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BIOCHEMISTRY

Evaluation of the Pharmacological Efficiency of a Lipid Extract from the Tunic of the Marine Hydrobiont *Halocynthia aurantium* (Pallas, 1774)

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Abstract—The effect of the lipid extract isolated from the tunic of the marine hydrobiont *Halocynthia aurantium* was studied. Its administration during hypothermia, hyperthermia, muscle load, introduction of hexenal, and the vertical fixation by the dorsal neck fold, as well as its embryotoxic and teratogenic effects, was evaluated. The impact of stress (vertical fixation of rats by the dorsal neck fold) was accompanied by an increase in the plasma levels of total lipids, total cholesterol, cholesterol/phospholipid ratios, and a decrease in the total phospholipids, as well as by a change in the quantitative characteristics of classes of neutral and phospholipids. A correction of the developed changes by a lipid extract of ascidia and a commercial reference preparation Omega-3 was carried out. The lipid extract of *H. aurantium* showed a higher efficiency in restoring the lipid composition of the blood plasma under stress impact compared to the preparation Omega-3 due to the wider range of neutral and phospholipid classes, polyunsaturated fatty acids of the n-3 and n-6 families. The tunic of ascidian can be used as a raw material for obtaining preparations with stress-protector and lipid-correcting properties.

Keywords: *Halocynthia aurantium*, lipid extract, hypothermia, hyperthermia, muscle load, embryotoxicity, teratogenicity, stress, blood plasma, lipids

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INTRODUCTION

Marine hydrobionts are promising sources of raw materials for the pharmaceutical industry. The study of the biological activity of substances isolated from marine aquatic organisms is associated with the presence of lipid structures in them, in particular phospholipids containing fatty acids, which are included in the n-3 and n-6 families. In the Far East of the Russian Federation, preparations obtained from sea cucumbers, cucumbers, bivalves, starfish, shrimp shells, and other hydrobionts are being studied intensively. Thus, in studies by T.P. Novgorodtseva et al. (2010), the presence of n-3 fatty acids and 1-*O*-alkyldiacylglycerol in the hepatopancreas of the Kamchatka crab was noted, which contribute to the normalization of the parameters of the immune, antioxidant, lipid transport, and hepatobiliary systems under high-fat load. A.A. Artyukov et al. (2012) found that a naphthoquinone pigment (Echinochrome A pharmaceutical substance) isolated from the shell of the sea urchin *Scaphechinus mirabilis* helped restore lipid and carbohydrate metabolism in patients with type-2 diabetes, had high antioxidant properties, and can be used as a means for the treatment and prevention of ischemic heart disease. In the work of N.M. Sanina et al. (2012), it was proven that the cucumarioside complex from the marine hydrobiont *Cucumaria japonica* with cholesterol and monogalactosyldiacylglycerol in a ratio of 3 : 2 : 6 can serve as a carrier of subunit antigens and be a highly effective adjuvant. In one study Hou et al. (2018) noted that sea urchin spinochromes have antioxidant, cytotoxic, and cardioprotective activity. The effectiveness of a mixture of oxygenated carotenoids (astaxanthin, lutein, and zeaxanthin) from starfish *Patiria pectinifera* has also been shown (Klimovich et al., 2018) when modeling skin carcinogenesis and allergic and inflammatory pathologies. There are works on studying the influence of extracts from seaweed of the Sea of Japan (*Saccharina* (=*Laminaria*) *japonica, Ulva lactuca, Sargassum pallidum,* and *Ahnfelthia tobuchiensis*) under experimental models of physical and chemical stress on laboratory animals (rats, mice) (Fomenko et al., 2019; Kushnerova et al., 2022; Sprygin et al., 2022).

Hydrobionts that are not used for food are of great scientific interest, in particular, the sea peach *Halocynthia aurantium*. According to zoological systematics, it belongs to the phylum of chordates (Chordata), subphylum of tunicates (Tunicata), class of ascidia (Ascidiae), order of folded ascidians (Stolidobranchiata) (Yavnov, 2010). They are common in the Far Eastern and Arctic seas at depths from 4 to 400 meters. In Peter the Great Bay of the Sea of Japan, there are individuals ranging in length from 6.5 to 27.5 cm and weighing from 52 to 950 g. The skin–muscle sac (tunic) has a thickness of 2.4 to 5.0 mm (Matrosova and Leskova, 2016). Ascidians are often found in fish bycatch. A promising direction may be its cultivation through mariculture (Bullard et al., 2013). The ascidian tunic contains a wide range of biologically active substances: lipid structures, proteins, carbohydrates, etc. A water–alcohol extract from the tunic in an experiment on laboratory animals (rats) contributed to the restoration of the leukocyte formula during radiation injury (Ponomareva, 2018), and experimental stress (Fomenko et al., 2012). Biologically active substances from marine hydrobionts help maintain reproductive health (Hoang et al., 2022), have antitumor and antioxidant properties (Jo et al., 2010; Krivoshapko and Popov, 2011), and are sources of amino acids (Tabakaeva and Tabakaev, 2017) and stimulants of the immune system (Motorya et al., 2009; Jang et al., 2022). At the same time, data on the lipid extract as a drug with pharmacological properties isolated from the tunic of the sea peach are limited. Fatty acids from the tunic of *H. aurantium* have been shown to have anti-inflammatory activity, which suppressed inflammation, and PGE2 production increased dose-dependently with their concentration (Monmai et al., 2018). The tunic may be an important raw material source for eicosapentaenoic and docosahexaenoic fatty acids, which are precursors of anti-inflammatory eicosanoids and docosanoids and can regulate anti-inflammatory activity (Lee et al., 2016). Such a multicomponent composition of a lipid extract isolated from the ascidian tunic led to a study of the spectrum of its pharmacological effects.

The purpose of this work is to study the lipid composition of the extract from the tunic of the sea peach and study some of its pharmacological effects.

MATERIALS AND METHODS

Sea peach (*Halocynthia aurantium*) specimens were collected during the summer period in Zapadnaya Bay, Popov Island, Peter the Great Bay (Sea of Japan). For this study, 50 specimens were used. The tunic was separated from the internal organs and base, rinsed with tap water, and dried at a temperature not exceeding 50°C. The dried tunic was ground in a laboratory mill to a size of 0.5–1.0 mm and extracted with a mixture of chloroform : methanol (2 : 1 by volume) (Bligh and Dyer, 1959). To obtain the value of the total lipids, the gravimetric method was used. Standardization of the lipid extract was carried out according to the value of the total lipids, and the dose of the administered drug was calculated in mg of total lipids per kilogram of animal weight (Novgorodtseva, 2010). The acute toxicity of the lipid extract (LD_{50}) was determined using the Kerber method (Mironov, 2012). All studies were carried out on outbred white male rats weighing 150 ± 5 g. Experiments to study the lipid extract of ascidians during pregnancy were performed on outbred white female rats weighing 150 ± 3 g. The animals were obtained from the nursery of the Stolbovaya Branch of the Federal State Budgetary Institution Scientific Center for Biomedical Technologies, Federal Medical and Biological Agency of Russia. Before the start of the experiment, the animals were adapted to the vivarium for seven days: their external condition was examined daily, and animals without health problems were taken into the experiment. Next, the rats were separated into experimental groups. In the free access mode, the animals were fed daily at the appointed time, and they received drinking water without restrictions.

The study of embryotoxic and teratogenic effects was carried out in accordance with the guidelines (Fisenko, 2000). Mature female rats with a regular estrous cycle were selected for the experiment, and vaginal smears were examined for 1–2 weeks. Males were placed with these rats for mating. The day of detection of sperm in the vagina was considered the first day of pregnancy. Twenty pregnant female rats were separated into two groups of ten animals each. Rats of group 1 (control) were intragastrically injected with a physiological solution at a dose of 1 g/kg animal weight on the 20th day of pregnancy. Animals of group 2 were administered intragastrically lipid extract of the ascidian squirrel during pregnancy at a dose of 1 g/kg animal weight. Rats of both groups were decapitated after 20 days of pregnancy (physiological pregnancy). The animals were dissected, and the ovaries, uterus, and fetuses were examined. The number of living fetuses, their weight, and the craniocaudal size characterized the embryotoxicity index. The number of fetuses with malformations characterized the teratogenic activity. Developmental defects (eyes, limbs, tail, anterior abdominal wall) were visually taken into account.

Stressful effects, such as running on an endless rope, an increase or decrease in temperature, and hexenal intoxication were studied according to the manual of A.N. Mironov (Mironov, 2012). For each stress exposure, the animals were separated into two groups of ten animals each. Into the first group (control), a physiological solution was injected intragastrically, and into the second group, ascidian lipid extract was injected intragastrically at a dose of 1 g/kg body weight. Sixty minutes after the administration of saline or lipid extract, the animals were subjected to a stress test.

The time it took the mice to run along an endless rope until they were completely tired determined the actoprotective effect. In the thermostat chamber TS-80-U4.2, the resistance of animals to overheating was studied (42°C). Rectal temperature measurements began one hour before the onset of overheating and after 120 minutes in the thermostat. Rectal temperature measurements were carried out with a Little Doctor LD-300 medical thermometer. From the onset of overheating to the moment of death of the animals, their life expectancy was assessed. In a thermostated refrigerator at a temperature of –8°C for 120 min, the rat resistance to hypothermia was noted. The rectal temperature was measured one hour before cooling and after 120 min in the refrigerating chamber. With intraperitoneal administration of hexenal (hexenal intoxication) at a dose of 50 mg/kg, the duration of sleep was assessed from its beginning until the animals left the lateral position (Mironov, 2012).

The next stage of the experiment was to study the effect of the lipid extract of ascidian and the reference drug Omega-3 on the parameters of lipid metabolism in the blood plasma of rats under conditions of stress–vertical fixation. The stress was modeled by fixing animals by the dorsal neck fold for 24 h (Kushnerova et al., 2005). The drugs were administered intragastrically through a tube immediately before vertical fixation and 6 h after the first administration. One gram of the Omega-3 preparation (Now Foods, United States) contains 350 mg of saturated fatty acids, 350 mg of monoenoic fatty acids, and 300 mg of Omega-3 fatty acids, represented by 180 mg of eicosapentaenoic acid and 120 mg of docosahexaenoic acid. The study consisted of four groups of ten animals each: the first group was the control (intact); the second group had stress (vertical fixation); the third group had stress + lipid extract of ascidian; and the fourth group had stress + Omega 3.

To obtain blood plasma, the generally accepted centrifugation method was used. Then extracts of total lipids were isolated from the blood plasma (Folch et al., 1957), in which the values of individual classes of phospholipids and neutral lipids were determined. The content of total lipids, total phospholipids, and total cholesterol in the blood plasma was determined using Olvex Diagnosticum diagnostic kits (Russia). Analysis of the phospholipid composition of the lipid extract of rat blood plasma was carried out using two-dimensional microthin layer chromatography (MTLC) on 6 × 6 cm glass plates with the KSK silica gel (Svetashev and Vaskovskii, 1972). Phospholipids were divided into classes in the solvent system (Vaskovskii and Terekhova, 1979); to reveal phospholipid classes on the plate, a molybdic acid reagent was used (Vaskovski et al., 1975). Specific detection of classes of phospholipids, which included an amino group (phosphatidylethanolamine, lysophosphatidylethanolamine, phosphatidylserine), was detected with a 0.2% solution of ninhydrin in acetone (Rouser et al., 1967), a choline group (phosphatidylcholine, lysophosphatidylcholine, sphingomyelin) with Dragendorff's reagent (Kates, 1975), and the hydroxyl group (phosphatidylinositol) is a periodate Schiff reagent (Kates, 1975). The values of individual classes of phospholipids were determined spectrophotometrically using a universal molybdate reagent (Vaskovskii et al., 1975). Neutral lipid classes were separated by one-dimensional microthin layer chromatography on silica gel (Amenta, 1964). To identify lipid stains, purified preparations of domestic production (Reakhim, Russia) were used. Calculation of the values of individual classes of neutral and phospholipids was expressed as a percentage of their total amount, respectively.

The composition and values of fatty acids in the lipid extract from the ascidian tunic were studied by gas–liquid chromatography (GLC). By transesterification of lipids, fatty acid methyl esters (FAMEs) were obtained (Carreau and Dubacq, 1978) and purified using MTLC. The study of FAMEs was carried out on an LHM-2000 gas chromatograph (OAO Chromatograph, Russia) with a flame ionization detector, capillary column HP-5-MS with 5% phenylmethylsiloxane (30 m × 0.25 mm, Agilent, United States). The carrier gas was helium, with the injector temperature 180°C and flow rate 50 mL/min. FAMEs were identified by comparing the retention times with known carbon number standards (Christie, 1988). The results were calculated as a percentage of the total fatty acids.

At the end of each experiment, the animals were decapitated 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 obtained data were processed using the statistical program Instat 3.0 (Graph Pad Software Inc., United States, 2005) with a built-in procedure for checking the compliance of the sample with the law of normal distribution. The statistical significance of differences in intergroup comparisons was determined using the nonparametric Mann–Whitney U test and the parametric Student t-test.

RESULTS

The lipid extract from the ascidian tunic is a yellow–brown oily mass. Table 1 shows the lipid composition of the extract. The amount of total lipids was 35.48 ± 0.70 mg/g dry tissue, which included 49.0% (17.38 ± 0.26 mg/g dry tissue) neutral lipids and 51.0% (18.1 ± 0.21% mg/g dry tissue) phospholipids. Among the neutral classes of lipids, triacylglycerols, waxes, and free fatty acids predominated in quantity (from 17 to 23%). The tunic lipid extract contains eight classes of phospholipids, which corresponds to the data known in the literature (Kostetskii et al., 2012). There

was a high level of phosphatidylcholine (PC), and the values of phosphatidylethanolamine (PE), phosphatidylserine (PS), and diphosphatidylglycerol (DPG) were in the range of 10 to 12%. These classes of phospholipids are essential in the metabolic reactions of the body. In the total lipids of the extract, saturated fatty acids accounted for 46.9%, and unsaturated fatty acids, for 53.1%, which is consistent with the literature data (Sanina et al., 2001). Of the individual FAs, palmitic (16 : 0), stearic (18 : 0), and α-linolenic (18 : 3 n-3) acids predominated in value. The extract contains a fairly large amount of polyunsaturated fatty acids (PUFA): acids of the n-3 family were 32.59%, which was three times higher than the content of PUFA of the n-6 family (9.92%). Based on the results obtained, it follows that the lipid extract from the ascidian tunic is a source of biologically active substances and, in the future, can be used to obtain effective medications and dietary supplements.

When studying the acute toxicity of the extract, it was determined that it is 2350 mg/kg, so this substance can be classified as toxicity class 4 (as low-hazard).

The results of the study on the effect of ascidian lipid extract on the body during physiological pregnancy and stress are presented in Tables 2 and 3.

Study of Embryotoxic and Teratogenic Effects

The introduction of ascidian lipid extract was accompanied by a statistically significant increase in the number of living fetuses relative to the control by 8% ($p < 0.05$) (Table 2). The average weight of live fetuses was 17% ($p < 0.001$) greater than in the control. The average craniocaudal size of the fetuses in this group of animals was also 3% larger ($p < 0.05$). The number of fetuses did not differ statistically significantly depending on gender, but a tendency towards more births of females than males was observed. With the introduction of ascidian lipid extract, there was also a decrease in the number of fetuses with deformities compared to the control by an average of 44% ($p < 0.05$). Anomalies of fetal development were in the form of cryptorchidism and incomplete ossification of the sternum.

Hypothermia. In animals of both groups (control and experiment), the rectal temperature decreased under the influence of hypothermia. When the ascidian extract was administered, the drop in rectal temperature was inhibited by 14% ($p < 0.001$).

Overheating. When in the thermostat chamber, the rectal temperature increased in rats of both groups. However, the differences between the groups were not statistically significant.

Overheat lifespan. When administered the ascidian lipid extract, there was an increase in the life expectancy of rats by an average of 7.4% ($p < 0.05$) compared to that in control animals.

Muscle load. Endless rope on installation time running of animals increased after administration of ascidian lipid extract on average by 47% ($p < 0.001$) compared to the control.

Hexenal intoxication (hexenal sleep). The duration of sleep in rats in the group with the administration of extract from the ascidian tunic decreased by 56% ($p < 0.001$) compared to that in the control.

Stress–vertical fixation. When studying biochemical parameters in the blood plasma, an increase in the amount of total lipids (TLs) was noted by 39% ($p < 0.001$). The level of total cholesterol was increased by 1.4 times ($p < 0.01$), and total phospholipids were reduced by 20% ($p < 0.001$) (Table 3). This caused a 1.8-fold increase ($p < 0.001$) in the cholesterol/phospholipid ratio. Thus, during stress–vertical fixation of animals, pronounced dyslipidemia was observed in the blood plasma. The values of individual classes of neutral lipids differed significantly from the control (Table 3). Thus, the level of triacylglycerols (TAG) was increased by 13% ($p < 0.001$). Free fatty acids (FFAs) and cholesterol (C) increased by an average of 15 and 19% ($p < 0.001$), respectively. At the same time, there was a decrease in the value of cholesterol esters (CE) by 12% ($p < 0.001$). Under the influence of stress, there is a mismatch in the values of phospholipid classes in the blood plasma. The level of phosphatidylcholine (PC) was reduced by 8% ($p < 0.001$); phosphatidylethanolamine (PE), by 18% ($p < 0.05$); and diphosphatidylglycerol (DPG), by 26% ($p < 0.001$). It is worth noting the increase in the value of lysoforms of phospholipids (PL) and lysophosphatidylcholine (LPC) by 28% ($p < 0.001$) and lysophosphatidylethanolamine (LPE) by 56% ($p < 0.001$). Against the background of an increase in the number of lysoforms, there was an increase in the LPC/PC ratio from 0.13 in the control to 0.18 under stress, and the LPE/PE from 0.31 in the control to 0.59 under stress. The level of sphingomyelin (SM) increased by 34% ($p < 0.001$), and the sum of metabolically active phospholipids (phosphatidylinositol and phosphatidylserine PI + PS), by 21% ($p < 0.01$).

The administration of ascidian lipid extract and the reference drug Omega-3 was accompanied by a tendency to relieve the state of dyslipidemia, but the degree of the normalizing effect varied. Thus, with the introduction of ascidian lipid extract, the amount of total lipids in the blood plasma decreased by 29% ($p < 0.001$), while with the introduction of Omega-3, by 20% ($p < 0.01$). The value of total phospholipids increased by an average of 29% ($p < 0.001$) (group 3) and by 24% ($p < 0.05$) (group 4). Cholesterol levels decreased by 28% ($p < 0.001$) and 20% ($p < 0.001$), respectively, which caused a decrease in the C/PL ratio by 44% ($p < 0.001$) with the introduction of ascidian lipid extract and by 35% ($p < 0.001$) with the introduction of Omega-3.

Table 1. Quantitative composition of neutral lipids, phospholipids, and fatty acids in the total lipids of the tunic of the sea peach ($M \pm m$, % of the total)

Biochemical parameters	Indicators
Neutral lipids, % of the sum of all classes	
Diacylglycerols	9.10 ± 0.92
Triacylglycerols	26.97 ± 1.45
Cholesterol	4.35 ± 0.70
Free fatty acids	16.98 ± 1.20
Methyl esters of fatty acids	7.26 ± 0.95
Fatty aldehydes	4.74 ± 1.10
Cholesterol esters	6.71 ± 0.12
Wax	23.89 ± 2.60
Phospholipids, % of the sum of all classes	
Phosphatidylcholine	50.80 ± 1.34
Lysophosphatidylcholine	3.00 ± 0.12
Sphingomyelin	6.11 ± 0.40
Phosphatidylethanolamine	10.53 ± 0.74
Lysophosphatidylethanolamine	2.94 ± 0.08
Phosphatidylinositol	4.79 ± 0.52
Phosphatidylserine	11.73 ± 2.41
Diphosphatidylglycerol	10.10 ± 1.95
Fatty acids, % of the total fatty acids	
12:0 (lauric)	1.10 ± 0.01
14:0 (myristic)	4.63 ± 0.99
16:0 (palmitic)	23.80 ± 2.10
18:0 (stearic)	17.37 ± 2.00
16:1 n-9 (palmitoleic)	5.70 ± 1.30
18:1 n-9 (oleic)	4.89 ± 0.40
18:2 n-6 (linoleic)	1.13 ± 0.10
20:3 n-6 (eicosatriene)	1.20 ± 0.40
20:4 n-6 (arachidonic)	4.69 ± 0.55
22:4 n-6 (docosatetraenoic)	2.90 ± 0.60
18:3 n-3 (α-linolenic)	15.85 ± 0.20
20:5 n-3 (eicosapentaenoic)	10.98 ± 0.55
22:6 n-3 (docosahexaenoic)	5.76 ± 0.54

The results of comparing the values of classes of neutral lipids in the blood plasma of groups 3 and 4 relative to group 2 (stress) showed that the administration of drugs was accompanied by a decrease in TAG levels by 12% (group 3, $p < 0.001$) and by 9% (group 4, $p < 0.001$); FFA, by 12 and 8% ($p < 0.001$); and C, by 20 and 13% ($p < 0.001$), respectively. CE values in the blood plasma of rats of both groups increased by an average of 14 and 9% ($p < 0.001$), respectively. Significant differences were noted when comparing the values of phospholipid classes in groups 3 and 4 with group 2. Thus, the number of PCs increased in both groups by an average of 7–10% ($p < 0.01$–0.001) with a simultaneous decrease in LPC by 23 and 12% ($p < 0.01$–0.001), and the ratio of LPC/PC values was 0.12 and 0.14, respectively. The value of sphingomyelin decreased by 28% with the administration of ascidian extract and by 21% ($p < 0.001$) with the administration of Omega-3. There was also an increase in PE levels by 15–18% ($p < 0.001$). At the same time, there was a decrease in the number of LPEs by 41% (group 3) and 25% (group 4) ($p < 0.001$), which led to a decrease in LPE/PE to 0.3 and 0.38, respectively. Among the metabolically active classes of phospholipids, noteworthy is the decrease in the value of the total PI + PS fraction by an average of 11–14% ($p < 0.05$), as well as

Table 2. Testing of ascidian lipid extract during physiological pregnancy and stress exposure ($M \pm m$)

Indicators	Group 1 Control (saline solution)	Group 2 Ascidian Lipid Extract
Number of fetuses on the 20th day of pregnancy, %		
Alive	84.0 ± 2.0	91.0 ± 2.3[1]
With deformities	16.0 ± 2.2	9.0 ± 1.7[1]
Average weight of live fetuses, g	2.4 ± 0.02	2.8 ± 0.05[3]
Average craniocaudal size of fetuses, cm	3.1 ± 0.02	3.2 ± 0.03[1]
Fetal gender		
Males	48 ± 2	44 ± 2
Females	52 ± 3	56 ± 3
The magnitude of the decrease in rectal temperature during hypothermia (−8°C, 120 min, deg)	−2.12 ± 0.07	−1.82 ± 0.03[3]
The magnitude of the increase in rectal temperature during overheating (42°C, 120 min, deg)	+3.93 ± 0.10	+3.82 ± 0.15
Life expectancy when overheated (42°C, min)	127.31 ± 2.70	136.79 ± 2.75[1]
Amount of muscle work on an endless rope, min	27.24 ± 1.13	40.17 ± 1.52[3]
Hexenal sleep, min	25.74 ± 1.66	11.33 ± 1.72[3]

Compared to the control [1] $p < 0.05$; [2] $p < 0.01$; [3] $p < 0.001$.

Table 3. Biochemical parameters of blood plasma under the influence of stress and its correction with a lipid extract from the ascidian tunic and Omega 3 ($M \pm m$)

Indicators	Group 1 Control	Group 2 Stress	Group 3 Stress + ascidian	Group 4 Stress + Omega 3
General lipids, g/L	4.73 ± 0.13	6.58 ± 0.20[3]	4.68 ± 0.18[c]	5.27 ± 0.15[1, c, *]
General phospholipids, mmol/L	2.50 ± 0.05	2.00 ± 0.04[3]	2.57 ± 0.06[c]	2.48 ± 0.04[c]
Total cholesterol, mmol/L	2.90 ± 0.02	4.17 ± 0.04[3]	3.00 ± 0.05[c]	3.36 ± 0.03[3, c, ***]
Cholesterol / Phospholipids	1.16 ± 0.03	2.08 ± 0.02[3]	1.16 ± 0.03[c]	1.35 ± 0.03[3, c, ***]
Neutral lipids				
TAG	24.00 ± 0.28	27.11 ± 0.29[3]	23.80 ± 0.31[c]	24.77 ± 0.26[c, *]
FFA	13.08 ± 0.25	15.00 ± 0.27[3]	13.20 ± 0.19[c]	13.77 ± 0.17[c, *]
C	15.00 ± 0.31	17.90 ± 0.30[3]	14.42 ± 0.18[c]	15.61 ± 0.14[c, ***]
CE	18.11 ± 0.21	16.00 ± 0.20[3]	18.20 ± 0.21[c]	17.47 ± 0.25[c, *]
Residual fraction	29.81 ± 0.70	23.99 ± 0.44	30.38 ± 0.62	28.48 ± 0.49
Phospholipids				
PC	63.08 ± 0.70	57.86 ± 0.87[3]	63.65 ± 0.72[c]	62.00 ± 0.69[b]
LPC	8.00 ± 0.20	10.20 ± 0.33[3]	7.88 ± 0.37[c]	8.97 ± 0.16[2, b]
SM	8.92 ± 0.24	11.94 ± 0.24[3]	8.64 ± 0.11[c]	9.38 ± 0.25[c]
PE	10.15 ± 0.63	8.37 ± 0.21[1]	9.89 ± 0.14[c]	9.66 ± 0.22[c]
LPE	3.17 ± 0.14	4.95 ± 0.13[3]	2.92 ± 0.11[c]	3.71 ± 0.11[3, b]
PI + PS	3.68 ± 0.13	4.45 ± 0.16[2]	3.98 ± 0.13[a]	3.84 ± 0.13[a]
DPG	3.00 ± 0.05	2.23 ± 0.03[3]	2.94 ± 0.07[c]	2.44 ± 0.05[3, c]

The differences are statistically significant when [1, a, *] $p < 0.05$; [2, b, **] $p < 0.01$; [3, c, ***] $p < 0.001$. Numbers mean compared to the control; letters mean compared to Group 2, asterisks mean compared to the Group 3. TAG, triacylglycerols; FFA, free fatty acids; C, cholesterol; CE, cholesterol esters; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; SM, sphingomyelin; PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; DPG, diphosphatidylglycerol. The residual fraction is waxes + hydrocarbons + methyl esters of fatty acids.

an increase in the level of DPG by 32% in group 3 ($p < 0.001$) and 9% ($p < 0.001$) in group 4.

It should be noted that, when the comparison drug Omega-3 was administered, statistically significant differences with the control were noted. This was manifested in an increased level of total lipids by 11% ($p < 0.05$) and of C and the C/PL ratio by an average of 16% ($p < 0.001$). Among the phospholipid classes, significant differences from the control are noteworthy in the values of LPC (increase by 14%, $p < 0.01$), LPE (increase by 27%, $p < 0.001$), and DPG (decrease by 17%, $p < 0.001$). Thus, the administration of the ascidian lipid extract and Omega-3 preparation under stress had a general tendency to restore the values of phospholipid classes.

DISCUSSION

Based on the results obtained, it follows that the ascidian lipid extract had low rates of acute toxicity, which makes it possible to characterize it as a harmless drug. This resulted in the absence of embryotoxic and teratogenic effects. There was a greater number of live fetuses and greater weight and size than in the control group. Thus, the pharmacological effect of the ascidian lipid extract is due to the participation of the lipid components of the drug in metabolic reactions to maintain homeostasis in the maternal body during pregnancy. Studies have shown the positive nature of the drug's action in the embryonic period. The prevention of the teratogenic effect is due, in our opinion, to the presence in the preparation of the main structural components of membranes (phospholipid classes) and metabolites (neutral classes of lipids) for the construction of organs and tissues of the growing organism. Experimental studies examining the pharmacological effects of a lipid extract from the ascidian tunic on models of overheating, hypothermia, muscle stress, and hexenal intoxication included in the stereotypical list of factors used in the study of stress-protective agents, showed a pronounced protective effect of the drug. Apparently, the biochemical mechanism of this phenomenon is due to increased energy metabolism due to the presence of energy-rich components of the drug (FFA, TAG, PUFA). The presence of metabolically active phospholipids (PI + PS), as well as DPG, necessary for the functioning of the electron transport chain in mitochondria, contributed to the restoration of metabolic pathways for energy production in the Krebs cycle. In the experiment with overheating, an increase in life expectancy was noted, which is due, in our opinion, to the preservation of metabolic reactions due to the metabolites and PUFAs of the n-3 and n-6 families included in the extract, which are bioregulators and precursors of biologically active eicosanoids. In addition, it is known that PUFA n-3 and n-6 are involved in the induction of enzymes of the glutathione antioxidant system (glutathione peroxidase and glutathione reductase) (Nieto et al., 1998), which is necessary under stress to protect against radical damage to membranes.

When conducting a hexenal test, preliminary administration of ascidian extract to animals significantly reduced sleep, which indicates the preservation of the antitoxic function of the liver.

In animals of group 2 "stress–vertical fixation," the formation of dyslipidemia was noted. A study of neutral lipid classes in plasma showed pronounced hypertriglycerinemia and hypercholesterolemia, as well as an increase in FFA and a decrease in CE. The results obtained are due to increased lipolytic processes in adipose tissue, which is associated with increased release of catecholamines from the adrenal glands (Solin et al., 2013). It is also known that liver glycogen is used extensively, which activates the FFA β-oxidation energy pathway. This leads to the formation of excess acetyl-CoA and the synthesis of C. The pathway of PL formation from TAG is also blocked, which is due to inhibition of the esterification reaction. As a result, fatty hepatosis is formed in the liver due to the accumulation of TAG, and the level of CE is reduced in the blood and liver (Momot et al., 2016). The decrease in the values of the main structural phospholipids (PC and PE) is caused by both an increase in the activity of phospholipases and the overoxidation of the fatty acids that make up their composition. As a result, the values of FFA and lyso compounds (LPC and LPE) increase. An increase in the amount of SM, which belongs to the group of choline-containing classes of phospholipids, in our opinion, is a replacement mechanism for reducing the PC value. The fact that PS + PI and DPG values decreased suggests an impairment of membrane-bound enzymes and the transport function.

The administration of ascidian lipid extract and the reference drug Omega-3 had a general tendency to normalize lipid metabolism. Apparently, this phenomenon is due to the incorporation of omega-3 PUFA into lysophospholipids. This led to an increase in the PC and PE values and a decrease in their lyso compounds, as well as of the LPC/PC and LPE/PE ratios. Of note is the ability of PUFA to esterify cholesterol and reduce phospholipase activity (Asztalos et al., 2016), which is confirmed by an increase in the CE values and total phospholipids from TAG.

The effectiveness of the ascidian lipid extract in our animal experiments had advantages over the commercial Omega 3 preparation. It is known that biologically active substances isolated from marine aquatic organisms are mainly due to the presence of n-3 PUFA in their composition. Thus, the commercial Omega-3 preparation, which is industrially produced from valuable fish species, contains eicosapentaenoic, docosahexaenoic, and monoenoic fatty acids. In our study, the PUFA content of both preparations was 300 mg per gram of total lipids. At the same time, the ascidian lipid extract contains a wider range of lipid structures:

seven classes of neutral lipids, which are metabolites for biochemical reactions, and eight classes of phospholipids that, along with monoenoic fatty acids, include PUFAs of the n-3 and n-6 families. This, in our opinion, may provide a higher biological activity of the extract than that of the Omega 3 drug.

CONCLUSIONS

The lipid extract isolated from the tunic of *H. aurantium*, containing neutral and phospholipid classes, PUFA n-3 and n-6, has a lipid-correcting and stress-protective effect. The ascidian extract in its biological effect corresponds to the commercial drug Omega 3, but in terms of the effect of restoring biochemical parameters in the blood plasma, it had the advantages of being able to normalize the state of dyslipidemia, preserve the metabolic reactions of the synthesis of PL from TAG, and maintain the quantitative values of neutral and phospholipid classes in the blood plasma. The ascidian tunic can be used as a raw material source for isolating a biologically active substance (extract) of a lipid nature, which has protective properties under stress, as well as for the correction of lipid metabolism disorders.

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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. 19 of April 12, 2022). 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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