INFLUENCE OF THE EXTRACTS FROM MARINE ALGAE ON THE ANTIOXIDANT SYSTEM STATUS AT ACUTE STRESS

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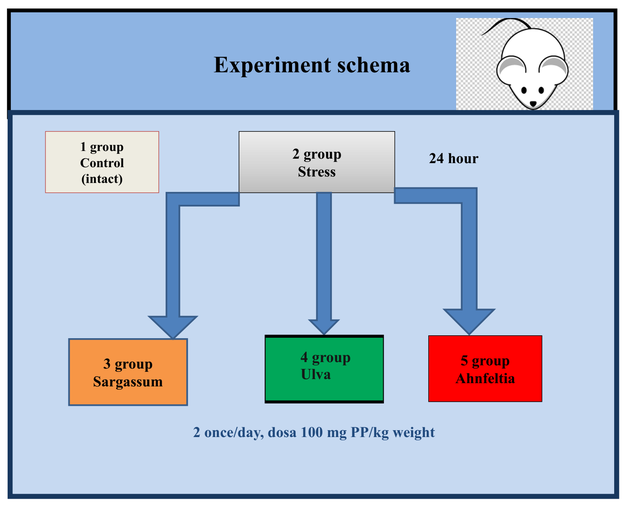
Marine algae are of great interest as a source of biologically active compounds. It is known that algae-derived preparations exhibit a wide range of pharmacological activities (antibacterial, antiviral, antitumor, antimicrobial, hepatoprotective, etc.). The algae composition includes easily digestible proteins, amino acids, lipids, polysaccharides, carotenoids, polyphenols, minerals, etc. Polyphenols as compounds with high antioxidant activity possess a great importance. Phlorotannins is a prevailing group of polyphenolic compounds in seaweed. It contains a large number of conjugated double bonds with high mobility and free hydroxyl groups, because of that, they have the ability to inactivate free radicals, neutralizing their damaging effects to the body.

In previous studies, we found that extracts of a number of marine macrophytes belonging to different taxonomic groups exhibited a pronounced protective effect under various experimental models (toxic hepatitis, hyperlipidemia, stress exposure).

The goal of this study was a comparative research of the preventive effect of water-ethanol extracts isolated from the thallus of brown alga - *Sargassum pallidum* (Turner) C. Agardh, green alga - *Ulva lactuca* Linnaeus, 1753, red alga - *Ahnfeltia tobuchiensis* (Kanno et Matsubara) Makienko on the indices of the antioxidant defense system of the liver and blood in mice under experimental stress exposure. The algae selected for the study are the most widespread in the seas of the Far East and are typical representatives of their divisions and the main mass species.

Material and methods

The algae were collected in August - September in the coastal waters of the Peter the Great Bay of the Sea of Japan at the marine experimental station of the POI FEB RAS (Popov Island). The air dry algae thallus was extracted with 70% ethyl alcohol by the method of repercolation. The yield of the extract made 1 liter from 1 kg of raw material. The obtained extract was evaporated under vacuum to dryness, and the total polyphenols (PP) contents was estimated by the Folin-Chocalteu method.



The experiment was carried out on white outbred male mice with a body weight 20-30 g that were kept under the standard conditions of a vivarium (see experiment schema). Acute stress was simulated by suspending animals in the upright position by their neck dorsal skin fold for 24 h. The control animals were also kept under the standard vivarium conditions. The preparations were introduced directly into the stomach through a feeding tube two times: immediately before the vertical suspension and 6 hours after the first administration. Prior to administration, the alcohol was removed from the extracts on a vacuum evaporator; the extract was then diluted to the required volume with distilled water. Preparations were administered as water suspension. The therapeutic dose made 100 mg of total PP / kg of body weight.

The animals from the "pure stress" group were treated with distilled water in a volume equal to that of the administered preparations.

Results and discussion

Stress impact has led to the formation of all characteristic attributes, such as adrenal hypertrophy, involution of the thymus and spleen, ulceration of the gastric and intestinal mucosa. The weight of the animals decreased by 24% ($p < 0.01$), while the Mass index of the internal organs (liver, spleen) decreased by an average of 20-23% ($p < 0.01$) (Fig. 1). The administration of algae extracts under exposure to the acute stress was accompanied by a decrease in the severity of destructive changes in the internal organs in comparison with the "pure stress" group. Ulcerative manifestations of the gastric mucous were absent.

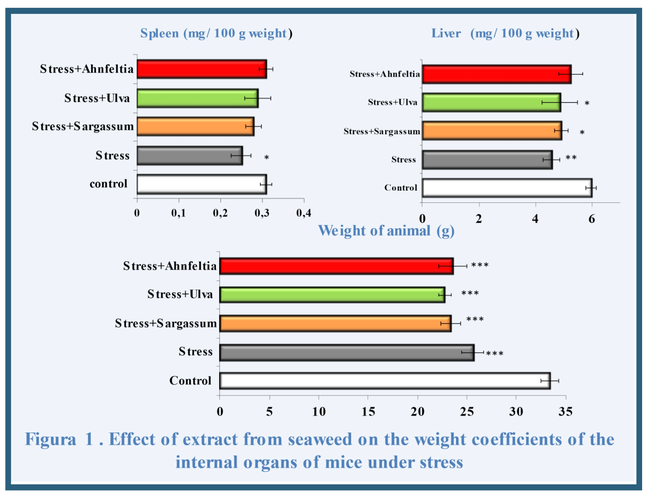


Figura 1 . Effect of extract from seaweed on the weight coefficients of the internal organs of mice under stress

Sargassum pallidum (Turner) C.Agardh
Phaeophyceae - Brown algae

While evaluation of the state of the antioxidant defense system of animals subjected to stress impact it was revealed a decrease in the level of antiradical activity (ARA) in blood plasma by 46%, the activity of superoxide dismutase (SOD) was by 40% below the control level (Fig.2). Impairment of the antioxidant defense system under stress impact were also manifested by an increase of malondialdehyde (MDA) level by 68%, which confirms the activation of lipids peroxidation and causes an increase of cell membranes permeability.

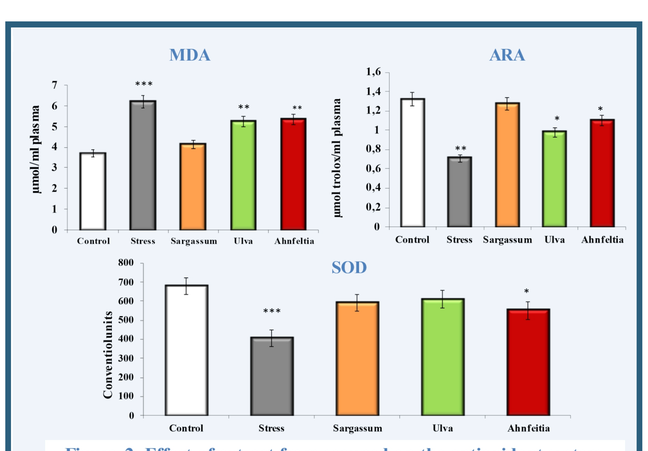
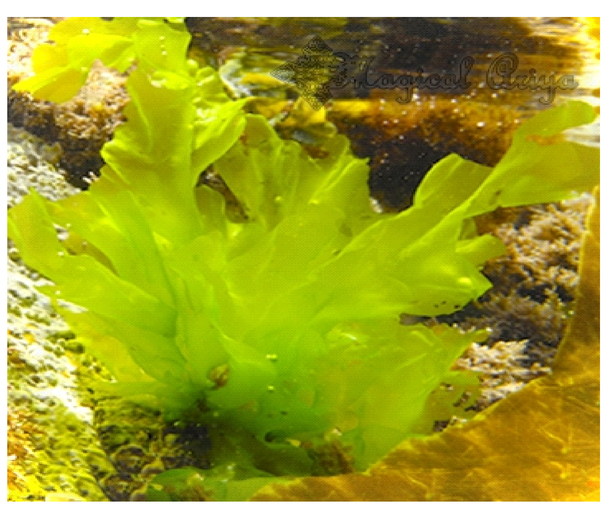


Figura 2. Effect of extract from seaweed on the antioxidant system indices of mice under stress



Ulva lactuca Linnaeus, 1753 [=*Ulva fenestrata*] ,
Chlorophyta – Green algae

The explored antioxidant parameters in all groups of animals taking algae extracts stabilized. In mice treated with S. pallidum extract, they approached the control values. When compared with the "pure stress" group significantly increased the level of ARA and SOD activity, while the content of MDA decreased.

There was also noted a decrease of reduced glutathione (G-SH) level in the liver by almost 2 times while the activity of glutathione reductase (GRx) declined by 26%. The activity of another key enzyme of the glutathione cycle, glutathione peroxidase (GPx), was also reduced by 35%. Such changes in the parameters of the antioxidant defense system might be defined as its depletion.

The introduction of extracts from seaweed was accompanied by an increase in the activity of antioxidant enzymes (GPx and GR) and the content of G-SH compared with the corresponding values in the "pure stress" group. However, parameters of the glutathione cycle in mice treated with extracts from *U. lactuca* and *A. tobuchiensis* remained significantly lower than similar parameters in the group of animals treated with *S. pallidum* extract.

The activity of GPx and GR in blood was lower, by 17 - 21% compared to ones in the group of mice receiving *S. pallidum* extract. Because the content of polyphenols in the extract of the brown alga *S. pallidum* is significantly higher than one in the extracts of *U. lactuca* and *A. tobuchiensis*, respectively, it has a higher level of antiradical activity. The greatest amount of polyphenols was found in the extract of the brown alga *S. pallidum*, which were 15–24 times higher than the corresponding values *in U. lactuca and A. tobuchiensis* (Table).

Ahnfeltia tobuchensis (Kanno et Matsubara)
Rhodophyta - Red algae

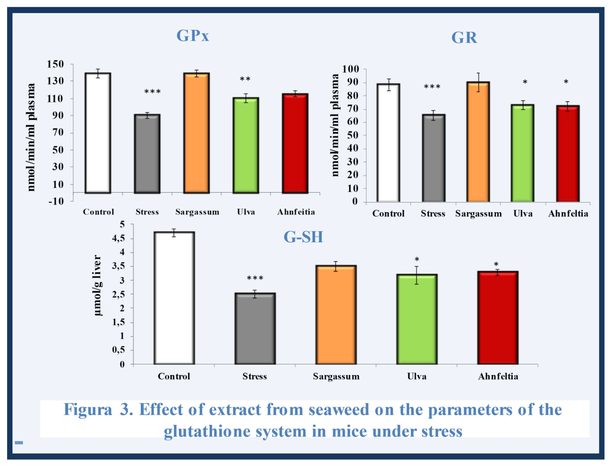


Figura 3. Effect of extract from seaweed on the parameters of the glutathione system in mice under stress

Table

Polyphenol content and antiradical activity of seaweed extracts (M± m)

Biochemical parameters	*Sargassum pallidum*	*Ulva lactuca*	*Ahnfeltia tobuchiensis*
Polyphenols (mg-equi. GA/100 g dry seaweed)	583,8±30,5	39,2±3,2	24,6±2,3
Polyphenols (mg-equi. GA/ g dry extract)	218,2±20,3	16,2±1,8	9,1±1,6
Antiradical Activity to $ABTS^+$ (µM Trolox/mg PP)	1,62±0,04	0,32±0,03	0,13±0,03
Antiradical Activity to peroxil radicals (µM trolox/ mg PP)	0,64±0,02	0,15±0,02	0,06±0,01

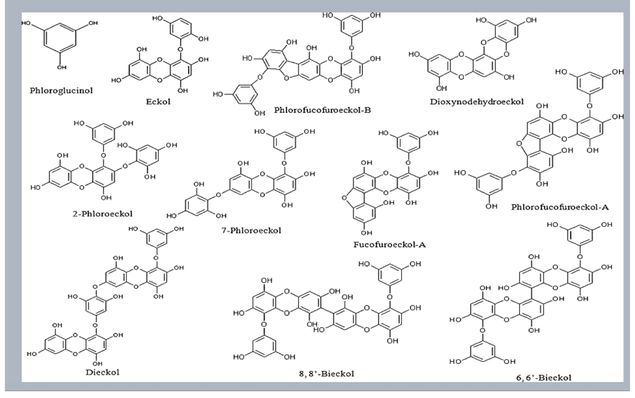
Note: PP - polyphenols, mg-equi. GA – mg equivalent of gallic acid

Accordingly, the extract of the S. pallidum is differed by a higher level of the antiradical activity, both is relation to the ABTS+ radical cation and to the peroxyl radical. This is confirmed by the data obtained when determined the content of total polyphenols and antiradical activity of extracts from seaweed.

Percolation device

The air dry algae thallus was extracted with 70% ethyl alcohol by the method of repercolation. The yield of the extract made 1 liter from 1 kg of raw material.

Chemical structures of phlorotannins from marine algae



Conclusions

1. **Under the conditions of acute stress exposure, metabolic reactions of the body were disturbed, which was accompanied by involution of the thymic-lymphatic system, the appearance of ulcerative manifestations of the gastrointestinal mucosa, as well as tension in the antioxidant defense system and activation of lipid peroxidation.**
2. **The prophylactic use of algal extracts under stress contributed to the preservation of the weight coefficients of the internal organs of animals (thymus, spleen, adrenal glands, liver), the absence of ulcers of the gastric mucosa.**
3. **The administration of the seaweed extracts demonstrated promising effect of diminishing the consequences of acute stress impact. This manifested in preservation of the parameters of the antioxidant defense system that is important for most of the vital processes in the body.**
4. **Data above propose seaweed S. pallidum, U. lactuca, and A. tobuchiensis are promising objects for creating drugs that can preserve the body antioxidant defense under acute stress preventing stress-induced disorders.**

РОССИЙСКАЯ ФЕДЕРАЦИЯ

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