Journal of Aquatic Biology and Fisheries Vol. 2/2014/ pp. 133 to 140

ANTIDIABETIC AND ANTIOXIDANT ACTIVITY OF *PADINA TETRASTROMATICA* IN HIGH CALORIE FED/STREPTOZOTOCIN TREATED RATS

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Received on: 10 October 2013, accepted on: 12 December 2013

Abstract: Type 2 diabetes mellitus is a complex, heterogeneous, and polygenic disease and the major sources of morbidity and mortality in diabetes mellitus can be attributed to the direct and indirect effects of chronic hyperglycemia on human vasculature. Chronic hyperglycemia condition produces multiple biochemical sequels and diabetes induced oxidative stress could play an important role in the onset and progression of the disease. The present study was designed to simulate the natural history and metabolic characteristics of human T2DM in male Wistar rats and its treatment with 80 % aqueous methanolic extract of *Padina tetrastromatica* (PME). The rats, except the controls were fed with high calorie/energy diet for two months and then intraperitoneally injected with streptozotocin (STZ) at a dose of 15 mg/kg body weight. Effect of oral administration of graded doses of PME viz, 150, 300, 450 and 600 mg/kg body weight for 45 days on body weight, fasting blood glucose, glycated haemoglobin, serum toxicity markers, hepatic and renal antioxidant enzymes and concentration of lipid peroxidation products in serum and tissues were evaluated using standard protocols. Treatment with the extract significantly decreased the blood glucose and glycated haemoglobin in a dose dependent manner. However a more significant effect was produced by treatment with 450 mg/kg, suggesting that it is the optimum dose for acquiring good glycemic control. Activities of various toxicity markers demonstrated the non toxic nature of the extract at these doses. PME restored the diabetes induced alterations in the activities of antioxidant enzymes as well as the levels of lipid peroxidation products. The present findings clearly demonstrated the *invivo* antidiabetic and antioxidant activities of PME. Since no work is available in this regard, further clinical studies are warranted to recommend *P. tetrastromatica* as a source of drug for the management of type II diabetes mellitus.

Key words: Type 2 diabetes mellitus, Insulin resistance, Oxidative stress, Antioxidant defence system, Lipid peroxidation, Edible brown seaweed

INTRODUCTION

Type 2 Diabetes mellitus accounts for 90 to 95% of all the diagnosed cases, affecting 10 to 20% of adults in many developed countries (Engelgau *et al*., 2004; Bell and Polonsky, 2001). T2DM results from a complex interaction of multiple factors, namely genetic and environmental components, such as high-energy diets and sedentary lifestyle (Conget, 2002). Diabetic abnormalities include post prandial glucose production by the liver (in an unregulated fashion), diminished glucose uptake by the skeletal muscle and liver, increased fatty acid generation (lipolysis) by the adipose tissue and increased levels of digestive enzymes and absorptive cells in the intestine (Bell and Polonsky, 2001). The risk to develop macro and microvascular complications in T2DM is very high, since in addition to hyperglycaemia, there is an abnormal lipid profile, (Smith, 2007; Boden and Pearson, 2000). Type 2 diabetes is characterised by resistance to the action of insulin (in liver, muscle and adipose tissue) that results in an impairment of glucose uptake, leading to insulin hyperstimulation and subsequently to a dysfunction of pancreatic β-cells (decreasing secretion of insulin) (Yki, 1995; Cheng and Fantus, 2005).

When high plasma glucose concentration persists for a long time, the physiological pathways of glucose (glycolysis and oxidative phosphorylation) saturate and glucose is shunted to other pathways such as polyol pathway, diacylglycerol pathway and intracellular advanced glycation end-products (AGE) pathway (Nishikawa *et al*., 2007; Robertson and Harmon, 2006). All of these pathways lead to the increase in the production of reactive species (reactive oxygen species – ROS and reactive nitrogen species – RNS) which

in turn increase oxidative stress and oxidative damage in proteins, lipids and DNA of the cells, particularly damaging pancreatic β-cells and endothelial cells (Robertson and Harmon, 2006; Jay *et al*., 2006). The control of postprandial hyperglycemia and inhibition of oxidative stress have often been suggested as important measures for the treatment of diabetes.

One therapeutic approach to decrease postprandial hyperglycemia is to retard the absorption of glucose via inhibition of carbohydrate-hydrolyzing enzymes, such as α-amylase and α-glucosidase, in the digestive organs (Yki 1990; Holman *et al*., 1999; Tewari *et al*., 2003). The negative effects of oxidative stress may be mitigated by antioxidants (Larson, 1995; Halliwell *et al*., 1995). Due to concerns on the toxic and carcinogenic effects of synthetic chemicals, the search for alternatives from natural sources has received much interest. Traditionally, due to ease of access terrestrial sources have provided humans with these much-needed cures. However, the oceans cover 70% of the earth's surface and are the habitat of an extremely rich and varied fauna and flora. Marine algae are exposed to an extreme environment in a combination of light and oxygen, but the absence of oxidative damage in their structural components (Matsukawa *et al*., 1997) and their stability to oxidation during storage (Ramarathnam *et al*., 1995) suggest they have good antioxidant defence system.

Padina tetrastromatica Hauck is a striped, yellowish brown, fan shaped alga, which grows in the coastal rocks of tropical areas. It is used as seasoning in dried flake form and as table salt replacement for high blood pressure patients (Novaczek and Athy, 2001). Studies from our laboratory reported that sulphated fucan from *P.tetrastromatica* possesses anti-oxidative and anti-inflammatory activities against carrageenan induced paw edema in rats (Mohsin *et al*., 2011). However, to the best of our knowledge, no reports are available regarding the antidiabetic and antioxidant activities of 80 % aqueous methanolic extract of *P.tetrastromatica* (PME). Moreover we found that PME possessed disaccharidase inhibitory as well as antioxidant activities *invitro* (unpublished data). So the present study was designed to evaluate the *invivo* antidiabetic and antioxidant activities of PME in experimental diabetes mellitus.

MATERIALS AND METHODS

Seaweed material: *Padina tetrastromatica* was collected freshly from the coastal rocks of Vizhinjam, Thiruvananthapuram, India (Lat. 8° 22' N; Long. 76°59' E) during the month of January-February. Immediately after collection, the algae were thoroughly washed in tap water to remove the epiphytes, sand and other extraneous matter. The specimen was identified by Dr. M.V.N Panikkar, (Department of Botany, S.N.College, Kollam, Kerala, India). A voucher specimen (KUBH 5804) was deposited in the Department of Botany, University of Kerala.

Chemicals: All the chemicals used were analytical grade reagents purchased from Sisco Research Laboratories Ltd, India.

Preparation of seaweed extracts: The extract was prepared according to the procedure of Kim *et al*. (2008). Briefly, the algal sample (1 kg) was soaked in MeOH: H_2O (8:2, v/v, 1l) and extracted under conditions where the solvent was heated until reflux began, this being maintained for 3 h. Each resulting residue was dissolved in MeOH: H_2O (8:2, v/v, 500 ml) and extracted twice as above, with the extracts individually filtered using Whatman No. 1 paper. Each combined supernatant was then concentrated under reduced pressure at a temperature of 40°C, defatted with hexane and again concentrated. The extract thus obtained was kept at -20°C and used for present study.

Experimental animals: Male Wistar rats from the same breed with a mean body weight of about 100 g were selected for the study. The animals were bred and kept in the animal house, Department of Biochemistry, University of Kerala. They were housed in polypropylene cages under standard environmental conditions (25±2°C; 12/12h light/ dark cycle). Prior to the beginning of the experiment, all rats were fed with basal diet at least for one week. Body weights of rats were recorded once in every two weeks. All studies were conducted after obtaining prior approval from the institutional ethics committee [IEAC-KU-22/ 2010-11 BC GMK (10)].

Experimental design: Rats were divided into six groups with six animals each. Normal control was fed with the standard laboratory diet throughout the experimental period. All other groups were fed with high calorie/ energy diet (HCD) prepared with a slight modification of Wang *et al*. (2007), by adding 20% sucrose (w/w) and 10% groundnut oil (w/ w) into the basal diet in order to develop insulin resistance. After two months of HCD feeding, streptozotocin (STZ) at a dose of 15 mg/kg (Zhang *et al*., 2003) in 0.1 M citrate buffer (pH4.6) was injected intraperitoneally to induce pancreatic β-cell damage. The diabetic rats were treated with graded doses of PME viz, 150, 300, 450 and 600 mg/kg body weight for 45 days. The groupings are as follows:

Group I	: Normal control
Group II	: HCD + STZ (Diabetic control)
Group III	: HCD + STZ + PME (150mg/kg body weight)
Group IV	: HCD + STZ + PME (300mg/kg body weight)
Group V	: HCD + STZ + PME (450mg/kg body weight)
Group VI	: HCD + STZ + PME (600mg/kg body weight)

At the end of the experimental period the rats were fasted overnight and sacrificed. Blood and tissues were removed to ice cold containers for the estimation of various biochemical parameters.

Biochemical estimations: Blood glucose was determined by glucometer (One Touch Horizon). Glycated hemoglobin (HbA_{1C}) was estimated according to the procedure of Trivelli *et al.* (1971) using kit purchased from Beacon Diagonostics Pvt. Ltd. India. Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) were assayed using commercial kits purchased from Agappe diagonostics, India according IFCC recommended procedure (Bergmeyer *et al*., 1976). Serum alkaline phosphatase (ALP) was estimated by DGKC-SCE procedure using the kit from Agappe diagonostics, India. Serum gamma glutamyl transferase (GGT) was assayed according to the procedure of Szasz, (1976) using commercial kits purchased from Reckon Diagnostics Pvt. Ltd. India. Serum urea was determined by enzymatic method using urease according to the procedure of Webster, (1977) using the kit from Accurex Biomedical Pvt. Ltd., India. Serum creatinine was determined by modified Jeffe's method using the kit from Agappe diagonostics, India (Allen *et al*., 1982). The activities of superoxide dismutase (SOD) and catalase (CAT) in the liver and kidney were assayed as described by Kakkar et al. (1984) and Maehly and Chance, (1954) respectively. The activity of Glutathione peroxidise (GPx) was determined by the method of Lawrence and Burk, (1976). Glutathione reductase (GRd) activity was determined by the method David and Richard, (1983). Thiobarbituric Acid Reactive Substances (TBARS) were estimated according to the procedures of Ohkawa *et al*. (1979).

Statistical Analysis: The SPSS 17 statistical programme was employed for analysis, and the results were evaluated using one way analysis of variance (ANOVA). The results were presented as mean value ± SD. Difference among the mean were assessed using Duncan's Multiple range test. $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

An ideal experimental model for human T2DM was developed following the procedures of Wang *et al*. (2007) and Zhang *et al*. (2003). In this model system, insulin resistance was created by feeding HCD for two months and the pancreatic β-cells were damaged by the intraperitoneal injection of streptozot.

Changes in body weight: In the present study we found that the high-energy feeding for two months increased the body weight of animals (Table 1). But after the intra peritoneal injection of STZ, the body weight decreased significantly. This was similar to the observations of Zhang *et al.* (2003). Diabetic rats showed marked reduction in their body weights when compared to normal rats, which could be due to poor glycemic control. The excessive catabolism of proteins to provide aminoacids for gluconeogenesis during insulin deficiency resulted in muscle wasting and weight loss in diabetic untreated rats (Babu *et al*., 2010). Treatment with graded doses of PME significantly increased the body weight of rats.

Table 1. Changes in body weight

Groups	Body weight (g)		
	Initial	After 2 months	Final
I	102.13±9.80[a]	202.41±19.42[a]	222.95±21.39[a]
II	104.08±9.98[a]	245.04±23.50[b]	193.71±18.58[b]
III	105.05±10.07[a]	247.65±23.76[b]	218.40±20.95[a,b]
IV	102.86±9.87[a]	254.47±24.41[b]	225.87±21.67[a]
V	104.81±10.05[a]	247.97±23.79[b]	240.82±23.10[a]
VI	104.56±10.03[a]	245.70±23.57[b]	245.70±23.57[a]

Values expressed as mean ± SD of 6 rats. Similar alphabets in the same column indicate no significant difference between groups.

The ability of the extract to restore body weight seems to be a result of its ability to reduce hyperglycemia.

Fasting blood glucose and glycated haemoglobin: Two months of high energy feeding resulted fasting blood glucose around 150mg/dL and upon STZ injection, the values shoot up to 300-400mg/dL. This may be due to the concerted action of insulin resistance and pancreatic β-cell damage. It was observed that treatment with graded doses of PME for 45 days reduced the fasting glucose to normal level (Table 2). The percentage of glycated haemoglobin also showed a similar pattern (Table 2). There was no statistically significant difference in the fasting blood glucose and glycated haemoglobin between the groups treated with 450mg/kg and 600 mg/kg PME, suggesting that 450mg/kg is the optimum dose for acquiring good glycemic control. The antihyperglycemic effect of PME can be attributed to the presence of phenolic compounds present in the extract. The phenolic compounds stimulates insulin secretion by the closure of K^+-ATP channels, membrane depolarization and stimulation of Ca^{2+} influx, an initial key step in insulin secretion from the remanent β-cells or from regenerated β-cells (Sunil *et al.*, 2012).

Table 2. Fasting blood glucose and glycated haemoglobin

Groups	Blood glucose (mg/dL)	Hb A_{1c} (%)
I	86.44±8.27[a]	4.450±0.22[a]
II	408.03±39.15[b]	10.46±0.52[b]
III	160.87±15.43[c]	7.710±0.38[c]
IV	133.93±12.22[d]	6.480±0.32[d]
V	114.73±11.01[d,e]	5.517±0.27[e]
VI	106.59±10.22[a,e]	5.450±0.27[e]

Values expressed as mean ± SD of 6 rats. Similar alphabets in the same column indicate no significant difference between groups.

Serum toxicity markers: The activities of hepatic toxicity markers like GOT, GPT, ALP and GGT were increased in serum of diabetic groups compared to control group (Table 3). The activities decreased significantly in PME treated groups when compared to diabetic group in a dose dependent manner. The concentration of renal toxicity markers urea and creatinine were increased in serum of diabetic groups as compared to control group (Table 4). Their concentration decreased significantly in PME treated groups when compared to diabetic group in a dose dependent manner.

Liver is the vital organ of metabolism, detoxification, storage and excretion of xenobiotics and their metabolites (Rej, 1978). Serum GOT, GPT, ALP and GGT are reliable markers of liver function. In STZ induced diabetic rats the liver was necrotized. An increase in the activities of these toxicity markers in serum might be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (Navarro *et al.*, 1993) which gives an indication of the hepatotoxic effect of STZ. Treatment of the diabetic rats with graded doses of PME reduced the

Table 3. Activity of hepatic toxicity markers

Groups	SGOT(U/L)	SGPT(U/L)	ALP(U/L)	GGT(U/L)
I	62.94±6.04[a]	38.77±3.72[a]	37.65±3.61[a]	1.50±0.15[a]
II	173.78±16.67[b]	67.20±6.44[b]	71.24±6.83[b]	5.60±0.53[b]
III	130.16±12.49[c]	50.19±4.81[c]	60.35±5.79[c]	4.17±0.40[c]
IV	96.97±9.30[d]	47.85±4.59[c,d]	53.32±5.11[d]	3.30±0.32[d]
V	81.24±7.79[e]	43.61±4.18[a,d]	47.08±4.52[e]	2.89±0.28[e]
VI	73.16±7.02[a,e]	41.05±3.94[a]	40.66±3.90[a]	2.25±0.21[f]

Values expressed as mean ± SD of 6 rats. Similar alphabets in the same column indicate no significant difference between groups

Table 4. Concentration of renal toxicity markers

Groups	Serum urea (mg %)	Serum creatinine (mg/dL)
I	21.25±2.04[a]	0.50±0.05[a]
II	42.12±4.04[b]	1.40±0.13[b]
III	33.26±4.05[c]	1.10±0.10[c]
IV	28.77±2.76[d]	0.90±0.08[d]
V	26.91±2.58[d,e]	0.80±0.07[d]
VI	24.35±2.33[a,e]	0.64±0.06[e]

Values expressed as mean ± SD of 6 rats. Similar alphabets in the same column indicate no significant difference between groups.

activity of these enzymes in serum when compared to the diabetic untreated group and consequently alleviated liver damage caused by STZ-induced diabetes. Diabetic rats showed significantly increased levels of urea and creatinine in the serum, which are considered as significant markers of renal dysfunction (Bethesda, 2001). In the present study, the significant reduction in the levels of serum urea and creatinine in the treated diabetic rats indicated that PME prevented the progression of renal damage in diabetic rats.

Activity of Antioxidant Enzymes

Table 5 and Table 6 show the activity of various antioxidant enzymes in liver and kidney respectively. Activity of SOD, CAT, GPx and GRd showed a similar trend with decreased activity in diabetic group. Treatment with PME significantly increased the activity of the scavenging enzymes in all groups in a dose dependent manner.

Hyperglycemia is a well-known cause for elevated free radical levels, followed by production of reactive oxygen species (ROS), which can lead to increased lipid peroxidation, alter antioxidant defense and further impair glucose metabolism in biological system (Balasubashini *et al.*, 2004). SOD has been postulated as the one of the most important enzymes in the enzymatic antioxidant defense system which catalyses the dismutation of superoxide radicals to produce H_2O_2 and molecular oxygen. Catalase is a hemoprotein which catalyses the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals. Previous studies have reported that the activity of SOD is low in diabetes mellitus (Coudary *et al.*, 1999). This is in agreement with our results of reduced activities of SOD and CAT in the liver and kidney of diabetic rats. Whereas, treatment with PME showed a significant increase in the hepatic renal SOD and CAT. GPx plays a primary role in minimizing oxidative damage. Glutathione peroxidase (GPx) is an enzyme which decomposes H_2O_2 or other organic hydroperoxides to non toxic products at the expense of reduced glutathione. Glutathione reductase is the major enzyme responsible for maintaining the glutathione level. Administration of PME significantly restored the activities of GPx and GRd in the liver and kidney of diabetic rats when compared to diabetic control. Thus PME improved the activities of all the antioxidant enzymes in diabetic rats which may be due to the free radical scavenging property of the extract.

Effect of PME on Lipid peroxidation: The level of lipid peroxidation products is measured as thio barbituric acid reactive substances (TBARS). The concentration of lipid peroxidation products in

Table 5. Activity of hepatic antioxidant enzymes

Groups	SOD[@]	CAT ($x10^{-3}$)[#]	GPx[$]	GRd[*]
I	22.96±2.20[a]	3.39±0.38[a]	66.04±6.34[a]	31.76±3.05[a]
II	7.10±0.68[b]	0.78±0.08[b]	17.48±1.67[b]	7.22±0.69[b]
III	10.18±0.98[c]	1.34±0.13[c]	25.45±2.44[c]	11.45±1.09[c]
IV	16.33±1.56[d]	3.66±0.35[a]	35.69±3.42[d]	13.57±1.30[c]
V	17.54±1.68[d]	3.74±0.34[a]	47.17±4.53[e]	19.37±1.86[d]
VI	21.32±2.04[a]	3.87±0.37[a]	54.27±5.14[f]	23.67±2.27[e]

Table 6. Activity of renal antioxidant enzymes

Groups	SOD[@]	CAT ($x10^{-3}$)[#]	GPx[$]	GRd[*]
I	66.17±6.35[a]	5.80±0.53[a]	47.23±4.53[a]	54.20±5.19[a]
II	21.06±2.02[b]	0.88±0.08[b]	8.46±0.81[b]	11.19±1.07[b]
III	34.78±3.33[c]	2.00±0.19[c]	22.13±2.10[c]	23.13±2.22[c]
IV	40.29±3.86[d]	2.72±0.26[d]	28.37±2.72[d]	29.65±2.82[d]
V	51.55±4.94[e]	3.99±0.38[e]	35.25±3.38[e]	39.94±3.83[e]
VI	59.13±5.67[f]	4.21±0.40[e]	43.64±4.18[a]	45.50±4.36[f]

Values expressed as mean ± SD of 6 rats. Similar alphabets in the same column indicate no significant difference between groups.

[@]: Units/mg protein. (Enzyme concentration required to inhibit the chromogen production (OD at 560) nm by 50% in 1 minute). [#]: Units /mg protein.[$, *]: Units/mg protein. (One unit - ìmol NADPH oxidised/min)

Table 7. Concentration of lipid peroxidation products

Groups	Level of TBARS			
	Serum[@]	Liver[#]	Kidney[#]	Pancreas[#]
I	2.95±0.27[a]	5.97±0.54[a]	5.02±0.46[a]	5.18±0.61[a]
II	16.42±1.49[b]	39.32±3.59[b]	58.28±5.31[b]	46.34±4.23[b]
III	12.81±1.17[c]	28.28±2.58[c]	42.53±4.43[c]	37.23±3.40[c]
IV	10.51±0.95[d]	18.36±1.67[d]	34.29±3.12[d]	33.95±3.25[d]
V	8.86±0.80[e]	19.41±1.77[d]	21.55±2.25[e]	21.74±1.43[e]
VI	5.58±0.50[f]	9.52±0.86[e]	10.69±0.97[f]	14.89±1.45[f]

Values expressed as mean ± SD of 6 rats. Similar alphabets in the same column indicate no significant difference between groups.[@]: mmol/100mL [#]: mmol/100g wt tissue

serum, liver, kidney and pancreas were increased in the diabetic control group. However there is a marked decrease in TBARS level in PME treated groups in a dose dependent manner (Table 7).

Lipid peroxidation of unsaturated fatty acids is a frequently used indicator of increased oxidative stress and subsequent oxidative damage (Hauggard, 1968). The high level of lipid peroxidation marker, TBARS, in the diabetic control rats is a reflection of insufficiency of antioxidant defenses in combating ROS-mediated damage. The present data also demonstrated that the treatment with PME reduced the concentration of TBARS in the liver, kidney, pancreas and serum of diabetic animals. This activity may be due to the free radical scavenging effect of PME.

CONCLUSIONS

From the present observations it was evident that PME possess significant anti hyperglycaemic as well as antioxidant effects in a dose dependent manner. However a more significant effect was produced by treatment with 450mg/kg. Activities of various toxicity markers demonstrate the non toxic nature of the extract at these doses. In short the effect of PME was dose dependent and the optimum dose which produced significant effect was 450mg/kg body weight. The molecular mechanisms behind the observed effect and the compound responsible for this require further investigations, which are in progress.

ACKNOWLEDGEMENTS

This research was supported by funding from the Kerala State Council for Science Technology and Environment (KSCSTE), Government of Kerala, India in the form of Junior Research Fellowship to Divya S. Mohan.

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