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ANALYSIS OF THE EFFICACY OF A COMPREHENSIVE USE OF A HEPATOPROTECTOR AND STATIN IN THE TREATMENT OF EXPERIMENTAL HYPERCHOLESTEROLEMIA WITH NONALCOHOLIC FATTY LIVER DISEASE IN RATS (experimental study)

Abstract. *Nonalcoholic fatty liver disease (NAFLD) is a pandemic disease spread in the whole world and is found among the population of different age groups. Nonalcoholic steatohepatitis (NASH) is a progressing condition of NAFLD often associated with fibrosis and can result in the development of cirrhosis or hepatocellular liver carcinoma. Hypercholesterolemia (HC) is one of the manifestations of NASH, and at the same time HC is one of the risk factors promoting the development of cardiovascular and cerebrovascular failures. Objective of the study was to investigate the efficacy of a ccomprehensive hypolipidemic therapy including statin (Rosuvastatin) and hepatoprotector (Hepadif) in modeling HC with NASH in the experiment conducted on animals. On the 60th day of the experiment in the III group of experimental animals receiving a comprehensive hypolipidemic therapy with administration of Rosuvastatin and hepatoprotector Hepadif resulted in the following: the level of the whole cholesterol 54% decreased, low density lipoproteins (LDL) 73% decreased, high density lipoproteins (HDL) 42% increased. Activity of the hepatic transaminase was characterized by a marked tendency to decrease: ALT activity 57% decreased, AST activity – 71% as much (p<0,05), as compared to the control group. Pathomorphological examination of the liver tissue in the III group of experimental animals found less pronounced signs of steatohepatitis.*
Key words: *hypercholesterolemia, nonalcoholic steatohepatitis, statin, hepatoprotector.*

Introduction. The issues of diagnostics, treatment and prevention of nonalcoholic fatty liver diseases (NAFLD) have become topical recently, since it assumes the nature of a pandemic disease spread among both young and old ages becoming the main cause of chronic inflammation and liver transplantation [1,5,6,9].

Due to a central participation of the liver in metabolic processes of lipids and carbohydrates, the liver becomes a target organ in case of NAFLD which is manifested by the development of insulin resistance (IR), cytological syndrome, hypercholesterolemia and other symptoms [1,3,6,7,9]. HC is known to be one of the main risk factors promoting development of cardiovascular and cerebrovascular diseases; and today vascular failure has gained the lead among sickness and mortality rates due to noninfectious causes in the whole world [2-4].

Often NAFLD is asymptomatic during several years, and in the course of advancing pathological process nonalcoholic steatohepatitis (NASH), liver cirrhosis and hepatocellular carcinoma at the late stage develop [1,3]. Therefore, NASH is a progressing stage of NAFLD.

Pathogenesis of NAFLD is closely associated with sedentary life, obesity, IR development, insufficiently controlled diabetes mellitus (DM) and metabolic syndrome (MS) [1-3,5,6]. With advancing of NAFLD membrane components of damaged liver cells and toxic metabolites of cells activate Kupffer cells which promote expression of cytokines and growth factors, such as platelet-derived growth factor (PDGF – β), transforming growth factor β1 (TGF - β1), tumor necrosis factor α (TNF – α) and reactive oxygen radicals, eventually resulting in the development of more severe histological form of NAFLD – NASH [1,5-9]. The disease is associated with an increased activity of hepatic transaminase in the blood serum, and morphological changes in the liver tissues: development of macro- and microvesicular steatosis, ballooning degeneration of the liver cells, lobular necrosis, pericellular and perivenular fibrosis [6,8]. Histological changes are caused by dysfunction of adipocytes and disorders of the peripheral ability of lipid

cumulation, resulting in release of free fatty acids (FFA) and overload of the liver tissue directly by lipid fraction [1,3,6,8]. Formation of large-panel fatty dystrophy is caused by suppression of mitochondrial oxidation, activation of transcriptional factors of hepatic lipogenesis, occurrence of IR and hyperinsulinemia, decreased secretion of very low density lipoproteins (VLDL). Therefore, hypercholesterolemia is one of the main conditions caused by the development of NAFLD, NASH [1,3,4,5].

Therapy of hypercholesterolemia includes non-pharmacological methods (correction of risk factors: give up smoking, control of arterial hypertension and diabetes mellitus, regular, individual dosed physical exercises, diet therapy) and pharmacological ones – intake of hypolipidemic means [1,2,4]. Statins are the drugs of the first choice in hypolipidemic therapy – 3-hydroxy-3-methyl coenzyme A (HMG-CoA) reductase inhibitors producing effect on a key enzyme of cholesterol synthesis [4]. Multicentric investigations demonstrated a high efficacy of statins, mainly, in decrease of atherogenic lipids – total cholesterol (TC) and low density lipoproteins (LDL) [3,4].

Objective of the study was to investigate the efficacy of a ccomprehensive hypolipidemic therapy including statin (*Rosuvastatin*) and hepatoprotector (*Hepadif*) in modeling HC with NASH in the experiment conducted on animals.

Materials and methods. The study was conducted on mature male Wistar rats aged 3-4 months with the body weight of 130-180 g, n=160. All the laboratory animals were divided into the following groups: I group – animals with experimental HC and NASH (n=50); II – animals with experimental HC and NASH receiving *Rosuvastatin* in the dose of 0,3 mg/kg once a day during 60 days (n=50); III – animals with experimental HC and NASH receiving therapy with *Rosuvastatin* with the same regimen similar to the animals from II group, and combined hepatoprotector *Hepadif* in the dose of 75 mg/kg twice a day during 60 days (n=50). The pathology was modeled during 90 days by means of changing standard forage in the vivarium to atherogenic diet with supplements of palm oil and pork lard in the dose of 50 g/kg. On the last day of pathology modeling, on the 30th and 60th days of therapy complete blood cell count (CBCC) and biochemical blood analysis were made: lipidogram including detection of the total cholesterol (TC), low density lipoproteins (LDL), high density lipoproteins (HDL), triglycerides (TG), calculation of atherogenic coefficient (AC), determination of hepatic transaminase in the blood serum – alanine aminotransferase (ALT), aspartate aminotransferase (AST); blood glucose; pathomorphological examination of the liver tissue by means of staining with hematoxylin-eosin (HE), Van Gieson, Sudan III, according to the standard methods using the light microscope Leica DLMS. The first day of therapy was considered to be the beginning of the experiment. The animals were taken from the experiment on the 30th and 60th days of therapy by means of dislocation of the cervical vertebra under ether anaesthesia. The organs were fixed in the buffer formalin 10% of concentration, embedded in the paraffin «Histomix»; sections 3-5 mcm were prepared followed by staining according to the standard methods. Laboratory animals were prepared to the experiment, and all the procedures were performed according to the International Bioethical Norms and Rules (European Communities Council Directives of 24 November 1986, 86/609/EEC).

Results. Examination of the results of CBCC in all the experimental groups during all the terms of observation did not find any statistically reliable differences.

Analysis of biochemical indices in I group of animals on the 90th day determined increased activity of the examined transaminase compared with the intact group: ALT – 147% as much, AST – 175%. The level of TC was 125% as high, LDL index increased 5 times as much, and the level of HDL was 26% lower than that of the intact group (Table 1).

In II group on the 30th day of the experiment LDL level 20% decreased in comparison with I group, and it was much higher than that of the intact group. Activity of the hepatic transaminase did not change much in comparison with I group.

LDL level in III group on the 30th day in comparison with I group 42% decreased, and in comparison with the intact group it was 145% higher ($p<0,05$) (Table 1). Analysis of the hepatic transaminase activity on the 30th day of therapy determined decreased ALT activity 52% as much in comparison with I group and it was 29% higher than that of the intact group; AST activity decreased 72% as much in comparison with I group and achieved a basic level in comparison with the intact group ($p<0,05$) (Table 1).

On the 60th day in II group of animals TC decreased 44% as much in comparison with the control group and it was 49% ($p<0,05$) higher than that of the intact group (Table 2). LDL content in II group 34% decreased concerning I group, but it appeared to be 2,5 times ($p<0,05$) higher than that of the intact group, while HDL level 28% increased as compared to I group, but it did not differ reliably from the indices of the intact group. An inconsiderable tendency to decrease activity of hepatic enzymes was found: ALT - 28% as compared to I group, which is 79% higher than that of the activity of the intact group; AST – 20% as compared to I group and 123% higher than that of the intact group (Table 2). The comparative analysis of findings in III group of animals receiving therapy with statin and hepatoprotector on the 60th day determined more marked decrease of TC – 54%

as much in comparison with I group (p<0,05), and in comparison with the intact group it was close to the level of referent values (Table 2). LDL level 73% decreased in comparison with I group, and it was 58% higher than that of the intact group; HDL level increased 42% as much in comparison with I group and it was 3% higher than that of the intact group (p<0,05) (Table 2). Decreased cytolysis was determined: ALT activity 57% decreased in comparison with I group and it remained 6% higher than that of the intact group (p<0,05), AST activity 71 % decreased in comparison with I group and remained within the level of the referent values (p<0,05) (Table 2)

Table 1

Comparative dynamics of biochemical indices of the blood serum in laboratory animals from I, II, III groups compared with the intact group

Index	Intact group n=10	Control group, n=50 90th day of modeling pathology	II group, n=50 30th day of therapy	III group, n=50 30th day of therapy
TC mmol/L	1,5±0,05	3,38±0,54*	3,15±0,71*	2,01±0,07*#
VLDL mmol/L	0,51±0,02	0,82±0,45	0,77±0,09*	0,54±0,09
LDL mmol/L	0,24±0,02	1,42±0,19*	1,17±0,13*	0,83±0,15*#
HDL mmol/L	0,71±0,04	0,53±0,05*	0,64±0,07	0,68±0,03
TG mmol/L	1,71±0,02	2,26±0,11*	1,95±0,28	1,84±0,37
AC st.un.	1,37±0,21	4,58±0,41*	3,17±0,42*	2,04±0,52#
ALT	1,70±0,2	4,21±0,12*	4,11±0,28 *	2,19±0,46#§
AST	1,30±0,15	3,58±0,09*	3,7±0,17*	1,17±0,08#§
Glucose, mmol/L	4,08±0,69	10,36±0,75*	7,02±0,52*	5,32±0,41#§

*Notes: the indices are presented in the following: M±m, where M – arithmetic mean, m – deviation from the mean; * - p<0,05 compared with the intact group, # - p<0,05 compared with I group; § - p<0,05 compared with II group.*

Table 2

Comparative dynamics of biochemical indices of the blood serum in experimental animals on the 60th day of therapy

Index	Intact group n=10	II group, n=50 60th day of therapy	III group, n=50 60th day of therapy
TC	1,5±0,05	2,24±0,15*	1,54±0,21#
VLDL	0,51±0,02	0,67±0,33	0,34±0,05*
LDL	0,24±0,02	0,95±0,23*	0,38±0,16#
HDL	0,71±0,04	0,68±0,17	0,75±0,08
TG	1,71±0,02	2,25±0,11	1,94±0,11*#
AC	1,37±0,21	2,29±0,37*	1,32±0,3#
ALT	1,7±0,2	3,05±0,59 *	1,81±0,19#
AST	1,30±0,15	2,9±0,25*	1,05±0,68#
Glucose	4,08±0,69	6,54±0,92*	4,47±0,42#

*Notes: the indices are presented in the following: M±m, where M – arithmetic mean, m – deviation from the mean; * - p<0,05 compared with the intact group, # - p<0,05 compared with II group.*

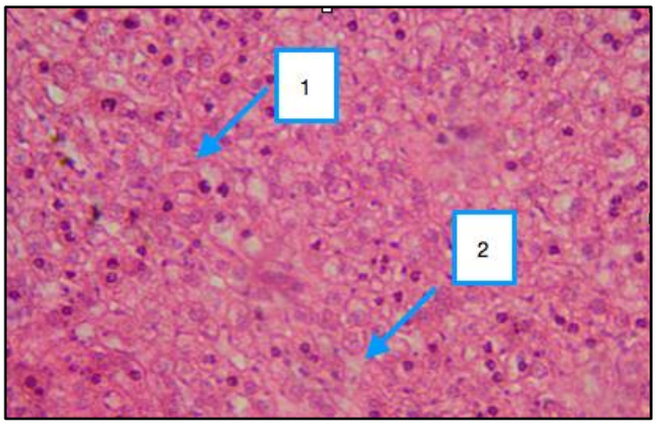


Fig.1 Liver tissue, I group, 90th day, HE, x100
1-protein dystrophy, 2-big-drop fatty dystrophy

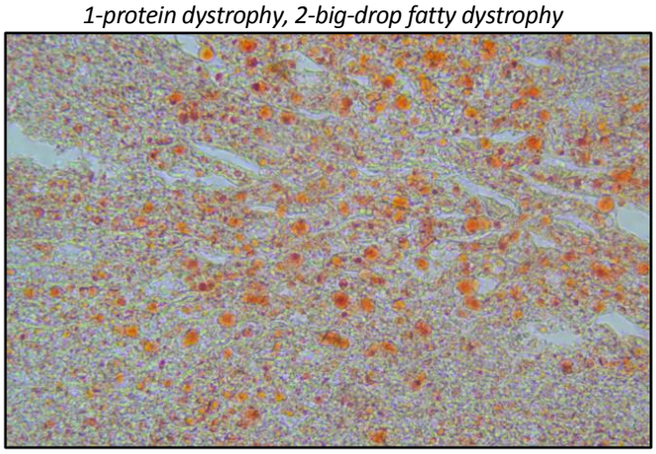


Fig. 2. Liver tissue, I group, 90th day, Sudan III, x40
Big-drop, small-drop fatty dystrophy fatty hepatosis III degree

was determined: protein dystrophy, areas of lympho-histiocytic infiltration, signs of perivascular fibrosis and diffuse central lobular small-drop fatty dystrophy, fatty hepatosis of I-II degree.

In III group of animals histological image was characterized by venous vascular plethora, foci of lympho-histiocytic infiltration, signs of small-drop fatty dystrophy, fatty hepatosis of 0-I degree (Fig. 3).

Administration of hepatoprotector *Hepadif* together with statin *Rosuvastatin* promoted a reliable decrease of lipidogram indices (TC, LDL) and increased level of HDL, as well as decreased activity of hepatic transaminase (ALT, AST), and it was associated with normalization of

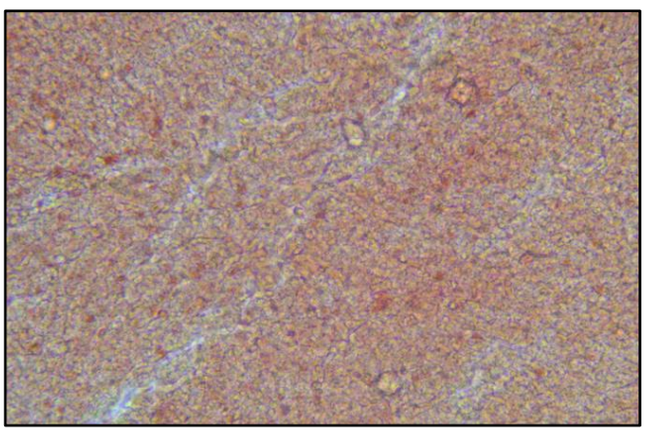


Fig. 3. Liver tissue, III group, 90th day, Sudan III, x40
Central lobe small-drop fatty dystrophy

Pathomorphological examination of animals in I group on the 90th day determined the following: marked protein dystrophy of the liver cells, perivascular lympho-histiocytic infiltration, and portions of central lobular fibrous formation (Fig. 1), diffuse central lobular big-drop and small-drop fatty dystrophy of the liver cells, fatty hepatosis of II-III degree (Fig. 2).

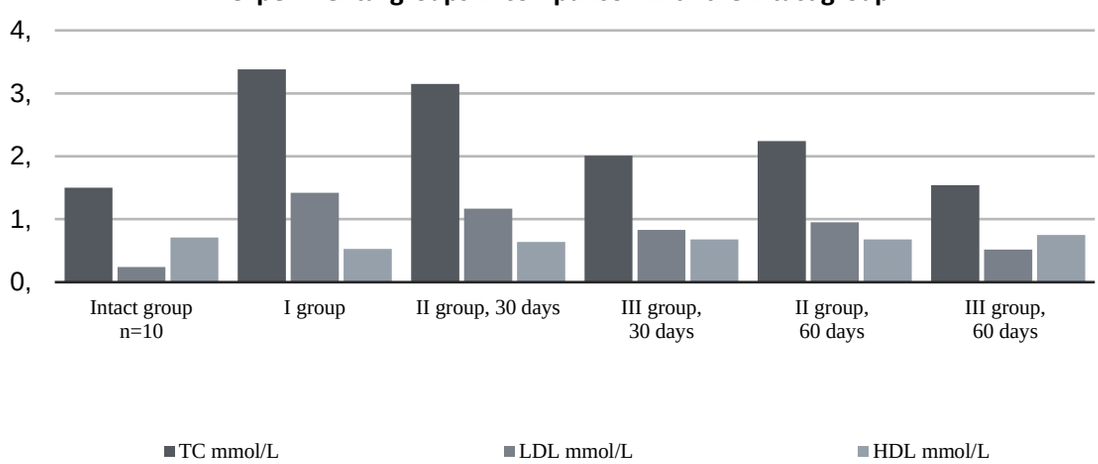
On the 60th day in animals from II group the following pathomorphological image of the liver tissue, which is indicative of a safe and effective method to stabilize pathological process.

Conclusions. 1. The use of atherogenic diet with supplements of palm oil and pork lard in the dose of 50 g/kg of the body weight during 90 days in experimental animals resulted in the formation of HC with NASH.

2. Administration of statin *Rosuvastatin* for the

Diagram

Dynamics of biochemical indices of total cholesterol, low density lipoproteins, high density lipoproteins in the experimental groups in comparison with the intact group



treatment of HC with NASH did not cause improvement of the morphological structure of the liver tissue in experimental animals.

3. Comprehensive hypolipidemic therapy including statin *Rosuvastatin* and hepatoprotector *Hepadif* results in a quick decrease of the atherogenic fraction content in the blood serum lipids, normalization of hepatic transaminase activity, as well as marked decrease of steatohepatosis manifestation and normalization of histioarchitectonics of the liver tissue in the animals with simulated HC with NASH.

Prospects of further studies. Further investigations are essential with the aim to study the duration of efficacy of the elaborated scheme of experimental therapy, the search of new, more effective and economically reasonable methods of correction of the morphofunctional condition of the liver tissue in case of hypercholesterolemia of various genesis associated with NASH.

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