Full Paper

EXTRACT OF *Sargassum echinocarpum* ALLEVIATES OXIDATIVE STRESS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Abstract

Oxidative stress occured in streptozotocin-induced diabetic rats. The ability of *Sargassum echinocarpum* to ameliorate the oxidative stress after treatment with streptozotocin was investigated in rats. Adult male rats were intraperitoneally injected with 45 mg/kg of streptozotocin to produce experimental oxidative stress characteristic of diabetes mellitus. Hyperglycemia was observed in blood serum after 10 days of streptozotocin treatment. There were a significant decrease in the activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxide (GPx) and a significant increase in the levels of malondialdehyde (MDA) in the serum of diabetic rats. It indicated that there were an increasing lipid peroxidation and oxidative stress in the diabetic rats. Providing *S. echinocarpum* extract for 90 days on diabetic rats significantly improved the oxidative stress evidenced by a decreasing of MDA serum level and an increasing SOD, CAT and GPx activities than streptozotocin-treated rats. These results showed that the extract might improve the clinical manifestation of diabetes mellitus and decrease the oxidative stress in the diabetic rats. This effects appear to be due to its antioxidant properties.

Key words: antioxidants, lipid peroxidation, *Sargassum echinocarpum*

Introduction

Diabetes mellitus is a common chronic disease. Although both genetic and environmental factors appear to play a role, the cause of diabetes mellitus is still not clear. A large number of studies have demonstrated that oxidative stress and nonenzymatic protein glycation are closely associated with the development of diabetes mellitus (Mehta *et al*., 2006). Excessive oxidative stress has been implicated in the pathology and complications of diabetes mellitus (Kuyvenhoven & Meinders, 1999). Hyperglycemia generates abnormally high levels of free radicals by a mechanism involving autoxidation of glucose, followed by oxidative degeneration and protein glycation. In addition, nonenzymatic glycosylation of those enzymes that normally detoxify free-radical species may exacerbate oxidative stress in diabetes (Sydow & Münzel, 2003). Thus, clinical complications in diabetes may be due partially to the inability of key antioxidant enzymes to function at normal levels (Jakus, 2000). Conversely, antioxidants are believed to be protective because they may help to protect the human body against damage by reactive oxygen singlet (ROS) (Halliwell, 2009). Antioxidants from natural sources are preferred by consumers due to concerns on the toxic and carcinogenic effects of synthetic antioxidants (Kranl *et al*., 2005).

Seaweeds have been habitually consumed on Indonesia, especially in coastal society. It has been reported that seaweeds contain a rich and largely untapped source of biologically active substances (Smit, 2004). Marine brown algae contain phloroglucinol phenolics (phlorotannins) (Koivikko *et al*., 2005, Singh & Bharate, 2006) which are probably good antioxidants, since plant phenolics can behave as ROS scavengers, metal chelators, enzyme modulators and prevent lipid peroxidation (Rice-Evans *et al*., 1997). The antioxidant activity of this seaweeds has been published (Anggadiredja *et al*., 1997; Lim *et al*., 2002; Mori *et al*., 2003; Wei *et al*., 2003; Kang *et al*., 2004; Okada *et al*., 2004; Iwashima *et al*., 2005), conversely, nowadays no experiment relating to the anti-oxidative stress activity of *S. echinocarpum* on diabetic animal model. The aim of this study was to investigate the anti-oxidative stress of *S. echinocarpum* extract on diabetic rats.

Material and Method

Material

S. echinocarpum was collected on the Coast of Talango Island, East Java. Indonesia, in April 2008. The alga was washed thoroughly with seawater, followed by tap water to remove sand and epiphytes, and then frozen at -20^{o}C. The specimen was identified

by Dr. Augy Syahailatua, Research Centre of Oceanography, Indonesian Institute of Sciences.

Drugs and Chemicals
All the drugs and biochemicals used in this study were purchased from Sigma Chemical Company, Inc., St Louis, MO, USA. The chemicals were of analytical grade.

Experimental Animals
Male Sprague-Dawley rats weighing 150-200 g were procured from Gadjah Mada University, Indonesia. All animal procedures were in accordance with the institutional guidelines for animal research, and approved by the animal research ethics committee of Brawijaya University, Indonesia. They were kept in clean and dry cages with a bedding of paddy husk, fed with standart diet (American Institute of Nutrition (AIN) -93) and water *ad libitum*.

Extraction of Extract
The dried of *S. echinocarpum* (2.0 kg) were ground to a fine powder and were macerated three times with methanol. The suspension was filtered and evaporated under reduced pressure and lyophilized. The green yellow of methanol extract (80 g) was obtained.

Animal Experiment
Animals were divided into 3 groups: normal control, diabetic control, and the diabetic animals were given *S. echinocarpum* extract (450 mg/kg body weight) by oral gavage. The experimental diabetic rats were obtained by single administration of streptozotocin (45 mg/kg, i.p.), dissolved in freshly prepared 0.1 M citrate buffer, pH 4.5. Diabetes was confirmed seven days latter in streptozotocin induced animals showing blood glucose levels > 200 mg/dl (11.1 mmol/l) as monitored in the blood from tail vein using glucometer. The extract treatment was given for 90 days.

Preparation of Serum Sample
After the 12 weeks treatment period, the whole blood was obtained by cardiac puncture from the sacrificed rat. The whole blood was allowed to clot for 2 h and further spin at 4000 rpm for 10 min to separate serum from the blood cells. The blood serum acquired was used for lipid peroxide and antioxidant enzymes assays.

Measurement of Lipid Peroxide in Serum
Lipid peroxide assay was determined based on Ohkawa *et al*. (1979). A volume of 200 µl Sodium dodecil sulfonate (SDS) was added with 50 µl buthylated hydroxy toluene (BHT), 50 µl Ethylene tetraacetic acid (EDTA), 1500 µl thiobarbituric acid (TBA) and 1250 µl serum. Solution was vortexed before added 1500 µl trichloro acetic acid (TCA), contunued by sentrifuged 500 rpm, for 10 min. Supernatan in reaction tube was heated on water bath at 80^{o}C, for 20 min. Malondialdehyde determined by absorbance on spectrophotometer at 532 nm.

Measurement of Superoxide Dismutase Activity in Serum
Superoxide dismutase activity was determined based on Misra and Fridovich (1972). A volume of 2800 µl sodium carbonate mixed with 100 µl serum and 100 µl epinephrine. Absorbance was measured at 480 nm after epinephrine addition.

Measurement of Catalase Activity in Serum
Catalase activity was determined based on Sinha (1972). A volume of 1000 µl serum was added with 2 ml potassium bichromate and boiled in water bath for 10 min. Absorbance was measured at 570 nm.

Measurement of Glutathione Peroxidase Activity in Serum
Glutathione peroxidase activity was measured by Paglia & Valentine method (1967). A volume of 2000 µl buffer phosphate 0.1 M pH 7.0 mixed with 200 µl serum, 200 µl glutathione 10 mM, and 200 µl glutathione reductase. After incubated at 37^{o}C, for 10 min, solution added with 200 µl NADPH 1.5 mM. Finally, 200 µl H_2O_2 1.5 mM mixed with solution after incubated at 37^{o}C, for 3 min. Absorbance of solution was measured at 340 nm.

Results and Discussion

Blood Glucose
The diabetic animals treated with *S. echinocarpum* showed significant decrease in the glucose level at 4th , 6th, 8th, 10th and 12th weeks as compared to the diabetic rats (Figure 1). Even though a significant antihyperglycemic effect was evident from 4th week onwards; a furthermore, the decrease in the blood glucose level was highly significant at 10th week as compared with the diabetic rats. The *S. echinocarpum* treatment however, could not restore glucose levels to normal.

There was significant increase in the glucose levels of diabetic rats throughout the experimental period. The treatment with *S. echinocarpum* extract showed decrease in the glucose level at different time interval as compared with the diabetic animals significantly. The hypoglycaemia effects of *S. echinocarpum* extract

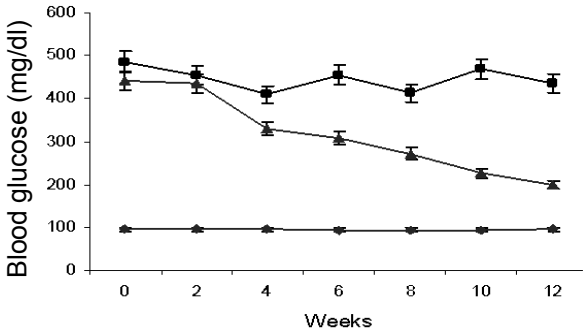

Figure 1. Glucose levels of normal (▮), diabetic (▲), and diabetic rats added *Sargassum echinocarpum* extract (★).

possibly done by inhibiting α glucosidase activity of gastrointestinal tract. Polyphenols are well known to bind to proteins in solution and form complexes whose properties depend on the structure of both the polyphenol and the protein. This complexation is responsible for the inhibition of digestive enzymes (Aguie-Beghin *et al.*, 2008).

Effect of Extract on the MDA Level

The highest MDA level of serum showed at diabetic rats. However the diabetic group of rats treated with *S. echinocarpum* extract, showed significant decrease in MDA level of serum (Table 1).

Table 1. Effects of *S. echinocarpum* extract on MDA levels in serum of rats**.**

Groups	MDA (nmol/ml)
Normal	1.07 ± 0.0474[a]
Diabetic	17.44 ± 0.4295[c]
Diabetic + *Sargassum*	2.87 ± 0.1305[b]

Values are expressed as mean ± SEM. Different letters indicate significant differences ($P < 0.05$).

The highest MDA level was obtained on diabetic rats. This suggests that lipid peroxidation is higher in diabetes and supported by earlier reports (Vessby *et al.*, 2002; Memisogullari *et al.*, 2003). Kuyvenhoven & Meinders (1999) suggested that hyperglycemia can increase lipid peroxidation. The excessive levels of glucose reaching the mitochondria lead to an overdrive of the electron transport chain, resulting in overproduction of superoxide anions, thus become hydrogen peroxide and hydroxyl radical. Interaction between hydroxyl radical and *polyunsaturated fatty acid* (PUFA) of membrane cell produces malondialdehyde. Lipid peroxidation of cellular structures, a consequence of free radical activity, is thought to play an important role complications of diabetes.

The diabetic rats with *S. echinocarpum* extract showed decrease in the MDA level as compared with the diabetic rats significantly. The treatment with *S. echinocarpum* extract showed significant prevention in increase in the levels of malondialdehyde. Several studies indicated that phlorotannin of brown algae is able to be protective against lipid peroxidation. The protective nature offered by the brown seaweed extracts may be due to the presence of phlorotannin having free radical scavenging properties (Mori *et al.*, 2003; Wei *et al.*, 2003; Kang *et al.*, 2004).

Effects of Extract on the Antioxidant Enzymes Activity

The antioxidant enzymes were decreased in serum of the diabetic rats as compared with the normal. However the diabetic group of rats treated with *S.* extract, showed significant increase in the activities of SOD, catalase and glutathione peroxidise in serum, respectively (Table 2).

The diabetic rats showed decrease in the antioxidant enzymes. The antioxidant enzymes such as SOD, CAT and GPx are the first line of defence against O_2^- and H_2O_2 mediated injury (Jakus, 2000). The levels of first line antioxidant enzymes were significantly depleted in diabetic rats. This deficient function of free radical scavenging enzymes leads to the accumulation of highly reactive free radicals and

Table 2. Effects of *Sargassum* extract on antioxidant enzymes activity in serum of rats.

	SOD[1]	Cat[2]	GPx[3]
Normal	32.08 ± 0.45[a]	184.46 ± 0.23[a]	109.95 ± 0.88[a]
Diabetic	6.99 ± 0.47[c]	79.70 ± 0.58[c]	41.53 ± 0.83[c]
Diabetic + *Sargassum*	25.18 ± 0.36[b]	150.99 ± 0.94[b]	92.44 ± 1.22[b]

[1] SOD activity expressed as U/min/ml
[2] Cat activity expressed as μmol H_2O_2/min/ml
[3] GPx activity expressed as mU/min/ml
Values are expressed as mean ± SEM. Different letters whithin coloumb indicate significant differences ($P < 0.05$).

consequent degenerative changes (Kuyvenhoven & Meinders, 1999). In the present study, the activities of SOD, CAT and GPx were significantly improved in diabetic rats treated seaweed extract, which may support the defensive nature of the *S. echinocarpum* extracts against diabetic-induced serum oxidative stress. The effects of phloroglucinol on cell viability might involve dual actions: direct action on oxygen radical scavenging, as shown by H_2O_2, OH radical scavenging and indirect action through induction of antioxidant enzymes. Antioxidant enzymes would be potential target molecules mediating antiapoptotic function of ERK pathway against oxidative stress (Kang *et al*., 2006).

Conclusion

This study investigated the anti-oxidative stress effect of *S. echinocarpum* extract on serum in diabetic rats and its possible mechanisms. The mechanism of this effect involves inhibition of glucose absorption, alleviation of the malondialdehyde level and enhancement of the antioxidant defence level; therefore, it rendered protection from oxidative stress which is usually associated with diabetes.

Acknowledgment

This study was financially supported by Directorate of Higher Education via Hibah Bersaing Project.

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