

Antioxidant and anti-inflammatory effects of *Padina pavonia* and *Turbenaria ornate* in streptozotocin/nicotinamide diabetic rats.

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Abstract: The present study was designed to evaluate the effect of two marine brown algae, *Padina pavonia* and *Turbenaria ornate*, on serum and adipose tissue tumor necrosis factor alpha (TNF- α) and oxidative stress in type 2 diabetic rats. Type 2 diabetes was induced by intraperitoneal injection of 120 mg/kg b.w. nicotinamide 30 minutes before injection of 50 mg/kg b. w. streptozotocin. Extracts of both *Padina pavonia* and *Turbenaria ornate* were orally and daily administered at a dose level of 100 mg/kg b.w. for 21 days to diabetic rats. In the diabetic control group, levels of glucose were significantly increased, while serum insulin level was decreased. Hepatic lipid peroxidation was significantly increased in diabetic rats as compared to normal ones. On the other hand, glutathione content and antioxidant activities were significantly decreased. Both tested algal extracts reversed these parameters nearly back to control values. In addition, both algae significantly down-regulated adipose tissue TNF- α mRNA expression in conjunction with decreased serum TNF- α . In conclusion, *Padina pavonia* and *Turbenaria ornate* extracts exert protection to type 2 diabetic rats through their antioxidant and anti-inflammatory efficacies.

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1. Introduction:

Diabetes mellitus is one of the common metabolic disorders diseases known to mankind, affecting at least 15 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders (Ogbonnia *et al.*, 2008). Abnormal regulation of glucose and impaired carbohydrate utilization that result from a defective or deficient insulin are the key pathogenic events in type 2 diabetes mellitus (T2DM) (Mahmoud *et al.*, 2012). The chronic hyperglycemic condition in diabetes is associated with long term damage, dysfunction, and failure of various organs, such as eyes, kidneys, nerves, heart, and blood vessels (Ramachandran *et al.*, 2011).

Tumor necrosis factor alpha (TNF- α) is a cytokine produced by a variety of immune cells including macrophages and lymphocytes (Antuna-Puente *et al.*, 2008) and is pleiotropic in nature (Vassiliou *et al.*, 2008). TNF- α is also thought to play a major role in the Metabolic Syndrome (MS), which is characterized by insulin resistance and inflammation (Yudkin, 2007). TNF- α can elicit an insulin-resistant state, characterized by an impaired ability of insulin to suppress hepatic glucose production and to stimulate peripheral glucose uptake (Lang *et al.*, 1992). Also, TNF- α is implicated to increase the circulating level of free fatty acids and thus indirectly contributes to the pathogenesis of insulin resistance (Ryden *et al.*, 2002).

The high cost, low availability, uncertainty of use during pregnancy and undesirable side effects of synthetic drugs such as undue weight gain, drug resistance and hypoglycaemia are several factors that leading to explore alternative medicine from natural product particularly of plant origin (Edem, 2009).

Over the past several decades, seaweeds have been reported to possess biological activity of potential medicinal value (Satoru *et al.*, 2003). Brown algae are rich sources of various bioactive compounds, including polyphenols, carotenoids and polysaccharides with different physiological effects on human health (Amsler and Fairhead, 2006). Reports regarding the antidiabetic effect and clinical significance of brown algae in diabetic rats are scanty in scientific literature. Therefore, the current study was designed to investigate the ameliorative potential of *Padina pavonia* and *Turbenaria ornata* extracts on proinflammatory cytokines and hyperglycemia-mediated oxidative stress in type 2 diabetic rats.

2. Materials and Methods:

Collection of algae and extract preparation:

The studied algae were collected from Red Sea, Egypt. The samples were washed three to remove the salt, sand, and epiphytes attached to the surface. Collected algae were air-dried and separately pulverized to a fine powder. The powdered materials were extracted by maceration in 80% aqueous ethanol until exhaustion at room temperature. After filtration,

the filtrate was concentrated under reduced pressure in a rotary evaporator and lyophilisation. The crude extracts were weighted and stored at -20 °C till used.

Chemicals:

Streptozotocin (STZ) and nicotinamide (NA) were purchased from Sigma Chemicals Co., St. Louis, MO, USA and all other chemicals were obtained from standard commercial supplies.

Experimental animals:

White male albino rats weighting about 130-150 g were used as experimental animals in the present investigation. The animals were housed in standard polypropylene cages (4 rats/cage) and maintained under controlled room temperature (22±2 °C) and humidity (55%±5%) with 12 h light and 12 h dark cycle and were fed a standard diet of known composition, and water *ad libitum*. The animals used in the present study were maintained in accordance with the principles and guidelines of the Canadian Council on Animal Care as outlined in the "Guide for the Care and Use of Laboratory Animals" (Canadian Council on Animal Care, 1993).

Induction of diabetes mellitus:

T2DM was experimentally induced in animals fasted for 16 hours by intraperitoneal injection of 120 mg/kg b.w. NA dissolved in NaCl solution (0.9%) 30 minutes before intraperitoneal injection of 50 mg/kg b. w. STZ dissolved in citrate buffer (pH 4.5) (Punitha *et al.*, 2005). Seven days after STZ injection, rats were screened for serum glucose levels. Overnight fasted animals were given glucose (3 g/kg b. wt.) by gastric intubation. After 2 h of oral administration, blood samples were taken from lateral tail vein, left to coagulate and centrifuged then serum glucose concentration was measured. Rats having serum glucose ≥ 200 mg/dl, after 2 h of glucose intake, were considered diabetic and selected for further subsequent studies.

Experimental design:

The experimental animals were divided into four groups, each group comprising six rats designated as follows:

Group 1: Normal rats

Group 2: Diabetic control rats

Group 3: Diabetic + *Padina pavonia* extract (100 mg/kg b.wt.) orally for 21 days

Group 4: Diabetic + *Turbenaria ornata* extract (100 mg/kg b.wt.) orally for 21 days.

Biochemical assays:

The level of serum glucose was estimated according to the method of Trinder (1969), using

commercial diagnostic kit (Spinreact, Spain). Serum insulin and TNF- α were determined using specific ELISA kit (R&D Systems, USA), according to the manufacturer instructions. Lipid peroxidation, reduced glutathione (GSH) content, and superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were measured in liver homogenate according to the methods of Preuss *et al.* (1998), Beutler *et al.* (1963), Marklund and Marklund (1974) and Kar and Mishra (1976), respectively.

RNA isolation and reverse transcriptase polymerase chain reaction (RT-PCR):

Total RNA was isolated from visceral adipose tissue using total RNA isolation kit (Fermentas, USA). First strand of cDNA was synthesized from 2 μ g of total RNA by using a high-capacity cDNA reverse transcription kit with RNase inhibitor (Applied Biosystems, USA). The produced cDNA was amplified using Green master mix (Fermentas, USA) using the following sets of primers:

Up 5'-AAATGGGCTCCCTCTCATCAGTTC-3' and Down 5'-TCTGCTTGGTGGTTTGCTACGAC-3' for TNF- α , and Up 5'-AAGTCCCTCACCTCCCAAAG-3' and Down 5'-AAGCAATGCTGTACCTTCCC-3' for β -actin. The reaction tubes were placed on a double heated led thermal cycler and the reaction series was performed as follows: initial denaturation at 95 °C for 2 min, then 40 cycles each was 95 °C (30 s), 59.5 °C (30 s), 72 °C (45 s).

PCR products were loaded in 1.5% agarose gel (Sigma) and electrical power was applied then the samples were left to migrate for suitable time. After migration, the cDNA bands were observed in the gel using UV transilluminator. Gel images were analyzed by scanning densitometry (Image J, NIH) and values were normalized to the quantity of β -actin, and presented as % mRNA relative to control.

Statistical analysis

Statistical analysis was performed using SPSS v.20. Results were articulated as mean \pm standard error (SE) and all statistical comparisons were made by means of one-way ANOVA test followed by Duncan's multiple range test post hoc analysis. A *P* value <0.05 was considered significant.

3. Results:

Figure 1 shows the effect of *Padina pavonia* and *Turbenaria ornata* extracts on the levels of both fasting and postprandial serum glucose in control and experimental rats. The diabetic control group of rats had a significantly (*P*<0.001) elevated serum fasting and postprandial glucose as compared with the normal control rats. Oral administration of both

extracts significantly improved the altered glucose levels. While the effect of *Padina pavonia* and *Turbenaria ornate* on fasting serum glucose was non-significantly different, *Padina pavonia* seemed to be more effective on decreasing postprandial blood glucose.

Serum insulin level exhibited an opposite pattern; it was significantly ($P<0.001$) decreased in STZ/NA diabetic rats as compared to normal ones and was significantly increased as a result of treatment with both *Padina pavonia* extract only. Treatment of diabetic rats with *Turbenaria ornate* produced a slight increase in serum insulin levels; however the produced increase was non-significant in comparison with diabetic control rats (Fig. 2).

Regarding TNF- α , diabetic rats exhibited a significant ($P<0.001$) increase in serum TNF- α level as compared with normal control rats. The

administration of both agents showed marked improvement of serum TNF- α concentration (Table 1). Similarly, adipose tissue TNF- α mRNA expression of diabetic control rats was significantly upregulated as compared to normal rats and significantly downregulated upon both algal extracts supplementation (Fig. 3), the extract of *Padina pavonia* seemed to be more effective.

The elevated level of lipid peroxides observed in liver of diabetic rats was potentially ($P<0.01$; LSD) improved by the treatment of diabetic groups of rats with both tested extracts. Conversely, hepatic GSH content and GPx and SOD activities were significantly declined in STZ/NA diabetic rats. Treatment of the diabetic rats with both algal extracts potentially increased ($P<0.01$; LSD) GSH as well as antioxidant enzymes activity (Table 2).

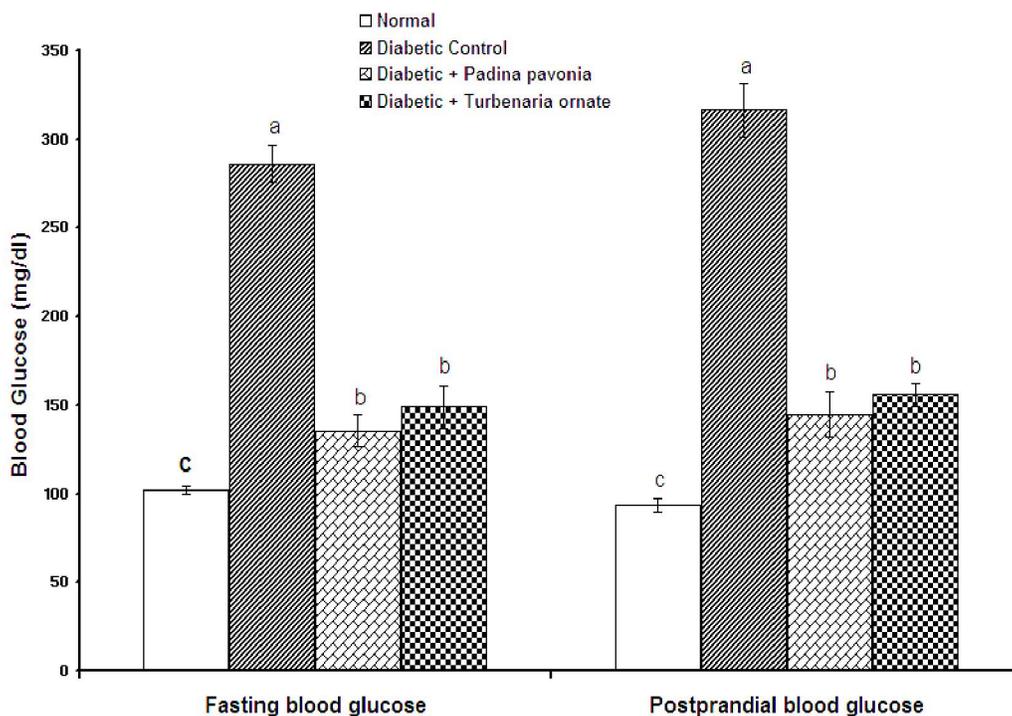


Fig. 1. Serum glucose of normal, diabetic control and diabetic treated rats.

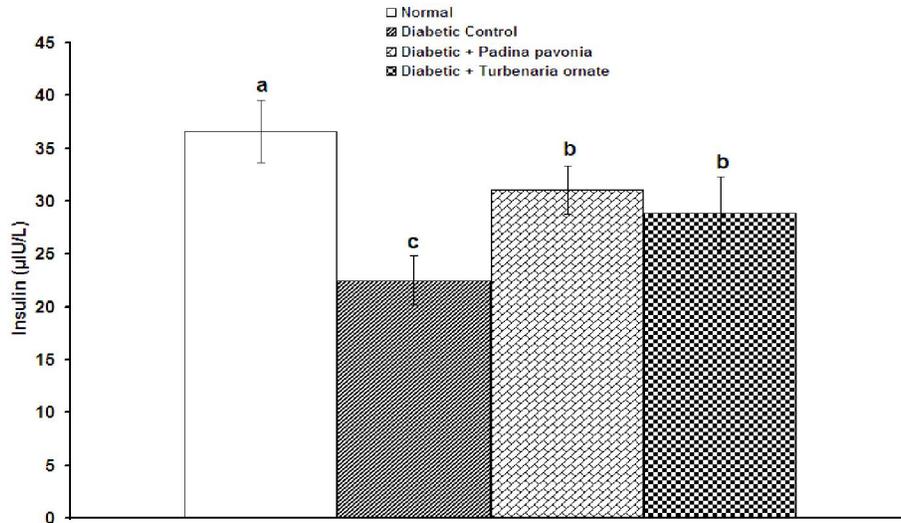


Fig. 2. Serum insulin of normal, diabetic control and diabetic treated rats.

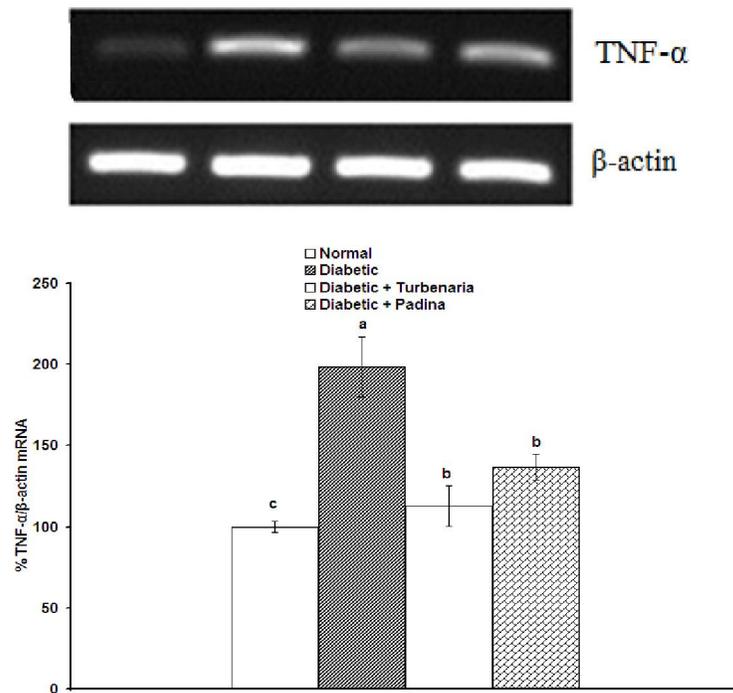


Fig. 3. RT-PCR analysis of adipose TNF-α and β-actin expression of normal, diabetic control and diabetic treated rats.

Table 1: Serum TNF-α of normal, diabetic and diabetic treated rats.

Group	Parameter	TNF-α (pg/ml)
Normal		63.31 ± 2.86 ^c
Diabetic control		144.98 ± 4.48 ^a
Diabetic + <i>Turbenaria ornate</i>		92.11 ± 3.21 ^b
Diabetic + <i>Padina pavonia</i>		101.56 ± 2.19 ^b
F- prob		<i>P</i> < 0.001

Data are expressed as Mean ± SE. Means which share different superscript symbol(s) are significantly different.

Table 2: Liver lipid peroxidation, glutathione and antioxidant enzyme activities of normal, diabetic and diabetic treated rats.

Group	Parameter	Lipid peroxidation (nmol MDA/100 mg tissue)	GSH (nmol/100 mg tissue)	GPx (U/g tissue)	SOD (U/100 mg tissue)
Normal		18.22 ± 1.43 ^d	63.77 ± 2.97 ^a	55.77 ± 3.02 ^a	40.15 ± 4.22 ^a
Diabetic control		51.33 ± 3.26 ^a	31.25 ± 2.62 ^d	24.36 ± 2.88 ^d	18.75 ± 3.41 ^c
Diabetic + <i>Turbenaria ornate</i>		29.15 ± 2.31 ^c	52.44 ± 1.87 ^b	35.07 ± 3.19 ^c	35.63 ± 6.37 ^b
Diabetic + <i>Padina pavonia</i>		33.18 ± 1.06 ^b	54.01 ± 3.29 ^b	46.12 ± 3.47 ^b	38.19 ± 3.99 ^a
F- prob		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.01$

Data are expressed as Mean ± SE. Means which share different superscript symbol(s) are significantly different

4. Discussion:

Type 2 diabetes mellitus is a complicated metabolic disease characterized by impairment of both insulin secretion and insulin sensitivity (Mahmoud *et al.*, 2013). Hyperglycemia plays an important role in development of T2DM and complications associated with the diseases such as micro-vascular and macro-vascular diseases (Baron, 1998). Therefore, the effective control of blood glucose level is the key for preventing or reversing diabetic complications and improving the quality of the life in diabetic patients (DeFronzo, 1999).

STZ/NA is a method currently used to induce diabetes in animals that resemble non obese T2DM in human (Tomonori *et al.*, 2006). The induction of diabetic rats with STZ increases the production of free radicals that damage the pancreatic DNA and thus affect insulin secretion (Oguri *et al.*, 2003). This is achieved by depleting NA which is a substrate of poly ADP ribose synthetase, an enzyme which involved in DNA repair. The pretreatment of NA into diabetic rats allows minor damage to pancreatic β -cell by inhibiting poly ADP ribose synthetase activity and prevents NAD depletion (LeDoux *et al.*, 1988). Data of the current study were consistent with Tahara *et al.* (2008) who revealed that administration of STZ/NA induces diabetes with moderate hyperglycaemia associated with loss of early-phase insulin. In addition, a study by Ahmed *et al.* (2010) demonstrated hyperglycemia and diminished serum insulin along with developing insulin resistance in STZ/NA type 2 diabetic rats. This form of diabetes is very common in people over 40 years of age (World Health Organization, 1999).

Moreover, data of the current study indicated that diabetic control rats exhibited significantly diminished serum insulin levels and treatment with both algal extracts potentially alleviated the diminished serum insulin. The possible mechanism by which both tested algal extracts brings about its hypoglycemic effect may be by increasing the insulin level from the protective effect of

pancreatic islets, to prevent the loss of β -cell mass, and stimulation of insulin secretion from the remaining β -cells.

An important finding of the present study was that both algal extracts attenuated production of the pro-inflammatory cytokine, TNF- α which is believed to play a significant role in the pathogenesis of DM. In addition, both tested agents significantly downregulated adipose tissue TNF- α mRNA expression. TNF- α affects intracellular insulin signaling in fat, skeletal muscle, endothelial cells, and other insulin-responsive tissues by inhibiting kinase activities in the insulin-signaling pathway (Rui *et al.*, 2001). TNF- α has been shown to increase plasma triglycerides and concentrations of very low density lipoproteins (Yudkin *et al.*, 1999), as well as lipolysis in mouse, rat, and human fat cells (Rosenstock *et al.*, 2001). TNF- α reduces insulin-stimulated receptor tyrosine kinase activity at low concentrations and can also decrease the expression of the insulin receptor IRS-1 and GLUT-4 at higher concentrations as well as increases the phosphorylation of serine 307 in IRS-1, thus impairing its ability to bind to the insulin receptor and initiate downstream signaling (Rui *et al.*, 2001). Hence, alleviation of the glycemic state following treatment of STZ/NA diabetic rats by the used algal extracts may be attributed to attenuating the production of TNF- α

Sustained hyperglycemia has been identified as a principle mediator of increased reactive oxygen species (ROS) generation in diabetes (Kumar *et al.*, 2008). Moreover, as a consequence of hyperglycemia, abnormally high levels of free radicals, decline of antioxidant defense systems (Maritim *et al.*, 2003) and lowered vitamin levels (West, 2000) have also been reported. Increased oxidative stress and impaired antioxidant defense mechanism are important factors in the pathogenesis and progression of DM and other oxidant-related diseases (Saravanan and Ponmurugan, 2011). The potentially antioxidative effects of brown algae could be confirmed in the present study. The administration

of both algal extracts to diabetic group of rats significantly reverted back the altered levels of malondialdehyde and the antioxidants, GSH, GPx and SOD, which in turn reveal their antioxidant potential.

The beneficial effects of *Padina pavonia* and *Turbenaria ornata* may be attributed to their rich contents of polyphenols, carotenoids and polysaccharides (Amsler and Fairhead, 2006), and they are an excellent source of vitamins such as A, B1, B12, C, D, E, riboflavin, niacin, pantothenic acid and folic acid as well as minerals such as Ca, P, Na and K (Dhargalkar and Pereira, 2005). In addition to potential role of polyphenols, Lewin (1974) have stated that, the lipids of algae comprise photosynthetic pigments-chlorophylls, carotenes and other compounds while carotenoids are powerful antioxidants. Moreover, Fucoxanthin is a major carotenoid of brown algae (Okuzumi *et al.*, 1993), has been found to improve alterations in lipid metabolism and insulin resistance induced by a high fat diet, at least in part, through reducing visceral fat mass, hyperinsulinemia, hepatic glucose production, and hepatic lipogenesis, and altering hepatic glucose-regulating enzymes activities as stated by Park *et al.* (2011).

In conclusion, biochemical findings of the present study indicate that the marine brown algae exert protection to STZ/NA diabetic rats against hyperglycemia-mediated oxidative stress. This could be due to the prevention or inhibition of lipid peroxidation and alleviating the antioxidant system in addition to their anti-inflammatory effect.

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