Prevention of endothelial dysfunction in streptozotocin-induced diabetic rats by Sargassum echinocarpum extract

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Abstract

Aim This study aimed to elicit the protective effect of Sargassum echinocarpum extract on endothelial dysfunction in thoracic aorta of streptozotocin-induced diabetic rats.

Methods The animals were divided into 5 groups. The first was normal, the second was diabetic non treated animals. The third to fifth groups were the diabetic animals which given Sargassum echinocarpum extract (150; 300, and 450 mg kg-1 body weight, respectively) by oral gavage and extract treatment was given for 12 weeks. Diabetes was induced by single administration of streptozotocin (45 mg kg-1, i.p.), dissolved in freshly prepared 0.1 M citrate buffer, pH 4.5. Diabetes was confirmed ten days latter in streptozotocin induced animals showing blood glucose levels > 200 mg dL-1 (11.1 mmol L-1) as monitored in the blood from tail vein using glucometer. After the treatment period, the blood serum acquired was used for antioxidant enzymes assays and the thoracic aorta was used for vasorelaxation assay.

Results There was a significant decrease in the activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-px) in diabetic rats (3.31±0.12;67.17±0.62;35.10±0.83) compared to control rats (9.97±0.12;185.31±0.23;116.38±0.88). Administration of Sargassum extract increased the activity of SOD, CAT, and GSH-px. The diabetic rats exhibit endothelial dysfunction as shown by loss of vasodilatory response to acetylcholine (ACH). This was restored by administration of Sargassum extract.

Conclusion Sargassum echinocarpum extract ameliorates oxidative stress and reverses the endothelial dysfunction associated with diabetes. This effect appears to be due to its antioxidant properties. (Med J Indones 2010; 19:32-5)

Key words: oxidative stress, sargassum echinocarpum, endothelium dependent relaxation, thoracic aorta

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Oxidative stress has been defined as a disturbance in the balance between antioxidants and pro-oxidants. Hyperglycemia can induce oxidative stress by various mechanisms; excessive levels of glucose reaching the mitochondria lead to an overdrive of the electron transport chain, resulting in overproduction of superoxide anions. When this radical scavenged by mitochondrial SOD fails, oxidative stress develops and it responsible for the activation of all major pathways underlying the vascular diabetic complications. The antioxidant defense may be lower in diabetes, which included reduced antioxidant status or free radical scavenging activity and this plays an important role in mediation of endothelial dysfunction accompanying diabetes. It seems reasonable to hypothesize that antioxidant treatment would prevent endothelial dysfunction in diabetes.

The relaxing response of aortic ring to Ach was decreased in untreated diabetic rats. Studies have clearly indicated that antioxidants may be beneficial in reducing oxidative stress and endothelial dysfunction in diabetes and eventually preventing the vasculopathy, which accounts for the majority of clinical complications in diabetes mellitus.

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. Marine brown algae contain phloroglucinol phenolics which are probably good antioxidants, since plant phenolics can behave as ROS scavengers, metal chelators and enzyme modulators and prevent lipid peroxidation. The antioxidant activity of this seaweeds has been published, conversely, nowadays no experiment has been reported the effect of Sargassum echinocarpum extract on endothelial dysfunction in diabetic animal model.

The aim of this study was to investigate the protective effect of Sargassum echinocarpum extract on endothelial dysfunction in diabetic rats.

METHODS

Male Sprague-Dawley rats weighing 150-200 g were procured from Gajah Mada University, Indonesia. All procedures for treating animal 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 normal diet and water ad libitum.

Brown alga and diabetic endothelial dysfunction

The animals were divided into 5 groups. The first was normal, the second was diabetic, and the others were diabetic which given extract. Diabetes was induced by single administration of streptozotocin (45 mg kg⁻¹, i.p.), dissolved in freshly prepared 0.1 M citrate buffer, pH 4.5. Diabetes was confirmed ten days latter in streptozotocin induced animals showing blood glucose levels > 200 mg dL⁻¹ (11.1 mmol L⁻¹) as monitored in the blood from tail vein using glucometer. The third to fifth groups were the diabetic animals which given Sargassum echinocarpum extract (150; 300, and 450 mg kg⁻¹ body weight, respectively) by oral gavage and extract treatment was given for 12 weeks.

After the twelve weeks treatment period, the whole blood was obtained by cardiac puncture from the sacrificed rat. Blood was allowed to clot for 2 h and further centrifuged at 1500 RPM for 10 min to separate serum from the blood cells. The blood serum acquired was used for antioxidant enzymes assays.

Afterwards, the thoracic aorta (from the arch of aorta to diaphragma) was quickly excised and placed in ice cold carbogenated (95% O₂ + 5% CO₂) Krebs Buffer (KB). The aorta was cleaned of adhering fat and tissue and cut into 3-4 mm segments in length. Due care taken not to stretch the tissue or damage the endothelium. The composition (mM) of KB was NaCl: 136, KCl: 5.4, MgSO₄: 0.5, KH₂PO₄: 2.7, KHPO₄: 0.45, NaHCO₃: 11.9 and glucose: 5.6 per liter.

Each ring was suspended by a pair of ‘s’ shaped stainless steel hooks in a water jacketed organ bath (10 mL capacity), filled with 10 mL of carbogenated KB maintained at 37°C. The segments were subjected to a tension of 1.5 ± 0.5 g (optimal resting tension), which was readjusted every 15 min during a 90-min equilibration period before drug administration. After a washout period, each segment was contracted with 1 µM noradrenaline (NA). Once a stable plateau was reached, a concentration-response curve to acetylcholine (Ach; 100 nM to 5 mM) was performed. Changes in isometric tension were recorded using a force displacement transducer (Grass FT 0.3, Quincy, MA, USA) connected to a Power Lab system 400 (ML 118, PowerLab, AD Instruments, Medford, MA) and stored in a computer. Responses were expressed as percentage relaxation of NA induced contraction.

The data were analyzed using SPSS version 16.0. Data are expressed as the mean ± s.e.mean. The statistical differences were determined by least square difference test, after a one-way analysis of variance, a probability level of P<0.05 being regarded as significant.
RESULTS

The antioxidant enzymes were decreased in serum of the diabetic rats as compared with the normal. The diabetic group of rats, however, treated with Sargassum extract showed a significant increase in the activities of SOD, catalase and glutathione peroxidise in serum, respectively (Table 1).

Addition of 1 µM NA to organ bath resulted in contraction of aorta of all groups. Isolated thoracic aorta rings precontracted with NA showed dose dependent relaxation to ACh (100 nM to 5 mM ). The magnitude of relaxations was significantly reduced in diabetic rats when compared with normal. The aortic rings of diabetic rats treated with Sargassum echinocarpum extract showed significant relaxation as compared to the diabetic aortic rings (Figure 1).

DISCUSSION

In this study, administration of Sargassum echinocarpum extract for 12 weeks exhibited anti oxidative stress and increased Ach-induced relaxation

The diabetic rats showed decrease in the antioxidant enzymes, meanwhile, the diabetic rats treated with Sargassum echinocarpum extract showed significant increase in the antioxidant enzymes (Table 1). The antioxidant enzymes such as SOD, CAT and GPx are the first line of defense against O$_2^-$ and H$_2$O$_2$. The levels of first line antioxidant enzymes were significantly depleted in diabetic rats. This deficient functioning of free radical scavenging enzymes leads to the accumulation of highly reactive free radicals and consequent degenerative changes. In the present study, the activities of SOD, CAT and GPx were significantly improved in diabetic rats that treated seaweed extract, which may support the defensive nature of the Sargassum echinocarpum extract 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$_2$O$_2$, OH radical scavenging and indirect action through induction of antioxidant enzymes. Antioxidant enzymes would be potential target molecules mediating anti-apoptotic function of ERK pathway against oxidative stress.

There is impairment of endothelium-dependent relaxation response to ACh in diabetic rat aorta. Several studies have indicated that the increased production of superoxide anions in vessels of diabetic animals and it is suggested that this active form of oxygen can inactivate NO to attenuate NO-dependent vasodilatory response in the diabetic rat aorta. Although the mechanisms underlying endothelial dysfunction may be multifactorial, there is a growing body of evidence that increased production of free radicals may considerably contribute to this phenomenon. Hyperglycaemia, being the characteristic feature of diabetes is one of the reasons for the increase the oxidative stress in diabetes.

We found that the aorta rings of the diabetic animals treated with Sargassum echinocarpum extract showed significant increase in the endothelial dependent relaxation when compared with that of the untreated diabetic animals. The relaxation was comparable with that of the controls. Extract apparent modulates NO bioavailability in aorta diabetic rats. This indicates its protective role against oxidative damage in diabetes.
Based on our findings, we suggest that *Sargassum echinocarpum* extract has the potential as a therapeutic component for treatment of diabetes and its complications. The mechanism of this effect involves enhancement of the antioxidant defence level and thus rendered protection from endothelial damage which is usually associated with diabetes.

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