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Research Article

ANTI-DIABETIC AND INSULIN SENSITIZING EFFECTS OF *PADINA PAVONIA* AND *TURBENARIA ORNATA* IN STREPTOZOTOCIN/NICOTINAMIDE DIABETIC RATS

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ABSTRACT

Objective: The current study was designed to investigate the hypoglycemic, hypolipidemic and insulin sensitizing effects of two marine brown algae, *Padina pavonia* and *Turbenaria ornata*.

Materials and Methods: Type 2 diabetes was induced by intraperitoneal injection of 120 mg/kg body weight nicotinamide 30 minutes before injection of 50 mg/kg b.wt. streptozotocin. Extracts of both *P. pavonia* and *T. ornata* were orally and daily administered at a dose level of 100 mg/kg b.wt. for 21 days to diabetic rats. At the end of the experimental period, blood, pancreas, and adipose tissue samples were taken for the subsequent studies.

Results: Both *P. pavonia* and *T. ornata* supplementation potentially ameliorated the elevated levels of glucose, aspartate aminotransferase, lactate dehydrogenase and creatine kinase-MB and the declined serum insulin levels of Type 2 diabetic rats. Furthermore, the tested algae increased the β-cell number in pancreata of diabetic rats. Both extracts were also found to alleviate the altered lipid profile and serum adiponectin level, as well as the insulin resistance indices, homeostasis model of insulin resistance and quantitative insulin-sensitivity check index. In addition, both algae significantly upregulated adipose tissue adiponectin messenger ribonucleic acid expression.

Conclusion: These experimental findings demonstrated that both *P. pavonia* and *T. ornata* exhibit anti-diabetic effects in a rat model of Type 2 diabetes by their insulinotropic and insulin sensitizing effects.

Keywords: Adiponectin, Insulin resistance, Brown algae, Diabetes.

INTRODUCTION

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1]. It is estimated that there are 171 million people in the world with diabetes in year 2000 and this is likely to increase up to 366 million by 2030 [2]. 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) [3]. Hyperglycemia and hyperlipidemia, as the most common features of diabetes mellitus, contribute to the development of microvascular and macrovascular complications, which cause the morbidity and mortality of diabetes [4].

Dysfunction in adipocytes or adipose tissue is associated with insulin resistance and T2DM as reported by Blüher [5] and Mahmoud [6]. Adiponectin is an adipokine first described just over a decade ago. Produced almost exclusively by adipocytes, adiponectin circulates in high concentrations in human plasma. Research into this hormone has revealed it to have insulin-sensitizing, anti-inflammatory and cardioprotective roles [7]. Evidence suggests that adiponectin has anti-atherogenic properties by improving endothelial function and having anti-inflammatory effects in the vascular wall [8].

In recent times, we have demonstrated the anti-oxidant and anti-inflammatory properties of the brown seaweeds, *Padina pavonia* and *Turbenaria ornata* in diabetic rats [9]. In addition, our recent study revealed that *T. ornata* ethanolic extract protected rats against cyclophosphamide-induced hepatotoxicity by potentiating the anti-oxidant defense system and alleviating serum adiponectin levels [10]. To the best of our knowledge, no studies have recorded the effect of brown seaweeds on plasma and adipose tissue adiponectin expression in diabetic rats. Thus, the current study was designed to demonstrate the effect of *P. pavonia* and *T. ornata* extracts on glucose intolerance, adiponectin expression and some biochemical parameters in streptozotocin (STZ)/nicotinamide (NA) diabetic rats.

MATERIALS AND METHODS

Collection of algal samples

Algae were collected from the Red Sea, between Quseir and Marsa-Alam, Egypt. The samples were washed 3 times with sea water followed by tap water to remove the salt, sand, and epiphytes attached to the surface.

Extract preparation

Samples were air-dried in shade, powdered and extracted by maceration in 80% aqueous ethanol. After filtration, the filtrate was concentrated under reduced pressure in a rotary evaporator and the crude extracts were weighted and stored at −20°C till used.

Experimental animals

Twenty-four male albino rats (*Rattus norvegicus*) weighing about 130-150 g were used as experimental animals in the present study. 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 hr light and 12 hr 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" [11].

Induction of diabetes mellitus

T2DM was induced in overnight fasted rats by a single intraperitoneal (i.p.) injection of 50 mg/kg STZ dissolved in citrate buffer (pH 4.5), 30 minutes after the i.p. administration of 120 mg/kg of NA [12]. Rats received 5% glucose in drinking water to overcome the hypoglycemia produced by STZ. One week after STZ injection, overnight fasted animals were given glucose (3 g/kg b.wt.) by gastric intubation. After 2 hr 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 hr of glucose intake, were considered diabetic and selected for subsequent investigations.

Experimental design

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

Group 1 : Rats served as normal control and received 1% carboxy methyl cellulose (CMC).

Group 2 : Served as diabetic control rats and received 1% CMC.

Group 3 : Served as diabetic rats administered *P. pavonia* extract (100 mg/kg b.wt.) suspended in 1% CMC orally for 21 days [9].

Group 4 : Served as diabetic rats administered *T. ornata* extract (100 mg/kg b.wt.) suspended in 1% CMC orally for 21 days [9].

The dosage was adjusted every week according to any change in body weight to maintain similar dose/kg body weight of rat over the entire period of study for each group. By the end of the experiment, animals were sacrificed and blood samples, pancreas and adipose tissue were obtained.

Biochemical assays

Oral glucose tolerance test was performed using blood samples obtained from lateral tail vein of rats deprived of food overnight. Successive blood samples were then taken at 0, 30, 60, 90 and 120 minutes following the administration of glucose solution. Blood samples were left to coagulate, centrifuged, and clear serum was obtained for determination of glucose concentration according to the method of Trinder [13], using reagent kit purchased from Spinreact Company (Spain).

Serum insulin and adiponectin levels were determined using specific enzyme-linked immunosorbent assay kit (R&D Systems, USA), according to the manufacturer instructions. Serum total cholesterol [14], triglycerides [15] and high density lipoprotein (HDL)-cholesterol [16] were assayed using commercial diagnostic kits (Spinreact, Spain). Serum very low-density lipoprotein (vLDL)-cholesterol concentration was calculated according to Nobert [17] formula (vLDL-cholesterol=triglycerides/5). Serum LDL-cholesterol level was calculated from Friedewald [18] formula (LDL-cholesterol=total cholesterol−triglycerides/5-HDL-cholesterol). Serum aspartate aminotransferase (AST) [19], lactate dehydrogenase (LDH) [20], and creatine kinase-MB (CK-MB) [21] activities were also estimated using commercial diagnostic kits. Cardiovascular indices were calculated according to Ross [22] as follows: Cardiovascular index 1=total cholesterol/HDL-cholesterol and cardiovascular index 2=LDL-cholesterol/HDL-cholesterol.

Determination of homeostasis model of insulin resistance (HOMA-IR) and quantitative insulin-sensitivity check index (QUICKI)

Insulin resistance was evaluated by HOMA-IR [23] and QUICKI [24] as follows:

- HOMA-IR=Fasting insulin (μU/ml)×Fasting glucose (mmol/L)/22.5.
- QUICKI=1/(log fasting insulin [μU/ml]+log fasting glucose [mmol/L]).

An increase in HOMA-IR and a decrease in QUICKI values indicate insulin resistance.

Ribonucleic Acid (RNA) isolation and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

RT-PCR of adipose tissue adiponectin was performed as previously described in our recent study [6]. Briefly, total RNA was isolated from visceral adipose tissue and first strand of complementary deoxyribonucleic acid (cDNA) was synthesized from 2 μg of total RNA by using cDNA reverse transcription kit with RNase inhibitor. The produced cDNA was amplified by Green master mix (Fermentas, USA) using the following sets of primers: Up 5′-AATCCTGCCCAGTCATGAAG-3′ and Down 5′-TCTCCAGGAGTGCCATCTCT-3′ for adiponectin, and Up 5′-AAGTCCCTCACCCTCCCAAAAG-3′ and Down 5′-AAGCAATGCTGTCACCTTCCC-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 minutes, then 35 cycles each was 95°C (30 sec), 59.5°C (30 sec), 72°C (45 sec). Polymerase chain reaction products were subjected to agarose gel electrophoresis and the cDNA bands were observed in the gel using ultra violet transilluminator. Gel images were analyzed by scanning densitometry (Image J, NIH) and values were normalized to the quantity of β-actin, and presented as percentage of messenger RNA (mRNA) relative to control.

Histological study

After sacrifice and dissection, pancreas was immediately excised from each animal, fixed in 10% neutral buffered formalin and transferred to Histopathology Department, Faculty of Veterinary Medicine, Beni-Suef University (Egypt) for preparation for blocking in paraffin wax and sectioning. Pancreas was stained with hematoxylin and eosin.

Statistical analysis

Statistical analysis was performed using SPSS version 16. Results were represented as mean±standard error and all statistical comparisons were made by means of one-way ANOVA test followed by Duncan's multiple range test *post-hoc* analysis. $p<0.05$ was considered to be significant.

RESULTS

The oral glucose tolerance curve of diabetic rats showed a highly significant elevation at fasting state and at 30, 60, 90 and 120 minutes after oral glucose loading when compared to normal rats. Oral administration of both *P. pavonia* and *T. ornata* significantly improved the altered glucose levels; *P. pavonia* seemed to be more effective on decreasing postprandial blood glucose (Fig. 1).

Serum insulin level exhibited an opposite pattern; it was significantly ($p<0.001$) decreased in STZ/NA diabetic rats when compared to normal ones and was significantly increased as a result of treatment with *P. pavonia* only. Treatment of diabetic rats with *T. ornata* produced a slight increase in serum insulin levels; however, the produced increase was non-significant in comparison with diabetic control rats as depicted in Table 1.

Diabetic rats showed a significant ($p<0.001$) elevation of HOMA-IR that was decreased significantly upon administration of either extracts

Table 1: Serum insulin levels, HOMA-IR and QUIKI of normal, diabetic control and diabetic treated rats

Parameter / Group	Insulin (μU/ml)	HOMA-IR	QUIKI
Normal	24.58±0.95^a	4.91±0.27^c	0.49±0.006^a
Diabetic control	18.35±1.33^b	11.13±0.75^a	0.41±0.005^c
Diabetic+*T. ornata*	20.65±2.01^b	6.49±0.53^b	0.46±0.009^b
Diabetic+*P. pavonia*	23.54±0.74^a	6.91±0.49^b	0.46±0.007^b
F-prob	p<0.001	p<0.001	p<0.001

Data are expressed as mean±standard error. Number of animals in each group is six. Means with different superscript symbol(s) are significantly different. HOMA-IR: Homeostasis model of insulin resistance, QUICKI: Quantitative insulin-sensitivity check index, *T. ornata: Turbenaria ornata, P. pavonia: Padina pavonia*

(Table 1). However, while both extracts have more or less similar effects, *P. pavonia* extract seemed to be more effective. QUICKI, on the other hand, was significantly ($p<0.001$) decreased in STZ/NA diabetic rats and significantly elevated following oral administration of both tested agents (Table 1).

Compared to normal rats, histopathological examination of pancreatic sections revealed degenerated and necrotic cells in pancreas of diabetic rats (Fig. 2a and b). The islets were present with a large proportion of islet cells though the mild degenerative changes present in pancreas of diabetic rats with *T. ornata* (Fig. 2c). On the other hand, in diabetic rats treated with *P. pavonia*, the islet architecture was found to be more organized and less disrupted (Fig. 2d).

Data described the effect of *P. pavonia* and *T. ornata* extracts on lipid profile of diabetic rats were represented in Table 2. Diabetic rats exhibited a highly significant increase ($p<0.001$) in serum total cholesterol, triglycerides, LDL-and vLDL-cholesterols as compared with the non-diabetic group. Conversely, HDL-cholesterol was affected in an opposite manner, as it was significantly decreased ($p<0.05$) in diabetic rats and significantly increased in response to both treatment agents. The administration of both tested agents led to marked amelioration of all parameters of the altered lipid profile. The ratios of total cholesterol and LDL-cholesterol to HDL-cholesterol were significantly ($p<0.001$) increased in STZ/NA diabetic rats as compared to normal control group. *P. pavonia* as well as *T. ornata* produced remarkable amelioration of these altered parameters.

Serum cardiac function biomarkers, AST, LDH and CK-MB, activities were deleteriously increased ($p<0.001$) in diabetic control rats. Moreover, treatment of diabetic animals with both algal extracts induced potential alleviation of these cardiac markers (Table 3).

Regarding adiponectin, diabetic rats exhibited a significant ($p<0.001$) decrease in serum adiponectin level as compared with normal control rats. The administration of both agents showed marked improvement of serum adiponectin concentration (Fig. 3). Similarly, adipose tissue adiponectin mRNA expression of diabetic control rats was significantly downregulated when compared to normal rats and significantly upregulated upon both algal extracts supplementation (Fig. 4), the extract of *P. pavonia* seemed to be more effective.

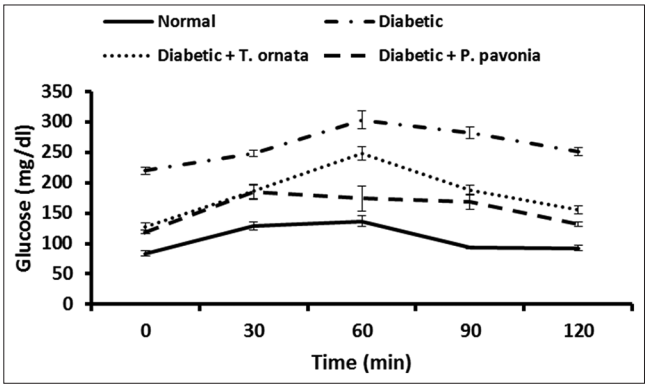

Fig. 1: Oral glucose tolerance test of normal, diabetic control and diabetic treated rats. Data are expressed as mean±standard error. Number of animals in each group is six, $p<0.001$

DISCUSSION

STZ/NA diabetes model is being increasingly used due to its similarity to human T2DM [25-27]. The cytotoxic action of STZ is associated with the generation of reactive oxygen species (ROS) causing oxidative damage that culminates in β-cell destruction through the induction of apoptosis and suppression of insulin biosynthesis as reported by Szkudelski [28] and Zhang *et al*. [29]. In addition, Tahara *et al*. [25] revealed that administration of STZ/NA induces diabetes with moderate hyperglycemia associated with loss of early-phase insulin secretion. The produced hyperglycemia plays an important role in development of T2DM and complications associated with the diseases such as micro-vascular and macro-vascular diseases [30].

In the current study, diabetic control rats exhibited significantly elevated blood glucose and HOMA-IR, accompanied with diminished serum insulin levels as well as decreased QUICKI. Hence, it is suggested that insulin resistance has been developed in these animals. These findings were in parallel to our previous study [31] where we

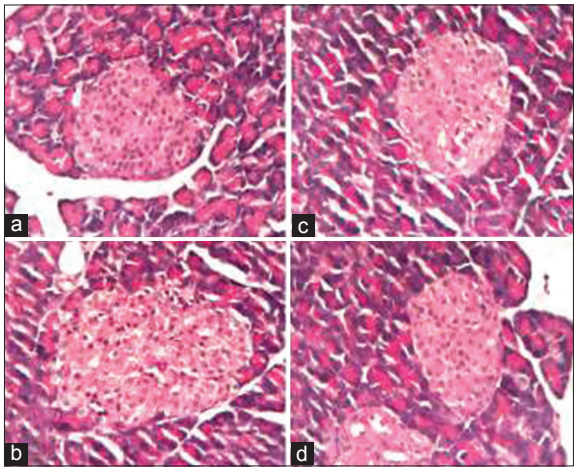

Fig. 2: Pancreas of normal, diabetic control and diabetic treated rats (×400). (a) Pancreatic tissue of normal rats showing normal histological structure of the islets. (b) Pancreas of diabetic control rats. Normal architecture of the islets is disrupted. Islets showing extreme necrosis and degeneration. (c) Pancreas of diabetic rats treated with *T. ornata*. The acinar cells are seen to be normal. The islets are present with a large proportion of islet cells though the mild degenerative changes present. (d) Pancreas of diabetic rats treated with *P. pavonia*. The islet architecture is more organized and less disrupted as compared with that of diabetic control

Table 2: Lipid profile of normal, diabetic control and diabetic treated rats

Parameter / Group	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	vLDL-cholesterol (mg/dl)	Total cholesterol/ HDL-cholesterol	LDL-cholesterol/ HDL-cholesterol
Normal	61.44±2.16^{d}	61.09±2.51^{d}	31.36±1.13^{a}	17.86±1.25^{c}	12.21±0.62^{d}	1.95±0.05^{c}	0.57±0.04^{c}
Diabetic control	142.08±3.56^{a}	179.91±2.14^{a}	29.14±1.71^{ab}	76.96±3.83^{a}	35.98±0.54^{a}	4.92±0.37^{a}	2.67±0.30^{a}
Diabetic+*T. ornata*	113.10±2.66^{b}	108.86±5.13^{b}	28.77±0.48^{ab}	62.55±2.85^{b}	21.77±1.28^{b}	3.91±0.08^{b}	2.17±0.11^{b}
Diabetic+*P. pavonia*	101.36±1.40^{c}	87.43±2.15^{c}	27.70±1.18^{b}	56.17±3.05^{b}	17.48±1.55^{c}	3.67±0.12^{b}	2.03±0.09^{b}
F-prob	p<0.001	p<0.001	p<0.05	p<0.001	p<0.001	p<0.001	p<0.001

Data are expressed as mean±standard error. Number of animals in each group is six. Means with different superscript symbol(s) are significantly different. HDL: High density lipoprotein, LDL: Low-density lipoprotein, vLDL: Very low-density lipoprotein, *T. ornata: Turbenaria ornata, P. pavonia: Padina pavonia*

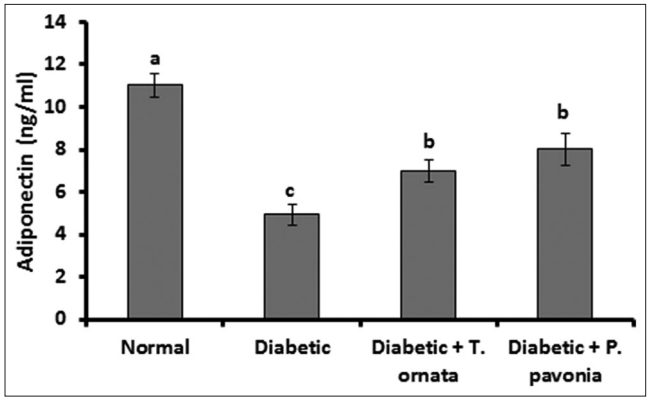

Fig. 3: Serum adiponectin of normal, diabetic control and diabetic treated rats. Data are expressed as mean±standard error. Number of animals in each group is six, p<0.001

Table 3: Serum AST, LDH and CK-MB of normal, diabetic control and diabetic treated rats

Parameter / Group	AST (U/L)	LDH (U/L)	CK-MB (U/L)
Normal	19.19±1.35^{b}	69.35±5.14^{b}	114.33±4.35^{c}
Diabetic control	40.11±4.68^{a}	138.47±7.45^{a}	290.03±8.02^{a}
Diabetic+*T. ornata*	26.09±2.06^{b}	48.88±2.34^{b}	204.20±6.52^{b}
Diabetic+*P. pavonia*	24.22±1.14^{b}	64.27±1.89^{b}	130.31±4.43^{c}
F-prob	p<0.01	p<0.001	p<0.001

Data are expressed as mean±standard error. Number of animals in each group is six. Means with different superscript symbol(s) are significantly different. AST: Aspartate aminotransferase, LDH: Lactate dehydrogenase, CK-MB: Creatine kinase-MB, *T. ornata: Turbenaria ornata, P. pavonia: Padina pavonia*

demonstrated hyperglycemia and diminished serum insulin along with developing insulin resistance in STZ/NA Type 2 diabetic rats. In addition, histopathological examination of pancreatic sections revealed degenerated and necrotic cells in diabetic rats. On the other hand, administration of both algal extracts significantly alleviated these abnormalities and increased β-cell mass. Therefore, the possible mechanism by which both tested algal extracts brings about its hypoglycemic effect may be through protecting pancreatic islets and stimulating insulin secretion from the remaining β-cells.

Diabetic dyslipidemia has long been shown to have a strong relation with coronary heart disease (CHD), which is the most dangerous and life-threatening complication of diabetes [32,33]. The current study revealed marked increase in total cholesterol, LDL-cholesterol, triglycerides and reduction in HDL-cholesterol in STZ/NA diabetic rats. These findings are in agreement with those of several previous studies [31,34]. The altered lipid and lipoprotein profiles were significantly reversed after 21 days of *P. pavonia* and *T. ornata* supplementation to the diabetic rats. The significant amelioration of the serum lipid levels in treated diabetic rats might have been due to the insulinotrophic and insulin sensitizing actions of both *P. pavonia* and *T. ornata*. The secreted insulin decreases triglyceride levels and increases HDL-cholesterol through activation of lipoprotein lipase (LPL) as revealed by Niemeijer-Kanters *et al*. [35]. In addition, it has been reported that dysfunction of LPL in insulin deficient state contributes to hypertriglyceridemia due to impaired catabolism of triglyceride-rich particles [36]. Moreover, insulin increases receptor-mediated removal of LDL-cholesterol and hence decreased activity of insulin during diabetes leads to increased levels of serum LDL-cholesterol and consequently hypercholesterolemia [37].

In addition, several atherogenic indices such as total cholesterol/HDL-cholesterol and LDL-cholesterol/HDL-cholesterol have been used to predict CHD risk [38]. Reduction of these indices in *P. pavonia*

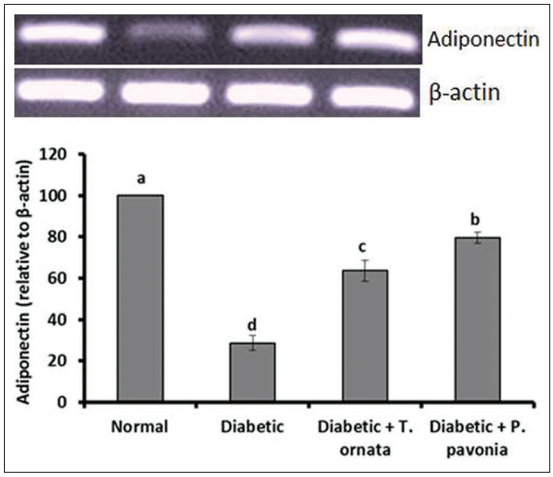

Fig. 4: Reverse transcriptase polymerase chain reaction analysis of adipose tissue adiponectin expression of normal, diabetic control and diabetic treated rats

and *T. ornata* treated diabetic rats strongly supported the notion that supplementation with either algal extracts may led to reduction in the risk of developing heart diseases. The observed cardioprotective effect of both tested algal extracts was further confirmed by the notably decreased serum cardiac markers, CK-MB, AST and LDH.

The administration of *P. pavonia* and *T. ornata* to diabetic rats produced a pronounced increase in serum adiponectin level as well as adipose tissue adiponectin mRNA expression and this may directly lead to marked amelioration of the lipid profile. In this context, Statnick *et al*. [39] revealed that serum levels of adiponectin were found to be in agreement with insulin sensitivity and its reduced levels are associated with the etiology of T2DM. By its insulin sensitizing effect, adiponectin has found to be involved in regulation of glucose metabolism through stimulation of adenine monophosphate-activated protein kinase [40], increase in muscle fat oxidation and glucose transport mediated through acetyl-CoA carboxylase inhibition [41], inhibition of hepatic gluconeogenesis through decrease in the expression of phosphoenolpyruvate carboxylase and glucose-6-phosphatase [40] and activation of peroxisome proliferator activated receptor-α leading to decreased triglyceride content in skeletal muscles and liver [42]. In addition, Woo *et al*., [43] demonstrated that the potential anti-obesity effect of fucothanxin, major carotenoid of brown seaweeds, might be mediated by improving plasma adiponectin level, down-regulating various lipogenic enzyme activities and fat production, up-regulating fatty acid β-oxidation activity and uncoupling protein gene expressions in visceral adipose tissues, suggesting that fucoxanthin might act as a regulator of lipid metabolism in fat tissues. Moreover, brown algae are rich in polyphenols, polysaccharides, vitamins such as A, B1, B12, C, D, E, riboflavin, niacin, pantothenic acid and folic acid, and minerals [44,45], which are powerful antioxidants. Yet, the insulin sensitizing effects of the tested algal extracts are mediated partly via increasing serum adiponectin level in conjunction with increased adipose tissue adiponectin mRNA expression.

CONCLUSION

The anti-hyperglycemic and anti-hyperlipidemic effects of both *P. pavonia* and *T. ornata* extracts may be mediated by preventing loss of β-cell mass, increase insulin secreting capacity and improve insulin sensitivity. In addition, the current study suggests that the anti-diabetic and cardioprotective effects of both tested algal extracts were mediated partially through enhancing adipose tissue adiponectin mRNA expression accompanied by increased serum adiponectin levels. Thus, it seems likely that both *P. pavonia* and *T. ornata* are promising anti-diabetic agents or pharmaceutical source that will be helpful for the improvement of T2DM.

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