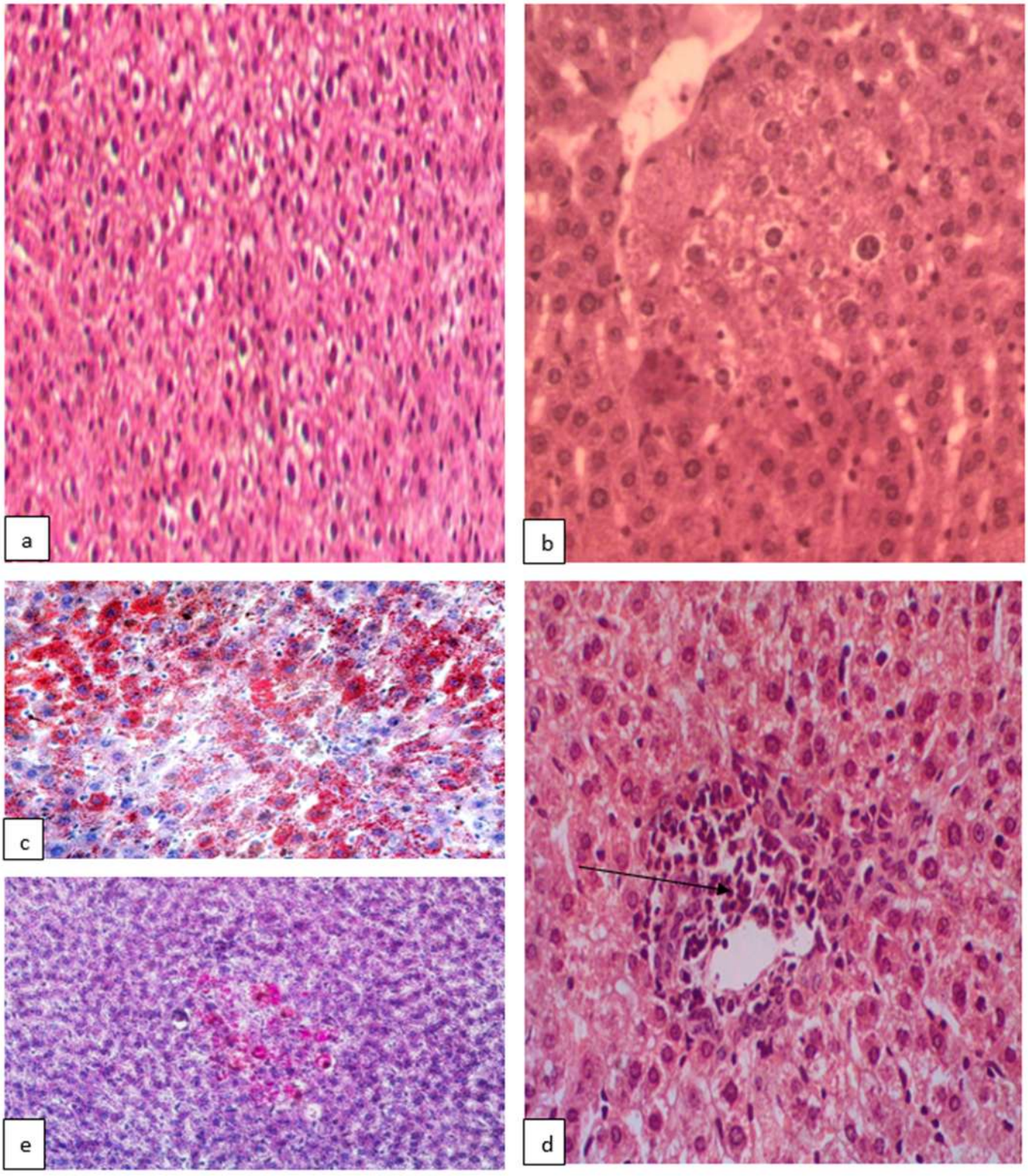

Figure 4. Liver of a rat at the background of EMS, which was injected with metformin: complete recovery of the liver parenchyma (a); small focal vacuolar dystrophy of hepatocytes (b); expansion of the Mall space, lymphocyte migration, formation of a temporary lymph node (c); no signs of fatty degeneration (d), a small cell of hepatocytes with accumulation of fat in the cytoplasm (e). a-b – hematoxylin-eosin, x250. c-d – Sudan IV-hematoxylin, x200.

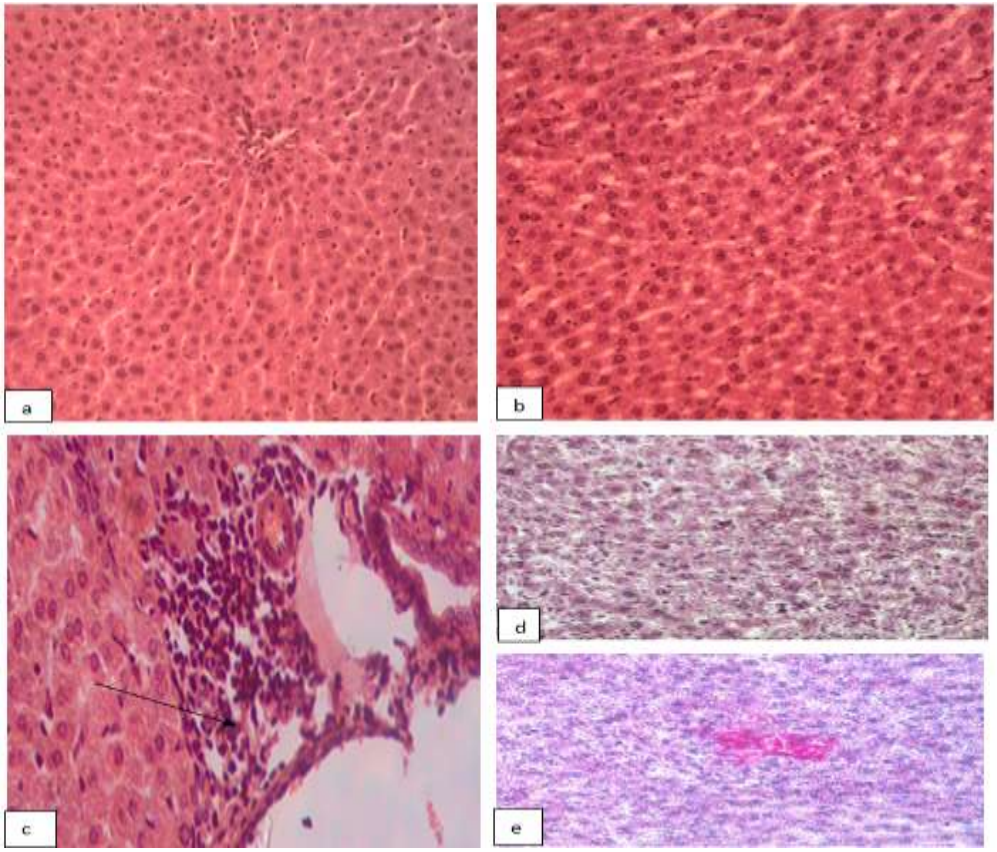

Figure 5. Liver of a rat at the background of EMS, which was injected with vitamin E: complete restoration of the normal state of the liver parenchyma (a); focal vacuolar dystrophy of hepatocytes (b); reduction of expansion of Mall's lymphatic space, migration of lymphocytes and macrophages in it (c); no signs of fatty degeneration (d), a small cell of hepatocytes with accumulation of fat in the cytoplasm (e). a-c –hematoxylin-eosin. d-e – Sudan IV-hematoxylin. a-b – x250, c – x400, d-e – x200.