Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Original article https://doi.org/10.12980/jclm.4.2016J6-228

Sargassum polycystum extract attenuates oxidative stress on diabetic rats

Muhamad Firdaus[1*], Setyawati Soeharto[2]

[1]*Laboratory of Biochemistry, Faculty of Fisheries and Marine Sciences, Brawijaya University, Malang-65145, Indonesia*

[2]*Laboratory of Pharmacology, Faculty of Medicine, Brawijaya University, Malang-65145, Indonesia*

ARTICLE INFO

Article history:
Received 28 Oct 2016
Accepted 10 Nov 2016
Available online 21 Nov 2016

Keywords:
Antioxidant enzymes
Brown seaweed
Diabetes mellitus
Lipid peroxidation

ABSTRACT

Objective: To evaluate hypoglycemic, free radical scavenging and improving antioxidant enzymes activities on diabetic-induced streptozotocin rats by *Sargassum polycystum* (*S. polycystum*) extract.
Methods: The seaweed extract was obtained by maceration, concentration and freeze drying, respectively. Acute toxicity was investigated on 25 rats. Forty eight of rats were used to study anti-stress oxidative of extract and divided into eight groups, where the first and fifth group were normal and diabetic control. The normal and diabetic treated groups were administered orally with extracts of *S. polycystum* for 28 days. The blood glucose and body weight of rats were observed each week. The blood was obtained for determination of malondialdehide, superoxide dismutase, catalase and glutathione peroxidase, respectively.
Results: Extract of *S. polycystum* revealed no mortality and it was grouped as relatively non-toxic substance. The normal rats revealed difference statistically to the diabetic rats. The diabetic rats treated extract showed decreasing of blood glucose level and increasing of body weight. The diabetic rats were treated with 450 mg/kg of extract showed the higher oxidative stress augmentation than other diabetic treatments. It was caused free radical scavenging and induction of antioxidant enzymes activity by brown seaweed extract.
Conclusions: The extract of *S. echinocarpum* reduce oxidative stress on diabetic-induced streptozotocin rats and demonstrate as candidate of antioxidant diabetic substances.

1. Introduction

Diabetes mellitus is metabolic disorder that characterized by high glucose level and produced excessively of free radical[1]. The chronically hyperglycemic able to causing complication for diabetics, such as endothelial dysfunction, retinopathy, nephropathy, stroke, neuropathy autonomy and heart disease[2,3]. In diabetes mellitus, free radical could be produced by many mechanisms, for example, autooxidation of glucose, induction and activation of various lipoxygenase enzymes, activation of glycation pathways, promotion of the interaction of nitric oxide with superoxide anions to produce peroxynitrite and hydroxyl radicals and reduction of the activity of the antioxidant defense mechanisms[4]. Insufficient of reduction blood glucose level and removal free radical might lead to lipid peroxidation and enzyme inactivation and finally inducing diabetic complications[2,5-6].

Lowering level of blood glucose and using antioxidant have been known as the best strategy to reduce free radical, oxidative stress and diabetic complication on diabetic[7,8]. Polyphenol is one of active compounds that it could used as hypoglycemic and antioxidant agent. Hypoglycemic effect of polyphenol is because of its glucosidase and amylase inhibition activities[9,10]. These enzymes are responsible to the degradation starch which become glucose on the upper gastro intestinal tract. The glucose-absorbed able to increase the glucose level in the blood. Another hypoglycemic capability of polyphenol was capacity to induce pancreas for insulin secretion. This hormone sensitize the cells of the body to uptake glucose from blood[11]. Antioxidant activity of polyphenols was caused their capability to proton donor, free radical

*Corresponding author: Muhamad Firdaus. Laboratory of Biochemistry, Faculty of Fisheries and Marine Sciences, Brawijaya University, Jl. Veteran, Malang-65145, Indonesia.
Tel: +62 341 553512
E-mail: muhamadfir@ub.ac.id

Foundation Project: Supported by Directorate of Higher Education, Ministry of Education and Culture, Republic of Indonesia via HibahBersaing Program (No.: 0636/023-04.2.16/15/2012).

All experimental procedures involving animals were conducted in accordance to Organization for Economic Co-operation and Development guidelines and approved by Brawijaya University Animal Care Committee.

The journal implements double-blind peer review practiced by specially invited international editorial board members.

scavenging and inducing of antioxidant enzymes activity and their gene expression[12-15].

Drinking of tea from brown algae have become a culture in the ancient Chinese imperial. King and kinsfolk of the imperial drank for their fitness and health and this benefit related to their polyphenol[17]. The polyphenol of brown seaweed have been studied their activity as hypoglycemic and antioxidant agent[4,9-16]. Hypoglycemic activity of *Sargassum* sp. on normal animals and extract of *Sargassum echinocarpum* on diabetic rats has been studied[4,18]. *Sargassum* sp. was belonged to brown seaweed and also contained polyphenol[19,20]. The objective of this research was to obtain the amelioration of oxidative stress on diabetic rats by extract of *Sargassum polycystum* (*S. polycystum*).

2. Materials and methods

2.1. Collection and preparation of the crude extract of S. polycystum

S. polycystum were collected in September, 2012 from Talango Island waters, Sumenep District of East Java, Indonesia and identified by botanist of the Biology Department of Brawijaya University, Indonesia. The plant material was dried at oven, 45 ˚C for 3 days and pulverized by blender and extracted into methanol (1/3; w/v) by maceration for 24 h three times at ± 4 ˚C. Supernatant was concentrated by rotary evaporation at 45 ˚C and freeze dried to obtain a yield of 30.4 g (15.2 %) extract from 200 g of the powdered material. The crude extract was stored in impervious amber bottle until used.

2.2. Chemicals and instrument

All reagents were of analytical grade and purchased from Sigma chemical company. The UV-vis spectrophotometer was Spectruquant Pharo 300 (Merck KGaA, Germany).

2.3. Animals

Twenty five Balb/c mice of male (18–20 g) were used for the acute toxicity study, forty eight Sprague-Dawley rats of male weighing between 165 and 180 g were used for the oxidative stress study. The use of animal was reviewed and approved by Brawijaya University Animal Care Committee. They were kept in a well-ventilated Laboratory Animal Physiology of the Faculty of Science and Technology of the State Islam University of Malang, Indonesia under standard laboratory condition (12:12 h dark/light cycle). The animals were allowed to acclimatize for a week during which they had free access to commercial pellet diet and water *ad libitum*.

2.4. Acute toxicity

The acute toxicity was studied according to the method of Organization for Economic Co-operation and Development guidelines[21]. The mice were randomly divided into one control and four test groups of 5 mice per group, and were fasted for 18 h before treatment. Four doses of the crude extract (625, 1 250, 2 500 and 5 000 mg/kg body weight) were administered orally by gastric probe; the control group received distilled water. Mortality was determined 24 h after administration and the LD_{50} was calculated. The animals were further observed for 2 weeks for possible delayed toxicity.

2.5. Induction of experimental diabetes

After the acclimatization period, diabetes was induced by a single intraperitoneal injection of freshly prepared streptozotocin (45 mg/kg body weight) in buffer citrate, pH = 4.5. Control rats received a similar volume of buffer. Diabetes was evident within 2 days after administration of streptozotocin. The rats with blood glucose level of above 200 mg/dL were considered diabetic and were involved in the study[4].

2.6. Extract administration and body observation

Fasted rats (12 h, 24 diabetic + 24 normal) were divided equally into 8 groups. Treatment with the extract started on the 10th day of streptozotocin treatment and then was continued daily for 28 days. The extract was given orally as a single dose in the morning. Group I (normal control) received aquadest, groups II to IV were given 150, 300 and 450 mg/kg body weight extract, respectively. Group V (diabetic control) received distilled water while groups VI to VIII (diabetic treated) received extract as in groups II to IV, respectively. The changes in body weight of the rats were observed during the treatment period.

2.7. Determination of blood glucose

Blood glucose was determined by biosensor of glucose oxidase method in glucometer (GlucoDr). The tail of rats incised by sterile scissor and a little of blood dripped on glucometer strip. Level of blood glucose was stated as mg/dL.

2.8. Lipid peroxidation

The assay principle is based on the fact that malondialdehyde (MDA), a secondary product of lipid peroxidation reacts with thiobarbituric acid in acidic medium to give a pink chromogen which is measured spectrophotometrically[22]. Two hundred microliter sodium dodecylsulfonate, 50 μL ethylene diamine tetraacetic acid, 1 500 μL thiobarbituric acid and 1 250 μL plasma were mixed into tube glass. After it were vortexed, 1 500 μL trichloroacetic acid was added and centrifuged 10 g for 10 min and supernatant separated. The supernatant was kept in a water bath at 80 ˚C for 20 min. The absorbance of the pink chromogen supernatant was measured at 532 nm and the thiobarbituric acid reactive substances produced were estimated using MDA standard curve and it were quantified as nmol/mL.

2.9. Determination of superoxide dismutase (SOD) activity

The capability of SOD inhibits epinephrine autooxidation to adenochrome and brown color of adenochrome is observed by spectrophotometer at 480 nm is the principle of this determination[23]. One hundred microliter of plasma or blank sample was mixed with 2 800 μL of sodium carbonate and 100 μL of epinephrine. Blank sample solution was created from aquadest. After the epinephrine solution was added, the absorbance of test solutions were measured at 480 nm, 30 °C. Inhibition (%) was determined by: $\frac{(\delta_{\text{absorbance of blank sample each minutes}} - \delta_{\text{abosrbance of plasma each minutes}})}{\delta_{\text{absorbance of blank sampleeach minutes}}} \times 100$.

One unit/mL of SOD was determined by formulation:

$$\frac{(\text{Inhibition}(\%) \times \text{dilution factor}}{(0.5 \times 0.1)}$$

2.10. Determination of catalase activity

The catalase activity was determined based on the disappearance of added hydrogen peroxide in the presence of enzyme source[24]. One milliliter plasma was added 2 mL of chromogen (5% potassium bichromate) and mixture solution was boiled in boiling water for 10 minutes. After cooling, the absorbance of solution was checked at 570 nm. The intensity of the absorbance was linearly related to the H_2O_2 concentration. The catalase activity was stated as μmol H_2O_2/min/mL.

2.11. Determination of glutathione peroxidase (GPx)

In this assay, GPx catalyzes the reduction of H_2O_2 to water thus oxidizing glutathione (GSH). The oxidized glutathione is then reduced by glutathione reductase to GSH and nicotinamide adenine dinucleotide phosphate to $NADP^+$. The rate of decreased in absorbance is directly proportional to GPx concentration and is measured at 340 nm[25]. In the procedure, two hundreds microliter of plasma, 200 μL of 0.1 mol/L of phosphate buffer pH 7.0, 200 μL of 10 mmol/L of glutathione and 200 μL of 2.4 IU of glutathione reductase were mixed. Mixture solutions were incubated for 10 min, at 37 ˚C. After that, the solutions were added 200 μL of 1.5 mmol/L of nicotinamide adenine dinucleotide phosphate and then the solutions were incubated again at the same temperature for 3 min. Finally, twenty microliter of 1.5 mmol/L of hydrogen peroxide were added into the mixture solutions and absorbance of solutions was observed at 340 nm. Glutathione peroxidase activity was expressed as mIU GSH-Px/mL/min.

2.12. Data and statistical analysis

Data are expressed as mean ± SD. Statistical analyses were performed using SPSS version 16.0 software. Analyses of the data were performed by One-way variance test and differences among treatments were analyzed by Duncan test. Values were considered to differ significantly if the *P* value was less than 0.05

3. Results

The acute toxicity study of the extract of *S. polycystum* on the mice was considered safe and no mortality was recorded. Normal control rats gained weight significantly throughout the study period. A significant decrease in body weight was observed in the diabetic control rats when compared to the normal rats ($P \leqslant 0.001$, Figure 1). The body weight reduction of diabetic rats was significantly attenuated following treatment with extract of *S. polycystum* in a dose dependent manner. The administration of the extract to the normal treated rats showed a steady increase in the body weight of the rats comparable to the normal control.

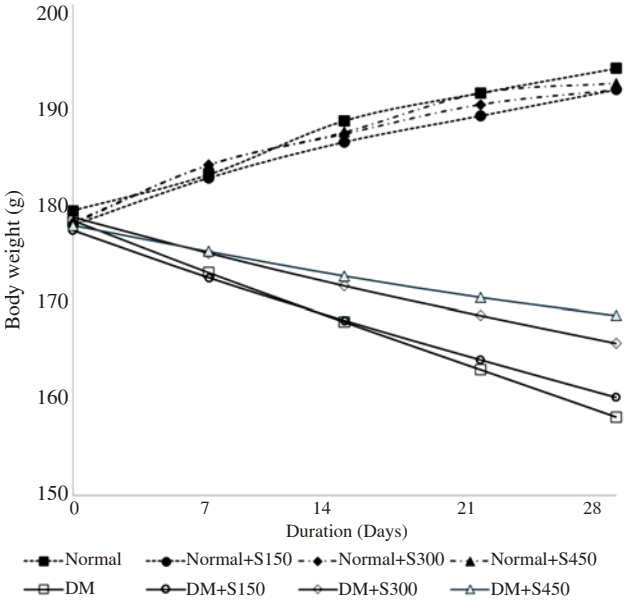

Figure 1. Body weight of normal and diabetic rats treated with *S. polycystum* extracts during the experiment.

Table 1
Blood glucose, MDA, SOD, catalase and GPx of normal and diabetic rats treated by *S. polycystum* extracts.

Treatments (Dose mg/kg)	Blood glucose (mg/dL)	MDA (nmol/mL)	SOD (IU/mL)	Catalase (μmol H_2O_2/min/mL)	GPx (mIU/min/mL)
Normal	110.67 ± 3.61^a	0.28 ± 0.003^a	38.94 ± 0.03^d	18.55 ± 0.11^e	2.63 ± 0.07^e
Normal + 150	109.00 ± 0.89^a	0.27 ± 0.001^a	39.07 ± 0.45^d	18.72 ± 0.06^f	2.69 ± 0.03^{ef}
Normal + 300	108.33 ± 1.37^a	0.26 ± 0.002^a	39.15 ± 0.12^d	18.92 ± 0.07^g	2.75 ± 0.03^f
Normal + 450	105.33 ± 1.37^a	0.26 ± 0.001^a	39.21 ± 0.04^d	18.92 ± 0.09^g	2.82 ± 0.05^g
Diabetic	435.67 ± 7.28^e	4.21 ± 0.010^e	15.99 ± 0.81^a	6.84 ± 0.01^a	0.88 ± 0.09^a
Diabetic + 150	425.00 ± 4.47^d	3.69 ± 0.005^d	16.42 ± 0.31^a	9.64 ± 0.16^b	1.12 ± 0.09^b
Diabetic + 300	409.67 ± 7.61^c	3.25 ± 0.134^c	23.09 ± 0.28^b	11.99 ± 0.15^c	1.54 ± 0.09^c
Diabetic + 450	330.67 ± 5.39^b	2.45 ± 0.086^b	27.22 ± 0.90^c	16.07 ± 0.14^d	1.94 ± 0.05^d

Values are expressed as mean ± SD, $n = 6$.

The levels of blood glucose and MDA were notably increased ($P \leqslant 0.001$) in the diabetic control compared to the normal control (Table 1). The extract of *S. polycystum* when administered to the diabetic rats significantly ($P \leqslant 0.05$) reduced the blood glucose and MDA levels. The effect was more pronounced in the diabetic group that received 450 mg/kg of the extract. The extract had little or no effect on the blood glucose and MDA of the normal treated group.

The activities of the enzymes, *i.e.* SOD, catalase and GPx in the normal or diabetic rats are presented in Table 1. These enzymes showed significant ($P \leqslant 0.001$) decrease in the diabetic control as compared to the normal control. Treatment with the extract of *S. polycystum* elevated the activities of these enzymes in the diabetic control in a dose dependent. There was no notable change in the activities of these enzymes in the normal group after treatment with the extract.

4. Discussion

The significant loss in body weight in the diabetic control could be due to accelerated gluconeogenesis of muscle and adipose tissue[26]. The weight gain in the diabetic treated group indicates improving of metabolism disturbance in the diabetic body. The previous study have been reported that polyphenol in the *Sargassum* extract contribute to the increasing of body weight in diabetic by insulin mimetic properties[11]. Polyphenol was able to increase the glucose uptake to muscle or adipose tissue by adenosine monophosphate kinase pathways activation. This activation caused increasing the translocation glucose transporter to cell membrane and then this transporter accessed glucose into the cell[27,28].

In diabetes mellitus, oxidative stress is mainly caused by over production free radical and reducing antioxidant enzymes capacity. Rats is induced to diabetic with streptozotocin injection, may exhibit oxidative stress[3,6]. There is decreasing of oxidative stress caused by free radicals in diabetes mellitus was corrected by antioxidant agent administration[8]. To elucidate the contribution of antioxidant agent to oxidative stress improving in diabetes mellitus, we studied the effect of *S. polycystum* extract on the level of lipid peroxidation product, MDA which results from attacking of free radical to membrane components of the cells[5]. The mean level of MDA increased significantly ($P \leqslant 0.05$) in the diabetic control compared to the normal group indicating increased oxidative stress in the diabetic rats. The significant decreased in the level of MDA to normal range in a dose dependent manner after treatment with the extract of *S. polycystum* suggests amelioration of the oxidative stress by the extract. This result coincides with the previous study of[4] who reported that the methanol extract of the *Sargassum echinocarpum* decreased the MDA level in the rat blood. It was caused by polyphenol in the extract capable to scavenge free radical and prevent the production of species oxygen reactive by proton donor or hydrogen abstraction[12].

In diabetes, high level of glucose reaching the mitochondria leads to an overdrive of the electron transport chain resulting in overproduction of superoxide free radicals (O_2-) usually scavenged by SOD. This enzyme converted anion superoxide to hydrogen peroxide and water. The failure of SOD to scavenge this free radical results in oxidative stress[2-4]. In this study, SOD activity was significantly reduced in the diabetic control compared to the non-diabetic control and may be attributed to hyperglycemia inactivating the enzyme by glycation[1,14]. The glycated SOD caused reduction activity and mRNA expression of this enzyme by the its DNA destruction[29]. The methanol extract of *S. polycystum* increased the activity of SOD dose dependently thus attenuating oxidative damage probably by preventing the potential glycation of the enzyme and an ultimate decrease in the enzyme activity. The increasing of SOD activity in diabetic rat treated may due to polyphenol in the extract induced SOD expression by activation of transcription factor AP-2, gene expression and mRNA of SOD[13], meanwhile, another theory stated that it caused by free radical scavenging and metal chelating activity of polyphenol[14].

Catalase is a hemeprotein found in all organs of living organisms and catalyzes decomposition of H_2O_2, a powerful and potentially harmful oxidizing agent to water and oxygen[30]. The significant reduction ($P \leqslant 0.05$) in catalase activities in the diabetic control compared to either the normal control or diabetic treated. It may be due to excessive generation of superoxide anion (O_2-) in diabetic state leading to inactivation of this enzyme as O_2- has been shown to reduce catalase activity[31]. The administration of the extract of *S. polycystum* to the diabetic and normal group increased catalase activity. This finding may suggest that the extract reduced the potential glycation of enzymes or they may reduce reactive oxygen free radicals and improve the activities of antioxidant enzymes. The previous study have been reported that polyphenol of brown seaweed could to reduce hydrogen peroxide level by induce increasing concentration and activity of catalase[4].

Glutathione peroxidase is found in cytoplasmic and mitochondrial fractions of cells and plays a primary role in minimizing oxidative damage[30]. It catalyzes the reduction of hydrogen peroxide and hydroperoxides formed from fatty acids to water thereby effectively removing toxic peroxides from living cells thus inhibiting the formation of free radicals. Oxidative damage to haemoglobin and cell membrane has been reported to reduce the activity of GPx[4,30]. Similarly in this study, there was reduction in the activity of this enzyme. The significant reduction ($P \leqslant 0.05$) in the GPx activities in the diabetic control compared to either the normal control or diabetic treated may be due to glycation of the enzyme and inactivation of the enzymes by reactive oxygen species, H_2O_2[32]. The earlier study has been shown that the administration extract of *Sargassum echinocarpum* to diabetic rats increased the GPx activity and its regarding antioxidant properties and inducing GPx expression by polyphenol in the brown seaweed extract[4].

Extract of *S. polycystum* able to prevent the increasing of free radical and preserve the antioxidant enzymes activity on diabetic rats and it provides basis for the utilization of *S. polycystum* extracts as candidate diabetic antioxidant agent.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This research was financially supported via Hibah Bersaing Program (No.: 0636/023-04.2.16/15/2012) by Directorate of Higher Education, Ministry of Education and Culture, Republic of Indonesia.

References

[1] American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2011; **34**(Suppl 1): S62-9.

[2] Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes* 1999; **48**: 1-9.

[3] Wiensperger NF. Oxidative stress: the special case of diabetes. *Biofactors* 2003; **19**: 11-8

[4] 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 Indonesia* 2010; **19**: 32-5.

[5] Owolabi MA, Jaja SI, Olatunji OJ, Oyekanmi OO, Adepoju S. Attenuation of oxidative damage in alloxan induced diabetic rats following administration of the extract of the leaves of *Vernonia amygdalina*. *Free Rad Antioxid* 2011; **1**: 94-101.

[6] Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 2007; **39**: 44-84.

[7] Araki E, Nishikawa T. Oxidative stress: a cause and therapeutic target of diabetic complications. *J Diabetes Investig* 2010; **1**: 90-6

[8] Nishikawa T, Araki E. Mechanism-based antioxidant therapies promise to prevent diabetic complications? *J Diabetes Investig* 2013; **4**: 105-7.

[9] Iwai K. Antidiabetic and antioxidant effects of polyphenol in brown alga *Ecklonia stolonifera* in genetically diabetic KK-A^y mice. *Plant Food Hum Nutr* 2008; **63**: 163-9.

[10] Lee SH, Park MH, Heo SJ, Kang SM, Ko SC, Han JS, et al. Dieckol isolated from *Ecklonia cava* inhibits alpha-glucosidase and alpha-amylase *in vitro* and alleviates postprandial hyperglycemia in streptozotocin-induced diabetic mice. *Food Chem Toxicol* 2010; **48**: 2633-7.

[11] Kim HK. *Ecklonia cava* inhibits glucose absorption and stimulates insulin secretion in streptozotocin-induced diabetic mice. *Evid Based Complement Alternat Med* 2012; **2012**: 439294.

[12] Nakai M, Kageyama N, Nakahara K, Miki W. Phlorotannins as radical scavengers from the extract of *Sargassum ringgoldianum*. *Mar Biotechnol (NY)* 2006; **8**: 409-14.

[13] Fernandez-Pachon MS, Berná G, Otaolaurruchi E, Troncoso AM, Martín F, García-Parrilla MC. Changes in antioxidant endogenous enzymes (activity and gene expression levels) after repeated red wine intake. *J Agric Food Chem* 2009; **57**: 6578-83.

[14] Rodrigo R, Miranda A, Vergara L. Modulation of endogenous antioxidant system by wine polyphenols in human disease. *Clin Chim Acta* 2011; **412**: 410-24.

[15] Shibata T, Ishimaru K, Kawaguchi S, Yoshikawa H, Hama Y. Antioxidant activities of phlorotannins isolated from Japanese Laminariaceae. *J Appl Phycol* 2008; **20**: 705-11.

[16] Chandini SK, Ganesan P, Bhaskar N. *In vitro* antioxidant activities of three selected brown seaweeds of India. *Food Chem* 2008; **107**: 707-13.

[17] Kim SH, Kim MS, Lee MS, Park YS, Lee HJ, Kang S, et al. Korean diet: characteristics and historical background. *J Ethnic Foods* 2016; **3**: 26-31.

[18] Lamela M, Anca J, Villar R, Otero J, Calleja JM. Hypoglycemic activity of several seaweed extracts. *J Ethnopharmacol* 1989; **27**: 35-43.

[19] Nakai M, Kageyama N, Nakahara K, Miki W. Phlorotannins as radical scavengers from the extract of *Sargassum ringgoldianum*. *Mar Biotech (NY)* 2006; **8**: 409-14.

[20] Noiraksar T, Ajisaka T. Taxonomy and distribution of *Sargassum* (Phaeophyceae) in the Gulf of Thailand. *J Appl Phycol* 2008; **20**: 963.

[21] Organisation of Economic Co-operation and Development. OECD guideline for testing of chemicals. Acute oral toxicity– up-and-down procedure No. 425. Paris: Organisation of Economic Co-operation and Development; 2001. [Online] Available from: http://www.oecd.org/chemicalsafety/risk-assessment/1948378.pdf [Accessed on 20th March, 2016]

[22] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; **95**: 351-8.

[23] Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem* 1972; **247**: 3170-5.

[24] Sinha AK. Colorimetric assay of catalase. *Anal Biochem* 1972; **47**: 389-94.

[25] Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967; **70**: 158-69.

[26] Chandra A, Mahdi AA, Ahmad S, Singh RK. Indian herbs result in hypoglycemic responses in streptozotocin-induced diabetic rats. *Nutr Res* 2007; **27**: 161-8.

[27] Quideau S, Deffieux D, Douat-Casassus C, Pouysegu L. Plant polyphenols: chemical properties, biological activities, and synthesis. *Angew Chem Int Ed Engl* 2011; **50**: 586-621.

[28] Zhang BB, Zhou G, Li C. AMPK: an emerging drug target for diabetes and the metabolic syndrome. *Cell Metab* 2009; **9**: 407-16.

[29] Flekac M, Skrha J, Hilgertova J, Lacinova Z, Jarolimkova M. Gene polymorphisms of superoxide dismutases and catalase in diabetes mellitus. *BMC Med Genet* 2008; **9**: 30.

[30] Jakus V. The role of free radicals, oxidative stress and antioxidant systems in diabetic vascular disease. *Bratisl Lek Listy* 2000; **101**: 541-51.

[31] Karasu C. Increased activity of H_2O_2 in aorta isolated from chronically streptozotocin-diabetic rats: effects of antioxidant enzymes and enzyme inhibitors. *Free Radic Biol Med* 1999; **27**: 16-27.

[32] Racz O, Nistiar F, Sipulova A, Riemerova M. Glutathione peroxidase in blood of diabetic and nondiabetic BB rats. *Arch Gerontol Geriatr* 1994; **19**: 207-16.