Prev. Nutr. Food Sci. 2019;24(2):150-158
https://doi.org/10.3746/pnf.2019.24.2.150
pISSN 2287-1098 · eISSN 2287-8602

Sargassum hystrix as a Source of Functional Food to Improve Blood Biochemistry Profiles of Rats under Stress

Amir Husni[1], Radipta Lailatussifa[1,2], and Alim Isnansetyo[1]

[1]Department of Fisheries Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia
[2]Sidoarjo Marine and Fisheries Polytechnic, Sidoarjo 61254, Indonesia

ABSTRACT: This study was conducted to determine the influence of *Sargassum hystrix* powder (SHP) as an alternative source of functional food for treating *in vivo* stress by measuring levels of glucose, triacylglycerol, total cholesterol, and cortisol, and liver histopathology. Wistar rats aged 3 months and weighing 150∼200 g were divided into 7 groups: normal control, fasting control, negative control (stress without adaptogen), and 4 experimental conditions (stress+0.18 mg/kg diazepam, stress+450 mg/kg pellet, stress+mixture of pellet with SHP 450 mg/kg, and stress+450 mg/kg of SHP). Intake of liquids and and body weight were measured daily. Blood samples were collected on day 0 (baseline), day 5, and day 10 to analyze levels of glucose, triacylglycerol, cholesterol, and cortisol. On day 10, rats were euthanized and livers were collected to observe the severity of inflammation. The results indicated that rats receiving SHP 450 mg/kg and the mixture of pellet with SHP 450 mg/kg showed a similar ability as those receiving diazepam 0.18 mg/kg to cope with stress, indicated by an improvement in all blood biochemistry parameters. Supplementation with SHP 450 mg/kg can be used as an alternative source of functional food for overcoming oxidative stress, as indicated by its ability to improve levels of blood glucose, triacylglycerol, total cholesterol, and cortisol, and to improve liver histology by decreasing severity of liver inflammation.

Keywords: anti-stress, brown algae, cortisol, liver inflammation, *Sargassum hystrix*

INTRODUCTION

Neuron diseases are relatively common in human, comprising approximately 6.3% of other illnesses, estimated to increase to 12% by 2030 according to the World Health Organization. Among neuron diseases, neurodegenerative diseases constitute the higher proportion, reaching 62% (Kalsi, 2015). One disease related to the nervous system is stress. Stress is a biological response that threatens and disturbs the homeostasis in an individual (Castro e Couto et al., 2015). Stress can originate from outside the body; for example, stress may result from physical activities such as swimming restraint (Lailatussifa et al., 2016). A type of stress that is included among the physical stressors is oxidative stress, which has a destructive characteristic.

Oxidative stress is a state of unbalance between prooxidants and antioxidants that results in oxidative damage (Guo et al., 2012). Oxidative stress is caused by free radicals (Chang et al., 2007). Oxidative damage has been implicated in several pathological conditions such as cell, tissue, and organ damage, including the liver, kidney, and heart (Valko et al., 2007). Oxidative stress is marked by dysfunction of the hypothalamus-pituitary-adrenal axis (HPA), therefore resulting in increased levels of cortisol and catecholamines (Castro e Couto et al., 2015).

Marine algae have anti-oxidant, anti-inflammatory, antidiabetic, and anti-cancer potential (Lailatussifa et al., 2016; Gotama et al., 2018). *Sargassum* sp. is a type of brown algae found readily in the South coastal district of Gunungkidul, Yogyakarta, Indonesia (Lailatussifa et al., 2016; Gotama et al., 2018; Husni et al., 2016). The safety of *Sargassum* sp. was reported by Tapia-Martinez et al. (2019), Nasmia et al. (2017), and Xiu et al. (2017). *Sargassum* sp. has alginate content, which is widely used by the food and pharmaceutical industries, in particular in the formation of ice cream, pills, and tablets (Holdt and Kraan, 2011). The bioactive compounds of *Sargassum* sp. possess pharmacological properties, such as anti-oxidant, anti-inflammatory, anti-hypertensive, anti-bacterial, and

Received 6 December 2018; Accepted 22 March 2019; Published online 30 June 2019

Correspondence to Amir Husni, Tel: +62-274-551218, E-mail: a-husni@ugm.ac.id
Author information: Amir Husni (Professor), Radipta Lailatussifa (Graduate Student), Alim Isnansetyo (Professor)

anti-viral activities. These pharmacological properties are a result of the biological activity of metabolites such as alkaloids, steroids, terpenoids, saponins, polyphenols, phlorotannin, and fucoidan (Liu et al., 2012). Firdaus et al. (2009) and Lailatussifa et al. (2016) reported that a 450 mg/kg dose of *Sargassum echinocarpum* and *Sargassum polycystum* brown algae for *in vivo* application (applied to animal tests) is most effective in inhibiting oxidative stress. Various studies on anti-stress oxidatives have been conducted, in which the *in vivo* adaptogen was still administered in the form of extracts and fractions, requiring expensive and complicated equipment to obtain the adaptogen so it can be applied to humans. Meanwhile, Chan et al. (2015) showed that 10% of red algae *Gracilaria changii* dry powder added to the pellet diet of rats was effective in decreasing cholesterol levels and improving the liver tissue, as a result of feeding with the high-fat compound. However, there are no recent studies on the effect of supplementation with *Sargassum hystrix* powder (SHP) on reducing oxidative stress through analyzing liver histopathology and the blood biochemistry levels in rats. Therefore, the present study was conducted to investigate the effect of supplementation with SHP as an alternative to functional food against oxidative stress by measuring the levels of blood glucose, triacylglycerol, cortisol, and total cholesterol, and analyzing liverhistopathology.

MATERIALS AND METHODS

Materials

The primary material used in this study was the brown seaweed *S. hystrix* obtained from the intertidal zone, the South coastal district of Gunungkidul, Yogyakarta, Indonesia. The general proximate composition of SHP used in this study was as follows: water content 13.43±0.02 %, fat content 0.05±0.02%, protein content 6.54±0.04%, and dietary fiber content 31.53±0.18%. In addition, a standard pellet diet (Japfa Comfeed, Jakarta, Indonesia) was used as a comparative diet. The general proximate composition of the standard pellet diet was as follows: water content 7.01±0.01%, fat content 4.28±0.04%, protein content 15.35±0.03%, and dietary fiber content 39.55%±0.09%. All other chemicals used in this study were of analytical grade.

Sample preparation

The fresh brown seaweed *S. hystrix* was collected, packed in a coolbox away from the sun, and brought to the laboratory of the Fisheries Departement of Universitas Gadjah Mada, Yogyakarta, Indonesia, where it was washed under flowing water. The sample was dried at room temperature (±26°C) for 5～7 days. The dried sample was cut into small pieces measuring ±0.5 cm using scissors and blended. Dried seaweed powder was stored in the freezer before *in vivo* use.

Animals

Female Wistar rats aged 3 months and weighing 150～200 g were obtained from the Integrated Research and Testing Laboratory, Universitas Gadjah Mada, Yogyakarta, Indonesia. Rats were housed under standard conditions of 20±3°C, a relative humidity of 70%, a 12-h light and 12-h dark cycle, and with access to water 100 mL/d. The animals were maintained for a week to adapt to the environment, during which their health status was checked and they were fed a homogenized diet. All experimental protocols involving animal studies were approved after scrutiny by the Institutional Animal Ethics Committee (IAEC) of the Integrated Research and Testing Laboratory, Universitas Gadjah Mada (Approval No. 00006/04/LPPT/IX/2016), Yogyakarta, Indonesia.

Experimental method

A swimming restraint stress model was used for evaluating antistress activities. Animals were divided into seven groups, eachconsisting of five animals. In the normal control group (group 1), rats did not receive any stressor and were fed a standard pellet diet. In the fasting normal control group (group 2), rats were fasted every day for 10 days, were provided with water 100 mL/d, but did not receive the swimming stress treatment. In the negative control group (group 3), rats were fasted and received the swimming stress treatment. In group 4, the rats received standard diazepam treatment at 0.18 mg/kg p.o.. This diazepam dose is the minimum dose limit obtained based on human body weight conversion of 70 kg to a rat body weight of 200 g. In group 5, the rats received the standard pellet diet 450 mg/kg p.o.. The standard pellet diet was the standard diet (ration) for rats, consisting of high water, fat, protein, and dietary fiber content. In group 6, the rats received a mixture of SHP and the standard pellet diet at a 1:1 ratio (450 mg/kg p.o.). In group 7, rats received 450 mg/kg p.o. SHP. SHP is a brown algae powder that consists of fat, protein, and dietary fiber, also alkaloids, steroids, terpenoids, phenols, tannins, and saponins (Lailatussifa et al., 2017). Stress was induced in all rats, except the normal control and the fasting normal control groups, by subjecting the fasting animals to swimming restraint stress with immobilization to water at a depth of 25 cm in a tub measuring 37×37×30 cm^3 for 5 min/d until 10 days. Stress was applied once-daily for 10 consecutive days. Body weight and drink intake were measured everyday. On day 10, the neck bones of the rats was dislocated, and the rats were dissected immediately after the application of stress.

Blood samples (1.5 mL) were collected in microtubes.

The blood was centrifuged (MPW-55, Eppendorf, Hamburg, Germany) at 10,000 rpm for 15 min at 4°C to obtain serum that was collected and stored at −20°C (GC-124GGFP, LG Electronics Inc., Seoul, Korea) before testing. Blood serum levels were analyzed to measure the biochemical parameters, including the levels of glucose, triacylglycerol, cholesterol, and cortisol. Glucose levels were measured using glucose oxidase-phenol 4-amino antipyrine (PAP), triacylglycerol levels were determined using glycerol-3-phosphate-oxidase, cholesterol levels were determined using cholesterol oxidase-PAP, and cortisol levels were measured using the cortisol enzyme-linked immunosorbent assay Fine Test kit (Wuhan Fine Biotech Co., Ltd., Wuhan, China).

The stomach of each animal was opened along the greater curvature and were qualitatively examined macroscopically for the presence of inflammation in the liver tissues. The liver tissue was stored in a 10% formalin solution before testing for inflammation. Inflammation in the liver was analyzed using the method described by Mossa et al. (2015).

Statistical analysis

The anti-stress effect was reported as mean±standard deviation (SD) and was assessed using analysis of variance (ANOVA). Differences between samples were determined based on Duncan's multiple range test using the Statistical Analysis System (SAS release 6.12, SAS Institute Inc., Cary, NC, USA). A value of $P<0.05$ was used to determine statistical significance.

RESULTS AND DISCUSSION

Body weight of rats

Rats in the normal control group showed a trend for increased body weight between day 0 and after the swimming restraint stress treatment, as assessed on the last day of treatment (day 10); the mean difference in body weight was 6.90 g. The optimum amount of SHP 450 mg/kg to prevent lowering of body weight was determined to be −30.17 g. Fig. 1 shows the changes in body weight between day 0 and after the final swimming restraint stress treatment (day 10).

Rats in the fasting control group showed a significant decrease body weight of −57.63 g between day 0 and the last day of swimming restraint stress treatment (day 10). Prolonged fasting promotes gluconeogenesis (glucose synthesis from non-carbohydrate substrates) and ketogenesis (ketone synthesis from acetyl Co-A) as the primary sources of fuel in the liver. Ketogenesis greatly relies on adipose tissue lipolysis to supply oxidized fatty acids to the liver, to generate ketogenic acetyl Co-A. The energy produced by the body during prolonged fasting is derived from conversion of glucose and fat into protein in the liver and muscle tissue, preventing deposition of fat around the body. This subsequently leads to significant weight loss (Goldstein and Hager, 2015). In the negative control group, rats showed a significant decrease in body weight of −66.76 g following treatment ($P<0.05$). This likely resulted from continuous fasting leading to a reduction of hepatic glucose in the body, and, when undertaking physical activity at the time of fasting, a shift in energy usage from sources of fatty acids to glucose and muscle glycogen in the muscles. Physical activity increases glucose transport into cells via glucose transporter-4, which leads to an increased activity of muscle adenosine monophosphate (AMP). AMP kinase induces changes in metabolism, including glucose metabolism hence why frequent but intense recreational activities are often fuelled by breakdown of carbohydrates (Sigal et al., 2004). Diazepam-treated rats showed no significant decreases in body weight after the stressor treatment, with a difference of −38.37 g. Diazepam has high lipophilicity and crosses the brain-blood barrier rapidly, and has a high affinity to gamma-aminobutyric acid (GABA) receptors, causing a sedative effect that stabilizes body weight (Brigo et al., 2016).

The body weights of rats in group 5 (standard pellet diet intake of 450 mg/kg) showed minor changes between

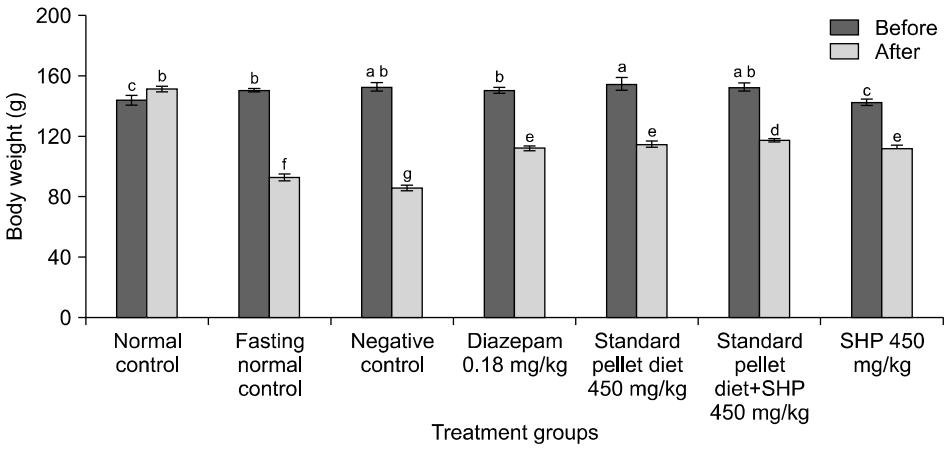

Fig. 1. Effect of *Sargassum hystrix* powder (SHP) and diazepam on swimming restraint stress in terms of body weight loss in Wistar rats. Values with different letters (a–g) indicate a significant difference ($P<0.05$).

day 0 and after the stress treatment (day 10), with a modest difference of −39.84 g. In groups 6 and 7, rats were fed with a mixture of standard pellet diet+SHP 450 mg/kg and SHP 450 mg/kg; after the stress treatment, rats in both groups showed minor decreases in body weight of −35.46 g and −30.17 g, respectively. The best treatment for preventing weight loss after stress treatment was SHP 450 mg/kg. Compounds present in SHP, such as saponins and alkaloids, may have increased levels of the central neurotransmitter, helping reduce stress (Ishola et al., 2012) and to adapt to the rats to cope with the stress (Joshi et al., 2012). In addition, tannins and a phenolic compound of *S. hystrix* can increase uptake of glucose through regulating cellular metabolism via AMP-activated protein kinase (AMPK) (Lailatussifa et al., 2016).

Drink intake

Drink intake of rats in all treatment groups decreases between day 1 and day 10 that they were subjected to the swimming stressor (Fig. 2). On day 1, when the stressor was given rats experienced shock and required a considerable amount of energy to stabilized their body condition during treatment. On day 6 and 10, rats subjected to stressors were able to better adapt, therefore water intake relatively stable so there was a decrease from day 1.

Drink intake on day 1 was highest in rats in the normal control group subjected to the stressors. Since rats in this group did not have a limit of food intake, rats could consume as much as possible, limited only by stomach size. Consumption of increased amounts of food is accompanied by increased intake of water to dissolved the food consumed. Throughout the 10 days of treatment, rats in the negative control group had the largest drink intake compared with any other group; the smallest drink intake was recorded for rats receiving SHP 450 mg/kg. The swimming restraint stress treatment caused exhaustion in rats, which resulted in an increased need to consume liquids as a source of energy during fasting. An increase in the amount of water lost from the body makes rats consume more water to maintain fluid levels. Water functions as a bond that can contribute hydrogen atoms, hydrogen ions, oxygen atoms, oxide ions, hydroxyl groups, or hydroxide ions to other reactants in the body, promoting biological reactions (Joshi et al., 2012). Interestingly, rats administered other supplements during fasting and induction of swimming stress treatment showed decreases in water intake. Rats receiving the standard pellet diet (450 mg/kg p.o.) were consuming small amounts of reverse osmosis drinking water since the rations orally given had been dissolved in distilled water; the rations also had a moisture content of 7.01±0.01% and a high dietary fiber content of 39.55±0.09%. Moreover, the brown seaweed *S. hystrix* has high concentrations of water-soluble minerals, notably Na and K, with 10∼20 times the amounts of minerals present in land plants; feeding rats with *S. hystrix* should therefore decrease water consumption (Garcés-Rimón et al., 2016).

Glucose level

Rats in the negative control group (rats treated and stressed without the adaptogen) showed significant increases in blood glucose levels after day 10 of stressor treatment, with glucose levels of 222.20±6.71 mg/dL (Table 1). Rats in both the fasting control groups [administered SHP 450 mg/kg, and a mixture of standard pellet diet+SHP (1:1) 450 mg/kg] showed significant (P <0.05) decreases in blood glucose levels, indicating rats experienced hyperglycemia and diabetes mellitus, shown by blood glucose levels of >200 mg/dL. Increased blood glucose levels are associated with the ability of cortisol to decrease insulin secretion and action, and activation of glucagon-secreting cells in the islets of Langerhans. Glucagon responds to decreases in blood glucose concentrations during fasting and due to physical activity. Furthermore, secreted glucagon binds to target receptors on hepatocyte cells, which activates adenyl cyclase to stimulate production of cyclic AMP (cAMP), resulting in glycogenolysis (breakdown of glycogen polymers into glucose monomers). During prolonged fasting, amounts of

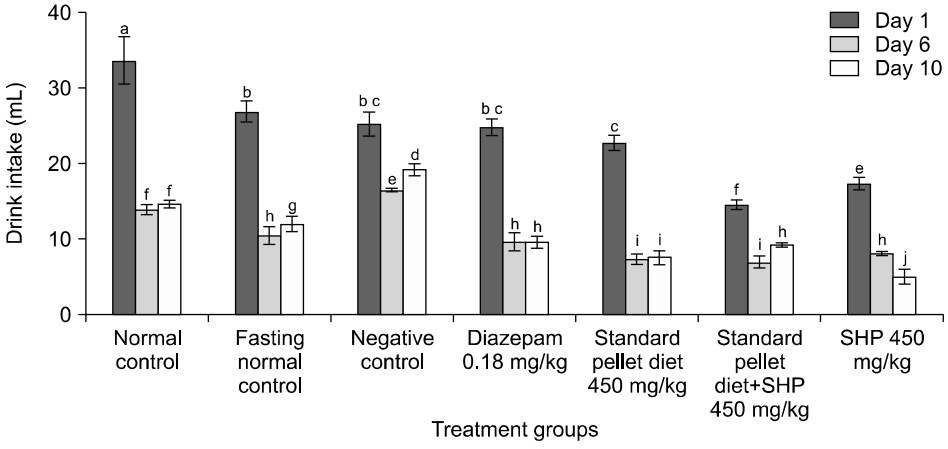

Fig. 2. Effect of *Sargassum hystrix* powder (SHP) and diazepam on the drink intake of swimming restraint stress-induced Wistar rats. Values with different letters (a–j) indicate a significant difference (P<0.05).

Table 1. Effect of *Sargassum hystrix* powder (SHP) and diazepam on swimming restraint stress-induced biochemical changes in fasting rats (unit: mg/dL)

Treatment groups	Glucose	Triacylglycerols	Cholesterol
Normal control	105.70±5.21[b]	89.93±3.82[c]	51.77±7.75[c]
Fasting normal control	78.90±8.00[a]	37.70±4.11[a]	33.77±7.04[b]
Negative control	222.20±6.71[d]	147.67±7.70[d]	60.60±2.92[c]
Diazepam 0.18 mg/kg p.o.	91.10±6.42[a]	72.30±6.77[b]	22.00±4.39[a]
Standard pellet diet 450 mg/kg p.o.	139.43±5.98[c]	64.93±6.93[b]	42.53±5.71[b]
Standard pellet diet+SHP 450 mg/kg p.o.	86.70±6.01[a]	89.43±9.15[c]	34.90±1.21[b]
SHP 450 mg/kg p.o.	88.77±7.48[a]	76.53±5.88[b]	35.40±4.10[b]

Values with different letters (a-d) in the same column indicate a significant difference (P<0.05).

glucose stored in the liver decreases as it is released into the bloodstream, allowing tissues to utilize free fatty acids and ketones for respiration. This increases gluconeogenesis (conversion of amino acids and fats into glucose, or the formation of glucose from non-carbohydrate sources such as lactate, alanine, and glycerol), resulting in release of large quantities of glucose into the blood (Güemes et al., 2015).

Both SHP and a mixture of SHP with the standard pellet diet contain complete sources of energy, which is required by rats to maintain stable glucose levels. However, saponins, terpenoids, and steroid compounds in SHP have an ability similar to that of diazepam (data not shown). SHP could therefore bind to certain receptors in the brain to increase synaptic transmission of GABA-ergic; this would increase chloride on postsynaptic membranes and decrease levels of norepinephrine, catecholamines, cortisol, serotonin, and others biogenic amines in the brain, allowing stable glucose levels to be maintained (Brigo et al., 2016). The SHP used in this study contains secondary bioactive forms of alkaloids, steroids, triterpenoids, saponins, phenols, and tannins, as shown by phytochemistry screening test results. Triterpenoid and saponin compounds could reduce levels of cortisol and adrenocorticotropic hormone (ACTH) during stress, increase levels of brain neurotropic factors and heat shock protein-70, eliminate effects of stress, and avoid hippocampal damage (Kim et al., 2014). Alkaloids, phenols, and tannins present in *S. hystrix* brown algae are able to reduce stress by donating hydrogen atoms from hydroxyl groups in compounds to stabilise free radicals (Li et al., 2011). However, activation of AMPK by a phenolic compound of algae can increase Akt activity, increasing glucose uptake via the glucose transporter into muscles, which further lowers the blood glucose levels (Güemes et al., 2015). Polyunsaturated fatty acids present in the algae impact insulin action through transformation of cellular membranes, such as by increasing the affinity of insulin receptor binding and increasing glucose uptake into cells. Moreover, foods containing high levels of algal fiber reduce the risk of diabetes. Soluble dietary fiber decreases the rate of stomach emptying, reducing postprandial glucose and blood glucose levels. The dietary fiber present in seaweed also decreases short-term energy requirements, stabilizes blood glucose levels, and improves insulin sensitivity (Sharifuddin et al., 2015).

Triacylglycerol level

Triacylglycerol levels of rats in the normal control group did not significantly (P>0.05) differ from those of rats fed with a mixture of standard pellet diet+SHP (1:1) 450 mg/kg. Moreover, triacylglycerol levels of rats in the diazepam 0.18 mg/kg group did not significantly differ from those of rats fed the SHP 450 mg/kg diet and the standard pellet diet 450 mg/kg. Triacylglycerol levels were significantly (P<0.05) different between rats in the negative control (fasting+swimming restraint stress-induced) group and the other treatment groups (Table 1).

Stress triggers an increase in triacylglycerol levels because it slows the synthesis of fatty acid and glycerols (lipogenesis process) in the blood (Neelima et al., 2014). The decrease in triacylglycerol levels after administration of adaptogens (diazepam, standard pellet diet, and SHP) was consistent with the study by Duraismi et al. (2010) who suggested that *Aegle marmelos* extract could decrease the triacylglycerol levels of bridle stress-induced mice. Kim et al. (2012) also reported that administration of *Sargassum yezoense* methanol extract at doses of 100 and 200 mg/kg to mice with diabetes mellitus could improve levels of triglycerides and decrease levels of plasma free fatty acids; this could help prevent hypertriglyceridemia by activating the transcriptional activity of the peroxisome proliferator-activated receptors (PPAR)α and PPARγ. PPARα and PPARγ are targets of metabolic syndrome and increase expression of the mitochondrial gene *UCP3* (uncoupling protein 3) in adipose tissue, which increases fat metabolism, leading to reduced total cholesterol and triglyceride levels in the blood. Moreover, administration of fucoidan (polysaccharide sulfate extract) isolated from *Fucus vesiculosus* brown algae has been shown to be effective in reducing and improving blood triglyceride levels by increasing lipoprotein lipase (LPL) secretion and increasing ApoC-II via activating LPL release into the blood, allowing free fatty acids to be stored

in adipose tissue, skeletal muscle, heart, and other tissues, and to be converted back into triacylglycerols. Administration of adaptogens with antioxidant activities can increase the activity of LPL, thereby decreasing blood triglyceride levels (Yokota et al., 2009).

Total cholesterol

Total cholesterol levels in the blood serum of rats did not significantly ($P>0.05$) differ between the fasting normal control group, the standard pellet diet 450 mg/kg group, the mixture of standard pellet diet+SHP 450 mg/kg group, and the SHP 450 mg/kg group. However, total cholesterol levels in these groups were significantly ($P<0.05$) different from those in the normal control and negative control groups (Table 1). Swimming and fasting stressors for 10 days induced increases in serum total cholesterol levels since stress stimulates HPA activity and for the sympathetic nervous system to produce cortisol and catecholamines (epinephrine and norepinephrine). Catecholamine hormone (epinephrine and norepinephrine) increase blood flow through adipose tissue and induce adenoceptor adipose B-2 stimulation, which induces adipose tissue to release free fatty acids into the blood through the lipolysis (the process of breaking down triacylglycerol into glycerol and free fatty acids). This results in formation and accumulation of free fatty acids in the blood circulation (Adekunle, 2011).

The lowest level of cholesterol was observed for rats treated with diazepam 0.18 mg/kg, followed by those receiving the mixture of standard pellet diet with SHP (1:1) at 450 mg/kg, and SHP 450 mg/kg. Supplementation of *S. hystrix* helped reduce cholesterol levels in the blood. This is because secondary bioactive compounds of algae (polyphenols, phlorotannins, and alkaloids) donate H^+ ions from hydroxyl groups to reactive oxygen species (ROS) during stress, which neutralizes ROS into stable forms (Lailatussifa et al., 2016). Moreover, the high fiber content in the standard pellet diet and SHP may increase excretion of bile salts and cholesterol in feces, which would decrease the amounts of bile salts in the enterohepatic cycle. Reduced amounts of bile salts and cholesterol entering the liver decrease liver cholesterol levels. This increases cholesterol levels in the blood that can be used to synthesise new bile salts, subsequently leading to reductions in blood cholesterol levels (Astuti et al., 2009). Secondary bioactive compounds, such as terpenoids, steroids, and saponins, play a role in facilitating inhibitory effects of neurotransmitters on the GABA systems (Joshi et al., 2012). Chan et al. (2015) suggested that 10% dried powder of *Gracilaria changii* algae added to the standard pellet diet of rats was effective in decreasing cholesterol levels that result from a high fat intake. Similar findings were reported by Makkar et al. (2016), who demonstrated that the metabolic ability and the beneficial effects of brown seaweeds have medicinal properties for intestinal disorders, such as hypocholesterolemic and hypoglycemic agents.

Cortisol hormone

Rats in the negative control group, who were fasted and subjected to swimming stress treatment (group 3), showed increased cortisol levels at day 5 and day 10. The normal control group also showed decreased cortisol levels after 10 days of treatment. Rats receiving the standard pellet diet with SHP 450 mg/kg and SHP 450 mg/kg showed decreased basal serum cortisol concentrations, suggesting both these treatments induced decreases in adrenal hormone secretion. Changes in cortisol hormone levels for all study groups are depicted in Fig. 3.

Cortisol exhibits significant fluctuations over time to regulate pulsatile episodic release of ACTH from the anterior pituitary and corticotrophin-releasing factor from the hypothalamus (Sharifuddin et al., 2015). Lowered adrenal function can result in insufficient release of adrenal hormones, which can lead to chronic fatigue. Triterpenoids, steroids, and saponin compounds of algae can decrease the levels of cortisol and ACTH during stress due to their abilities to inhibit GABA neurotransmitters (Joshi et al., 2012). Supplementation of rats with brown seaweed *Aschophyllum nodosum* decreases levels of plasma

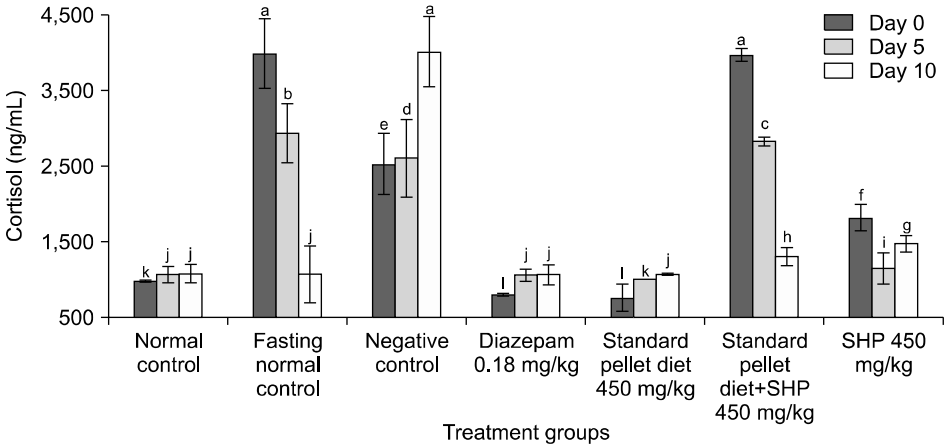

Fig. 3. Effect of *Sargassum hystrix* powder (SHP) and diazepam on the levels of cortisol (ng/mL) in swimming restraint stress-induced Wistar rats. Values with different letters (a–l) indicate a significant difference ($P<0.05$).

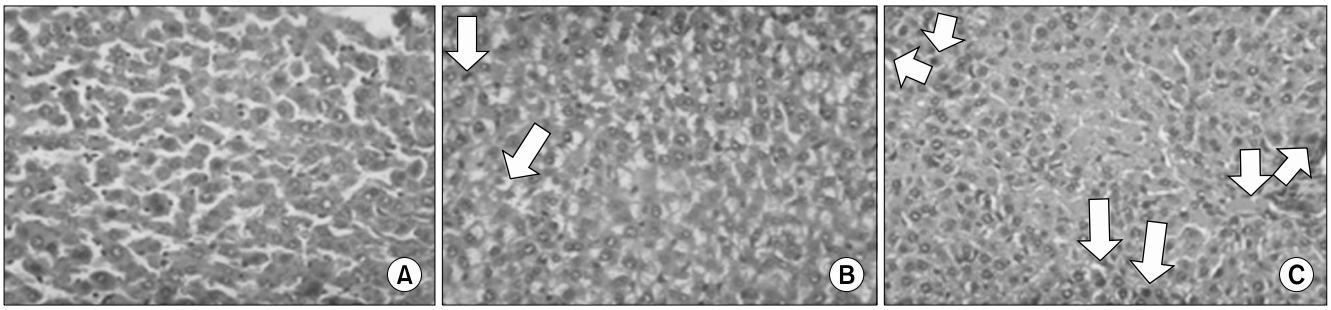

Fig. 4. Effect of *Sargassum hystrix* powder (SHP) and diazepam on liver inflammation in swimming restraint stress induced Wistar rats. (A) Severity 0%, (B) severity 1%～25%, and (C) severity 26%～75%.

cortisol and aldosterone (Neelima et al., 2014). Kannan et al. (2007) also showed that the brown seaweed *A. nodosum* may decrease levels of cortisol in goats under transportation stress. In addition, sulphated polysaccharides from the brown seaweed *Laminaria japonica* may regulate and stabilize plasma cortisol levels in rats subjected to psychological stress for 10 days (Li et al., 2014).

Liver inflammation

In Fig. 4, normal liver tissue (0% severity level) is indicated by the arrangement and shape of normal liver cells. Hepatocyte cells appear to be lined radially in liver lobules, in polyhedral-shaped with six or more surfaces containing one or two round nuclei, and form layers consisting of one to two cells, similar to brick arrangement. The cell plate leads from the edges of the lobules to the center and abrasively freezes to form structures such as labyrinth and foam. The gaps between these plates contain capillaries known as sinusoids of the liver. The sinusoid liver is a capillary blood vessel branching from the portal vein and the hepatic artery. The sinusoid shaped channel is tortuous and widened, coated in endothelial cells, has an irregular diameter, and is not intact (Makkar et al., 2016). Low-severity liver tissue (1%～25% severity level) is indicated by a slight degree in parenchymal degeneration, characterized by slight changes in hepatocyte form, slight infiltration of lymphocytes, presence of vacuole degeneration, and cell nuclei shifting to the edges. Liver tissue with moderate severity (26%～75% severity level) is indicated by red tissue because of the presence of several erythrocytes, a considerable amount of parenchymal degeneration with some form of hepatocyte changes, changes to eosinophils, sliding of cell nuclei to the edges, lymphocyte infiltration, irregularly arranged sinusoids, and occurrence of sinusoid constriction (Junquiera, 2007). Rats treated with diazepam 0.18 mg/kg, SHP 450 mg/kg, and the mixture of the standard pellet diet and SHP 450 mg/kg showed reduced liver inflammation (Table 2). The severity of liver inflammation and the effect of each treatment on the histopathological changes of liver hepatocytes are shown in Fig. 4 and Table 2.

Table 2. Effect of *Sargassum hystrix* powder (SHP) and diazepam on liver inflammation in swimming restraint stress-induced Wistar rats

Treatment group	Severity of liver inflammation (Replication)				
	1	2	3	4	5
Normal control	—	—	—	—	—
Fasting normal control	+	++	+	++	+
Negative control	++	++	++	++	++
Diazepam 0.18 mg/kg p.o.	—	—	+	+	+
Standard pellet diet 450 mg/kg p.o.	+	+	+	+	+
Standard pellet diet+SHP 450 mg/kg p.o.	-	+	—	+	+
SHP 450 mg/kg p.o.	+	—	—	+	+

—, 0%; +, low severity (1%～25%); ++, severity (26%～75%).

Hepatic injury is always accompanied by inflammation (Han et al., 2016). Swimming and fasting stressors increases the energy needs of an organism, thus increasing levels of ROS. Excessive accumulation of ROS due to stress will reduce the body's immune system. This activates Toll-like receptor 4 (TLR4), which plays an important role in activating the innate immune system through downstream signalling pathways, such as activation of p38 mitogen-activated protein kinase (MAPK). p38 MAPK plays an important role in signalling pathways involved in generating proinflammatory cytokines and downstream signals related to inflammation. Signalling via p38 MAPK pathways activate cAMP downstream element that binds proteins (CREB). CREB further regulates genes associated with inflammation through increasing nuclear factor (NF)-κB expression in the nucleus. NF-κB rapidly induces expression of inflammatory genes, including the inflammatory cytokines interleukin (IL)-1β, IL-6, tumor necrosis factor-α, and cyclooxygenase-2, resulting in liver inflammation (Liu et al., 2016). Diazepam has a hypnotic-sedative effect to regulate cortisol secretion, which reduces glucose consumption and the supply of glucose and fat to the liver tissues, effectively preventing liver inflammation (Pifarré et al., 2015). Saponins, steroids, terpenoids, and alkaloids present in SHP also have functions like those of diazepam in facili-

tating the inhibitory effect of the neurotransmitter GABA (data not shown). These compounds also reduce levels of adrenaline and noradrenaline in the brain, and reduce the cerebral metabolic rate of both oxygen and cerebral blood flow, thus reducing fatigue and helping the body adapt to stress (Joshi et al., 2012; Pifarré et al., 2015). In addition, phenolic compounds in SHP possess radical-scavenging activity, which prevent oxidative stress by transferring hydroxyl groups, therefore helping prevent liver inflammation (Firdaus et al., 2009). It has been demonstrated that polyphenol extracts of hawthorn peels exhibit hepatoprotective capacities through inducing release of pro-inflammatory cytokines, such as IL-1β and IL-6, and genetic biomarkers involved in the inflammatory response (Han et al., 2016).

Diet composition may be affected by the physiology of animals. Malta et al. (2014) reported that low-protein diets induces weak hallmarks of metabolic malfunctions in rats later in life. Barros et al. (2018) also suggested that a protein-restricted diet during pregnancy does not alter reproductive, biochemical, and hematological parameters of Wistar rats.

In conclusion, supplementation of SHP 450 mg/kg can be used as an alternative source of functional food for overcoming oxidative stress; SHP improves blood glucose, triacylglycerol, total cholesterol, and cortisol levels and liver histology by decreasing the severity of inflammation. SHP 450 mg/kg was the best experimented diet supplement for preventing oxidative stress, indicated by decreases in liver inflammation severity (ranging from low to none, 0%～25%), decreases in fasting blood glucose levels to 86.70 mg/dL, a stable triacylglycerol level of 89.43 mg/dL, decreases in total cholesterol level to 34.90 mg/dL, and a significant decrease in cortisol levels to 1,308.31 ng/mL verses initial values prior to treatment.

ACKNOWLEDGEMENTS

The authors would like to thank the Directorate General of Higher Education of the Republic of Indonesia. This research was supported by Research Grants Flagship of Universitas Gadjah Mada through DIPA UGM 2015, number 244/LPPM/2015. The authors would like also to thank Radipta Lailatussifa for collecting and analysing the data.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

REFERENCES

Adekunle AS. Dyslipidemic and atherogenic effects of academic stress. Afr J Biochem Res. 2011. 5:356-359.

Astuti P, Sarmin, Kusumawati A, Airin CM, Maheswari H, Sjahfirdi L. Comparison level of cortisol and ratio of neutrophil /lymphocytes as acute stress marker to long road transportation of bligon bucks. Proceedings of International Seminar on Zoonotic and Tropical Diseases. 2009 Jun 26-27. Yogyakarta, Indonesia. p 57-60.

Barros MAV, Andrade EB, Barros RGN, Costa IKM, Costa ICL, Vitorino GFA, et al. Low-protein diet does not alter reproductive, biochemical, and hematological parameters in pregnant Wistar rats. Braz J Med Biol Res. 2018. 51:e6602.

Brigo F, Bragazzi NL, Bacigaluppi S, Nardone R, Trinka E. Is intravenous lorazepam really more effective and safe than intravenous diazepam as first-line treatment for convulsive status epilepticus? A systematic review with meta-analysis of randomized controlled trials. Epilepsy Behav. 2016. 64:29-36.

Castro e Couto T, Brancaglion MYM, Cardoso MN, Bergo Protzner A, Garcia FD, Nicolato R, et al. What is the best tool for screening antenatal depression?. J Affect Disord. 2015. 178:12-17.

Chan PT, Matanjun P, Yasir SM, Tan TS. Histopathological studies on liver, kidney and heart of normal and dietary induced hyperlipidaemic rats fed with tropical red seaweed *Gracilaria changii*. J Funct Food. 2015. 17:202-213.

Chang CY, Wu KC, Chiang SH. Antioxidant properties and protein compositions of porcine haemoglobin hydrolysates. Food Chem. 2007. 100:1537-1543.

Duraisami R, Mohite VA, Kasabe AJ. Anti stress, adaptogenic activity of standardized dried fruit extract of aegle marmelos against diverse stressors. Asian J Pharm Clin Res. 2010. 3:1-3.

Firdaus M, Astawan M, Muchtadi D, Wresdiyati T, Waspadji S, Karyono SS. Prevention of endothelial dysfunction in streptozotocin-induced diabetic rats by *Sargassum echinocarpum* extract. Med J Indones. 2009. 19:32-35.

Garcés-Rimón M, González C, Uranga JA, López-Miranda V, López-Fandiño R, Miguel M. Pepsin egg white hydrolysate ameliorates obesity-related oxidative stress, inflammation and steatosis in Zucker fatty rats. PLoS One. 2016. 11: e0151193.

Goldstein I, Hager GL. Transcriptional and chromatin regulation during fasting—the genomic era. Trends Endocrinol Metab. 2015. 26:699-710.

Gotama TL, Husni A, Ustadi. Antidiabetic activity of *Sargassum hystrix* extracts in streptozotocin-induced diabetic rats. Prev Nutr Food Sci. 2018. 23:189-195.

Güemes M, Rahman SA, Hussain K. What is a normal blood glucose?. Arch Dis Child. 2015. 101:569-574.

Guo CH, Chen PC, Lin KP, Shih MY, Ko WS. Trace metal imbalance associated with oxidative stress and inflammatory status in anti-hepatitis C virus antibody positive subjects. Environ Toxicol Pharmacol. 2012. 33:288-296.

Han X, Li W, Huang D, Yang X. Polyphenols from hawthorn peels and fleshes differently mitigate dyslipidemia, inflammation and oxidative stress in association with modulation of liver injury in high fructose diet-fed mice. Chem Biol Interact. 2016. 257:132-140.

Holdt SL, Kraan S. Bioactive compounds in seaweed: functional food applications and legislation. J Appl Phycol. 2011. 23:543-597.

Husni A, Anggara FP, Isnansetyo A, Nugroho AE. Blood glucose level and lipid profile of streptozotozin-induced diabetic rats treated with *Sargassum polystum* extract. Int J Pharm Clin Res. 2016. 8:445-450.

Ishola IO, Chatterjee M, Tota S, Tadigopulla N, Adeyemi OO, Palit G, et al. Antidepressant and anxiolytic effects of amentoflavone

isolated from *Cnestis ferruginea* in mice. Pharmacol Biochem Behav. 2012. 103:322-331.
Joshi T, Sah SP, Singh A. Antistress activity of ethanolic extract of *Asparagus racemosus* Willd roots in mice. Indian J Exp Biol. 2012. 50:419-424.
Junquiera LC, Carneiro J. Basic histology: text & atlas. 10th ed. Mc Graw-Hill, New York, NY, USA. 2007. p 320.
Kalsi DS. What is the effect of fasting on the lifespan of neurons?. Ageing Res Rev. 2015. 24:160-165.
Kannan G, Saker KE, Terrill TH, Kouakou B, Galipalli S, Gelaye S. Effect of seaweed extract supplementation in goats exposed to simulated preslaughter stress. Small Rumin Res. 2007. 73: 221-227.
Kim M, Kim SO, Lee M, Park Y, Kim D, Cho KH, et al. Effects of ginsenoside Rb1 on the stress-induced changes of BDNF and HSP70 expression in rat hippocampus. Environ Toxicol Pharmacol. 2014. 38:257-262.
Kim SN, Lee W, Bae GU, Kim YK. Anti-diabetic and hypolipidemic effects of *Sargassum yezoense* in db/db mice. Biochem Biophys Res Commun. 2012. 424:675-680.
Lailatussifa R, Husni A, Isnansetyo A. Antioxidant activity and proximate analysis of dry powder from brown seaweed *Sargassum hystrix*. Jurnal UGM. 2017. 19:29-37.
Lailatussifa R, Husni A, Nugroho AE. Anti-stress activity of *Sargassum polycystum* extracts using a cold restraint stress model. Food Sci Biotechnol. 2016. 25:589-594.
Li J, Wang S, Yang X, Pang G, Zheng H, Shen B, et al. Effect of sulfated polysaccharides from *Laminaria japonica* on vascular endothelial cells in psychological stress rats. J Ethnopharmacol. 2014. 151:601-608.
Li YX, Wijesekara I, Li Y, Kim SK. Phlorotannins as bioactive agents from brown algae. Process Biochem. 2011. 46:2219-2224.
Liu CM, Ma JQ, Liu SS, Feng ZJ, Wang AM. Puerarin protects mouse liver against nickel-induced oxidative stress and inflammation associated with the TLR4/p38/CREB pathway. Chem Biol Interact. 2016. 243:29-34.
Liu L, Heinrich M, Myers S, Dworjanyn SA. Towards a better understanding of medicinal uses of the brown seaweed *Sargassum* in Traditional Chinese Medicine: a phytochemical and pharmacological review. J Ethnopharmacol. 2012. 142:591-619.
Makkar HPS, Tran G, Heuzé V, Giger-Reverdin S, Lessire M, Lebas F, et al. Seaweeds for livestock diets: a review. Anim Feed Sci Technol. 2016. 212:1-17.
Malta A, de Oliveira JC, Ribeiro TA, Tófolo LP, Barella LF, Prates KV, et al. Low-protein diet in adult male rats has long-term effects on metabolism. J Endocrinol. 2014. 221:285-295.
Mossa ATH, Swelam ES, Mohafrash SMM. Sub-chronic exposure to fipronil induced oxidative stress, biochemical and histopathological changes in the liver and kidney of male albino rats. Toxicol Rep. 2015. 2:775-784.
Nasmia, Natsir S, Rosyida E, Rusaini, Yala ZR. Toxicity of liquid extract of seaweed *Sargassum* sp. on the growth of microalgae *Skeletonema costatum*. AACL Bioflux. 2017. 10:247-253.
Neelima N, Sudhakar M, Lakshmi BVS. Adaptogenic activity of ethanolic extract of *Emblica officinalis* fruits. Int J Adv Pharm Sci. 2014. 5:1859-1865.
Pifarré P, Simó M, Gispert JD, Plaza P, Fernández A, Pujol J. Diazepam and Jacobson's progressive relaxation show similar attenuating short-term effects on stress-related brain glucose consumption. Eur Psychiatry. 2015. 30:187-192.
Sharifuddin Y, Chin YX, Lim PE, Phang SM. Potential bioactive compounds from seaweed for diabetes management. Mar Drugs. 2015. 13:5447-5491.
Sigal RJ, Kenny GP, Wasserman DH, Castaneda-Sceppa C. Physical activity/exercise and type 2 diabetes. Diabetes Care. 2004. 27:2518-2539.
Tapia-Martinez J, Hernández-Cruz K, Franco-Colín M, Mateo-Cid LE, Mendoza-Gonzalez, C, Blas-Valdivia, V, et al. Safety evaluation and antiobesogenic effect of *Sargassum liebmannii* J. Agardh (Fucales: Phaeophyceae) in rodents. J Appl Phycol. https://doi.org/10.1007/s10811-019-1752-y.
Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007. 39:44-84.
Xiu L, Zhong G, Liu D, Chen S, Liu H, Chen F. Comparative efficacy and toxicity of different species of *Sargassum* in Haizao Yuhu Decoction in PTU-induced goiter rats. Evid Based Complement Alternat Med. 2017. 2017:1-10.
Yokota T, Nagashima M, Ghazizadeh M, Kawanami O. Increased effect of fucoidan on lipoprotein lipase secretion in adipocytes. Life Sci. 2009. 84:523-529.