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BIOCHEMISTRY

The Effect of a Lipid Extract from the Marine Green Algae *Codium fragile* (Suringar) Hariot 1889 on Metabolic Reactions under Acute Stress

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Abstract—The effect of a lipid extract isolated from the marine green algae *Codium fragile* (Suringar) Hariot on the liver and blood biochemical indicators in mice under the impact of acute stress (vertical fixation by the dorsal neck fold) was studied. The pharmacological effect of the *C. fragile* lipid extract was manifested in the restoration of lipid and carbohydrate metabolism, as well as in the normalization of the indicators of the endogenous antioxidant defense system under the effect of stress. The biological activity of the lipid extract of *C. fragile* is, probably, due to the action of its constituent polyunsaturated fatty acids of the ω-3 and ω-6 families. The lipid extract of *C. fragile* was not inferior to the reference Omega-3 preparation in restoring the body's metabolic reactions caused by the impact of the stress; however, it showed higher antioxidant activity.

Keywords: lipid extract, *Codium fragile*, Omega-3, stress, lipids, antioxidant defense, mice

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INTRODUCTION

Seaweed is a source of a variety of compounds with high biological activity, which creates the prerequisites for their potential use in food, cosmetics, pharmaceuticals, and other industries. Although algae are not the main source of energy, it is known that they have nutritional and pharmacological value due to the content of proteins, carbohydrates, lipids, minerals, vitamins, and other compounds (Ortiz et al., 2009). It was found that the daily human need for vitamins A, B_2, and B_{12} and two-thirds of the vitamin C requirement can be met by consuming 100 g of seaweed (Chapman, V.J. and Chapman, D.J., 1980). An important group of compounds among the secondary metabolites that make up seaweeds is the class of lipids that participates in the course of most biochemical processes vital for the body (Kushnerova et al., 2020). At the same time, seaweed is considered a natural source of long-chain polyunsaturated fatty acids (PUFAs) of the ω-3 and ω-6 families, such as eicosapentaenoic acid, docosohexaenoic acid, arachidonic acid, etc. (Khotimchenko et al., 2002), which may reduce the risk of developing cardiovascular disease. Numerous scientific studies show that consuming seafood products containing PUFAs can prevent the risk of developing thrombosis and atherosclerotic plaques in blood vessels and can reduce triglycerides, cholesterol in the blood, and blood pressure levels (Jamp et al., 2015; Khan and Makki, 2017).

However, in everyday life, a deficiency of these ingredients in food, as well as disturbances in the metabolic processes of lipid–carbohydrate metabolism caused by exposure to various stress factors (physical, biological, toxic, mechanical, emotional), can contribute to the development of various diseases. According to J. Chrousos (2009), one of the main reasons the development of diseases, including diseases of the hepatobiliary, circulatory, neuroendocrine, and immune systems, is considered the effect of stress on the body, especially chronic stress.

Previous studies in rat models of acute stress showed that marine green algae extract *Ulva lactuca,* enriched with the lipid fraction, has a hepatoprotective effect, normalizes liver lipid metabolism, and reduces lipid peroxidation products (Fomenko et al., 2016). In the studies of N.F. Kushnerova et al. (Kushnerova et al., 2020), it was shown that the lipid complex isolated from the red algae *Ahnfeltia tobuchiensis* is not inferior to the phospholipid drug Essentiale® by the ability to normalize the blood lipid profile and the ratio of the phospholipid fractions in erythrocyte membranes under experimental stress conditions.

Among seaweeds, green algae of the Codiaceae family are of great interest as a raw material source of lipid complexes, a widespread representative of which

is *Codium fragile* (Suringar) Hariot 1889. Codium grows in the lower littoral zone and in the upper sublittoral zone on muddy, rocky, pebble, and muddy–sandy soils, near open and semi-protected coasts. It is distributed in temperate and subtropical waters of the World Ocean, off the coast of the countries of the Asia–Pacific region (China, Taiwan, Japan, Korea, Russia, Indonesia) (Titlyanov and Titlyanova, 2012). However, recently *C. fragile* has been considered an established invader in marine ecosystems around the world (Ortiz et al., 2009). It is classified as an invasive (alien) species, introduced into other regions where these algae were not previously found (Pereira et al., 2021). Due to its high reproductive capacity and simplicity, *C. fragile* is more competitive than local species, which contributes to its spread and increase in biomass. *C. fragile* has been used since ancient times in Japan and Korea as an edible plant; in oriental medical manuals, it is registered as a remedy for the treatment of enterobiasis, dropsy, dysuria, etc. (Ahn et al., 2021).

As part of the thallus, *C. fragile* includes a relatively small percentage of lipid substances (4.4–5.3 mg/g wet weight) (Khotimchenko, 2003). However, the high content of PUFAs of the ω-3 and ω-6 families, which are important components of the glycolipid and phospholipid fractions, determines the high pharmacological activity of the lipid complex (Ortiz et al., 2009). Due to the ability of seaweed to produce PUFA C_{18} and C_{20}, they have attracted the attention of researchers from all over the world. Lipid extracts obtained from several species of the genus *Codium* sp. exhibit antibacterial, antiviral, antifungal, and cytotoxic activity (Goecke et al., 2010). In the lipid fraction of *C. fragile*, the compound clerosterol (a cholesterol derivative) was isolated, which exhibits antioxidant properties because it helps reduce oxidative damage in keratinocytes HaCaT of human cells caused by UV radiation (Lee et al., 2013). In recent experimental studies (Seo et al., 2022), it has been shown that the extract of *C. fragile* is effective against corpulence and obesity. It effectively induced weight loss, reduced total triglycerides and liver cholesterol, and suppressed adipocyte differentiation in white adipose tissue in mice fed a high-fat diet. Introduction of the extract of *C. fragile* significantly altered the gut microbiota in obese mice, increasing the proportion of beneficial bacteria (Kim et al., 2020). All of the above indicates the high pharmacological effect of the lipid extract isolated from *C. fragile*. However, codium is not used as a source of raw materials for the production of food ingredients, medicinal preparations, or dietary supplements in the domestic food and pharmaceutical industries.

Today, the problem of stress remains of high medical and social significance. In modern unfavorable conditions caused by all kinds of stressful situations, the use of lipid extract from *C. fragile*, as a possible stress protector, seems very relevant.

As a model of stress in laboratory studies on small rodents (mice, rats), vertical fixation by the dorsal neck fold is used (Kushnerova et al., 2005). Regardless of the nature of the stress-inducing effect, the body reacts with a constant set of biochemical and physiological reactions, such as hyperemia and hypertrophy of the adrenal cortex, degradation of the thymic–lymphatic system, and the appearance of ulcerations in the gastrointestinal tract. In addition, intense stress leads to an increase in the formation of reactive oxygen radicals, which is accompanied by peroxidation of cell membrane lipids (Sahin and Gümüşlü, 2007). As a result, the formation of polar lipid hydroperoxides and an imbalance in the ratio of phospholipid fractions of membranes occurs, which leads to a change in their permeability and possible damage (Fomenko et al., 2013). Thus, stress has an adverse effect on all metabolic processes in the blood, liver, and other organs, which makes it relevant to develop drugs based on lipid complexes and PUFAs.

In this regard, the use of the lipid extract of *C. fragile*, containing phospho- and glycolipids of marine origin in combination with PUFAs, will help restore the lipid matrix of cell membranes and normalize metabolic processes, thereby improving the general condition of the body during pathological processes and stress. The combination of high biological activity, high reproductive capacity, and the rapid self-renewal of biomass defines the marine green algae *C. fragile* as a source of raw materials for creating effective pharmacological agents and nutritional supplements.

The purpose of this work is to evaluate the composition of the lipid extract isolated from the thallus of the marine green algae *C. fragile* and its effects on the metabolic processes of the liver and blood of mice under conditions of acute stress.

MATERIALS AND METHODS

All algae specimens of *C. fragile* were collected by hand during the summer months in shallow water (<2 m) on Popov Island in Peter the Great Bay, Sea of Japan. Preprocessing of the collected material was carried out at the research station, where the algae were washed in sea water, then in fresh water, to remove sand, epiphytes, zoobenthos, and various contaminants as much as possible. Next, the raw material were transported fresh in a refrigerator to the institute's laboratory, where all subsequent analytical procedures were performed. To inhibit enzyme activity, purified macrophyte specimens were immersed in boiling water for no more than two minutes. Then they were wrung out and dried under natural conditions to a residual humidity of ~30–40%. The dried raw materials were crushed using a blender and stored at a temperature of –20°C for further use and processing. Isolation of the lipid fraction was carried out according to the method of Bligh and Dyer (Bligh and Dyer, 1959). To do this, one kilogram of crushed algae powder was extracted

with 1.5 L of a mixture of chloroform : methanol (1 : 2 by volume) and left overnight. To separate the phases, 500 mL of chloroform and distilled water were added to the mixture, then the mixture was stirred gently. The upper water–methanol layer was separated and removed, and the lower chloroform layer containing the lipid fraction was concentrated on a vacuum evaporator (Type 349/2, Unipan, Poland) at a temperature not exceeding 37°C. The content of total lipids in the extracts was determined by weighing aliquots of the extract dried to a constant weight.

The chromatographic distribution of lipids was carried out using microthin layer chromatography (TLC) on glass plates coated with a layer of silica gel of the KSK brand (Labkhimos LLC, Russia). To separate plant glycolipids, a solvent system was used: acetone : benzene : water in the ratio 91 : 30 : 8 (by volume) (Vaskovskii and Khotimchenko, 1982). Glycolipids were detected in chromatograms using the anthrone reagent (Van Gent et al., 1973). Determination of the amount of total phospholipids in the algal extract was carried out according to the method of V. Vaskovskii et al. (Vaskovskii et al., 1975). The phospholipids were separated into fractions using two-dimensional TLC (Svetashev and Vaskovskii, 1972) in solvent system: in the first direction is a mixture of chloroform : methanol : 28% ammonia in the ratio of 65 : 35 : 5 (by volume), and in the second is a mixture of chloroform : acetone : methanol : glacial acetic acid : water in a ratio of 50 : 20 : 10 : 10 : 5 (by volume). Phospholipid fractions separated in chromatograms were detected with a 10% solution of sulfuric acid in methanol, followed by heating the plates on a closed electric stove. The content of individual phospholipid fractions was calculated as a percentage of their total amount.

The chromatographic distribution of neutral lipids was carried out by one-dimensional TLC (Amenta, 1964) in a solvent system of hexane : sulfuric ether : glacial acetic acid in a ratio of 80 : 20 : 1 vol/vol or 90 : 10 : 1 vol/vol. Samples after chromatography were detected with iodine vapor.

The content of individual fractions was expressed as a percentage of the total amount of neutral lipids.

The composition of fatty acids in the algal lipid extract was analyzed by gas–liquid chromatography (GLC). For this purpose, fatty acid methyl esters (FAMEs) were obtained by transesterification of lipids according to the method of Carreau and Dubacq (Carreau and Dubacq, 1978). The resulting FAMEs were purified by TLC using benzene in the system, then eluted from silica gel with hexane, and the isolated eluate was evaporated. FAMEs were redissolved in a certain volume of hexane and analyzed by GLC on an LKhM-2000 chromatograph (JSC Chromatograph, Russia) with a flame ionization detector. Fatty acids were identified by comparison of retention times (Rt) with standards and carbon number values (Christie, 1988). The results were calculated as a percentage of the total fatty acids.

An experiment to simulate stress exposure was carried out on outbred white male mice eight weeks of age weighing 25–30 g. During the acclimatization period for one week, the animals were kept in vivarium conditions at room temperature 22 ± 2°C (in cages of five individuals) on a basic diet, without water restriction. Then the mice were randomly divided into the control and experimental mice, ten animals in each group. Animals of the experimental groups were subjected to vertical stress fixation at the dorsal neck fold for 24 hours. Immediately before the experiment, mice in the two experimental groups were administered preparations *per os*; six hours after the first introduction, the preparations were administered again. A lipid extract of codium (LEC) and an Omega-3 lipid complex were administered at a dose of 1 g/kg animal weight. The choice of dose used is based on data from the literature (Novgorodtseva et al., 2010), as well as our own research. Animals in the control group and the "stress" group were injected with an equivolume amount of 0.9% NaCl solution according to a similar scheme. The introduction of saline solution does not affect the results of the experiment, but at the same time it eliminates research errors, since any external irritation is stress for the body. Standardization of the codium lipid extract was carried out based on the amount of total lipids. Pharmacy-grade Omega-3 was used as the reference preparation for comparison. The active components of the Omega-3 preparation are PUFAs, such as docosohexaenoic acid (120 mg) and eicosapentaenoic acid (180 mg), which are part of the natural fish oil concentrate obtained from anchovies.

In the experiment, mice were divided into the following groups: Group 1 was the control; Group 2 underwent stress (vertical fixation); Group 3 experienced stress + LEC; and the 4th group had stress + Omega-3. All animals were weighed at the beginning and end of the study. Also, at the end of the experiment, the internal organs of all tested mice were weighed to calculate the mass index (MI, mg organ mass per 100 g body weight) of the liver, spleen, and thymus. Blood was drawn using the technique of bleeding from the orbital venous plexuses of the head and neck. Animals were removed from the experiment by decapitation under light ether anesthesia in compliance with the principles and international recommendations set out in the European Convention for the Protection of Vertebrate Animals used for Experimental or Other Scientific Purposes (European Convention, 1986).

To assess the effect of preparations administered under stress conditions, the following parameters were used: weight coefficients (weight of mice, MI of liver and spleen) and biochemical parameters characterizing the state of lipid–carbohydrate metabolism and the state of the antioxidant system of the liver and

blood of animals. The content of total cholesterol (TC), triacylglycerols (TAG), and blood glucose was determined enzymatically using sets of reagents from the Olvex Diagnosticum company (Russia). To determine the content of neutral lipids in the liver tissue, a lipid extract was prepared using the traditional method of J. Folch et al. (1957). The amount of total lipids in the liver extract was determined by the gravimetric method. Nonpolar lipids were separated into fractions by one-dimensional TLC (Amenta, 1964). The content of individual fractions of neutral lipids was calculated as a percentage of their total amount.

To assess the potential of antioxidant protection of the animal body, the following indicators were used: the value of total antiradical activity (ARA) in relation to the radical cation $ABTS^+$ (Re et al., 1999), glutathione peroxidase (GP) activity in the blood plasma (Burk et al., 1980), and the level of reduced glutathione (G-SH) in the liver tissue (Karpishchenko et al., 2013).

The obtained quantitative data were expressed as the arithmetic mean ± standard error meaning, which were analyzed using analysis of variance ($P < 0.05$). The treatment was carried out using the statistical package Instat 3.0 (GraphPad Software Inc., United States, 2005). The statistical significance of differences in mean values was determined by Student's *t*-test after checking the normality of the distribution of the studied values.

RESULTS AND DISCUSSION

The chemical composition of the lipid fraction of the thallus of the green algae *C. fragile* is presented in Table 1. The total content of isolated lipids was 13.92 ± 0.22 mg/g dry tissue, of which the largest amount was glycolipids (44%) and neutral lipids (40%), while the share of phospholipids was 16%. The main fractions among neutral lipids were TAG (41.55 ± 2.15%) and sterols (15.16 ± 0.74%). The remaining fractions had approximately the same content: monoacylglycerols + diacylglycerols (8.94 ± 0.31%), sterol esters (9.47 ± 1.90%), and free fatty acids (11.21 ± 0.41%). Analysis of the content of polar lipids in the LEC showed the presence of the following representatives of the phospholipid class: phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), which is confirmed by data obtained by S. Khotimchenko (Khotimchenko, 2003). Moreover, PC, PG, and PE are some of the main components of the phospholipid fraction; their content was in the range of 21–31% of the total amount of phospholipids. As is known, these phospholipids are structure-forming and functional components of all biological membranes. Fatty acids in seaweeds are more diverse than in terrestrial plants. With the relatively low lipid content in marine macrophytes, the amount of PUFAs in them prevails significantly compared to plants (Sanchez-Machado et al., 2004). Study of the composition and ratio of fatty acids in the LEC showed (Table 1) that PUFAs were prevalent as a percentage (more than 50%) of the total fatty acids. At the same time, the proportion of saturated fatty acids (SFA) in the lipid extract was 34% and that of monounsaturated fatty acids (MUFA), 12%. In terms of the quantitative composition, palmitic acid (16 : 0) (28.38%) predominated in the lipid fraction of codium, which is the most common, followed by α-linolenic acid (18 : 3 ω-3) (19.7%), hexadecatrienic acid (16 : 3 ω-3) (12.2%), oleic (18 : 1 ω-9) (10.72%), etc. It is important to note that algae of the Chlorophyta division, including *C. fragile,* is characterized by the presence of significant amounts of C_{16} and C_{18} PUFA. For the algae family Codiaceae, the genus *Codium* sp. is characterized by a high content of PUFA 16 : 3, which is a taxonomic feature of this genus (Goecke et al., 2010). In turn, C_{18} PUFAs (α-linolenic and linoleic), classified as essential, are very important for nutrition, since they are not formed in the body of humans and animals, and can only be obtained from products containing them (river and marine fish, vegetables, seaweed). At the same time, algae of the genus *Codium* sp. are also capable of synthesizing long-chain C_{20} PUFA (arachidonic and eicosapentaenoic acids) and C_{22} PUFA. The results obtained on the content of fatty acids in the lipid fraction of *C. fragile* are consistent with research materials presented in domestic and foreign literary sources (Khotimchenko, 2003; Ortiz et al., 2009; Goecke et al., 2010).

The next stage of the experimental study was to study the effect of the LEC and the reference preparation Omega-3 on the physiological and biochemical parameters of animals under stress—vertical restraint conditions. When determining the specific gravity of the internal organs of mice exposed to stress, there was a decrease in the liver MI by 16% ($p < 0.01$) and in the spleen by 23% ($p < 0.01$) (Fig. 1). At the same time, the weight of the animals decreased by 18% ($p < 0.05$), and the appearance of ulcerative lesions of the gastric mucosa was noted (2.4 ± 0.1 pcs/animal, and in the control, 0). The resulting changes in the weight coefficients and the appearance of ulcerations of the mucous membranes of the gastrointestinal tract (GIT) are considered indicative signs of stress in animals.

The impact of stress was accompanied by changes in the indicators of fat and carbohydrate metabolism (Fig. 2), which were characterized by a decrease in the amount of TAG by 3.5 ($p < 0.001$) times and an increase in blood glucose levels by 1.3 times ($p < 0.001$). It is known that during acute stress, compared to chronic stress, partial depletion of the TAG content in the bloodstream occurs against the background of mobilization of the carbohydrate reserves of cells, which increases the release of glucose into the blood (Gurskaya et al., 2017). In emergency situations, the central nervous system uses glucose as the most quickly mobilized and preferred energy source to pro-

Table 1. Chemical composition of the lipid fraction of the thallus of *Codium fragile* Suringar (Hariot) 1889

Biochemical parameters	Indicators
Total lipids (mg per 1 g dry tissue)	13.92 ± 0.22
Total glycolipids (mg per 1 g dry tissue)	6.12 (44%)
Total phospholipids (mg per 1 g dry tissue)	2.23 (16%)
Total neutral lipids (mg per 1 g dry tissue)	5.57 (40%)
Neutral lipid fractions (% of the sum of all fractions)	
Diacylglycerols + monoacylglycerols	8.94 ± 0.31
Free sterols	15.16 ± 0.74
Free fatty acids	11.21 ± 0.41
Triacylglycerols	41.55 ± 2.15
Fatty acid esters	4.15 ± 0.41
Sterol esters	9.47 ± 1.90
Residual fraction	9.52 ± 0.76
Phospholipid fractions (% of the sum of all fractions)	
Phosphatidylcholine	31.80 ± 0.76
Phosphatidylglycerol	29.28 ± 0.52
Phosphatidylethanolamine	21.14 ± 0.48
Phosphatidylinositol	7.40 ± 0.17
Phosphatidylserine	10.38 ± 0.33
Fatty acids (% of the sum of all fractions)	
14 : 0 (myristic acid)	1.7 ± 0.02
16 : 0 (palmitic acid)	28.38 ± 1.45
16 : 1 n-7 (palmitoleic acid)	1.6 ± 0.01
16 : 2 n-6	2.6 ± 0.12
16 : 3 n-3 (hexadecatrienoic acid)	12.2 ± 0.56
18 : 0 (stearic acid)	0.9 ± 0.03
18 : 1 n-9 (oleic acid)	10.72 ± 0.46
18 : 2 n-6 (linoleic acid)	9.0 ± 0.36
18 : 3 n-3 (α-linolenic acid)	19.7 ± 0.64
20 : 4 n-6 (arachidonic acid)	6.2 ± 0.23
20 : 5 n-3 (eicosapentaenoic acid)	4.3 ± 0.32
22 : 0 (behenic acid)	2.7 ± 0.04

vide cells with energy. The content of the total cholesterol (TC) increased by 38% ($p < 0.01$) compared to the control, which is probably a sign of the proatherogenic effect of stress.

Study of the lipid profile of the liver of mice under stress (Table 2) revealed a statistically significant decrease in the amount of free fatty acids (FFA) by 12% and an increase in the level of TAG by 15% and of cholesterol by 13% compared to the control. The resulting changes in the lipid parameters are associated with the stimulating effect of glucocorticoid hormones on adipocytes, which is accompanied by the mobilization of TAG and esterification of FFA (Solin et al., 2013). Under stress conditions, the release of catecholamines from the adrenal glands increases, under the influence of which peripheral lipolysis is activated, releasing non-esterified fatty acids from adipose tissue. As a result, excess FFA enters the bloodstream into the liver, where, together with glycerol, it is used for the synthesis of newly formed TAG, which subsequently leads to fatty degeneration of the liver.

In addition, fatty acids, due to the inhibition of their mitochondrial oxidation, are actively used in the form of acetyl-CoA for the synthesis of cholesterol, which may be associated with an increase in its level in the liver. Also, an increase in the amount of cholesterol may be due to inhibition of its breakdown in the liver due to the accumulation of lipid peroxides, which inhibit the enzyme 7-α-hydroxylase, involved in the

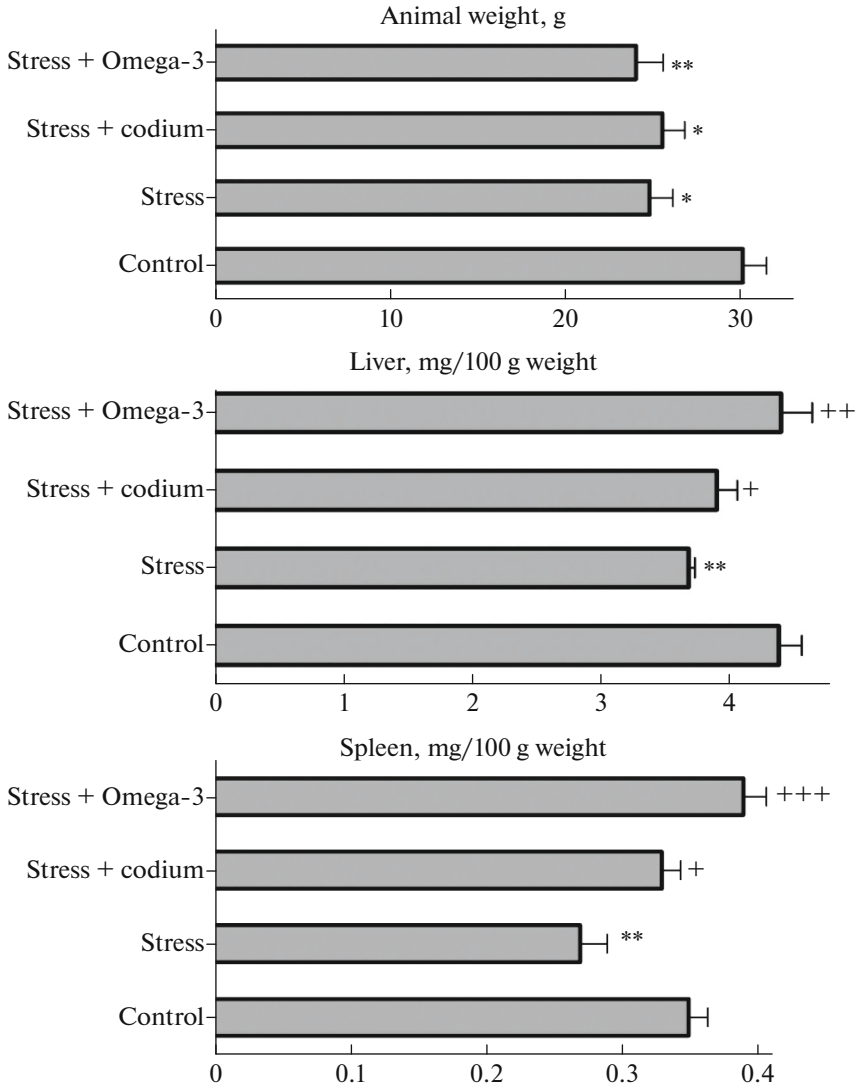

Fig. 1. Effect of the codium lipid extract and Omega-3 on the weight parameters of mice under stress. The changes are statistically significant: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ when compared with the control; $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$ when compared with group 2 (stress) for Figs. 1–3.

catabolism of cholesterol and its conversion into bile acids (Hulbert et al., 2005).

Analysis of indicators characterizing the state of the endogenous antioxidant system indicates the accumulation of reactive oxygen species in the blood and liver of animals under the influence of stress. In the blood plasma, there was a significant drop in the level of ARA (1.4 times; $p < 0.001$) and a decrease in the activity of the key antioxidant defense enzyme GP (by 26%; $p < 0.001$) compared to the control (Fig. 3). The G-SH content in the liver under stress also decreased by 36% ($p < 0.001$).

A decrease in GP activity may indicate an increase in the amount of fatty acid hydroperoxides and hydrogen peroxide (H_2O_2), which in turn react with superoxide radicals, leading to an uncontrolled increase in lipid peroxidation processes and the development of oxidative stress. Depletion of the hepatic G-SH pool, which is involved in many enzymatic and non-enzymatic antioxidant pathways, also indicates a mismatch and imbalance in the pro-oxidant–antioxidant system.

With the introduction of LEC (group 3) and Omega-3 (group 4) against a background of stress, there was a pronounced tendency towards normalization of both the weight characteristics and the biochemical parameters of the liver and blood of animals. This is evidenced by the absence of significant differences from the control in the indicators of the liver and spleen MIs in animals of the 3rd and 4th groups that received the preparations (Fig. 1). However, the weight of these animals still remained significantly lower than the control values, on average by 15% in the

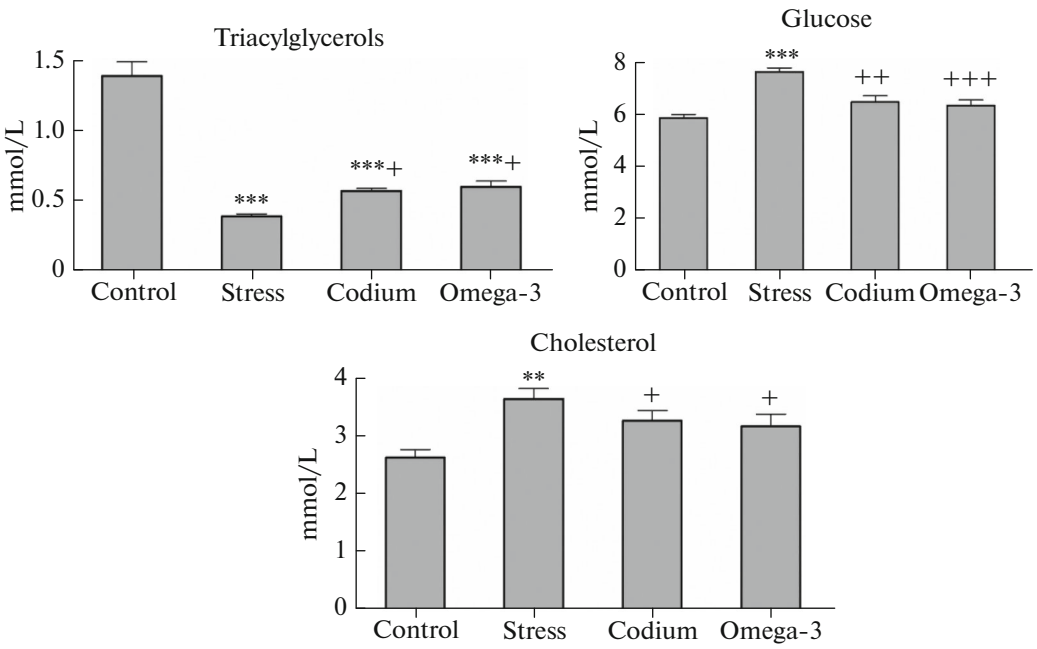

Fig. 2. The effect of the codium lipid extract and Omega-3 on the biochemical parameters of the blood plasma of mice under stress.

3rd group (Codium) and by 20% in the 4th group (Omega-3). At the same time in mice receiving the preprations, no ulcerations of the mucous membranes along the gastrointestinal tract were recorded.

When comparing the parameters of lipid metabolism in the blood plasma of mice of the 3rd (codium) and 4th (Omega) groups with those in the 2nd group (stress), significant differences were noted (Fig. 2). In animals treated with LEC (3rd group), the content of circulating TAG in the blood plasma increased by 45% ($p < 0.001$), and in those receiving Omega-3 (group 4), by 53% ($p < 0.001$), while the amount of cholesterol decreased by 16% ($p < 0.001$) and 13% ($p < 0.001$), respectively. Under the influence of the administered preparations, blood glucose levels decreased by 15% ($p < 0.001$) in group 3 (Codium) and by 17% ($p < 0.05$) in group 4 (Omega-3). According to the literature (Komal et al., 2020), the introduction of ω-3 PUFAs as part of fish oil helps to reduce the level of glucose in the blood of rats by increasing the sensitivity of the insulin signal. It has been noted that ω-3 PUFAs increase the level of the hormone adiponectin, which is responsible for reducing the fasting glucose levels and leading to increased peripheral insulin sensitivity (Ravussin, 2002). The decrease in the level of cholesterol in the blood plasma under the influence of the administered lipid preparations is possibly associated with a decrease in the activity of HMG-CoA reductase, a key enzyme in the biosynthesis of cholesterol. In the works of Khan and Makki (Khan and Makki, 2017), it is noted that the use of Omega-3 in experimental hypercholesterolemia reduces the cholesterol content in serum rat blood, which may be due to inhi-

Table 2. Effect of the codium lipid extract and Omega-3 on the content of neutral lipids in the liver of mice under stress ($M \pm m$)

Neutral lipids	Group1 Control	Group 2 Stress	Group 3 Stress + Codium	Group 4 Stress + Omega-3
Cholesterol	15.44 ± 0.67	17.48 ± 0.50*	15.04 ± 0.27[3]	15.74 ± 0.15[1]
Free fatty acids	15.86 ± 0.26	13.95 ± 0.36***	15.70 ± 0.18[3]	14.82 ± 0.08
Triacylglycerols	20.85 ± 0.47	24.02 ± 0.64***	21.08 ± 0.16[2]	20.76 ± 0.36[2]
Fatty acid esters	15.89 ± 0.51	14.05 ± 0.65	14.16 ± 0.33	14.47 ± 0.19
Cholesterol esters	16.04 ± 0.55	16.84 ± 0.78	17.39 ± 0.33*	17.74 ± 0.38*
Residual fraction	15.92 ± 0.26	13.66 ± 0.79	16.63 ± 0.21	16.47 ± 0.23

The changes are statistically significant: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ when compared with the control. [1] $p < 0.05$; [2] $p < 0.01$; [3] $p < 0.001$ when compared with group 2 (stress).

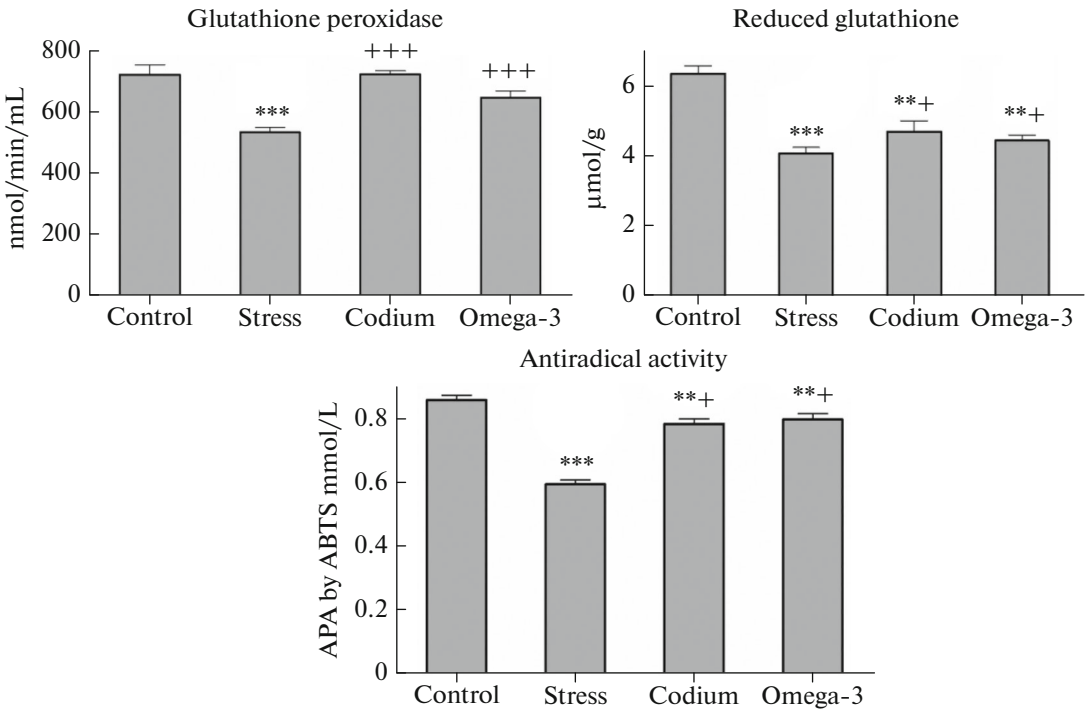

Fig. 3. The effect of the codium lipid extract and Omega-3 on the indicators of the antioxidant system in the blood plasma and liver of mice under stress.

bition of the process by the conversion of hydromethylglutarate to mevalonate (an intermediate substance in the formation of cholesterol).

The noted changes in the lipid metabolism parameters of the liver of mice of the 3rd and 4th groups were characterized by a pronounced tendency to restore the studied parameters against the background of stress and had practically no significant differences between the groups. Thus, administration of LEC to animals led to a decrease in the TAG content by 12% ($p < 0.01$) and in cholesterol by 14% ($p < 0.001$) compared to group 2 (stress). At the same time, under the influence of Omega-3, the level of TAG decreased by 14% ($p < 0.001$), and the amount of cholesterol, by 10% ($p < 0.05$). Among other lipid fractions of the liver of mice in groups 3 and 4, there was a significant increase in the concentration of cholesteryl esters (CE) by an average of 8–10% relative to group 2 (stress).

Thus, under the influence of the administered lipid complexes in the blood and liver of animals, there is a characteristic tendency to relieve the state of dyslipidemia and suppress fatty infiltration. This effect is due to the ability of ω-3 PUFAs included in the composition lipid complexes of marine origin to reduce TAG synthesis in the liver by inhibiting the enzyme acyl-CoA:1,2-diacylglycerol acyltransferase (Harris et al., 2008). At the same time, peroxisomal and/or mitochondrial β-oxidation of fatty acids increases and thereby the active entry of FFA into the liver decreases. A decrease in cholesterol levels with a simultaneous increase in its esters in the liver of animals treated with LEC and Omega-3 against the background of stress, is due, as already noted, to the hypocholesterolemic effect of ω-3 PUFAs, as well as the restoration of the esterifying function of the liver. In this case, mainly unsaturated fatty acids participate in the formation of ECS; the esterification of cholesterol is catalyzed by the liver enzyme Acyl-CoA: cholesterol acyltransferase (ACAT). Based on the above, it follows that the introduction of LEC and Omega-3 under stress helps restore the balance that determines the functioning of carbohydrate–lipid metabolism in the liver and blood.

LEC and the Omega-3 preparation exhibited a pronounced antioxidant effect against the background of stress. It is known that PUFAs of the ω-3 family are effective targeted antioxidants that can "quench" free radicals (Richard et al., 2008). This fact is confirmed by the noted increase in the value of ARA in blood plasma by an average of 32–34% ($p < 0.001$). It has been shown that ω-3 PUFAs restore GP activity (Refaat et al., 2022) and some low molecular weight antioxidants, including G-SH (Patten et al., 2013), which was noted when analyzing the results obtained.

Although under the influence of the preparations administered under stress conditions, the indicators of the glutathione system in animals of the 3rd and 4th groups were characterized by positive dynamics, there were significant differences in the severity of the normalizing effect. Thus, with the introduction of

LEC, the level of liver G-SH increased by 15% ($p < 0.05$) in comparison with group 2, and with the introduction of Omega-3, by only 9% ($p < 0.05$).

Indicators of GP activity in the blood plasma of animals of group 3 (codium) did not have significant differences from the control values, which may indicate suppression of lipid peroxidation processes. At the same time, in animals of group 4 (Omega-3), GP activity remained significantly lower than the control by 10%. When compared with group 2 (stress), GP activity in mice of group 3 increased by 35% ($p < 0.001$), and in group 4, by 21% ($p < 0.001$). The data obtained indicate that the effect of LEC on the state of the glutathione redox system showed greater effectiveness compared to the Omega-3 preparation. This LEC effect may be due to the combined action of the ω-3 and ω-6 PUFAs, which are capable of activating antioxidant defense enzymes (Nieto et al., 1998), including enzymes of the glutathione link, thereby protecting cells and organ tissues from damage.

CONCLUSIONS

Study of the composition of the lipid extract *C. fragile* showed its multicomponent nature, in particular the presence of nonpolar and polar lipids with a high content of PUFAs of the ω-3 and ω-6 families. From the data obtained on the study of metabolic reactions in the body of mice under stress conditions, it follows that the lipid extract of *C. fragile* has hypolipidemic and antioxidant effects. This effect of LEC is manifested in the restoration of the weight coefficients (MI of the liver and spleen), the content of lipids and carbohydrates in the liver and blood (CS, TAG, FFA, glucose), and the parameters of antioxidant protection of the animal body (ARA, G-SH, GP).

The lipid extract of the green algae *C. fragile* is not inferior to the commercial comparison supplement Omega-3 in restoring the body's metabolic reactions caused by acute stress, and at the same time it showed higher antioxidant activity. The biological effect of LEC is probably explained by its diverse composition, which contains a class of neutral lipids that are metabolites for biochemical reactions, a class of phospholipids responsible for the functioning of all biomembranes, and mainly the presence of PUFAs of the ω-3 and ω-6 families. Based on the research conducted, it can be concluded that prophylactic use of lipid extracts isolated from marine macrophytes, in particular from *C. fragile*, has great prospects for creating drugs with stress-protective and lipid-correcting properties.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The experiments with animals were conducted in compliance with the NIH Guidelines for the care and use of laboratory animals (http://oacu.od.nih.gov/regs/index.htm). Experimental protocols were approved by the Ethics Committee of Ilyichev Pacific Oceanological Institute (Protocol No. 21 of November 10, 2022).

CONFLICT OF INTEREST

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

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