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RESEARCH ARTICLE

Effectiveness of Brown Algae Extract to Reduce Serum Malondialdehyde and Protein Carbonyl Levels in Streptozotocin-Induced Sprague Dawley Rats

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ABSTRAK
Pendahuluan: Alga coklat *(Sargassum duplicatum)* mengandung komponen bioaktif yang berpotensi sebagai sumber antioksidan diharapkan dapat menghambat proses peroksidasi lipid dan oksidasi protein pada diabetes melitus. **Tujuan:** untuk membuktikan bahwa pemberian ekstrak alga coklat *(Sargassum duplicatum)* dosis 150mg/kgbb, 300mg/kgBB dan 450mg/kgBB dapat menurunkan kadar MDA dan PCO serum tikus *Sprague dawley* yang diinduksi streptozotocin.
Metode: Penelitian ini menggunakan rancangan *post test only control group design.* Dua puluh delapan (28) tikus *Sprague dawley* diinduksi dengan *Streptozotocin* (STZ) 40 mg/kgBB (i.p) dibagi secara acak menjadi 4 kelompok (n=7). Satu kelompok tidak diberikan perlakuan (kontrol), ketiga kelompok lainnya diberikan perlakuan berupa pemberian ekstrak alga coklat dengan dosis masing-masing 150mg/kgBB, 300mg/kgBB dan 450mg/kgBB selama 30 hari. Kadar MDA dan PCO serum diperiksa menggunakan metode ELISA. Analisis data dilakukan dengan uji *one way ANOVA* dengan tingkat kemaknaan p<0,05.
Hasil: Tidak ditemukan perbedaan yang bermakna pada kadar MDA serum antar kelompok penelitian (p=0,405). Kadar PCO serum pada kelompok pemberian ekstrak alga coklat dosis 450mg/kgBB lebih rendah bermakna dibanding dengan kelompok kontrol (p=0.001), kelompok pemberian ekstrak alga coklat dosis 150mg/kgBB (p=0.001) dan dosis 300mg/kgBB (p=0,037).
Kesimpulan: Pemberian ekstrak alga coklat tidak dapat menurunkan kadar MDA serum. Pemberian ekstrak alga coklat dosis 450mg/kgBB dapat menurunkan kadar PCO serum secara bermakna.

ABSTRACT
Introduction: *Brown algae (Sargassum duplicatum) contains bioactive compound with natural antioxidant that may potentially decrease lipid peroxidation and protein oxidation process in diabetes mellitus.* ***Objective:*** *to prove the effectiveness of brown algae extract administration (150mg/kgBW; 300mg/kgBW and 450 mg/kGBW respectively) in reducing serum MDA and PCO levels in streptozotocin-induced sprague dawley rat.*
Methods: *This experiment study used the post test only control group design. Twenty eight (28) Sprague dawley rats induced with Streptozotocin (STZ) 40 mg/kgBW (i.p) were divided into four groups (n=7). The first group was diabetic non treated group (control). The second to fourth groups were the diabetic rats which given brown algae extract (150mg/kgBW; 300mg/kgBW and 450 mg/kgBW respectively) for 30 days. Serum MDA and PCO levels were examined by using ELISA method. Data were analyzed using one way ANOVA.*
Results: *There were no significant differences in serum MDA levels among groups (p=0,405). However, serum PCO level in group administered with 450mg/kgBW of brown algae extract decreased significantly compared to control group (p=0.001), group administered with 150mg/kgBW (p=0.001) and 300mg/kgBW (p=0,037).*
Conclusion: *Administration of brown algae extract did not decrease serum MDA levels significantly. Administration of 450mg/kgBW brown algae extract is effective to decrease serum PCO level significantly.*

Keywords: *brown algae, diabetes mellitus, MDA, PCO, Sargassum duplicatum*

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder suffered by 8.3% of the adult global population (about 382 million people) and this number will continue to increase to more than 592 million people in less than 25 years (Aguiree *et al.,* 2013). Persistent hyperglycemia in diabetes causes increasing production of reactive oxygen species (ROS) through mitochondrial dysfunction, glucose auto oxidation and protein glycosylation (Moussa, 2008).

Mitochondrial dysfunction causes increased production of superoxide ($O2.^-$) which transformed

by Haber-Weiss and Fenton reaction in to hydroxyl radical (HO.) and hydroperoxyl ($HO.^2$). These radicals are two main ROS that have the ability to damage lipid membrane structure in the process of lipid peroxidation (Giacco & Brownlee 2010; Tiwari *et al*. 2013). Lipid peroxidation is a series of reactions that take place in three phases where the final phase will generate toxic aldehyde secondary products such as malondialdehyde (MDA) (Ayala *et al*., 2014).

Other important biomolecules which is also one of the potential targets of ROS is protein. The reaction between ROS and proteins may occur either directly or indirectly through some of the oxidative pathway that produces carbonyl derivatives (protein carbonyl/PCO) (Dalle Donne & Aldini, 2006). Protein carbonyl represents a form of irreversible process of protein modification which was formed at the beginning of oxidative stress, and not caused by the work of a particular oxidant so that this molecules can be used as a biomarker which represents a whole process of protein oxidation (Weber *et al*., 2015).

Brown algae (Sargassum duplicatum) is one type of seaweed found abundance and widespread in tropical waters, including Indonesia. Brown algae contains bioactive compounds such as polyphenol, flavonoid and phlorotannin that potential as a natural sources of antioxidant (Meenakshi & Gnanambigai, 2009; Samee *et al*., 2009). Hardoko *et al* conducted a study on brown algae S. duplicatum antidiabetic activity using α-glucosidase enzyme resulted that the fraction laminaran and fucoidan from S. duplicatum has a potential as anti-type 2 diabetes.(Hardoko *et al*., 2014) Experimental study performed by Aulanni'am *et al* showed that brown algae (S.duplicatum) extract can reduce MDA levels in a rat model of inflammatory bowel disease (IBD) (Aulanni'am *et al*., 2012). Other experimental study on a rat model of lung cancer conducted by Botutihe *et al* showed a decrease in MDA levels of rat treated with S. duplicatum extract compared with control group (Botutihe & Aulanni'am, 2010). The effects of brown algae (S. duplicatum) extract on diabetic rat model has not been reported and this study aimed to investigate the effect of brown algae (S. duplicatum) extract on serum MDA and PCO levels of streptozotocin-induced diabetic rat.

METHODS

Research Design

This study was a laboratory experimental research with post test only control group design using experimental animals as research objects.

Animal Models

Male Sprague dawley rats aged 2-3 months weighing 100-150 grams obtained from *Laboratorium Penelitian dan Pengujian Terpadu - Layanan Penelitian Pra Klinik Pengembangan Hewan Percobaan* (LPPT-LP4HP) unit IV Gajah Mada University, Jogjakarta. Animals were acclimatized for 7 days in a cages, at 25-28°C with 70-75% relative humidity and a 12 hours light/12 hours dark cycle. Animals were feed with AD II pellet (PT. Japfa Comfeed Indonesia Tbk.) and reverse osmosis drinking water ad libitum.

Preparation of Brown Algae (S. duplicatum)

Brown algae (S. duplicatum) obtained from the intertidal zone of Sepanjang beach, Gunung Kidul, Jogyakarta. S.duplicatum were washed using fresh water to remove dirt, mud and sand that are still attached. Brown algae were dried in an oven at 40°C for 48 hours, ground to powder, sieved and kept in an airtight container until extraction.

Extraction of Brown Algae (S. duplicatum)

A total of 950 grams of brown algae (S.duplicatum) powder macerated in 4000 ml of 70% ethanol, stirred for 30 minutes, allowed to stand for 24 hours, then filtered to obtain extracts and lees (the process is repeated twice). Extracts containing solvent is separated from the solvent by evaporation using a rotary vacuum evaporator in 70°C water bath until thick blackish brown extract obtained.

Streptozotocin Induction

All the experimental animals were induced by 40mg/kgBW streptozotocin injection (intraperitoneal) dissolved in a citrate buffer (0.1 M; pH 4.5). Fasting plasma glucose levels were measured seven days after the induction of STZ. Rats were diagnosed with diabetes when the fasting blood glucose level after STZ induction are ≥200 mg/dl.

Treatment in Experimental Animals

Twenty-eight (28) male diabetic Sprague dawley rats were randomly divided into four groups (n=7): Diabetic control group (C); Diabetic group treated with 150mg/kgBW brown algae (S. duplicatum) extract (SD150); Diabetic group treated with 300mg/kgbw brown algae (S. duplicatum) extract (SD300); Diabetic group treated with 450mg/kgbw brown algae (S. duplicatum) extract (SD450). All experimental animals received their relevant treatments once daily for 30 days by oral gavage.

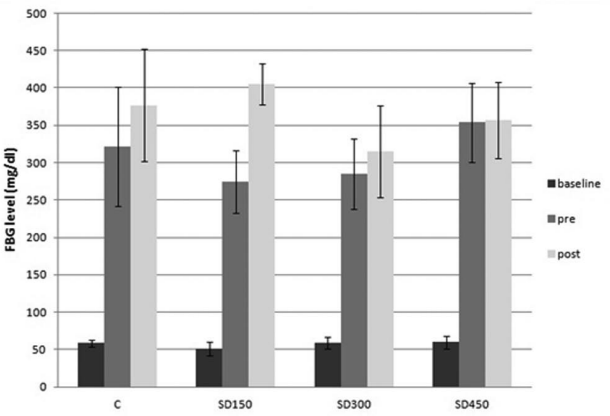

Figure 1. Fasting blood glucose levels (mg/dl). Values are expressed as mean ± SD. C, diabetic control group; SD150, diabetic group treated with 150mg/kgbw brown algae (S. duplicatum) extract; SD300, diabetic group treated with 300mg/kgbw brown algae (S. duplicatum) extract; SD450, diabetic group treated with 450mg/kgbw brown algae (S. duplicatum) extract. Baseline, fasting blood glucose levels at the beginning of the study; Pre, fasting blood glucose levels after STZ induction/before treatment; Post, fasting blood glucose levels after treatment.

Laboratory Analysis

Fasting plasma glucose levels were measured by using glucometer Nesco Multicheck N-01. Serum PCO levels were determined by using enzyme linked immunosorbent assay (ELISA) methods with a commercial kit from Bioassay Technology Laboratory. Serum MDA levels were measured with a commercial kit from Elabscience (No. Catalog E-EL-0060) by ELISA.

Statistic Analysis

Data analysis was performed by one way ANOVA test followed by analysis of Least Square Difference test using SPSS software version 20.0. p value <0.05 was considered as significant.

Ethical Clearance

The treatment given to the experimental animals was in accordance with the instructions given by medical research ethics committee (KEPK) Faculty of Medicine Diponegoro University and Kariadi Hospital Semarang (No. 632/EC/FK-RSDK/2015).

RESULTS

General overview of the experimental animals

During the treatment period, there were four dropout rats, one from each group. Seven days after the induction of STZ, the level of fasting blood glucose in each group increased to more than 200 mg/dl, it indicates that the induction of STZ was successful. Although the levels of fasting blood glucose after 30 days treatment with brown algae (S. duplicatum) extract is still increased as seen in figure 1, but the group received 450 mg/kgbw extract show improvements compared with other groups.

Table 1. Serum MDA and PCO levels after 30 days treatment with brown algae S.duplicatum extract (mean ± SD)

Experimental groups	MDA	PCO
Control (C)	139,23 ± 113,74	40,58 ± 2,83
Treatment 1 (SD150)	138,80 ± 71,91	40,57 ± 2,20
Treatment 2 (SD300)	218,48 ± 126,26	37,47 ± 3,41
Treatment 3 (SD450)	132,80 ± 72,70	32,03 ± 6,40 *

Serum MDA levels expressed as ng/ml
Serum PCO levels expressed as ng/ml
Values are expressed as mean ± SD
*** p<0,05 vs. C, SD150 and SD300**

Serum MDA and PCO Levels

There wasn't significant difference in serum MDA levels among groups (p=0,405). However, serum PCO level in group administered with 450mg/kgbw of brown algae (S. duplicatum) extract decreased significantly compared to control group, group administered with 150 mg/kgbw and 300 mg/kgbw brown algae (S. duplicatum) extract (table 1).

DISCUSSION

Fasting blood glucose levels seven days after STZ induction were increased 4-5 fold from the baseline. Akbarzadeh *et al* stated that one of the characteristics found in rats induced by STZ is increased blood glucose levels up to 4-5 folds compared with normal rats (Akbarzadeh *et al.,* 2007). Streptozotocin have a toxic properties that can cause an irreversible cell necrosis on pancreatic beta cells (Lenzen, 2008). The damage that occurred on pancreatic beta cells caused interference in insulin secretion which has a major role in glucose uptake mechanism. Disruption in the process of secretion of insulin will cause glucose unable to enter the cells and finally buildup in the blood (hyperglycemia), this condition resembles the process that occurred in type 1 diabetes (Giacco & Brownlee, 2010). A study conducted by Hardoko *et al*. showed that the fraction laminaran and fucoidan from brown algae (S. duplicatum) extract had the ability to inhibit the action of the α-glucosidase enzyme which played

a vital role in the conversion of carbohydrates into glucose (Hardoko *et al.*, 2014). Although administration of brown algae (S. duplicatum) extract in this study had not been able to lower blood glucose levels, but it appeared that the administration of 450 mg/kgbw of brown algae (S. duplicatum) had the potential to inhibit the increase in blood glucose levels.

In this study, administration of 450 mg/kgbw brown algae (S. duplicatum) extract effective to decrease serum PCO level. Phytochemical examination shows that brown alga (S. duplicatum) extract contains bioactive compounds as a source of natural antioxidants such as flavonoid and phlorotanin (Putranti, 2013; Septiana & Asnani, 2012; Firdaus *et al.*, 2009). Flavonoid and phlorotanin have more than one phenol ring structure, and in each rings there is a hydroxyl group (OH) and double conjugate bonds that plays an important role in the process of free radicals scavenging, especially for the hydroxyl radical (HO.) and peroxyl radicals (ROO.) (Aulanni'am *et al.*, 2012). Flavonoid/ phlorotanin donated an atom (H) from the hydroxyl group (OH) of phenolic ring to react with free radicals that produce flavonoid fenoksil radical/phlorotanin fenoxyl radical (FlO./FrO.) that have a lower reactivity. Flavonoid fenoxyl radical/phlorotanin fenoxyl radical has a conjugated double bond and a stable resonance structure so that electrons can be delocalized and the effects of free radicals can be removed (Fauziah & Mahdi, 2013). Meanwhile, the administration of 150, 300 and 450 mg/kgbw brown algae (S. duplicatum) extract were not able to reduce levels of serum MDA. This is likely caused by several factors that affect the antioxidant effect of brown algae (S.duplicatum) extract used in this study. Phenol compound is easily oxidized and sensitive to heat treatment, so that the drying process by using direct heat energy can reduce the content of phenolic compounds present (Guihua Xu, 2007). Masduqi AF *et al* comparing total phenols levels contained in brown algae Sargassum extract using different method: drying under direct exposure with sun, drying with an oven and drying with winds. It is indicate that the content of total phenols highest obtained by using the drying with winds method (Masduqi *et al.*, 2014). Research conducted by Budhiyanti SA *et al* showed that the antioxidant activity of brown algae (Sargassum sp.) is also influenced by the season when the algae harvested. The antioxidant content of brown algae (Sargassum sp.) harvested in summer (April) have a better antioxidant content compare with brown algae (Sargassum sp.) harvested during the rainy season (October) (Budhiyanti & Raharjo, 2012). Andrew and Cornelius said that the levels of phenolic compounds contained in Sargassum sp. affected by its exposure to UV radiation. It is a physiological mechanism owned by algae to address the damaging effects of the sun radiation (Plouguerné *et al.*, 2006; Holzinger & Lütz, 2006). Brown algae (S. duplicatum) used in this study were harvested in November (the rainy season) and dried using an oven with a temperature of ± 40°C. Both of these factors can affect the phenolic content in the extract used so that the highest dose used in this study amounted to 450mg/kgbw can only provide significant effects on protein oxidation process, but do not have any influence on the process of lipid peroxidation that occurs.

This study also have several limitations. Standard deviation value of serum MDA levels in each group tends to be high, especially in the control group and the SD300 group. This indicates that the variation in serum MDA levels between one subjects to another subjects in one group were too large. Ansarin *et al* stated that the examination of MDA levels in biological samples can not provide valid data, because the molecules of MDA is unstable and has a tendency very high to react with other molecules (Ansarin K, Jouyban A., 2015). In order to obtain more accurate and sensitive results of MDA levels, Hamdy *et al* recommend the use of HPLC (High Performance Liquid Chromatography) method (Hamdy *et al.*, 2013). Serum MDA levels in this study examined by using ELISA (Enzyme linked immunosorbent assay) method. It is considered easier to apply as well as the facilities and equipment needed to carry out checks is available in the laboratory where the research was conducted. This study also did not examined another stress oxidative biomarker that can still be explored, such as superoxide dismutase (SOD); catalase (CAT); and glutathione peroxidase (GSH-Px). Examination by using a wider biomarker will be able to provide a clearer picture about the ability of brown algae S. duplicatum against oxidative stress conditions.

In conclusion, administration of brown algae S. duplicatum extract did not decrease serum MDA levels significantly. Administration of 450mg/kgBW brown algae extract effective to decrease serum PCO level significantly. Further research we suggested to explore another stress oxidative biomarker such as superoxide dismutase (SOD); catalase (CAT); and glutathione peroxidase (GSH-Px) to provide more explanation about the ability of brown algae S. duplicatum against oxidative stress conditions.

Conflict of Interest

The authors affirm no conflict of interest in this study.

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