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ASSESSMENT OF ANTIOXIDANT ACTIVITY OF SEAWEED EXTRACTS FROM THE SEA OF JAPAN *IN VITRO* AND *IN VIVO*

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Seaweeds are a source of important biologically active substances: lipids, amino acids, phenolic compounds, polycarbohydrates, *etc.* Polyphenolic compounds are one of the perspective groups of constituents of marine origin with high antioxidant activity; those play a key role in the life of marine macrophytes, allowing them to quickly respond to external stress and to perform protective functions. At the same time, the multicomponent composition of the phenolic fraction of the seaweed extract provides a wide spectrum of its pharmacological activity, *inter alia* a regulatory effect on numerous homeostasis disorders occurring during pathological processes in humans and animals. Wherein, the available opportunities for the practical use of seaweed extracts have not yet been depleted, and this is of undoubted interest for modern science. The aim of the work was to carry out a comparative assessment of the antioxidant activity of hydroalcoholic extracts isolated from the thalli of three classes of algae [brown (*Sargassum pallidum*), green (*Ulva lactuca*), and red (*Ahnfeltia fastigiata* var. *tobuchiensis*)] and to analyze their effect on indices of the endogenous antioxidant system of liver and blood in mice under experimental stress. Seaweeds were sampled in summer in the coastal waters of the Peter the Great Bay (the Sea of Japan). Sampled seaweeds were dried at a temperature of about +50 °C, grinded in a laboratory mill to particles 0.5–1 mm in size, and extracted with 70% ethanol *via* repercolation. In the extract of the brown alga *S. pallidum*, the highest content of polyphenols was recorded – (218.2 ± 20.3) mg-Eq GA·g^{-1} dry weight. In the extract of the green alga *U. lactuca*, the value was (16.2 ± 1.8) mg-Eq GA·g^{-1} dry weight; in the extract of the red alga *A. fastigiata* var. *tobuchiensis*, (9.1 ± 1.6) mg-Eq GA·g^{-1} dry weight. Accordingly, the antiradical activity of *S. pallidum* extract towards the cation radical ABTS^{+} and the alkyl peroxyl radical was significantly higher than that of *U. lactuca* and *A. fastigiata* var. *tobuchiensis* extracts. The effect of these seaweed extracts on the antioxidant defense indices of liver and plasma in mice under acute stress was studied experimentally. Weight indicators (weight of animals and weight coefficients of their internal organs) and biochemical indices (level of antiradical activity, malondialdehyde and reduced glutathione content, and activity of antioxidant enzymes) were established. The experiment was carried out on white outbred male mice (weight of 20–30 g). To model conditions of acute stress, mice were fixed vertically by the dorsal neck crease for 24 h. Alcohol-free seaweed extracts were injected into mice stomachs as an aqueous suspension (a dose of 100 mg of total polyphenols *per* kg of body weight) through a tube twice: right before vertical fixation and in 6 h. Into stomachs of the animals of the control and the "stress" groups, distilled water was injected in a volume equal to that of the injected extracts. In this model, all the attributes of stress manifested themselves: adrenal hypertrophy, involution of the thymus and spleen, and ulceration of the gastric and intestinal mucosa. Moreover, disturbances of the antioxidant defense system were recorded: a decrease of antioxidant enzymes activity in blood plasma, a drop in reduced glutathione

content in liver, and an increase of the malondialdehyde level. Under the effect of the extracts, in all the groups of animals under stress, a tendency to stabilization of the studied antioxidant defense indices was observed. Interestingly, the values in mice receiving *U. lactuca* and *A. fastigiata* var. *tobuchiensis* extracts were inferior to those in the group of animals receiving *S. pallidum* extract. In the latter group of mice, there were no significant differences from the control values in terms of antioxidant defense indices. This is due to the fact the main components of the polyphenolic fractions of green and red algae are monomeric flavonoids, while brown algae contain high molecular weight phlorotannins. The latter ones are characterized by higher antioxidant activity than low molecular weight polyphenolic fractions of green and red algae.

Keywords: seaweeds, polyphenols, antioxidant activity, stress, mice

An important component of marine ecosystems and a key link in food chains of many species of marine organisms are seaweeds serving as a source of organic matter and energy. Due to their diverse composition, they are used as a raw material for production of several substances with beneficial properties. Specifically, seaweeds contain easily digestible proteins, amino acids, lipids, polysaccharides, carotenoids, minerals, polyphenolic compounds, *etc.* [Michalak, Chojnacka, 2015].

Among secondary metabolites that make up seaweeds, an important group of substances are polyphenolic compounds with pronounced antioxidant properties. These compounds are produced by seaweeds and seagrasses to perform protective, structural, and reproductive functions [Pradhan et al., 2021]. Polyphenols are involved in growth and reproduction processes of seaweed cells and in formation and early development of cell walls, forming a complex with alginic acid, a structural polysaccharide of a cell wall [Imbs, Zvyagintseva, 2018]. Polyphenols are capable of protecting macrophytes from damage by pathogenic bacteria, grazing by herbivores, and UV exposure. According to international classification, polyphenolic compounds include various subclasses: phenolic acids, flavonoids, lignans, stilbenes, and so on [Zhong et al., 2020]. In composition of brown algae and some species of red algae, a special group of phenolic compounds was found – phlorotannins, which are oligomers of phloroglucinol (1,3,5-trihydroxybenzene) [Ragan, Glombitza, 1986]. Phlorotannins are the main cytoplasmic components of seaweeds; those are contained in specific organelles – physodes [Ragan, Glombitza, 1986]. Unlike other polyphenolic compounds, phlorotannins are characterized by the fact that about 90% of their total amount is in free form [Bogolitsyn et al., 2018]. These compounds are accumulated mainly in outer layers of the epidermis and in cortical layer of the thallus [Shibata et al., 2004], which allows them to quickly respond to external stress and to perform protective functions.

A rising interest in seaweeds is due to the content of bioactive components in them, which can be used as pharmaceuticals, nutraceuticals, and food additives. As known, preparations derived from seaweeds exhibit a wide range of pharmacological properties: antibacterial, antiviral, antitumor, antimicrobial, hepatoprotective, *etc.* [Cotas et al., 2020; Manach et al., 2004].

Health benefits of seaweeds for humans and animals are largely due to the ability of their polyphenolic compounds to scavenge free radicals, which may help in reducing oxidative stress [Zhong et al., 2020]. The mechanism of active binding of free radicals involved in the development of several pathological processes in the body is based on the presence of a branched structure of conjugated double bonds of high mobility and a large number of free hydroxyl groups in macrophyte polyphenols.

In previous studies, we found that extracts isolated from a number of marine macrophytes representing different classes [brown, *Sargassum pallidum* (Turner) C. Agardh, 1820; green, *Ulva lactuca* Linnaeus, 1753; and red, *Ahnfeltia fastigiata* var. *tobuchiensis* (Kanno & Matsubara) Skriptsova

& Zhigadlova, 2022] have shown a pronounced protective effect in various experimental models. Thus, an extract from the brown alga *S. pallidum*, enriched with polyphenolic compounds, had a hepatoprotective effect in modelling hepatosis in rats [Sprygin et al., 2017]. The lipid fraction of an extract from the green alga *U. lactuca* showed a preventive effect under acute stress, which manifested itself in the preservation of carbohydrate–lipid metabolism in liver and a decrease in the level of lipid peroxidation [Fomenko et al., 2019]. The pharmacological effect of an extract from the red alga *A. fastigiata* var. *tobuchiensis* was expressed in the ability to restore the lipid composition of blood and the ratio of phospholipid fractions in erythrocyte membranes [Kushnerova et al., 2020]. As a continuation of the studies carried out, it seems relevant to acquire new knowledge on the biological activity of the investigated seaweed extracts and to clarify the prospects for their use as antioxidant agents.

The aim of the work is to compare antioxidant activity of hydroalcoholic extracts isolated from thalli of the brown alga *Sargassum pallidum*, green alga *Ulva lactuca*, and red alga *Ahnfeltia fastigiata* var. *tobuchiensis* and to determine their effect on antioxidant defense indices of liver and blood plasma of mice under experimental stress.

MATERIAL AND METHODS

The objects of the study were seaweeds:

- *Ulva lactuca* [= *Ulva fenestrata*], division Chlorophyta, class Ulvophyceae, order Ulvales, family Ulvaceae;
- *Sargassum pallidum*, division Phaeophyta, class Cyclosporophyceae, order Fucales, family Sargassaceae;
- *Ahnfeltia fastigiata* var. *tobuchiensis* [= *Ahnfeltia tobuchiensis*], division Rhodophyta, class Florideophyceae, order Ahnfeltiales, family Ahnfeltiaceae [Skriptsova, Zhigadlova, 2022].

The selected seaweeds are the most widespread in the seas of the Far East and are the main, mass species.

Seaweeds were sampled in August–September 2021 in the Peter the Great Bay coastal waters (the Sea of Japan). A sample included 20 thalli of each species. All thalli were cleaned of epiphytes and zoobenthos, washed with seawater and then distilled water, and dried. A thallus in the air-dried state was grinded using a laboratory mill to 0.5–1-mm particles and extracted with 70% ethanol *via* repercolation. The extract yield was 1 L *per* 1 kg of dry raw material. Extraction with ethanol is an effective method of seaweed processing: during it, most mineral and organic substances exhibiting biological activity are extracted, and ethanol, due to its low toxicity, is the most preferable for the extraction of phenolic compounds among all solvents [Cotas et al., 2020].

Seaweed extracts were evaporated in a vacuum until ethanol was completely removed; then, those were extracted with chloroform to scavenge lipophilic substances and pigments – in accordance with the technique described earlier [Sprygin et al., 2013]. The resulting aqueous fraction containing polyphenols was evaporated to dryness in a vacuum and resuspended in water to obtain stock solutions (10 $mg \cdot mL^{-1}$), in which total polyphenols (hereinafter PP) and antiradical activity (hereinafter ARA) were preliminarily determined. All biochemical studies were carried out on a Shimadzu UV-2550 spectrophotometer (Japan). Total PP were determined using the Folin–Ciocalteu reagent at a wavelength of 765 nm [Parys et al., 2007]. Gallic acid (GA) was used as a reference standard; total PP were expressed in mg-Eq $GA \cdot g^{-1}$ dry extract. The level of ARA was also assessed spectrophotometrically towards the cation radical $ABTS^{+}$ (λ = 734 nm) [Re et al., 1999] and the alkyl peroxyl

radical (λ = 414 nm) [Bartosz et al., 1998]. When determining ARA, Trolox (a water-soluble analog of vitamin E) was used as a reference standard. ARA was expressed in μmol Trolox·mg^{-1} PP. The results were statistically processed with Instat 3.0 software package and GraphPad Prism program, with the second one including the function of checking a sample compliance with the normal distribution law. To determine the statistical significance of differences depending on the distribution parameters, the parametric Student's *t*-test or the nonparametric Mann–Whitney *U*-test were used.

The experiment was carried out on white outbred male mice (weight of 20–30 g). Animals were kept under vivarium conditions in cages of 5 individuals on a standard diet, with natural light, and at a constant air temperature of +20…+22 °C.

To model conditions of acute stress, mice were fixed vertically by the dorsal neck crease for 24 h. This stress model is used in laboratory animals in experimental studies [Kushnerova et al., 2005]. Alcohol-free seaweed extracts were injected into mice stomachs as an aqueous suspension (a dose of 100 mg of total PP *per* kg of body weight) through a tube twice: right before vertical fixation and in 6 h. This concentration corresponds to a known therapeutic dose for polyphenolic hepatoprotectors [Vengerovsky et al., 1999]. Into stomachs of the animals of control and "stress" groups, distilled water was injected in a volume equal to that of the injected extracts.

In the course of the study, there were five groups of animals, 10 mice each: 1, control (intact); 2, "stress" (vertical fixation by the dorsal neck crease); 3, "stress + *Sargassum* extract"; 4, "stress + *Ulva* extract"; and 5, "stress + *Ahnfeltia* extract." Animals were taken out of 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 Experimental and Other Scientific Purposes [1986].

After exposure to acute stress, weight of animal, the mass index of internal organs (mg of organ weight *per* 100 g of body weight), and the number of ulcerations of the gastric mucosa were determined. The latter ones were counted visually: we determined the number of formed ulcerative lesions. The design of the study was approved by the ethics committee of V. I. Il'ichev Pacific Oceanological Institute FEB RAS.

Blood for research was sampled from neck vein of animals into vacuettes with 1% heparin solution. To separate plasma, blood was centrifuged for 10 min at 3,000 rpm. Then, plasma samples were frozen at a temperature of −80 °C for further determination of biochemical parameters. After extraction, liver was washed in physiological saline and frozen in a refrigerator at −80 °C as well. The state of the antioxidant system was assessed in blood plasma of animals by a spectrophotometric method by the value of total ARA (λ = 734 nm) [Re et al., 1999], the level of malonic dialdehyde (hereinafter MDA) (λ = 532 nm) [Buege, Aust, 1978], activity of superoxide dismutase (hereinafter SOD) (λ = 550 nm) [Paoletti et al., 1986], and glutathione enzymes – glutathione reductase (hereinafter GR) [Goldberg, Spoone, 1983] and glutathione peroxidase (hereinafter GPx) (λ = 340 nm) [Burk et al., 1980], as well as by the value of the level of reduced glutathione (hereinafter G-SH) in liver tissue (λ = 412 nm) [Ellman, 1959].

RESULTS

Prior to conducting the experiment aimed at analyzing the state of the antioxidant defense system of an animal body under stress, total PP and ARA were determined in samples of extracts of the macrophytes studied. Evaluation of the quantitative composition of PP in them showed that their content

varies significantly in different macrophyte species (Table 1). The highest amount of PP was registered in the extract of the brown alga *S. pallidum*, and the value was 13.5 times higher than in *U. lactuca* and 24 times higher than in *A. fastigiata* var. *tobuchiensis*.

Table 1. Polyphenol content and antiradical activity of the seaweed extracts ($M \pm m$)

Seaweed	Total polyphenols, mg-Eq GA·g^{-1} dry extract	Antiradical activity towards ABTS^{+}, μmol Trolox·mg^{-1} PP	Antiradical activity towards alkyl peroxyl radicals, μmol Trolox·mg^{-1} PP
Sargassum pallidum	218.2 ± 20.3	1.62 ± 0.04	0.64 ± 0.02
Ulva lactuca	16.2 ± 1.8	0.32 ± 0.03	0.15 ± 0.02
Ahnfeltia fastigiata var. *tobuchiensis*	9.1 ± 1.6	0.13 ± 0.03	0.06 ± 0.01

An important aspect of investigating the antioxidant potential of the studied seaweed extracts is the assessment of their ARA towards the cation radical ABTS^{+} and the alkyl peroxyl radical. The level of ARA in the extracts of the macrophytes varied significantly, as well as PP content. Specifically, *S. pallidum* extract was characterized by a higher level of ARA towards ABTS^{+}: the value was 5 times higher than the corresponding value in *U. lactuca* and 12.5 times higher than in *A. fastigiata* var. *tobuchiensis.* A similar trend was observed for ARA indices towards the alkyl peroxyl radical. Alkyl peroxyl radicals are formed in the body during lipid peroxidation and are one of the main initiators of free radical reactions. In *S. pallidum* extract, this index was 4 times higher than the corresponding value for *U. lactuca*. An even lower level of APA towards the alkyl peroxyl radical was recorded in *A. fastigiata* var. *tobuchiensis*: its value was 2.5 times less than that for *U. lactuca* and 10 times less than that for *S. pallidum*.

The next stage of the experimental studies was the investigation of the effect of the seaweed extracts on the state of the antioxidant defense system in animals under stress. Their vertical fixation by the dorsal neck crease for 24 h was accompanied by the manifestation of all known attributes of stress, such as adrenal hypertrophy, involution of the thymus and spleen, and ulceration of the gastric and intestinal mucosa. These alterations were recorded in all animals subjected to stress (the groups 2–5). However, there were significant differences between the groups in terms of severity. Thus, in the group 2 ("stress"), weight of animals decreased by 23% ($p < 0.01$) with a simultaneous decrease in the mass index of internal organs (liver and spleen) by an average of 19–23% ($p < 0.05$) (Fig. 1). In the group 2, under stress, the number of recorded ulcerations of the gastric mucosa was (2.6 ± 0.1) pcs *per* animal; in the control, it was 0.

When assessing the state of the antioxidant system of animals under stress, a drop in ARA value of blood plasma by 46% was revealed ($p < 0.001$) compared to the value in the control. At the same time, there was a decrease in the activity of one of key enzymes of the antioxidant defense system, SOD, by 40% ($p < 0.001$) (Fig. 2). The level of G-SH in liver dropped by almost 2 times (Fig. 3), while the activity of GR, an enzyme which plays the main role in maintaining a certain concentration of G-SH inside the cell, decreased by 26% ($p < 0.001$). The activity of another key enzyme of the glutathione unit, GPx, which catalyzes the reduction of hydrogen peroxide (H_2O_2) and organic peroxides in the presence of G-SH, was reduced by 35% ($p < 0.001$). Such changes in the antioxidant defense indices can be

defined as its weakening. Disturbances in the functioning of the antioxidant defense system under stress conditions also manifested themselves in an increase in MDA content by 68% ($p < 0.001$) (Fig. 3), which is a biomarker of oxidative stress.

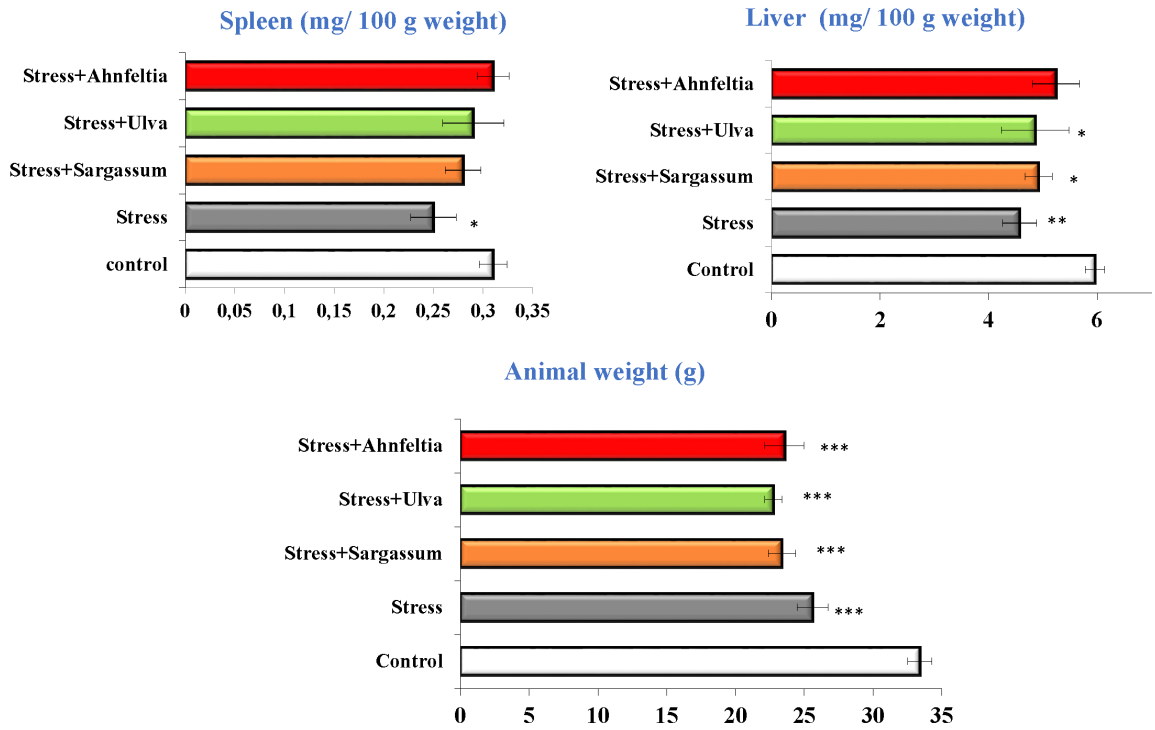

Fig. 1. The effect of the seaweed extracts on the total weight of mice and weight coefficients of their internal organs under stress. Differences are statistically significant compared to the control: $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***)

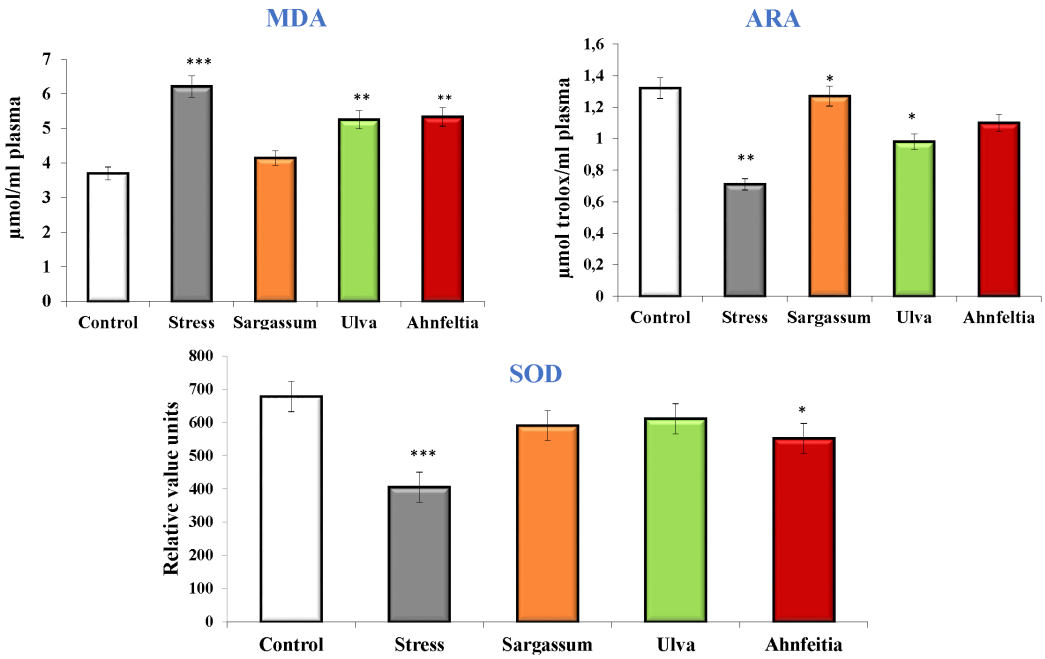

Fig. 2. The effect of the seaweed extracts on the antioxidant defense indices of mice under stress. MDA, malondialdehyde; ARA, antiradical activity; SOD, superoxide dismutase. Differences are statistically significant compared to the control: $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***)

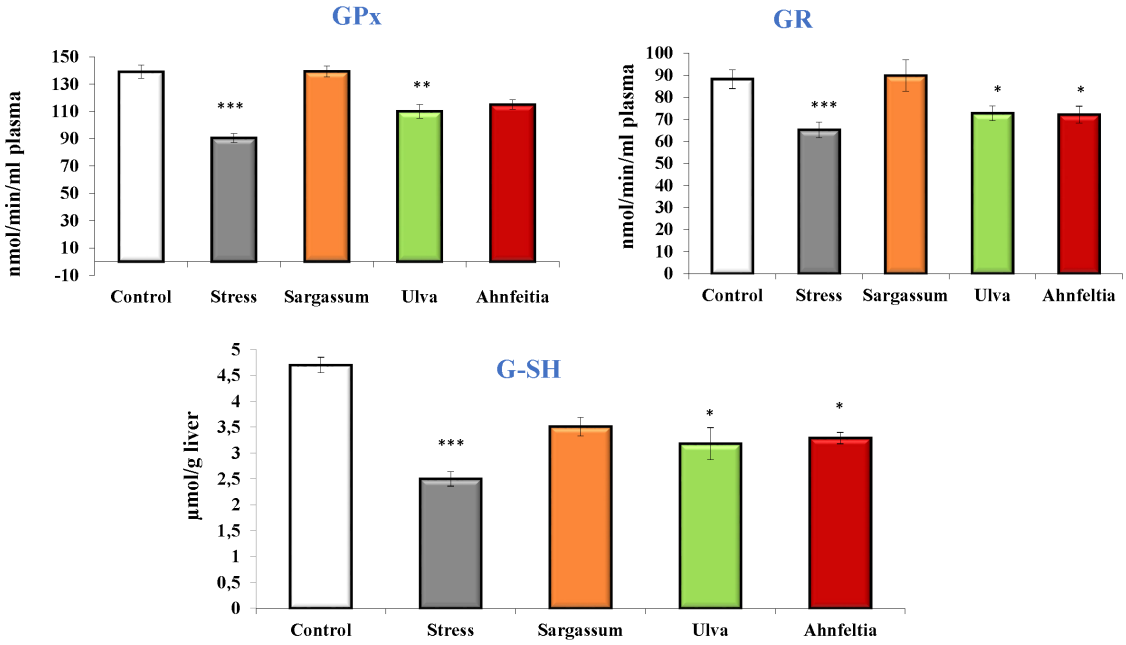

Fig. 3. The effect of the seaweed extracts on the glutathione system indices in mice under stress. GPx, glutathione peroxidase; GR, glutathione reductase; G-SH, reduced glutathione. Differences are statistically significant compared to the control: $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***)

The administration of the seaweed extracts against the backdrop of acute stress (the groups 3–5) was accompanied by a tendency to reduce the severity of involutional alterations in internal organs compared with their severity in the group 2 ("stress"). Specifically, in the groups of mice treated with *U. lactuca* and *S. pallidum* extracts, the relative mass of liver increased by an average of 6–8% ($p < 0.05$), while in the group 5, under the effect of *A. fastigiata* var. *tobuchiensis* extract, the value was 15% higher ($p < 0.05$). Moreover, in mice receiving the seaweed extracts, the relative mass of spleen increased by an average of 12–24% ($p < 0.001$). As noted, the injection of the seaweed extracts did not lead to the complete restoration of the relative mass of internal organs, but contributed to a significant increase in these indicators compared to the group 2 (see Fig. 1). In terms of the body weight parameters of mice in the groups 3–5, these indicators significantly differed from the control. Importantly, these animals had no ulcerations of the gastric mucosa.

In all groups of animals treated with the seaweed extracts, against the backdrop of stress, there was a tendency to stabilize the studied antioxidant defense indices (Figs 2, 3). Thus, in the group 3 (*S. pallidum* extract), the values corresponded to the control ones. The comparison with the group 2 ("stress") revealed as follows: in these mice, the level of MDA in blood plasma decreased by 33% ($p < 0.001$), ARA value increased by 1.8 times ($p < 0.001$), and the activity of SOD rose by 46% ($p < 0.001$). Under the effect of *S. pallidum* extract, there was also an increase in the level of G-SH in liver tissue by 40% ($p < 0.001$), while the activity of antioxidant enzymes, GPx and GR, in blood plasma rose by an average of 38–54% ($p < 0.001$).

The state of the antioxidant system in animals of the groups 4 and 5 (mice treated with *U. lactuca* and *A. fastigiata* var. *tobuchiensis* extracts under acute stress) was characterized by positive dynamics as well. However, the studied biochemical parameters still differed significantly from the control. At the same time, when compared with the group 2 ("stress"), it was recorded as follows: in blood plasma of mice of the groups 4 and 5, there was an increase in the level of ARA by 37% ($p < 0.001$)

and 54% ($p < 0.001$), respectively. The activity of SOD in animals of the group 4 (*U. lactuca*) rose by 51% ($p < 0.001$); in mice of the group 5 (*A. fastigiata* var. *tobuchiensis*), it rose by 36% ($p < 0.001$). Meanwhile, MDA in blood plasma of these animals decreased on average by 14–16% ($p < 0.001$). In terms of the level of G-SH in liver tissue and the activity of glutathione enzymes, there was positive dynamics as well. Specifically, the use of *U. lactuca* and *A. fastigiata* var. *tobuchiensis* extracts was accompanied by an increase in G-SH content by 27 and 32%, respectively ($p < 0.05$). In its turn, the activity of GPx in animals in these groups rose on average by 22–27% ($p < 0.05$), and the activity of GR, by 12–20% ($p < 0.05$).

DISCUSSION

From the results obtained, it follows that under conditions of acute stress, the complex functional balance of organs and systems of the whole organism is disturbed. This is evidenced by a drop in the relative mass of internal organs (liver and spleen). Interestingly, a significant decrease in the spleen mass index results from the involution of the lymphatic system, which is associated with increased secretion of steroid hormones by the adrenal cortex causing the breakdown of lymphocytes and inhibition of metabolic processes in cells [Chrousos, 2009]. The antioxidant defense system is stressed due to overproduction of free radicals under certain exposure [Şahın, Gümüşlü, 2007]. As a result, the antioxidant system of the body is incapable of coping with their excessive production, while the activity of antioxidant enzymes (SOD, GPx, and GR) and G-SH content decrease. This phenomenon underlies the violations of many metabolic reactions in the body. Evidence of increased generation of free radicals is a significant decrease in ARA value along with a rise in the level of MDA in blood plasma of mice; it is characterized by high activity of peroxidation of fatty acids making up membrane lipids and is accompanied by a rise in the permeability of cell membranes in various tissues [Şahın, Gümüşlü, 2007]. Subsequently, the lack of antioxidant defense factors leads to an uncontrolled increase in lipid peroxidation processes and to development of oxidative stress.

The administration of the seaweed extracts against the backdrop of stress was accompanied by a rise in the activity of antioxidant enzymes and G-SH content with a simultaneous drop in the level of MDA. However, in the groups 4 and 5, as it was noted earlier, the values of the antioxidant defense indices (MDA, G-SH, GPx, and GR) still differed from the control ones. At the same time, the values of the antioxidant defense indices in mice treated with *U. lactuca* and *A. fastigiata* var. *tobuchiensis* extracts were inferior to those in animals of the group 3 (*S. pallidum*). This fact is confirmed by the calculation of statistical significance between the values of the studied biochemical parameters in blood plasma and liver tissue of mice of the groups 3–5. Thus, the values of the activity of GPx and GR for blood plasma in animals treated with *U. lactuca* and *A. fastigiata* var. *tobuchiensis* extracts (the groups 4 and 5, respectively) were lower on average by 17–21% ($p < 0.05$) compared with the values in the group 3 (*S. pallidum* extract). Significant differences between these groups were also revealed for other indices: the level of MDA was higher by 27–28% ($p < 0.01$); G-SH content was lower by 7–9% ($p < 0.05$); and ARA was lower by 13–23% ($p < 0.001$).

In our opinion, this effect is driven by the fact that metabolic activity of polyphenols in the extract of the brown alga *S. pallidum* is noticeably higher than in the extracts of *U. lactuca* and *A. fastigiata* var. *tobuchiensis*. Accordingly, *S. pallidum* extract has a higher level of ARA, and this is confirmed by the data obtained (see Table 1). As known, the main components of the polyphenolic fractions of green and red algae are monomeric flavonoids [Alagan et al., 2017; de Quirós et al., 2010]. In turn, high molecular weight

phlorotannins of brown algae and their extracts enriched with phlorotannins exhibit high antioxidant activity [Ferreres et al., 2012; Wang et al., 2012], in contrast to low molecular weight polyphenolic fractions of green and red algae. According to the researchers [Agregán et al., 2018], seaweed extracts with high content of polyphenolic compounds have a pronounced antioxidant potential.

Based on the data obtained, it can be concluded as follows: under stress, the administration of seaweed extracts contributed to the restoration of the indices of the antioxidant defense system, which plays a key role in the course of most vital processes.

Conclusions:

1. In mice, under conditions of experimental acute stress, a violation of metabolic reactions of the body was registered, which was accompanied by involution of the lymphatic system, appearance of ulcerations of the gastric mucosa, decrease in the weight of internal organs, stress in the antioxidant defense system, and activation of lipid peroxidation reactions.
2. The administration of seaweed extracts contributed to the stabilization of the antioxidant defense system, which is involved in the course of most vital processes.
3. A prophylactic use of seaweed extracts enriched with polyphenolic compounds under stress conditions contributed to the restoration of weight coefficients of internal organs of animals (liver and spleen) and the absence of ulcerations of the gastric mucosa.
4. Seaweeds *Sargassum pallidum*, *Ulva lactuca*, and *Ahnfeltia fastigiata* var. *tobuchiensis* are a promising raw material for the production of drugs capable of increasing the potential of the endogenous antioxidant defense system of the body under conditions of stress-induced disorders.
5. The predominant effect of the extract of the brown alga *S. pallidum* under stress is determined by the high-molecular structure of phlorotannins, which provides higher antioxidant activity compared to that of monomeric flavonoids in *U. lactuca* and *A. fastigiata* var. *tobuchiensis* extracts.

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REFERENCES

1. Bogolitsyn K. G., Druzhinina A. S., Ovchinnikov D. V., Kaplitsyn P. A., Shulgin E. V., Parshina A. E. Polyphenols of brown algae. *Khimiya rastitel'nogo syr'ya*, 2018, no. 3, pp. 5–21. (in Russ.). https://doi.org/10.14258/jcprm.2018031898
2. Vengerovsky A. I., Markova I. V., Saratikov A. S. Doklinicheskoe izuchenie gepatozashchitnykh sredstv. *Vedomosti farmakologicheskogo komiteta*, 1999, no. 2, pp. 9–12. (in Russ.)
3. Imbs T. I., Zvyagintseva T. N. Phlorotannins are polyphenolic metabolites of brown algae. *Biologiya morya*, 2018, vol. 44, no. 4, pp. 217–227. (in Russ.). https://doi.org/10.1134/S0134347518040010
4. Kushnerova N. F., Sprygin V. G., Fomenko S. Ye., Rakhmanin Yu. A. Impact of stress on hepatic lipid and carbohydrate metabolism, prevention. *Gigiena i sanitariya*, 2005, no. 5, pp. 17–21. (in Russ.)
5. Kushnerova N. F., Fomenko S. E., Sprygin V. G., Momot T. V. The effects of the lipid complex of extract from the marine red alga *Ahnfeltia tobuchiensis* (Kanno et Matsubara) Makienko on the biochemical parameters of blood plasma and erythrocyte membranes during experimental

stress exposure. *Biologiya morya*, 2020, vol. 46, no. 4, pp. 269–276. (in Russ.). https://doi.org/10.31857/S0134347520040051

6. Sprygin V. G., Kushnerova N. F., Fomenko S. E., Sizova L. A., Momot T. V. The hepatoprotective properties of an extract from the brown alga *Saccharina japonica*. *Biologiya morya*, 2013, vol. 39, no. 1, pp. 50–54. (in Russ.)
7. Sprygin V. G., Kushnerova N. F., Fomenko S. E., Drugova E. S., Lesnikova L. N., Merzlyakov V. Y., Momot T. V. The influence of an extract from the marine brown alga *Sargassum pallidum* on the metabolic reactions in the liver under experimental toxic hepatitis. *Biologiya morya*, 2017, vol. 43, no. 6, pp. 444–449. (in Russ.)
8. Fomenko S. E., Kushnerova N. F., Sprygin V. G., Drugova E. S., Lesnikova L. N., Merzlyakov V. Yu. Lipid composition and membranoprotective action of extract from marine green algae *Ulva lactuca* (L.). *Khimiya rastitel'nogo syr'ya*, 2019, no. 3, pp. 41–51. (in Russ.). https://doi.org/10.14258/jcprm.2019035116
9. Agregán R., Munekata P. E. S., Franco D., Carballo J., Barba F. J., Lorenzo J. M. Antioxidant potential of extracts obtained from macro- (*Ascophyllum nodosum*, *Fucus vesiculosus* and *Bifurcaria bifurcata*) and micro-algae (*Chlorella vulgaris* and *Spirulina platensis*) assisted by ultrasound. *Medicines*, 2018, vol. 5, iss. 2, art. no. 33 (9 p.). https://doi.org/10.3390/medicines5020033
10. Alagan V. T., Valsala R. N., Rajesh K. D. Bioactive chemical constituent analysis, *in vitro* antioxidant and antimicrobial activity of whole plant methanol extracts of *Ulva lactuca* Linn. *British Journal of Pharmaceutical Research*, 2017, vol. 15, no. 1, pp. 1–14. https://doi.org/10.9734/BJPR/2017/31818
11. Bartosz G., Janaszewska A., Ertel D., Bartosz M. Simple determination of peroxyl radical-trapping capacity. *Biochemistry and Molecular Biology International*, 1998, vol. 46, iss. 3, pp. 519–528. https://doi.org/10.1080/15216549800204042
12. Buege J. A., Aust S. D. Microsomal lipid peroxidation. In: *Biomembranes. Part C, Biological Oxidants, Microsomal, Cytochrome P-450, and Other Hemoprotein Systems* / F. Sidney, P. Lester (Eds). New York : Academic Press, 1978, pp. 302–310. (Methods in Enzymology ; vol. 52). https://doi.org/10.1016/s0076-6879(78)52032-6
13. Burk R. F., Lawrence R. A., Lane J. M. Liver necrosis and lipid peroxidation in the rat as the result of paraquat and diquat administration: Effect of selenium deficiency. *The Journal of Clinical Investigation*, 1980, vol. 65, iss. 5, pp. 1024–1031. https://doi.org/10.1172/JCI109754
14. Chrousos G. P. Stress and disorders of the stress system. *Nature Reviews Endocrinology*, 2009, no. 5, pp. 374–381. https://doi.org/10.1038/nrendo.2009.106
15. Cotas J., Leandro A., Monteiro P., Pacheco D., Figueirinha A., Gonçalves A. M. M., da Silva G. J., Pereira L. Seaweed phenolics: From extraction to applications. *Marine Drugs*, 2020, vol. 18, iss. 8, pp. 384–431. https://doi.org/10.3390/md18080384
16. de Quirós A. R.-B., Lage-Yusty M. A., López-Hernández J. Determination of phenolic compounds in macroalgae for human consumption. *Food Chemistry*, 2010, vol. 121, iss. 2, pp. 634–638. https://doi.org/10.1016/j.foodchem.2009.12.078
17. Ellman G. L. Tissue sulfhydryl group. *Archives of Biochemistry and Biophysics*, 1959, vol. 82, iss. 1, pp. 70–77. https://doi.org/10.1016/0003-9861(59)90090-6

18. *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes.* Strasbourg : Council of Europe, 1986, 11 p. (European Treaty Series ; no. 123). URL: https://rm.coe.int/168007a67b [accessed: 28.12.2021].
19. Ferreres F., Lopes G., Gil-Izquierdo A., Andrade P. B., Sousa C., Mouga T., Valentão P. Phlorotannin extracts from Fucales characterized by HPLC-DAD-ESI-MS*n*: Approaches to hyaluronidase inhibitory capacity and antioxidant properties. *Marine Drugs*, 2012, vol. 10, iss. 12, pp. 2766–2781. https://doi.org/10.3390/md10122766
20. Goldberg D. M., Spoone R. J. Assay of glutathione reductase. In: *Methods of Enzymatic Analysis.* Vol. 3: Enzymes 1. Oxidoreductases, transferases. 3rd edition / H. U. Bergmeyer (Ed.). Weinheim : Verlag Chemie, 1983, pp. 258–265.
21. Manach C., Scalbert A., Morand C., Rémésy C., Jiménez L. Polyphenols: Food sources and bioavailability. *The American Journal of Clinical Nutrition*, 2004, vol. 79, iss. 5, pp. 727–747. https://doi.org/10.1093/ajcn/79.5.727
22. Michalak I., Chojnacka K. Algae as production systems of bioactive compounds. *Engineering in Life Sciences*, 2015, vol. 15, iss. 2, pp. 160–176. https://doi.org/10.1002/elsc.201400191
23. Paoletti F., Aldinucci D., Mocali A., Caparrini A. A sensitive spectrophotometric method for the determination of superoxide-dismutase activity in tissue extracts. *Analytical Biochemistry*, 1986, vol. 154, iss. 2, pp. 536–541. https://doi.org/10.1016/0003-2697(86)90026-6
24. Parys S., Rosenbaum A., Kehraus S., Reher G., Glombitza K.-W., König G. M. Evaluation of quantitative methods for the determination of polyphenols in algal extracts. *Journal of Natural Products*, 2007, vol. 70, iss. 12, pp. 1865–1870. https://doi.org/10.1021/np070302f
25. Pradhan B., Patra S., Behera C., Nayak R., Jit B. P., Ragusa A., Jena M. Preliminary investigation of the antioxidant, anti-diabetic, and anti-inflammatory activity of *Enteromorpha intestinalis* extracts. *Molecules*, 2021, vol. 26, iss. 4, pp. 1171–1187. https://doi.org/10.3390/molecules26041171
26. Ragan M. A., Glombitza K. W. Phlorotannins, brown algal polyphenols. In: *Progress in Phycological Research*. Bristol : Biopress Ltd, 1986, vol. 4, pp. 129–241.
27. Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 1999, vol. 26, iss. 9–10, pp. 1231–1237. https://doi.org/10.1016/s0891-5849(98)00315-3
28. Şahın E., Gümüşlü S. Stress-dependent induction of protein oxidation, lipid peroxidation and anti-oxidants in peripheral tissues of rats: Comparison of three stress models (immobilization, cold and immobilization–cold). *Clinical and Experimental Pharmacology and Physiology*, 2007, vol. 34, iss. 5–6, pp. 425–431. https://doi.org/10.1111/j.1440-1681.2007.04584.x
29. Shibata T., Kawaguchi S., Hama Y., Inagaki M., Yamaguchi K., Nakamura T. Local and chemical distribution of phlorotannins in brown algae. *Journal of Applied Phycology*, 2004, vol. 16, pp. 291–296. https://doi.org/10.1023/B:JAPH.0000047781.24993.0a
30. Skriptsova A. V., Zhigadlova G. G. A revision of the red algal genus *Ahnfeltia* on the Russian coast of the North Pacific. *Phycologia*, 2022, vol. 61, iss. 4, pp. 396–402. https://doi.org/10.1080/00318884.2022.2061154

31. Wang T., Jónsdóttir R., Liu H., Gu L., Kristinsson H. G., Raghavan S., Ólafsdóttir G. Antioxidant capacities of phlorotannins extracted from the brown algae *Fucus vesiculosus*. *Journal of Agricultural and Food Chemistry*, 2012, vol. 60, iss. 23, pp. 5874–5883. https://doi.org/10.1021/jf3003653

32. Zhong B., Robinson N. A., Warner R. D., Barrow C. J., Dunshea F. R., Suleria H. A. R. LC-ESI-QTOF-MS/MS characterization of seaweed phenolics and their antioxidant potential. *Marine Drugs*, 2020, vol. 18, iss. 6, pp. 331–352. https://doi.org/10.3390/md18060331

ОЦЕНКА АНТИОКСИДАНТНОЙ АКТИВНОСТИ ЭКСТРАКТОВ ИЗ МОРСКИХ ВОДОРОСЛЕЙ ЯПОНСКОГО МОРЯ *IN VITRO* И *IN VIVO*

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Морские водоросли являются источником важных биологически активных соединений — липидов, аминокислот, фенолов, полисахаридов и др. Перспективную группу веществ морского происхождения составляют полифенольные соединения, обладающие высокой антиоксидантной активностью, которые играют ключевую роль в жизнедеятельности морских макрофитов, что позволяет им быстро реагировать на внешний стресс и выполнять защитные функции. В то же время многокомпонентный состав фенольной фракции экстракта из водорослей обусловливает широкий спектр её фармакологической активности, включающей регулирующее влияние на многочисленные нарушения гомеостаза при патологических процессах в организме животных и человека. При этом имеющиеся возможности практического использования экстрактов из водорослей ещё не исчерпаны, что представляет несомненный интерес для современной науки. Цель работы — выполнить сравнительную оценку антиоксидантной активности водноспиртовых экстрактов, выделенных из талломов представителей трёх классов водорослей [бурых (*Sargassum pallidum*), зелёных (*Ulva lactuca*) и красных (*Ahnfeltia fastigiata* var. *tobuchiensis*)], а также проанализировать их влияние на показатели антиоксидантной защиты печени и плазмы крови мышей при экспериментальном стрессе. Водоросли собирали в летние месяцы в прибрежных водах залива Петра Великого Японского моря, затем сушили при температуре около +50 °С, измельчали на лабораторной мельнице до частиц размером 0,5–1 мм и экстрагировали 70%-ным этиловым спиртом методом перколяции. Наибольшее количество полифенолов отмечено в экстракте бурой водоросли *S. pallidum* — (218,2 ± 20,3) мг-экв ГК·г$^{-1}$ сухого веса. В экстракте зелёной водоросли *U. lactuca* значение этого показателя составляло (16,2 ± 1,8) мг-экв ГК·г$^{-1}$ сухого веса, в экстракте красной водоросли *A. fastigiata* var. *tobuchiensis* — (9,1 ± 1,6) мг-экв ГК·г$^{-1}$ сухого веса. Соответственно, антирадикальная активность экстракта *S. pallidum* по отношению к катион-радикалу 2,2'-азино-бис(3-этилбензотиазолин-6-сульфоновой кислоты) ($ABTS^+$) и алкилпероксильному радикалу была существенно выше, чем таковая экстрактов *U. lactuca* и *A. fastigiata* var. *tobuchiensis*. Проведена экспериментальная проверка с целью определить влияние исследуемых экстрактов водорослей на показатели антиоксидантной защиты печени и плазмы мышей в условиях острого стресса. В задачи эксперимента входило установление весовых показателей (вес животных, индекс массы внутренних органов) и биохимических параметров (уровень антирадикальной активности, содержание малонового диальдегида и восстановленного глутатиона, активность антиоксидантных ферментов). Эксперимент по стрессовому воздействию проводили на белых беспородных мышах-самцах массой 20–30 г. Острый стресс моделировали путём вертикальной фиксации животных за дорсальную шейную складку на 24 ч. Освобождённые от спирта экстракты водорослей вводили в виде водной взвеси в дозе

100 мг общих полифенолов на кг массы тела в желудок мышам через зонд дважды — непосредственно перед вертикальной фиксацией и спустя 6 ч. Животным контрольной группы и группы «стресс» вводили дистиллированную воду в объёме, равном объёму вводимых препаратов. В данной модели проявились все атрибуты стресса: гипертрофия надпочечников, инволюция тимуса и селезёнки, изъязвления слизистой желудка и кишечника. Также были отмечены нарушения системы антиоксидантной защиты, которые выражались в снижении активности антиоксидантных ферментов в плазме крови, уменьшении содержания восстановленного глутатиона в печени и увеличении уровня малонового диальдегида. Под действием экстрактов во всех группах животных на фоне стресса прослежена тенденция к стабилизации исследуемых показателей антиоксидантной защиты. При этом показатели у мышей, получавших экстракты из *U. lactuca* и *A. fastigiata* var. *tobuchiensis*, уступали аналогичным параметрам в группе животных, получавших экстракт *S. pallidum*. В группе животных, получавших экстракт *S. pallidum*, в показателях антиоксидантной защиты не было выявлено достоверных отличий от контрольных значений. Данный факт обусловлен тем, что основными компонентами полифенольных фракций зелёных и красных водорослей являются мономерные флавоноиды, тогда как в бурых водорослях присутствуют высокомолекулярные флоротаннины, которые проявляют более высокую антиоксидантную активность, чем низкомолекулярные полифенольные фракции зелёных и красных водорослей.

Ключевые слова: морские водоросли, полифенолы, антиоксидантная активность, стресс, мыши