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Short Communication

Anti-stress Effect of Brown Seaweed *Sargassum hystrix* J. Agard Ethanol Extract on Cold-Induced Stress in Wistar Rats

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ABSTRACT

Oxidative stress is a potential cause of cell damage, which has deleterious impact on the entire body. *Sargassum hystrix* contains polyphenols with potential antioxidant properties. Therefore, the objective of this study was to evaluate the effect of the ethanol extract of *S. hystrix* on the body weight, glucose, total cholesterol, triglyceride and cortisol levels of Wistar rats with cold-induced stress. In this experiment, the rats were orally administered with 150, 300, and 450 mg/kg of *S. hystrix* extract (SHE), while the standard anti-stress drug used was diazepam, at a dose of 0.18 mg/kg. The result showed that administeration of *S. hystrix* ethanol extract at various doses was able to lower the glucose, cholesterol and cortisol levels in the Wistar rats. The extract was also shown to cause a reduction in triglyceride levels similar to diazepam at doses of 300 and 450 mg/kg, while the dose of 300 mg/kg was shown to produce the most optimum anti-stress effect in Wistar rats with cold-induced stress.

Keywords: Anti-stress, Ethanol extract, *Sargassum hystrix*, Marine algae, Cortisol.

Introduction

According to Ravindran *et al*.,[1] the phenomenon of stress is commonly experienced by humans and is now a common occurrence in modern life. Meanwhile, the condition of oxidative stress is characterized by more free radicals, compared to antioxidants.[2] In cases where free radical production is higher than the rate of neutralization by intracellular antioxidants, the surplus free radicals are potential causes of damage to cells. This is commonly called oxidative damage, the destruction of cell biomolecules due to reaction with free radicals.[3] A study by Khotari *et al*.[4] reported these free radicals to be alleged causes of apoptosis, lipid peroxidation in cell membrane, and damage to DNA (deoxyribo nucleic acid), while a report by Manisha *et al*.[2] showed this tends to have consequent impacts on the body, including cancer and other serious ailments.

Marine algae is a significant traditional food in Japan, Korea, and other eastern countries.[5] Meanwhile, the brown algae *Sargassum* sp. is common to the southern beaches of Gunungkidul, in Yogyakarta, Indonesia. This alga contains bioactive compounds with antitumour, antioxidant, antifungal, antihypertensive, antiviral, and antidiabetic properties.[6-10] A study by Lailatussifa *et al*.[11] reported *Sargassum polycystum* to contain polyphenols which is able to inhibit oxidative stress due to cold, while Budhiyanti *et al*.[12] stated *S. hystrix* possesses polyphenols with antioxidant properties. Therefore, marine algae are able to reduce oxidative stress.

Bali and Jaggi[13] reported numerous pre-clinical models for inducing stress, including restraint stress in animals, while Di-Cerbo *et al*.[14] reported cold restraint stress to be widely used in evaluating the anti-stress effects exhibited by the extracts of *Borago officinalis*, *S. polycystum* extract[11] and *Eugenia caryophyllys* buds.[15]

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This research was therefore conducted to determine the effect of *S. hystrix* ethanol extract on the levels of cholesterol, glucose, cortisol and triglyceride, as significant markers of oxidative stress.

Materials and Methods

Materials

The sample used was the brown seaweed *S. hystrix*, which was collected from Sepanjang Beach, Gunungkidul, Yogyakarta, Indonesia, in May 2016, and identified at the Plant Systematics Laboratory, Faculty of Biology, Universitas Gadjah Mada. A voucher specimen (BS-S03) was deposited at the Fisheries Department, Agriculture Faculty, Universitas Gadjah Mada (Yogyakarta, Indonesia). The seaweed was extracted using ethanol (Merck KGaA, Darmstadt, Germany), and all the other reagents utilized in this research, were of analytical grade.

Extraction of seaweed

This was performed to acquire polyphenol rich extracts, using a modified version of the technique reported by Azizah *et al*.[16] The seaweeds were thoroughly washed and dried for 3-4 days at room temperature (26°C), prior to extraction. Subsequently, the dried seaweeds were cut into small pieces (approx. 0.5 cm) with scissors, and about 200 g of the pieces were measured and placed in an Erlenmeyer flask. This was then macerated with 1,875 mL of 96% ethanol, covered with aluminum foil, and left to stand at room temperature for 48 h. The extract obtained was then filtered with a Whatman filter paper, and heated in a rotary evaporator (RV 10 basic, IKA) at 60°C and 135~150 rpm, to evaporate the ethanol. This was followed by freeze-drying (Virtis; SP Scientific) and storage at -20°C (GC-124GGFP; LG Electronics Inc.), before the evaluation of anti-stress activity.

Animals

Female albino Wistar rats of weights between 150 and 200 g, were bought from the Universitas Gadjah Mada's Integrated Research and Testing Laboratory, in Yogyakarta, Indonesia. They were housed under standard conditions of 22.5 ± 1°C temperature, 65 ± 10% relative humidity, with a 12 h light-12 hn darkness cycle, and fed with a standard diet of pellets, with water *ad libitum*. The experiment was

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performed after approval was obtained from the Institutional Animal Ethics Committee (IAEC) of the Universitas Gadjah Mada's Integrated Research and Testing Laboratory (Approval Number 00006/04/LPPT/IX/2016).

Experimental design

This research applied a model of cold-restraint stress to evaluate anti-stress activity, and shared the rats into 6 groups of 5 rats each. The normal control (group 1) received no stress or treatment, while the stress control (group 2), received no treatment. The third group was administered diazepam at a dose of 0.18 mg/kg, while groups 4, 5, and 6, were administered *S. hystrix* extract at doses of 150, 300, and 450 mg/kg, respectively. With the exception of the normal control group, all the rats were subjected to cold restraint stress with immobilization in a cylindrical tub (90 cm x 50 cm x 70 cm (LxWxH) cage) with a 30cm water height, at 7°C, for 10 minutes daily, for fourteen consecutive days. The rats were also weighed daily and the body mass were recorded.

On the fourteenth day, the rats were anesthetized using ether, then dissected instantly after stress was applied. The rats' blood were collected in microtubes, and centrifuged (Centrifuge 5810 R; Eppendorf) at 1,610×g at 4°C for 20 min, to obtain serum. Subsequently, the serum was obtained and stored at -20°C (GC-124GGFP; LG Electronics Inc.), prior to analysis. The blood serum was then subjected to analysis with a commercially available cholesterol testing kit, to assess the biochemical parameters, including glucose, cholesterol, and triglycerides. Also, the cortisol hormone levels, was assessed based on the enzymatic photometric "CHOD-PAP" test (FineTest, Wuhan Fine Biotech Co., Ltd., Wuhan, China). This analysis was done with a microplate reader (ZENIX-320, Taoyuan, Taiwan), following the instructions of the manufacturer.

Statistical analysis

The results (anti-stress effect) were expressed as mean ± SD (standard deviation) and subjected to ANOVA (analysis of variance). Subsequently, the significant changes among the specimens were evaluated based on Duncan's multiple range tests, using the SAS (Statistical Analysis System) release "6.12" program, while a *P* value greater than 0.05 was regarded as statistically significant difference.

Results and Discussion

Rat body weight

Figure 1 shows the changes in body weights of the rats before (day 0) and after (final day 14) the swimming restraint stress treatment. Normal control, stress control, Diazepam, and SHE-treated rats demonstrated no significant decrease in body weight after the stressor treatment. *S. hystrix* ethanol extract supplementation did not interfere with body weight, compared to the control group. A report by Chakraborty *et al.*[17] stated that stress due to cold temperature is able to cause weight loss, and this is possibly due to adaptive changes resulting from treatment with long term administration of anti-stress drugs. Meanwhile, Firdaus *et al.*[18] reported *Sargassum echinocarpum* extract to contain an anti-oxidative stress agent, with the ability to increase glucose uptake, through cellular metabolism regulation, by adenosine monophosphate-activated protein kinase (AMPK) stimulated by the phlorotannins present in algae.

Glucose level

As shown in Table 1, all the groups, except the stress control, exhibited a reduction in blood glucose. The blood glucose rise in the stress control group indicated possible hyperglycemia. This condition is able to further impair the secretion of insulin and increase insulin resistance, thus, creating a vicious circle of increased hyperglycemia and reduced insulin production.[19]

In the diazepam-treated group, blood glucose levels was discovered to be normal, because diazepam acts as a pain reliever, sedative, and CNS (central nervous system) depressant.[20] The SHE treatment groups were also found to exhibit normal blood glucose (<100 mg/dL) and differ significantly from the stress control group, suggesting that the SHE was able to prevent hyperglycemia. This is in line with the study by Lailatussifa *et al.*[11] who reported that *S. polycystum* methanol extract is able to stabilize blood glucose levels of rats with induced stress and inhibit stress due to cold temperatures, because the antioxidants present inhibit the ability of enzymes in converting carbohydrates into glucose.[7]

Triglyceride levels

As shown in Table 1, all the Wistar rats exhibited normal triglyceride levels (31.53 ± 2.01 - 66.47 ± 3.18 mg/dL). The stress control group exhibited triglyceride levels significantly different ($P<0.05$) from that of the diazepam-treated group. Meanwhile, the group treated with 150 mg/kg SHE did not differ significantly ($P>0.05$) from the stress control group. This is possible because rats move aggressively while stressed, thus reducing the body's energy reserves. The applied stressor causes oxidative stress, and inhibits insulin formation. Consequently, fatty acids and glycerol synthesis takes longer, and lipogenesis (fat deposition) is inhibited. This reduces triglyceride accumulation within the body.[15] In addition, the 300 mg/kg and 450 mg/kg SHE groups exhibited no significant difference compared to the diazepam group. A report by Lailatussifa *et al.*[11] reported optimum triglyceride levels in Wistar rats after being administered with 450 mg/kg of *S. polycystum*, as a treatment for cold temperature stress.

Total cholesterol

Table 1 shows the Wistar rats' total cholesterol levels. The stress control treatment group were found to exhibit the highest cholesterol level, and differed significantly from the other treatment groups. Under stress, cortisol stimulates gluconeogenesis within the liver, and this causes the release of fatty tissue cholesterol deposits, thus increasing the blood's total cholesterol level. On the other hand, administration of diazepam and SHE could reduce levels of cholesterol. This finding was reinforced by the work of Lailatussifa *et al.*[11] who stated that the administration of diazepam and extracts of *S. polycystum* had the ability to reduce cholesterol levels in cold stress treated-Wistar rats. A study by Singh *et al.*[15] described that *E. caryophillus* extracts had the ability to reduce cholesterol level in Wistar rats with induced voice stress, as administering a drug adaptogen is able to inhibit the sympathetic nervous system stimulation and prevent fat mobilization, consequently, reducing cholesterol synthesis.[11]

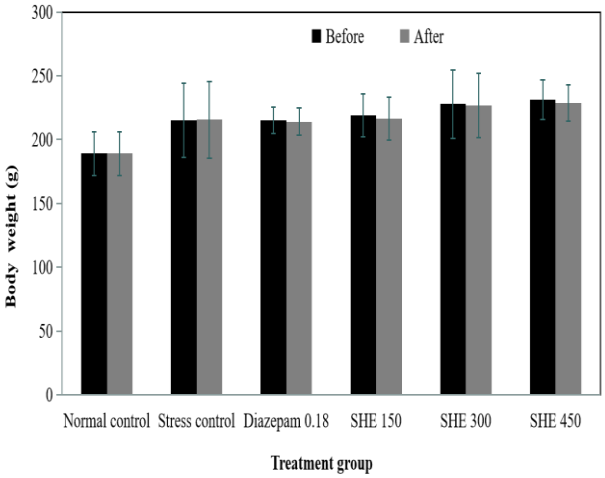

Figure 1: The impact of *Sargassum hystrix* extract (SHE) and diazepam on changes in the rats' body weight by cold restraint stress, before (day 0) and after (final treatment, day 14) the experiment.

Cortisol

The hormone cortisol plays a significant role in blood pressure, host defense mechanisms, basal metabolism, and response to stress in humans. Table 1 shows the cortisol levels in each Wistar rat treatment

group. The stress control group was observed to have the highest cortisol levels (170 µg/dL), and differ significantly from the other treatment groups. Meanwhile, the cortisol level (110 µg/dL) in the diazepam group did not differ significantly from that in the 150 mg/kg (100 µg/dL), 300 mg/kg (90 µg/dL), and 450 mg/kg (100 µg/dL) SHE treatment groups.

Therefore, administering SHE extract has similar effect as the administration of 0.18 mg/kg diazepam. The compounds identified in the *S. hystrix* ethanol extract using GC-MS (data not shown), are useful for stress treatment. Also, palmitic and oleic acids have antioxidant properties,[21] while 5,8,11,14-eicosatetraenoic acid (ETYA) has the ability to inhibit lipoxigenase activity as a trigger of inflammation, and is associated with the inhibition of adrenocorticotropic release (ACTH).[22] In addition, alkaloids are able to inhibit oxidative damage to tissue and restore endogenous antioxidant enzyme activity,[23] while steroids, phenols, and tannins, have antioxidant properties.[24]

Table 1: The effects of *Sargassum hystrix* extract (SHE) and diazepam on blood glucose, triglyceride, cholesterol, and cortisol changes by cold restraint stress in Wistar rats

Treatment groups	Glucose (mg/dL)	Triglyceride (mg/dL)	Cholesterol (mg/dL)	Cortisol (µg/dL)
Normal control	52.77 ± 3.80^{a}	66.47 ± 3.18^{c}	48.37 ± 9.45^{a}	1.08 ± 0.12^{c}
Stress control	130.30 ± 7.98^{c}	31.53 ± 2.01^{a}	71.13 ± 9.85^{b}	170.00 ± 50.00^{b}
Diazepam 0.18 mg/kg	80.77 ± 6.47^{b}	47.93 ± 5.44^{b}	53.67 ± 5.27^{a}	110.00 ± 20.00^{a}
SHE 150 mg/kg	79.90 ± 7.99^{b}	33.13 ± 2.11^{a}	51.20 ± 8.78^{a}	100.00 ± 30.00^{a}
SHE 300 mg/kg	81.73 ± 8.06^{b}	42.17 ± 4.66^{b}	46.60 ± 5.36^{a}	90.00 ± 20.00^{a}
SHE 450 mg/kg	84.40 ± 7.60^{b}	41.63 ± 5.40^{b}	48.10 ± 4.85^{a}	100.00 ± 20.00^{a}

Note: A statistically significant difference in the values is indicated by different letters within one column ($p<0.05$)

Conclusion

The findings from the present study shows that the ethanol extract of *S. hystrix* supplementation did not interfere with the body weight of Wistar rats suffering from cold-restraint stress. However, the extract was able to reduce glucose, cortisol, as well as cholesterol levels at doses of 150, 300, and 450 mg/kg, and also cause a reduction in triglyceride levels, similar to diazepam, at doses of 300 and 450 mg/kg.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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