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Lipid-Correcting and Antioxidant Effects of the Lipid Complex from the Red Marine Algae *Ahnfeltia tobuchiensis* under the Conditions of a High-Fat Diet

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Abstract—The influence of the lipid complex isolated from the thallus of the red marine algae *Ahnfeltia tobuchiensis* (LCA) on the metabolic parameters of the blood and liver of rats under a high-fat diet was studied. It was shown that the administration of LCA had a pronounced lipid-correcting and antioxidant effect, which was superior to that of the "Omega 3-6-9" reference preparation in terms of its ability to restore lipid metabolism, the ratio of lipoprotein fractions, and the indices of the endogenous antioxidant protection system; in addition it prevented the development of hepatosis. The lipid-correcting and antioxidant effect of LCA is specified by the action of n-3 polyunsaturated fatty acids, in particular eicosapentaenoic acid, which are part of the structure of phospholipids and glycolipids of marine origin and make up the main part of the studied lipid complex.

Keywords: *Ahnfeltia tobuchiensis*, marine lipids, n-3 PUFA, lipid metabolism, antioxidant system, dyslipidemia, high-fat diet

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INTRODUCTION

The modern human diet is characterized by excessive consumption of animal fat with a high cholesterol content (C), which contributes to the development of dyslipidemia with consequences in the form of obesity (Öngün Yılmaz, 2018). The resulting pathologies, such as coronary heart disease, stroke, hypertension, and nonalcoholic steatohepatitis and liver cirrhosis, have become a serious socio-economic problem.

The main remedies used to solve this problem are synthetic lipid-lowering drugs—statins. In addition, there are known drugs that belong to the group of substances that prevent the reabsorption of bile acids, a factor regulating plasma cholesterol homeostasis. Also, to correct lipid metabolism and, in particular, to reduce the level of triacylglycerols (TAG) and increase the concentration of high-density lipoproteins (HDLs), fibrates are used. The main disadvantage of the listed lipid-lowering medications is their high cost and the presence of contraindications in some cases. With their long-term use, especially in large doses, they can cause a number of quite serious adverse side effects (Dyadyk et al., 2018). In this regard, it seems relevant to search for alternative products of natural origin that are free from the disadvantages listed. Promising in this regard are biologically active complexes from marine raw materials, which have become the subject of research in the field of combating obesity and dyslipidemia-related diseases. These include lipid complexes from marine aquatic organisms containing polyunsaturated fatty acids (PUFAs) of the n-3 family.

Experimental studies have shown that dietary n-3 supplementation plays a significant role in the prevention of atherosclerosis (Torres et al., 2015). In contrast to statins, n-3 PUFAs are able to influence a number of key cellular processes associated with the development of atherosclerosis, including a decrease in the intensity of monocyte migration and their adhesion to endothelial cells, inhibition of the process of uptake of modified low-density lipoproteins (LDL) by macrophages, and suppression of migration of smooth muscle cells, changing the ratio of n-6/n-3 fatty acids in membrane phospholipids towards its decrease (Ramji, 2019). These properties make it possible to use complexes of marine hydrobionts that are rich in n-3 PUFAs for the prevention and treatment of dyslipidemias of various types. Of great interest in this regard are benthic forms of hydrobionts, namely, various types of seaweed, which are the primary producers of n-3 PUFAs in the food chain of marine organisms (Mišurcová et al., 2011). Lipid complexes isolated from a number of algae contain significant amounts of

essential phospholipids (PLs) and glycolipids (GLs) that are rich in PUFAs (Susanto et al., 2019). Moreover, some drugs, such as a complex of lipids from microalgae of the *Schizochytrium* species, have an inhibitory effect on HMG-CoA reductase, reducing the level of cholesterol biosynthesis (Chen et al., 2011). The lipid-correcting effect of the lipid extract of *Schizochytrium* was as effective in reducing the level of TAG and cholesterol as that isolated from fish (Komprda et al., 2015).

In the early stages and for the prevention of cardiovascular pathologies, the use of docosahexaenoic acid (DHA) has been shown to be effective in increasing high-density lipoprotein cholesterol (HDL-C) (Bernstein et al., 2012). Experimental studies have been published that investigated the hypolipidemic effect of lipid extracts from the brown algae *Sargassum pallidum*, green algae *Ulva fenestrate,* and sea grass *Zostera marina* under conditions of experimental hyperlipidemia caused by a single administration of Triton-1339 (Krivoshapko et al., 2012). In addition, a lipid extract obtained by alcohol extraction of the crushed dry brown algae Japanese sea tangle (*Laminaria japonica*) has lipid-correcting properties (Kushnerova, 2018). These drugs also include the lipid complex from the thallus of the sea red algae *Ahnfeltia tobuchiensis* (LCA). Marine red algae are known to be characterized by a high content of eicosapentaenoic acid (EPA) (Khotimchenko, 2004), which is a key component of marine n-3 PUFA complexes. EPA is one of the main carriers of the biological effects of n-3 acids, which determine their effectiveness in correcting lipid metabolism disorders. This suggests a high pharmacological activity of red algae lipids and, in particular, their lipid-correcting effect.

The chemical composition of LCA was described by us earlier (Fomenko et al., 2019). The fatty acid composition of the LCA contains a high percentage of eicosapentaenoic (20.20%) and arachidonic (26.24%) acids, which is a characteristic distinctive feature of red algae (Khotimchenko, 2004). It is necessary to note the appreciable content of monounsaturated fatty acids (MUFAs) of the n-9 family—oleic and palmitoleic acids (22.6%), which play an important role in the normalization of lipid metabolism (Johnson and Bradford, 2014). The ratio of arachidonic and eicosapentaenoic acids or n-6/n-3 in the LCA was 1.3, which is shown by the literature data to be an important indicator reflecting the lipid-correcting potential of the lipid complex and, when this coefficient is less than 4, we can talk about the effectiveness of their use for normalizing the parameters of lipid metabolism and prevention of the development of cardiovascular pathologies (Liu et al., 2016). However, data on red algae as a source of lipids with lipid-correcting properties, in particular those belonging to the genus *Ahnfeltia*, are very limited. Thus, it seems relevant to expand knowledge about the biological activity of the lipid fraction of the commercial marine red alga *Ahnfeltia tobuchiensis* (Kanno et Matsubara) Makienko, which is common in the seas of the Far East (Podkorytova et al., 2019), since it has not previously been studied in this aspect.

The goal of this work was to study the lipid-correcting properties of a lipid complex isolated from the thallus of the marine red alga *A. tobuchiensis* (Kanno et Matsubara) Makienko under the conditions of a hypercholesterol diet with a fat load.

MATERIALS AND METHODS

Samples of the algae *A. tobuchiensis* were collected in August–September 2021 in the Peter the Great Bay of the Sea of Japan (Stark Strait, Popov Island). The algae sample consisted of 100 thalli. After being collected, thalli were cleaned of epiphytes and bottom benthos in the laboratory of the Popov Island Marine Experimental Station, washed first with sea water and then with distilled water. After this, the raw materials were squeezed out and transported to the laboratory. Next, the algae were immersed in boiling water for two minutes to inactivate the enzymes. Then, the algae were dried to a residual moisture content of ~40% and placed in a freezer (–80°C) for two hours to increase fragility, after which they were crushed using a laboratory mill to a particle size of ~1 mm. Lipids were extracted using the Bligh and Dyer method (Bligh and Dyer, 1959). One kilogram of crushed algae was extracted with 1.5 L of a chloroform : ethanol mixture (1 : 2), stirred thoroughly and left for two hours; this was followed by adding 0.5 L of chloroform and 0.5 L of water after thorough mixing. Next, the extract was separated and settled to separate the phases. The lower phase containing lipids was evaporated on a rotary evaporator (Type 349/2, Unipan, Poland) ($T < 37$°C) until there was no chloroform odor. The resulting lipid extract was a green–brown oily mass. The lipid yield was 2.2% of the algae weight in terms of dry-air raw material. The content of total lipids was determined by the gravimetric method.

Standardization of the lipid complex of *A. tobuchiensis* was carried out according to the sum of metabolically active fractions (the sum of GL and PL) that are the main source of PUFAs, which amounted to 55% (8.6 mg/g dry tissue). The acute toxicity of the LCA was determined using the Kerber method (Fisenko, 2000); it was more than 2000 mg/kg, which allows the resulting substance to be classified as toxicity class 4 (low-hazard).

The experiment was carried out on outbred white male rats weighing 146 ± 3 g, which were obtained from the nursery of the Stolbovaya Branch of the Scientific Center for Biomedical Technologies, Federal Medical–Biological Agency of Russia. The animals were adapted to the vivarium for seven days before the start of the experiment. During this period, the external condition of the rats was examined daily. Animals

with no signs of health problems were taken into the experiment. The animals were kept placing each specimen in an individual plastic cage on sawdust bedding at 20–22°C and a lighting regime of 12/12 h. The animals received drinking water without restrictions and food daily at the same time in the free access mode. After adaptation, the rats were randomly separated into intact rats (control), which consumed a standard general vivarium diet throughout the experiment, and rats in which nutritional dyslipidemia was modeled. The development of nutritional dyslipidemia was achieved by feeding animals a high-fat diet (HFD) for 30 days, which consisted of a basic standard diet with the addition of 2% cholesterol and 20% beef tallow from the total composition of the diet (Ryzhenkov et al., 2012). In the experimental group of rats, the HFD was supplemented with the LCA at a dose of 1 g/kg of animal weight in terms of total lipids (Novgorodtseva et al., 2010), which corresponded to 0.6 g/kg of the metabolically active fraction.

As a reference drug, we used the commercial complex Super Omega 3-6-9 (hereinafter referred to as Omega) containing a mixture of saturated (20%), monounsaturated (20%), and polyunsaturated (60%) fats, including a complex of n-3 PUFAs (6% EPA (n-3), 3% DHA (n-3), 8% gamma-linolenic (n-6) (GLA) acids) and n-9 MUFAs (3% oleic (n-9) acid). Animals in the comparison group were given the Omega complex in their diet at a dose of 1 g/kg body weight, which corresponds to 0.6 g/kg PUFA ethyl esters and is comparable to the dose of the metabolically active LCA fraction. The choice of the lipid complex Super Omega-3-6-9 as a reference drug is due to the similar set of n-6 and n-3 PUFAs and n-9 MUFAs as in the LCA under study and, accordingly, the proposed mechanism of action.

The animals were separated into the following groups of ten rats in each: group 1, control (intact, standard basic diet); group 2, HFD; group 3, HFD + LCA; and group 4, HFD + Omega. The animals were removed from the experiment by decapitation under light ether anesthesia in compliance with the "Rules and International Recommendations of the European Convention for the Protection of Vertebrate Animals Used for Experiments or for Other Scientific Purposes" (Strasbourg, 1986). The experimental protocol was approved by the Ethics Committee of the Il'ichev Pacific Oceanological Institute, Far East Branch, Russian Academy of Sciences (protocol no. 16 of April 15, 2021).

The effectiveness of the drugs studied was assessed by their effect on the weight characteristics (body weight, liver weight) and the biochemical parameters of the liver and blood plasma. The levels of cholesterol, LDL-C, HDL-C, and TAG in the blood plasma were determined using biochemical reagent kits from the Olvex Diagnosticum company (Russia). Extracts of total lipids from blood plasma and liver tissue were prepared according to the Folch method (Folch et al., 1957).

Analysis of the composition of neutral lipids (NLs) in the liver was carried out using microthin layer chromatography (TLC) on 6 × 6 cm glass plates with KSK brand silica gel. A suspension of silica gel and plates were prepared according to the method developed by Svetashev and Vaskovsky (1972). Fractional separation of NL was carried out using the Amenta method (Amenta, 1964). The solvent systems used were hexane : sulfuric ether : acetic acid in a ratio of 90 : 10 : 1 v/v. Identification of NL spots (cholesterol, free fatty acids, triacylglycerols) by TLC was carried out using purified preparations of domestic production (Reakhim, Russia). The content of individual fractions was expressed as a percentage of the total amount of NL.

The state of the antioxidant system was assessed by the level of antiradical activity (ARA) of blood plasma in relation to alkyl-peroxyl radicals (Bartosz et al., 1998), the level of malondialdehyde (MDA) (Buege and Aust, 1978), glutathione enzymes—glutathione reductase (GR) (Goldberg and Spooner, 1983) and glutathione peroxidase (GP) (Burk et al., 1980) in the blood plasma, as well as by the level of reduced glutathione (G-SH) (Karpishchenko, 2013) and superoxide dismutase (SOD) activity (Paoletti et al., 1986) in the liver. Protein concentration was determined using the Bradford method (Bradford, 1976).

Statistical processing of the results was carried out using the Instat 3.0 application package (Graph Pad Software Inc., United States, 2005), which includes a function for checking the compliance of the sample with the law of normal distribution. To determine the statistical significance of differences for intergroup comparisons depending on the distribution parameters, Dunnett's parametric multiple comparisons t-test or the nonparametric Mann–Whitney U test, as well as the Student t-test, were used.

RESULTS

Long-term consumption of high-fat food (high-fat diet) caused the development of nutritional dyslipidemia in rats, which was characterized by high levels of TAG, cholesterol, and LDL-C and low levels of HDL-C. To conduct studies to evaluate the lipid-correcting effects of various drugs, the HFD model that causes persistent dyslipidemia and the development of obesity is widely used (Sirichaiwetchakoon et al., 2020). HFD that lasted 30 days was accompanied by an increase in the weight of animals by 29% ($p < 0.001$), which amounted to 185 ± 4 g compared to 143 ± 3 g in the control, while the specific weight of the liver increased by 72% (7.39 ± 0.32/100 g body weight versus 4.30 ± 0.12/100 g body weight in the control; $p < 0.001$). During visual inspection, a solid granularity of fatty inclusions was noted in the liver; that is, pronounced fatty hepatosis had formed.

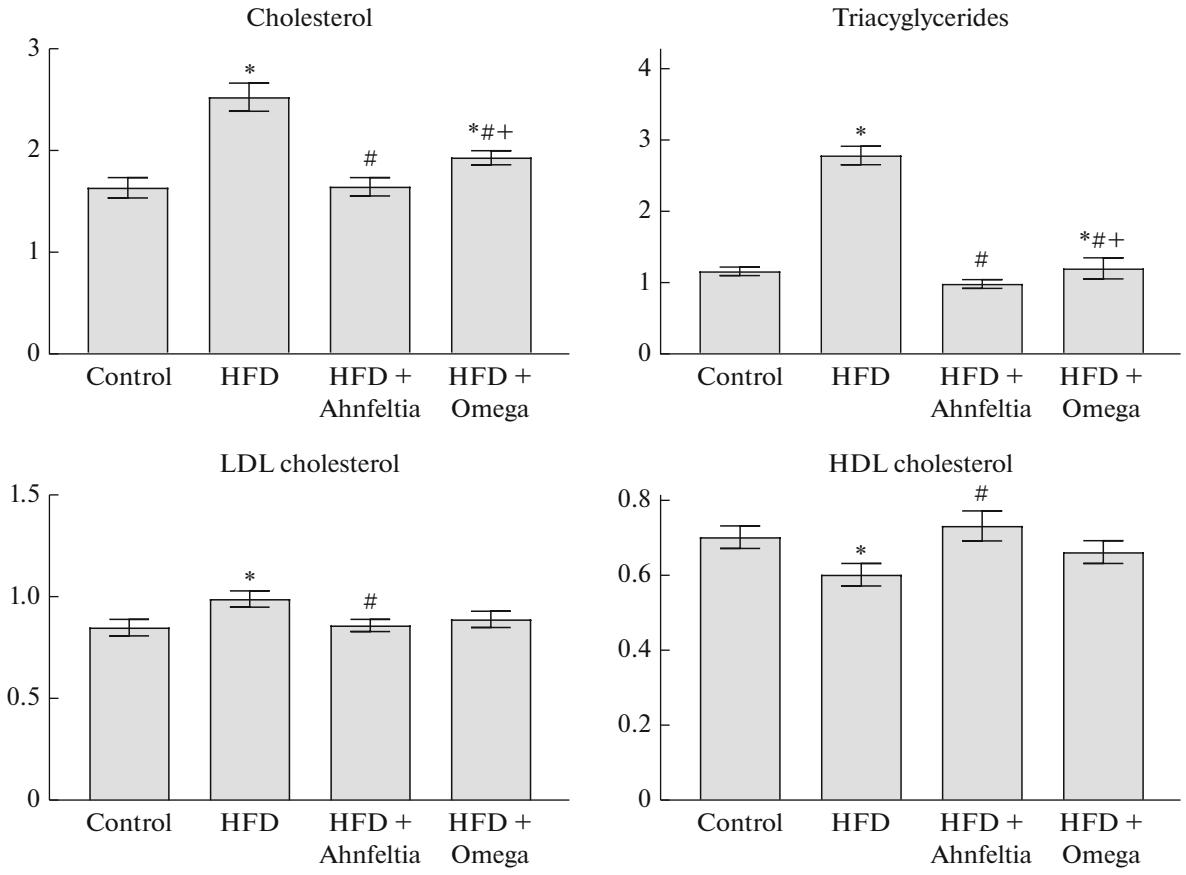

Fig. 1. The content of lipid fractions (mmol/L) in the blood plasma of rats on a high-fat diet (HFD) with the administration of the lipid complex from *A. tobuchiensis* and the Omega drug. The differences are significant ($p < 0.05$) compared with the control *, with the HFD group #, with the HFD + Ahnfeltia group +.

Biochemical indicators of lipid metabolism in blood plasma in experimental animals under the conditions of HFD indicated the development of severe dyslipidemia. Thus, the amount of total lipids in the blood plasma increased by 36% ($p < 0.001$), which was 6.21 ± 0.19 versus 4.56 ± 0.21 g/L in control animals. There was an increase in cholesterol levels by 55% ($p < 0.05$); the TAG value increased more than 2.5 times ($p < 0.05$), and the quantitative ratio of lipoprotein fractions also changed (Fig. 1). The level of atherogenic LDL-C increased by 16% ($p < 0.05$), while the value of HDL-C decreased by 14% ($p < 0.05$). As a result, the calculated atherogenic coefficient in animals of group 2 was 2.4 times higher than the control value (3.2 versus 1.34 in the control).

A study of lipid metabolism indices in the liver of rats under the conditions of HFD use revealed significant deviations in the content of NL fractions (Fig. 2). The level of TAG, cholesterol, and free fatty acids (FFAs) significantly increased by 16 ($p < 0.05$), by 28 ($p < 0.05$), and by 19% ($p < 0.05$) compared to the control, respectively. There was a 21% decrease ($p < 0.05$) in the content of cholesteryl esters (CEs), which are one of the constituent components in the assembly of lipoproteins in the liver.

An analysis of the state of the body's antioxidant defense (AOD) under the conditions of HFD revealed the accumulation of secondary products of lipid peroxidation (LPO) in the blood. The level of MDA increased by two times ($p < 0.001$) with a simultaneous decrease in plasma ARA by 16% ($p < 0.05$) (Table 1). Against the background of an increase in the activity of free radical processes, a mismatch in the indicators of the AOD system was noted both at the level of enzymes and in the pool of low molecular weight antioxidants. SOD activity in the liver was reduced by 53% ($p < 0.001$), and the G-SH content was reduced by 41% ($p < 0.001$) relative to the control group (Table 1). At the same time, the activity of GP, which catalyzes the reduction of hydrogen peroxide (H_2O_2) and organic peroxides to water in the presence of G-SH, increased by 18% ($p < 0.001$). The activity of another enzyme of the glutathione unit, GR, decreased by 13% ($p < 0.05$), which is apparently due to its increased consumption during the reduction of oxidized glutathione.

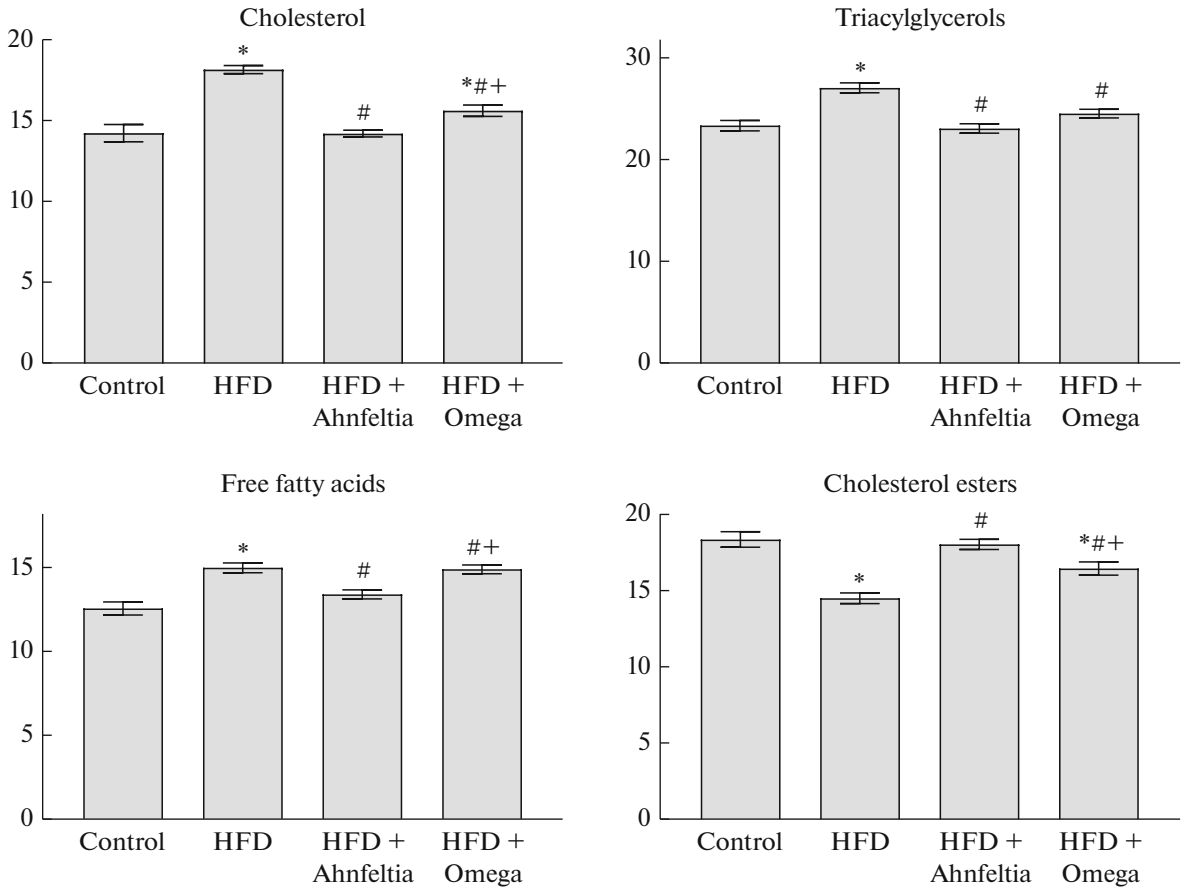

Fig. 2. The content of neutral lipid fractions (% of the sum of fractions) in the liver of rats under the conditions of a high-fat diet (HFD) with the introduction of a lipid complex from *A. tobuchiensis* and the Omega drug. The differences are significant ($p < 0.05$) compared with the control *, with the HFD group #, with the HFD + Ahnfeltia group +.

When the lipid complex from *A. tobuchiensis* or the Omega reference preparation (groups 3 and 4, respectively) was introduced into the high-fat diet, there was a pronounced tendency towards normalization of both the weight characteristics and the biochemical parameters of the liver and blood (Table 1, Figs. 1, 2). However, significant differences were revealed for a number of indicators. The body weight of group 3 rats (LCA) (146 ± 3 g) corresponded to the control level, while it was lower by 29% compared to group 2 (HFD) ($p < 0.001$). The specific weight of the liver (4.42 ± 0.14/100 g weight) was also at the control level, but it was 40% lower compared to group 2 ($p < 0.001$). In the group of animals receiving the Omega drug (group 4), body weight exceeded the control by 11% (158 ± 3 g, $p < 0.01$) and, compared with group 2, it was lower by 15% (HFD) ($p < 0.001$). The specific weight of the liver exceeded that in the control group by 29% (5.56 ± 0.33/100 g of weight, $p < 0.01$), and, compared to this indicator in group 2, it was lower by 25% ($p < 0.001$).

When comparing the parameters of lipid metabolism in the blood plasma of rats of the 3rd (LCA) and 4th (Omega) groups with those in the 2nd group (HFD), there was a decrease in total lipids by 22 ($p < 0.05$) and 14% ($p < 0.05$), which was 4.86 ± 0.21 and 5.19 ± 0.19 g/L, respectively. The cholesterol level was lower by 35 ($p < 0.05$) and 24% ($p<0.05$), and that of TAG was lower by 65 ($p < 0.05$) and 57% ($p < 0.05$) (Fig. 1). The LDL-C value in the blood plasma of rats of the 3rd group and in the 4th group was lower by 13 ($p < 0.05$) and by 10% ($p < 0.05$), respectively. At the same time, the level of HDL-C in the blood plasma of rats of the 3rd group was higher by 22% ($p < 0.05$), whereas in the 4th group it did not differ significantly from that in the 2nd group. When calculating the atherogenic index, it was revealed that its value did not differ from the control in rats receiving LCA (1.26 versus 1.34 in the control), while in animals of the 4th group (Omega) the atherogenic index was 1.93. When comparing these values with those in the 2nd group, the atherogenic index in the 3rd group (LCA) and in the Omega group was lower by 61 and 40%, respectively.

The introduction of LCA and Omega into the high-fat diet was accompanied by significant changes

Table 1. Indicators of the antioxidant system in the blood plasma and liver of rats upon administration of a lipid complex from *A. tobuchiensis* and the Omega drug under conditions of a high-fat diet (HFD) ($M \pm m$)

Indicators	Group 1 Control	Group 2 HFD	Group 3 HFD + *Ahnfeltia*	Group 4 FHD + Omega 3-6-9
Malondialdehyde (nmol/mL) plasma	3.48 ± 0.03	6.91 ± 0.09^{3}	$4.38 \pm 0.03^{3, c}$	$4.65 \pm 0.073^{3, c, ***}$
Antiradical activity (μmol/mL plasma)	0.260 ± 0.015	0.219 ± 0.008^{1}	0.277 ± 0.010^{c}	$0.245 \pm 0.007^{a, *}$
Superoxide dismutase (activity units/mg protein)	659 ± 25	312 ± 14^{3}	642 ± 26^{c}	619 ± 16^{c}
Reduced glutathione (μmol/g liver)	6.24 ± 0.13	3.66 ± 0.17^{3}	$5.96 \pm 0.23^{3, c}$	$4.94 \pm 0.26^{3, c}$
Glutaione reductase (nmol/min/mL plasma)	20.23 ± 0.48	17.28 ± 0.43^{3}	20.21 ± 0.39^{c}	$18.85 \pm 0.37^{1, a, *}$
Glutathione peroxidase (nmol/min/mL plasma)	623 ± 20	734 ± 19^{2}	643 ± 21^{a}	$573 \pm 16^{c, *}$

The differences are statistically significant at $^{1, a, *}p < 0.05$; $^{2, b, **}p < 0.01$; $^{3, c, ***}p < 0.001$. Numbers, comparison with control; letters, comparison with group 2; * comparison with group 3. HFD is the high fat diet.

in the quantitative content of NL fractions in the liver of rats (Fig. 2). In group 3, there was a decrease in the level of TAG by 15% ($p < 0.05$), FFA by 11% ($p < 0.05$), and cholesterol by 22% ($p < 0.05$). In group 4, with the administration of the Omega drug, the decrease in TAG and cholesterol levels was 9 and 14% ($p < 0.05$), respectively, and the FFA content was higher than in the control by 11% ($p < 0.05$). The CE content in groups 3 and 4 exceeded that in group 2 by 24 ($p < 0.05$) and 14% ($p < 0.05$), respectively. Consequently, LCA more effectively contributed to the normalization of the values of the studied NL parameters in the liver against the background of HFD.

Changes in the indicators of the antioxidant system and free radical processes in animals of groups 3 and 4 were characterized by a pronounced tendency towards the restoration of the studied indicators against the background of HFD (Table 1). This indicates a decrease in LPO activity. Despite the fact that the level of MDA in the blood of animals of groups 3 and 4 exceeded the control values by 26 and 34% ($p < 0.001$), it was significantly lower in relation to group 2 by 37 and 33% ($p < 0.001$), respectively. Against the background of a decrease in LPO activity, there was an increase in the blood plasma level of ARA by 27% ($p < 0.001$) and 12% ($p < 0.05$) compared with those indicators in group 2. It should be noted that the activity of the antioxidant enzymes SOD and GP in the animals receiving lipid complexes did not differ significantly from the control. But compared to group 2, SOD activity was two times higher ($p < 0.001$), which also confirms the decrease in lipid peroxidation processes under the influence of lipid complexes introduced into the diet. This fact is also supported by the GP activity being lower compared to group 2; it was lower by 12 and 21% ($p < 0.05$), respectively. The activity of GR that is the enzyme responsible for the production of the G-SH pool was at the level of control indicators in the blood plasma of group 3, but compared to group 2, it exceeded this level by 20% ($p < 0.001$), while the activity of GR in animals of group 4 exceeded it by 9% ($p < 0.001$). Against the background of a decrease in the level of free radical processes and an increase in GR activity, there was a significant increase in the G-SH pool in the liver of animals of groups 3 and 4 by 63 and 35% ($p < 0.001$), respectively, compared to group 2. It should be noted that the G-SH content in the liver of animals receiving the Omega drug was significantly lower than that in relation to the control group (by 21%, $p < 0.001$), which may be due to lower GR activity in these animals.

When analyzing the experimental data obtained, a statistically significant difference was revealed between a number of corresponding values of the studied biochemical blood plasma and liver parameters in rats when comparing the indicators of the 3rd and 4th groups (LCA and Omega) (Fig. 1). The amount of total lipids in the blood plasma in the case of the administration of Omega was higher by 6.2% ($p < 0.05$) than that in case of the introduction of LCA, and there was also a higher level of TAG (by 23%, $p < 0.05$) and cholesterol (by 18%, $p < 0.05$). This fact, as well as the lower (by 10%) value of HDL-C in the blood of animals of group 4, determined the higher atherogenic index (by 54%). A similar pattern was observed when assessing the quantitative indicators of NL fractions in the liver (Fig. 2). Thus, in animals of the 4th group, the cholesterol level was 10% ($p < 0.05$) higher than that in the animals receiving LCA, and the CE content was 9% lower ($p < 0.05$). At the same time, the content of TAG and FFA in the animals receiving Omega was higher than in group 3 by 11 and 6.3% ($p < 0.05$),

respectively. The higher efficiency of LCA compared to Omega is also supported by the values of the endogenous AOP system. Thus, in the liver of animals receiving LCA, the G-SH value exceeded that in animals from the Omega group by 17% ($p < 0.01$) and GR activity was higher by 7% ($p < 0.05$). This indicates a lower level of free radical oxidation, which is supported by a lower level of MDA (by 6%, $p < 0.05$) and a higher value of ARA (by 12%, $p < 0.05$) in the blood plasma of rats in group 3 compared with similar indicators in the Omega group animals. Based on the above, it follows that the LCA was more effective as a lipid-correcting and antioxidant agent than the Omega drug.

DISCUSSION

A high-fat diet for 30 days in rats is accompanied by an increase in body weight and the relative liver weight, a violation of the ratio of lipid classes in the blood and liver, and the formation of dyslipidemia. According to the literature (Novgorodtseva et al., 2011), in the case of HFD, hepatosteatosis characterized by fatty hypertrophy of hepatocytes already develops within 30 days due to the accumulation of excess fat in them. Meanwhile, there is a significant increase in the blood plasma level of TAG, cholesterol, and LDL cholesterol, while the value of HDL cholesterol decreases. Consequently, excessive introduction of cholesterol and saturated fats into the diet contributes to increased formation of atherogenic LDLs that are the main risk factor for the development of atherosclerosis. Thus, increased consumption of fat rich in TAG, cholesterol, and saturated fatty acids leads to the formation of obesity and the development of fatty hepatosis. An increase in the content of cholesterol, TAG, and FFA in the liver is also observed against the background of a decrease in the level of CEs, its main transport form in the composition of lipoproteins. Thus, a hypercholesterol diet with a fat load leads not only to fatty hepatosis, but also to the accumulation of a non-esterified form of cholesterol in the liver. The results indicate a violation of lipid metabolism in the blood plasma under the influence of HFD, which is a risk factor for the development of metabolic syndrome and cardiovascular diseases.

It is known that disturbances in lipid metabolism due to HFD (alimentary dyslipidemia, metabolic syndrome) are accompanied by the development of oxidative stress (Francisqueti et al., 2017) expressed in an imbalance of the lipid peroxidation–antioxidant defense system (LPO–AOD). Lipid accumulation in the liver activates molecular pathways associated with oxidative stress and inflammation, which are important pathogenetic consequences of fatty liver disease (Ghezelbash et al., 2020). The liver shows an increase in the level of MDA that is one of the LPO markers indicating the state of oxidative stress in the cell against the background of a decrease in SOD activity, the G-SH level, and GR activity against the background of an increase in GP activity. According to some authors, such a change in GP activity under stress conditions against the background of HFD is explained by the formation of an excess amount of peroxidation products (Jimoh et al., 2018), which is observed in the blood plasma of animals on the 30th day of the experiment. Thus, the emerging metabolic imbalance is both a consequence of a significant intake of fat components from the diet and the body's stress response to the fat load (Noeman et al., 2011). Being activated under stress conditions, lipolysis in adipose tissue leads to an increase in the flow of fatty acids into the blood and liver, which are the initial components in the synthesis of TAG and cholesterol. As a result, this leads to their accumulation in the liver and suppression of the activity of LDL receptors, which in turn reduces the flow of atherogenic lipoproteins into the liver and causes an increase in their blood concentration (W.E. Connor and S.L. Connor, 1989). Our results on the mismatch between the components and the direction of changes in the activity of individual enzymes of the AOD system under conditions of HFD are consistent with the data previously obtained by other authors (Bodur et al., 2019). All this indicates the depletion of the body's adaptive capacity to resist the developing pathology. Thus, the experimental model of combining a hypercholesterol diet with a fat load for 30 days was accompanied by the development of dyslipidemia with characteristic signs of oxidative stress.

The introduction of the LCA and the Omega reference drug contributed to the correction of disorders caused by the use of the HFD. Since the lipid extract from *A. tobuchiensis* is a complex natural complex, its biological effect must be considered as the result of the sum of all its components. Meanwhile, it is considered that the active base of lipid complexes from marine hydrobionts is n-3 PUFAs, and in particular EPA, which is dominant in the preparations we studied. It was previously shown that the addition of EPA to the diet under the conditions of fat loading helps to normalize the content of TAG and cholesterol both in the blood plasma and in the liver of rats (Hirotani et al., 2015), which is consistent with our data. Several studies have shown that n-3 PUFAs directly inhibit enzymes such as acetyl-CoA carboxylase, hormone-sensitive lipase, and diacylglycerol acetyltransferase, which are involved in fatty acid biosynthesis, TAG biosynthesis, and their mobilization (Shibabaw, 2021). In addition, the normalization of lipid profile parameters under the influence of PUFAs is associated with increased beta-oxidation of fatty acids in the liver (Yanagita and Nagao, 2008). The decrease in the level of atherogenic LDL under the influence of the lipid complexes studied may be a consequence of both a decrease in the availability of TAG in the liver for the collection of very low density lipoproteins, LDL precursors (Shibabaw, 2021), and activation of LDL

receptors in the liver due to a decrease in the levels of TAG and cholesterol according to the feedback principle (W.E. Connor and S.L. Connor, 1989). The normalization of the ratio of the LDL and HDL fractions against the background of a decrease in the blood level of cholesterol and the corresponding decrease in the atherogenic index, which we observed when lipid complexes were introduced into the HFD is believed by us to be due to the n-3 PUFAs included in their composition, in particular, EPA. It is known that a decrease in the blood level of cholesterol may be the result, on the one hand, of a decrease in the activity of HMG-CoA reductase under the conditions of modification of hepatocyte membranes by EPA (Murthy et al., 1988) and, on the other hand, the result of a direct increase in the amount of HDL in the blood (Balk et al., 2006). It is also known that n-3 PUFAs, modifying HDL, increase their affinity for cholesterol, which leads to an increase in the reverse transport of cholesterol to the liver (Burillo et al., 2012).

The *Ahnfeltia* lipid complex and the Omega drug exhibited a pronounced antioxidant effect against the background of the development of oxidative stress under the conditions of a HFD. It is known that n-3 PUFAs and, in particular, EPA, are effective direct-acting antioxidants capable of absorbing peroxyl radicals (Richard et al., 2008), which was confirmed by the increase in blood plasma ARA and the decrease in secondary lipid peroxidation products that we detected. It was shown that n-3 acids activate the induction of SOD (Garrel et al., 2012) and restore the activity of GR (Refaat et al., 2022) and the pool of low molecular weight antioxidants, such as G-SH (Patten et al., 2013), which is what we observed when analyzing the results.

CONCLUSIONS

The results show that the lipid complex from the marine red algae *A. tobuchiensis* that contains a metabolically active fraction of marine phospho- and glycolipids with a high content of PUFAs of the n-3 family and, in particular, eicosapentaenoic acid, has a pronounced lipid-correcting and antioxidant effect in conditions of a high-fat diet. Metabolic changes observed when using the lipid complex of *A. tobuchiensis* and the Omega drug contribute to an increase in HDL cholesterol and a decrease in the level of atherogenicity in the blood plasma, as well as normalization of the quantitative content of NL fractions in the liver. The ability of exogenous "marine" lipids to be involved in metabolism suggests their active influence on most vital processes. The consequences of oxidative stress are minimized, which is reflected in the restoration of the parameters of the AOP system, including the normalization of the activity of SOD, GP, and GR and the preservation of the G-SH pool, as well as a decrease in the level of lipid peroxidation and the restoration of ARA in the blood plasma.

The studied complex of lipids from *Ahnfeltia* is not only not inferior in its effectiveness to the reference Omega 3-6-9 PUFA complex, but also surpasses it in its ability to normalize the metabolic parameters of lipid metabolism in the blood and the main fractions of NL in the liver and the body's ability to resist to oxidative stress factors under the conditions of a high fat load and the formation of dyslipidemia. According to the authors, the higher biological activity of the lipid complex of *A. tobuchiensis* compared to Omega-3-6-9 is explained by both the wider range of PUFAs of the n-3 family and their being contained in the form of "marine" phospho- and glycolipids, which is more effective compared to ethyl esters of fatty acids in Omega 3-6-9.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Animal studies were carried out in accordance with the order of the Ministry of Health and Social Development of Russia of April 1, 2016, no. 199n "On Approval of the Rules of Laboratory Practice" and the requirements of GOST R 53434-2009 "Principles of Good Laboratory Practice." This study was approved by the Bioethics Commission of the Il'ichev Pacific Oceanological Institute of the Far Eastern Branch of the Russian Academy of Sciences (protocol No. 16 dated April 15, 2021). The experiments were performed in accordance with the requirements of the Federation of European Laboratory Animal Science Associations (FELASA) in compliance with the "Regulations and International Recommendations of the European Convention for the Protection of Vertebrate Animals Used for Experiments or Other Scientific Purposes" (Strasbourg, 1986).

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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