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Spirulina versicolor improves insulin sensitivity and attenuates hyperglycemia-mediated oxidative stress in fructose-fed rats

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ABSTRACT

Aim: The current study aimed to investigate the anti-hyperglycemic, anti-hyperlipidemic and insulin sensitizing effects of the cyanobacterium *Spirulina versicolor* extract in fructose-fed rats. **Materials and Methods:** Rats were fed 30% fructose solution in drinking water for 4 weeks. Animals exhibited hyperglycemia and hyperinsulinemia were selected for further investigations. Diabetic and control rats were orally supplemented with 50 mg/kg body weight *S. versicolor* extract for 4 weeks. **Results:** At the end of 8 weeks, fructose-fed rats showed a significant increase in serum glucose, insulin, cholesterol, triglycerides, cardiovascular risk indices and insulin resistance. Treatment of the fructose-fed rats with *S. versicolor* extract improved this metabolic profile. Fructose feeding produced a significant increase in serum tumor necrosis factor alpha and a decrease in adiponectin levels. In addition, fructose-fed rats exhibited a significant increase in liver, kidney and heart lipid peroxidation levels, and declined antioxidant defenses. Supplementation of the fructose-fed rats with *S. versicolor* extract reversed these alterations. **Conclusion:** *S. versicolor* attenuates hyperglycemia-mediated oxidative stress and inflammation, and is thus effective in improving insulin sensitivity in fructose-fed rats.

KEY WORDS: Diabetes, fructose, inflammation, insulin resistance, oxidative stress, *Spirulina*

INTRODUCTION

Type 2 diabetes mellitus is a metabolic disease characterized by the presence of chronic hyperglycemia that results from defective or deficient insulin [1,2]. It accounts for more than 90% of all diabetic patients [3]. According to the International Diabetes Federation, the number of patients with diabetes mellitus in 2015 was estimated to be 415 million, and is expected to increase to 642 million by 2040 [4]. Type 2 diabetes and its complications constitute a major public health problem [5]. Several lifestyle factors such as physical inactivity [6], sedentary lifestyle [7], alcohol consumption [8] and smoking [9] are of key importance to the development of Type 2 diabetes. In addition, diet is a modifiable risk factor for Type 2 diabetes. The consumption of fructose has been enormously increased in the last few centuries because of the high increase in using sucrose and high fructose syrup [10]. Previous studies have demonstrated that high fructose intake is hazardous for human beings and animals [11,12], and results in hyperlipidemia, fatty liver, and insulin resistance [13]. The metabolism of fructose in the liver increases *de novo* lipogenesis [14], and an increase in high fructose corn syrup consumption has been linked to a rise in obesity and metabolic disorders [11]. Fructose feeding has also been shown to provoke oxidative damage and exert disturbing effects by diminishing antioxidant defenses, and increasing generation of free radicals [15]. Thus, the use of antioxidants could offer protection against fructose-induced metabolic alterations.

Currently, there is growing interest in the usefulness of algae for the treatment of diabetes. The cyanobacterium *Spirulina* is gaining a more attention as a nutraceutical and as a source of potential pharmaceutical. Studies have revealed the potential properties of *Spirulina* including antigenotoxic, anti-carcinogenic, immunostimulants, anti-inflammatory, anti-hepatotoxic, anti-diabetic and anti-hypertensive. *Spirulina* is a well-known source of anti-oxidant and anti-inflammatory molecules [16] such as c-phycocyanin, vitamins, β-carotene, phenolic compounds γ-linolenic acid and minerals [17,18]. *Spirulina maxima* (*Arthrospira maxima*), *Spirulina platensis* (*Arthrospira platensis*) and *Spirulina fusiformis* (*Arthrospira fusiformis*) are the most intensively investigated species of

Spirulina [17,19,20]. Recently, the preliminary anti-diabetic effect of *Spirulina versicolor* was reported in the study of AbouZid *et al*. [21] in streptozotocin/nicotinamide-induced diabetic mice. The authors reported that *S. versicolor* exerts anti-hyperglycemic effect, depending on assaying fasting and postprandial blood glucose levels in diabetic mice. To the best of our knowledge, nothing has yet been reported on the beneficial effects of *S. versicolor* in fructose-fed rats. Therefore, the current study was undertaken to investigate the anti-hyperglycemic, insulin sensitizing, anti-hyperlipidemic and antioxidant effects of *S. versicolor* in high fructose-fed rats. This investigation could provide an understanding of the anti-diabetic mechanism of *S. versicolor*.

METHODS

Preparation of *S. versicolor* extract

S. versicolor was purchased from Harraz medicinal plant company, Cairo, Egypt (www.harrazegypt.com). The algae was ground to a fine powder and extracted by 80% aqueous ethanol. Following filtration, the filtrate was concentrated under reduced pressure in a rotary evaporator and was stored at −20°C until use.

Experimental Animals

Male Wistar rats weighing 130-150 g, obtained from animal house of the National Research Centre (El-Giza, Egypt), were included in the present investigation. The animals were housed in plastic well-aerated cages at a normal atmospheric temperature (25±2°C) and normal 12 h light/dark cycle. Rats had free access to water and were supplied daily with laboratory standard diet of known composition. All animal procedures were undertaken with the approval of Institutional Animal Ethics Committee of Beni-Suef University (Egypt).

Experimental Design

About 24 rats were allocated into 4 groups, each consisting of six ($n = 6$) animals and were subjected to the following treatments:

Group 1 (Control): Received the vehicle 1% carboxymethylcellulose (CMC) and served as control rats.

Group 2 (Control + *S. versicolor*): Received 50 mg/kg b.wt. *S. versicolor* extract suspended in 1% CMC and served as drug control.

Group 3 (Diabetic): Received 30% fructose in tap water.

Group 4 (Diabetic + *S. versicolor*): Received 30% fructose in tap water and 50 mg/kg b.wt. *S. versicolor* extract suspended in 1% CMC.

Rats were fed 30% fructose solution in drinking water for 4 weeks, and biochemical parameters were estimated. Rats exhibited hyperglycemia and hyperinsulinemia were selected for further subsequent studies. *S. versicolor* extract has been administered by oral gavage for 4 weeks. The doses were balanced consistently as indicated by any change in body weight to keep up the comparable dosage for every kg body weight over the entire period of study.

Samples Preparation

By the end of the experiment, overnight fasted animals were sacrificed, and blood samples were collected, left to coagulate and centrifuged at 3000 rpm for 15 min to separate serum. Liver, kidney, and heart samples were immediately excised and perfused with ice-cold saline. Frozen samples (10% w/v) were homogenized in chilled saline, and the homogenates were centrifuged at 3000 rpm for 10 min. The clear homogenates were collected and used for subsequent assays.

Biochemical Study

Oral glucose tolerance test (OGTT)

On the day before sacrifice, OGTT was performed using blood samples obtained from lateral tail vein of rats deprived of food overnight. Successive blood samples were then taken at 30, 60, 90 and 120 min following the administration of glucose solution (3 g/kg b.wt.). Blood samples were left to coagulate, centrifuged, and clear sera were obtained for determination of glucose concentration according to the method of Trinder [22] using reagent kit purchased from Spinreact (Spain).

Determination of Serum Insulin, Adiponectin and Tumor Necrosis Factor Alpha (TNF-α)

Serum levels of insulin, adiponectin and TNF-α were determined using specific ELISA kits (R&D systems) following the manufacturer's instructions. The concentrations of assayed parameters were measured specrophotometrically at 450 nm. Standard curves were constructed by using standard proteins and concentrations of the unknown samples were determined from the standard plots.

Determination of Homeostasis Model of Insulin Resistance (HOMA-IR)

The insulin resistance was evaluated by homeostasis model assessment estimate of insulin resistance (HOMA-IR) [23] as follows:

$$\text{HOMA} - \text{IR} = \frac{\text{Fasting insulin } (\mu\text{U/ml}) \times \text{Fasting blood glucose} (\text{mmol/L})}{22.5}$$

Determination of Lipid Profile and Cardiovascular Risk Indices

Serum total cholesterol [24], triglycerides [25] and high density lipoprotein (HDL)-cholesterol [26] were assayed

using commercial diagnostic kits (Spinreact, Spain). Serum very low density lipoprotein (vLDL)-cholesterol concentration was calculated according to the following formula [27]: vLDL-cholesterol = triglycerides/5. Serum LDL-cholesterol level was calculated from the formula [28]: LDL-cholesterol = Total cholesterol - ([Triglycerides/5] + HDL-cholesterol]. Cardiovascular risk indices were calculated according to Ross [29] as follows: Cardiovascular risk index 1 = Total cholesterol/HDL-cholesterol and cardiovascular risk index 2 = LDL-cholesterol/HDL-cholesterol. Antiatherogenic index (AAI) was determined according to the following equation [30]: AAI = HDL-cholesterol × 100/Total cholesterol - HDL-cholesterol.

Assay of Serum Enzymes

Serum aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatine kinase (CK-MB) activities were assayed using reagent kits purchased from Biosystems (Spain) following the methods of Schumann and Klauke [31], Teitz and Andresen [32] and Kachmar and Moss [33], respectively.

Assay of Lipid Peroxidation and Antioxidant Defenses

Lipid peroxidation levels in liver, kidney, and heart homogenates were assayed by measurement of malondialdehyde (MDA) formation according to the method of Preuss *et al*. [34]. Reduced glutathione (GSH) content and activity of the antioxidant enzymes superoxide dismutase (SOD) and GSH peroxidase (GPx) were measured according to the methods of Beutler *et al*. [35] Marklund and Marklund [36] and Matkovics *et al*. [37], respectively.

Statistical Analysis

Data were analyzed using Graph Pad Prism 5 software and all statistical comparisons were made by means of the one-way ANOVA test followed by Tukey's test *post hoc* analysis. Results were articulated as mean ± standard error of the mean (SEM) and a P value < 0.05 was considered significant.

RESULTS

S. versicolor Represses Hyperglycemia and Insulin Resistance in Fructose-fed Rats

OGTT of the fructose-induced diabetic rats showed significantly ($P < 0.001$) elevated glucose levels and at all points of the OGTT when compared with the normal control rats [Figure 1a]. Oral supplementation of *S. versicolor* extract to fructose-fed rats significantly alleviated the blood glucose levels. The OGTT area under the curve (AUC) showed non-significant ($P > 0.05$) difference between the control and *S. versicolor* supplemented control rats. On the other hand, fructose-induced diabetic rats exhibited a significant ($P < 0.01$) increase in AUC when compared with the control rats. Treatment of the diabetic rats with *S. versicolor* markedly ($P < 0.01$) decreased OGTT AUC when compared with the diabetic control rats, as depicted in Figure 1b.

Serum insulin level was significantly ($P < 0.001$) increased in fructose fed rats compared with the control group as depicted in Figure 1c. Oral treatment with *S. versicolor* markedly ameliorated serum insulin levels in the fructose-induced diabetic rats. Similarly, diabetic rats exhibited a significant ($P < 0.001$) increase in HOMA-IR, an effect that was reversed by oral administration of *S. versicolor* to fructose-induced diabetic rats [Figure 1d].

S. versicolor Exerts Anti-hyperlipidemic, Cardioprotective and Anti-atherogenic effects in Fructose-fed Rats

Data represented in Table 1 show the effect of *S. versicolor* on lipid profile, cardiovascular risk indices, heart marker enzymes and antiatherogenic index of control and diabetic rats. Compared with the control group, rats supplemented with *S. versicolor* exhibited non-significant ($P > 0.05$) changes in all lipid profile parameters. On the other hand, fructose-induced diabetic rats exhibited significant increase in serum total cholesterol ($P < 0.001$), triglycerides ($P < 0.01$), LDL-cholesterol ($P < 0.001$) and vLDL-cholesterol ($P < 0.01$) when compared with the control group. Serum levels of HDL-cholesterol showed a non-significant ($P > 0.05$) difference between all studied groups. In addition, diabetic rats showed significantly ($P < 0.001$) increased HDL-cholesterol/T. cholesterol and LDL-cholesterol/HDL-cholesterol. In addition, the antiatherogenic index was significantly ($P < 0.05$) declined in diabetic rats. By comparison, the oral supplementation of *S. versicolor* extract to diabetic rats potentially ameliorated the altered serum lipid profile as well as cardiovascular risk indices.

Serum AST, CK-MB and LDH activities were significantly increased in the fructose-induced diabetic rats when compared with the control group [Table 1]. Treatment of the diabetic rats with *S. versicolor* extract significantly ameliorated serum activities of AST ($P < 0.05$), CK-MB ($P < 0.05$) and LDH ($P < 0.001$).

S. versicolor Increases Circulating Adiponectin and Decreases TNF-α in Fructose-fed Rats

Fructose-fed rats exhibited markedly ($P < 0.01$) declined serum adiponectin levels when compared with the control group, as represented in Figure 2a. Treatment of the fructose-induced diabetic rats with *S. versicolor* extract significantly ($P < 0.01$) alleviated serum adiponectin levels. The effect of *S. versicolor* on serum levels of TNF-α in control and fructose-induced diabetic rats showed a significantly ($P < 0.01$) increased levels of TNF-α ($P < 0.001$) in diabetic rats and potential ($P < 0.05$) alleviation following treatment with *S. versicolor* extract [Figure 2b].

S. versicolor Attenuates Hyperglycemia-induced Oxidative Stress in Liver, Kidney and Heart of Fructose-fed Rats

Fructose-induced diabetic rats showed significantly increased MDA levels in liver ($P < 0.001$), kidney ($P < 0.01$) and heart

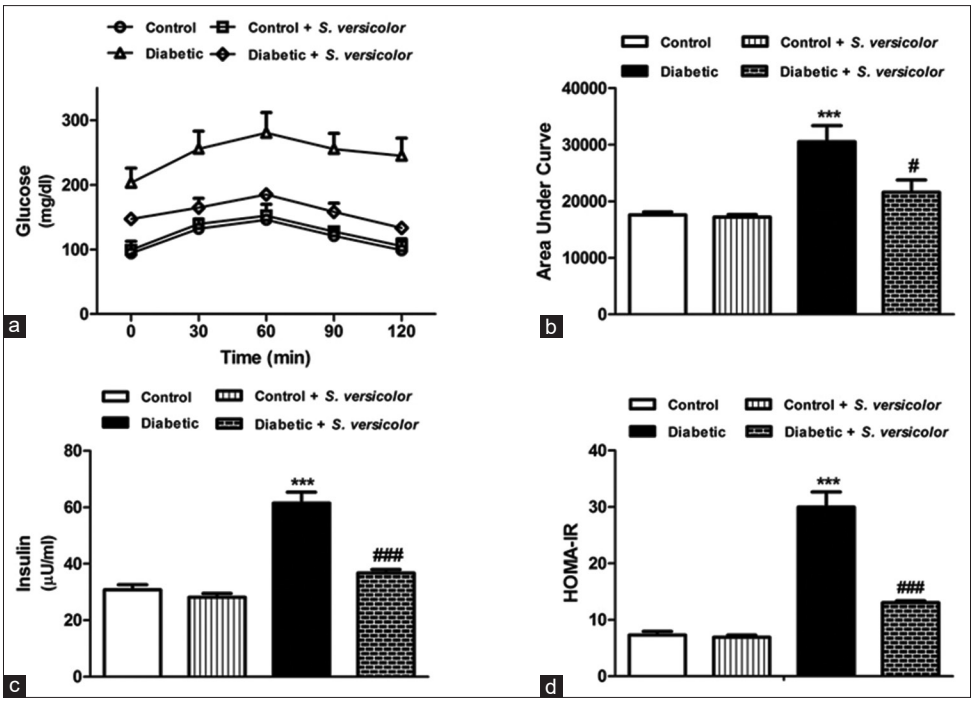

Figure 1: Effect of *Spirulina versicolor* on (a and b) glucose tolerance, (c) serum insulin and (d) homeostasis model of insulin resistance. Results are mean ± standard error of the mean ($n = 6$). ***$P < 0.001$ versus control, and $^{\#}P < 0.05$, and $^{\#\#\#}P < 0.001$ versus diabetic group

Table 1: Effect of *S. versicolor* on serum lipid profile, cardiovascular risk indices and antiatherogenic index in control and fructose-fed rats

Parameter	Control	Control+*S. versicolor*	Diabetic	Diabetic+*S. versicolor*	*P* value
Total cholesterol (mg/dl)	83.62±6.70	83.85±8.88	171.80±8.04***	111.90±3.36$^{\#\#\#}$	$P<0.001$
Triglycerides (mg/dl)	110.40±5.71	95.40±6.08	249.8±41.19**	110.60±8.37$^{\#\#}$	$P<0.001$
HDL-cholesterol (mg/dl)	39.25±3.44	38.23±2.05	29.22±4.63	44.88±1.39$^{\#}$	$P<0.05$
LDL-cholesterol (mg/dl)	27.85±6.34	26.54±6.65	92.64±6.87***	44.92±3.65$^{\#\#\#}$	$P<0.001$
vLDL-cholesterol (mg/dl)	22.07±1.14	19.08±1.22	49.96±8.24**	22.12±1.67$^{\#\#}$	$P<0.001$
Total cholesterol/HDL-cholesterol	2.18±0.23	2.18±0.16	6.32±0.79***	2.49±0.05$^{\#\#\#}$	$P<0.001$
LDL-cholesterol/HDL-cholesterol	0.61±0.21	0.67±0.15	3.39±0.40***	1.00±0.08$^{\#\#\#}$	$P<0.001$
Antiatherogenic index (%)	98.80±6.95	94.41±14.79	20.90±3.71*	67.21±2.56$^{\#}$	$P<0.01$
AST (U/L)	19.20±1.35	19.77±0.79	30.45±1.09***	24.22±0.69$^{\#}$	$P<0.001$
LDH (U/L)	56.95±4.53	21.14±3.17	138.50±10.54**	18.27±2.67$^{\#\#\#}$	$P<0.001$
CK-MB (U/L)	185.70±8.40	234.79±17.84	290.00±8.02**	268.91±11.86$^{\#}$	$P<0.01$

Data are expressed as mean±SEM. Number of rats in each group is six, *$P<0.05$, **$P<0.01$, ***$P<0.001$ versus control. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ versus diabetic, SEM: Standard error mean, CK: Creatine kinase, LDH: Lactate dehydrogenase, AST: Aspartate aminotransferase, LDL: Low density lipoprotein, HDL: High density lipoprotein, vLDL: Very low density lipoprotein, *S. versicolor: Spirulina versicolor*

($P < 0.01$) when compared with the control rats [Figure 3a]. Treatment of the fructose-induced diabetic rats with *S. versicolor* extract significantly alleviated liver ($P < 0.01$), kidney ($P < 0.001$) and heart ($P < 0.001$) lipid peroxidation levels. Oral supplementation of *S. versicolor* to normal rats produced significant ($P < 0.05$) decrease in kidney MDA content, with no effect exerted on liver and heart.

On the contrary, fructose supplementation significantly decreased liver ($P < 0.001$), kidney ($P < 0.05$) and heart ($P < 0.001$) GSH content when compared with the control group, as represented in Figure 3b. Similarly, SOD activity was significantly decreased in the liver ($P < 0.05$), kidney ($P < 0.05$) and heart ($P < 0.01$) of fructose-induced diabetic rats when compared with the control group [Figure 3c]. GPx activity showed a similar pattern where it was significantly decreased in the liver ($P < 0.01$), kidney ($P < 0.001$) and heart ($P < 0.05$) of fructose-induced diabetic rats, as depicted in Figure 3d. On the other hand, treatment of the fructose-induced diabetic rats with *S. versicolor* extract potentially ameliorated GSH content as well as activities of SOD and GPx in the liver, kidney and heart.

DISCUSSION

Several studies have demonstrated the deleterious effects of fructose on insulin sensitivity and glucose metabolism [38]. In the present study, fructose-fed rats showed significantly impaired glucose tolerance accompanied with hyperinsulinemia and increased HOMA-IR. Therefore, it is suggested that insulin resistance has been developed in these animals. This would closely reflect the natural history and metabolic characteristics of human diabetes, and it is further sensitive to pharmacological

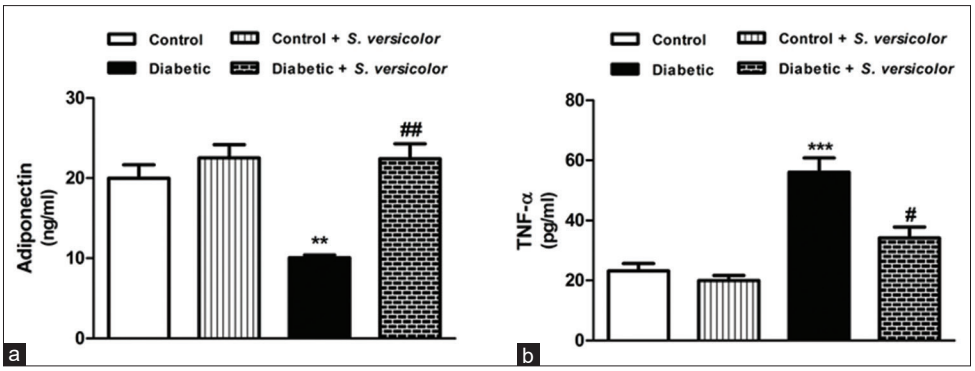

Figure 2: Effect of *Spirulina versicolor* on (a) serum adiponectin and (b) tumor necrosis factor alpha. Results are mean ± standard error of the mean ($n = 6$). $^{**}P < 0.01$, and $^{***}P < 0.001$ versus control, and $^{\#}P < 0.05$, and $^{\#\#}P < 0.01$ versus diabetic group

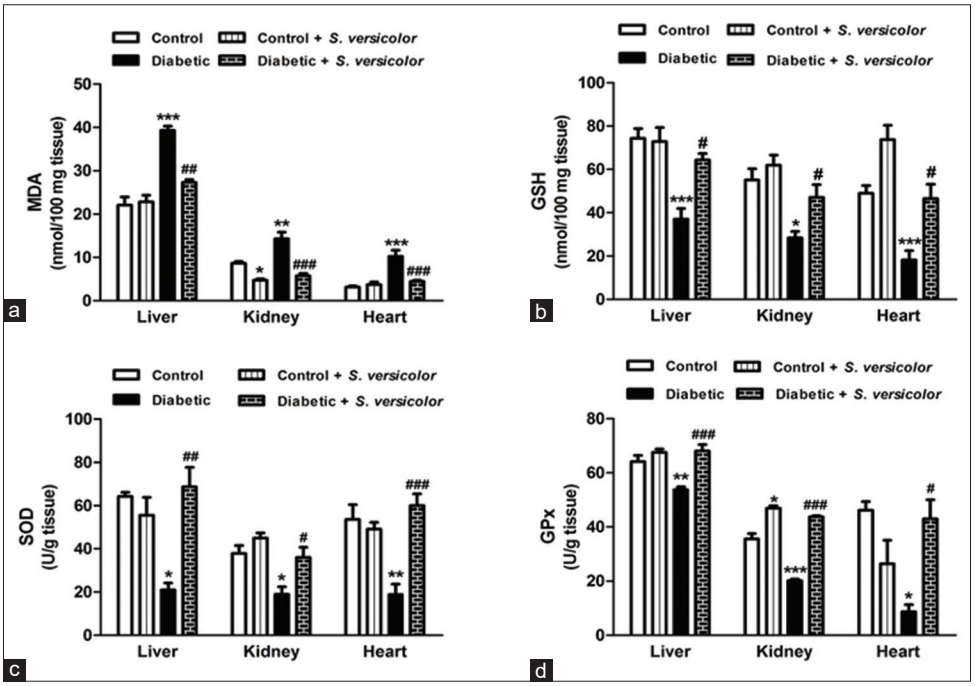

Figure 3: Effect of *Spirulina versicolor* on (a) lipid peroxidation, (b) reduced glutathione, (c) superoxide dismutase and (d) glutathione peroxidase in liver, kidney and heart. Results are mean ± standard error of the mean ($n = 6$). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ versus control, and $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ versus diabetic group

testing [2]. Long term fructose feeding has been demonstrated to induce diabetes associated with insulin resistance in experimental animals [38-41]. The fructose-induced insulin resistance may be linked to alteration of insulin signaling. In this context, high fructose feeding has been reported to decrease insulin receptor substrate (IRS)-1 phosphorylation in rat skeletal muscles [42]. In addition, fructose-induced hyperlipidemia [43] and fat deposition [44] may generate lipid-derived metabolites which reduce insulin signaling via increasing serine/threonine phosphorylation of IRS-1 [45]. Oral supplementation of *S. versicolor* extract markedly reduced blood glucose and improved insulin sensitivity in fructose-fed rats. Although the anti-hyperglycemic effect of different *Spirulina* species has been previously reported, studies demonstrating the anti-diabetic efficacy of *S. versicolor* are scarce. In this context, Mani *et al*. [46] showed a significant decrease in the fasting blood sugar level of patients received 2 g/day *Spirulina* for 21 days, and Layam *et al*. [47] proved the same effect in diabetic rats treated with 15 mg/kg *Spirulina* for 45 days. The hypoglycemic effect of *Spirulina* could perhaps attributed to its high fiber content that diminish glucose absorption [48], or to the possible action of peptides generated by the digestion of *Spirulina* proteins [49].

Insulin resistance in Type 2 diabetes is also associated with hyperlipidemia and atherosclerosis [50]. Fructose-fed rats in the present investigation exhibited hypercholesterolemia and hypertriglyceridemia. The fructose-induced hyperlipidemia may be attributed to the increased *de novo* hepatic lipogenesis through providing large amounts of hepatic triose-phosphate for fatty acid synthesis [14]. In addition, fructose increases the expression of key lipogenic enzymes and induces the expression of sterol regulatory element binding protein-1c which is the principal inducer of hepatic lipogenesis [51,52]. Moreover,

fructose has been demonstrated to activate carbohydrate-responsive element binding protein (ChREBP), leading to up-regulated expression of hepatic fatty acid synthase and acetyl-CoA carboxylase [53]. Activation of ChREBP may be attributed to the fructose-induced expression of glucose-6-phosphate dehydrogenase and intermediary substrates of the hexose-monophosphate shunt [54].

The elevated triglycerides and cholesterol levels in the fructose-induced diabetic rats represent atherogenic lipid profile. The recorded values of atherogenic indices in the present study showed the bad impact of fructose-induced dyslipidemia on the cardiovascular system. These findings were confirmed by the elevated serum levels of AST, CK-MB, and LDH. Treatment of the diabetic rats with *S. versicolor* extract significantly ameliorated the altered lipid profile and atherogenic indices. Reduction of these indices in treated fructose-fed rats strongly supported the notion that dietary supplementation with *S. versicolor* may reduce the risk of developing heart diseases. These findings were further confirmed by the significantly decreased serum activities of the cardiac markers, CK-MB, LDH and AST, in *S. versicolor* treated fructose-fed rats. The anti-hyperlipidemic effects of *Spirulina sp.* have been demonstrated in animal [55,56] and human studies [57-59].

The beneficial effects of *S. versicolor* extract in fructose-induced diabetic rats might be explained, at least in part, through its ability to increase serum adiponectin levels. Serum level of adiponectin is in agreement with insulin sensitivity and its reduced levels are associated with insulin resistance [60]. Adiponectin regulates glucose metabolism [61], increases muscle fat oxidation and glucose transport mediated [62], inhibits hepatic gluconeogenesis [63] and activates peroxisome proliferator activated receptor-α leading to decreased triglyceride content in skeletal muscles and liver [64]. We also assume that suppression of the release of TNF-α following *S. versicolor* administration could be a direct result of increased serum adiponectin levels. Adiponectin is well known to inhibit the expression of the pro-inflammatory cytokine TNF-α in various tissues [65]. TNF-α diminishes the ability of insulin to stimulate peripheral glucose uptake and to suppress hepatic glucose production [66], and increases circulating free fatty acids; thus contributes to the pathogenesis of insulin resistance [67]. In the present study, treatment of the fructose-induced diabetic rats with *S. versicolor* markedly decreased serum levels of TNF-α, confirming its anti-inflammatory efficacy.

Oxidative stress has been implicated in fructose-induced insulin resistance and Type 2 diabetes in rats [13]. Oxidative stress can cause oxidation and damage to many cellular components such as DNA, lipids and proteins [68]. Reactive oxygen species (ROS) in diabetes could react with polyunsaturated fatty acids leading to lipid peroxidation [69]. In addition, high levels of free radicals and the simultaneous decline in endogenous antioxidants can lead to damage of cellular organelles, and development of insulin resistance [70]. Hence, it was recommended by Mahmoud *et al.* [2] that therapy with antioxidants represents a useful pharmacologic overture to the management of diabetes. The present findings showed significant elevation in lipid peroxidation levels in liver, kidney and heart of fructose-administered rats. Treatment of the fructose-fed rats with *S. versicolor* extract significantly decreased lipid peroxidation levels, reflecting its radical scavenging property.

In contrary, GSH and the antioxidant enzymatic defenses showed a simultaneous decrease in the liver, kidney and heart of fructose-induced diabetic rats. Antioxidant defenses are known to decrease under hyperglycemia [71] and oxidative stress [72]. Treatment of diabetic rats with *S. versicolor* significantly increased levels of GSH and activity of the antioxidant enzymes SOD and GPx. GSH is an endogenous antioxidant that protects against oxidative stress-induced cellular damage by reacting with oxidants or as a substrate for GPx. SOD and GPx provide a defense system against ROS-induced cellular damage [73]. The antioxidant effect of *Spirulina* and their constituents has been previously demonstrated. Ahmed *et al.* [74] reported that *S. versicolor* extract protected against diethylnitrosamine-induced hepatocarcinogenesis through potentiating the antioxidant defense system.

Several studies have reported the *in vitro* and *in vivo* antioxidant and/or anti-inflammatory efficacies of *Spirulina* and its extracts, suggesting the beneficial effects of *Spirulina* in managing insulin resistance and diabetes. The antioxidant and anti-inflammatory effects of *Spirulina Sp.* could be attributed to its active constituents. *Spirulina* contains a relative high concentration of β-carotene, provitamin A, vitamin B, vitamin C, vitamin D, vitamin E, ω-3 and ω-6 polyunsaturated fatty acids, and phycocyanin [75]. Phycocyanin has the ability to scavenge free radicals, decrease nitrite production, suppress inducible nitric oxide synthase expression, and inhibit liver microsomal lipid peroxidation. In addition, phycocyanin has been reported to inhibit pro-inflammatory cytokine formation, suppress cyclooxygeanase-2 expression and decrease prostaglandin E2 production [76-78]. Another constituent, β-carotene, has been reported to have antioxidant and anti-inflammatory activities [79,80].

CONCLUSION

The current findings provide new information on the antidiabetic mechanism of *S. versicolor* in fructose-fed rats. High fructose feeding induces insulin resistance, inflammation and oxidative stress. Oral administration of *S. versicolor* ameliorates insulin sensitivity, increases serum adiponectin, and attenuates oxidative stress and inflammation in diabetic rats. Our findings suggest that *S. versicolor* extract could be used as a dietary supplement in diabetes management, pending further studies to trace out its exact mechanistic pathways.

REFERENCES

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2010;33 Suppl 1:S62-9.
2. Mahmoud AM, Ashour MB, Abdel-Moneim A, Ahmed OM. Hesperidin and naringin attenuate hyperglycemia-mediated oxidative stress and proinflammatory cytokine production in high fat fed/streptozotocin-induced Type 2 diabetic rats. J Diabetes Complications 2012;26:483-90.

3. Tripathi BK, Srivastava AK. Diabetes mellitus: Complications and therapeutics. Med Sci Monit 2006;12:RA130-47.
4. International Diabetes Federation (IDF). IDF Diabetes Atlas. 7th ed. Brussels, Belgium: International Diabetes Federation; 2015. Available from: http://www.idf.org/diabetesatlas.
5. Wu Y, Ding Y, Tanaka Y, Zhang W. Risk factors contributing to Type 2 diabetes and recent advances in the treatment and prevention. Int J Med Sci 2014;11:1185-200.
6. Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG, *et al.* Diet, lifestyle, and the risk of Type 2 diabetes mellitus in women. N Engl J Med 2001;345:790-7.
7. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. Nature 2001;414:782-7.
8. Cullmann M, Hilding A, Östenson CG. Alcohol consumption and risk of pre-diabetes and Type 2 diabetes development in a Swedish population. Diabet Med 2012;29:441-52.
9. Manson JE, Ajani UA, Liu S, Nathan DM, Hennekens CH. A prospective study of cigarette smoking and the incidence of diabetes mellitus among US male physicians. Am J Med 2000;109:538-42.
10. Regy J, Padmaja G. Comparative studies on the production of glucose and high fructose syrup from tuber starches. Int Res J Biol Sci 2013;2:68-75.
11. Bray GA, Nielsen SJ, Popkin BM. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. Am J Clin Nutr 2004;79:537-43.
12. Johnson RJ, Segal MS, Sautin Y, Nakagawa T, Feig DI, Kang DH, *et al.* Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease. Am J Clin Nutr 2007;86:899-906.
13. Roncal-Jimenez CA, Lanaspa MA, Rivard CJ, Nakagawa T, Sanchez-Lozada LG, Jalal D, *et al.* Sucrose induces fatty liver and pancreatic inflammation in male breeder rats independent of excess energy intake. Metabolism 2011;60:1259-70.
14. Tappy L, Lê KA. Metabolic effects of fructose and the worldwide increase in obesity. Physiol Rev 2010;90:23-46.
15. Faure P, Rossini E, Lafond JL, Richard MJ, Favier A, Halimi S. Vitamin E improves the free radical defense system potential and insulin sensitivity of rats fed high fructose diets. J Nutr 1997;127:103-7.
16. Chu WL, Lim YW, Radhakrishnan AK, Lim PE. Protective effect of aqueous extract from *Spirulina platensis* against cell death induced by free radicals. BMC Complement Altern Med 2010;10:53.
17. Gershwin ME, Belay AE. *Spirulina* in Human Nutrition and Health. Boca Raton, London, New York: CRC Press Taylor & Francis Group; 2008.
18. Kelly Moorhead K, Capelli B, Cysewski GR. *Spirulina* Nature's Super Food. Hawaii: Cyanotech Corporation; 1993. p. 1-63.
19. Khan Z, Bhadouria P, Bisen PS. Nutritional and therapeutic potential of *Spirulina*. Curr Pharm Biotechnol 2005;6:373-9.
20. Karkos PD, Leong SC, Karkos CD, Sivaji N, Assimakopoulos DA. *Spirulina* in clinical practice: Evidence-based human applications. Evid Based Complement Alternat Med 2011;2011:531053.
21. AbouZid SF, Ahmed OM, Ahmed RR, Mahmoud A, Abdella E, Ashour MB. Antihyperglycemic effect of crude extracts of some Egyptian plants and algae. J Med Food 2014;17:400-6.
22. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem 1969;6:24-7.
23. Haffner SM. Coronary heart disease in patients with diabetes. N Engl J Med 2000;342:1040-2.
24. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974;20:470-5.
25. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem 1982;28:2077-80.
26. Burstein M, Scholnick HR, Morfin R. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. J Lipid Res 1970;11:583-95.
27. Tietz NW. Clinical Guide to Laboratory Tests. Philadelphia: W. B. Saunders Co.; 1995.
28. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.
29. Ross R. The pathogenesis of atherosclerosis. In: Braunwald E, editor. Heart Disease: A Textbook of Cardiovascular Medicine. 4th ed. Philadelphia, PA: W. B. Saunders; 1992. p. 1106-24.
30. Guido S, Joseph T. Effect of chemically different calcium antagonists on lipid profile in rats fed on a high fat diet. Indian J Exp Biol 1992;30:292-4.
31. Schumann G, Klauke R. New IFCC reference procedures for the determination of catalytic activity concentrations of five enzymes in serum: Preliminary upper reference limits obtained in hospitalized subjects. Clin Chim Acta 2003;327:69-79.
32. Tietz NW, Andresen BD. Textbook of Clinical Chemistry. Philadelphia: W. B. Saunders; 1986.
33. Kachmar JF, Moss DW. Fali naslov poglavlja. In: Tietz NW, editor. Fundamentals of Clinical Chemistry. 2nd ed. Philadelphia, PA: W. B. Saunders Co.; 1976. p. 682.
34. Preuss HG, Jarrell ST, Scheckenbach R, Lieberman S, Anderson RA. Comparative effects of chromium, vanadium and gymnema sylvestre on sugar-induced blood pressure elevations in SHR. J Am Coll Nutr 1998;17:116-23.
35. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963;61:882-8.
36. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974;47:469-74.
37. Matkovics B, Szabo L, Varga IS. Determination of enzyme activities in lipid peroxidation and glutathione pathways (in Hungarian). Lab Diagn 1998;15:248-9.
38. Mahmoud AM, Hozayen WG, Soliman HA, Mostafa SR. *Enteromorpha flexuosa* improves insulin sensitivity and metabolic control in fructose-induced diabetic rats. J Endocrinol Diabetes Obes 3(2):1072.
39. Nakagawa T, Hu H, Zharikov S, Tuttle KR, Short RA, Glushakova O, *et al.* A causal role for uric acid in fructose-induced metabolic syndrome. Am J Physiol Renal Physiol 2006;290:F625-31.
40. Veerapur VP, Prabhakar KR, Thippeswamy BS, Bansal P, Srinivasan KK, Unnikrishnan MK. Antidiabetic effect of *Dodonaea viscosa* (L). Lacq. aerial parts in high fructose-fed insulin resistant rats: A mechanism based study. Indian J Exp Biol 2010;48:800-10.
41. Padiya R, Khatua TN, Bagul PK, Kuncha M, Banerjee SK. Garlic improves insulin sensitivity and associated metabolic syndromes in fructose fed rats. Nutr Metab (Lond) 2011;8:53.
42. Eiffert KC, McDonald RB, Stern JS. High sucrose diet and exercise: Effects on insulin-receptor function of 12- and 24-mo-old Sprague-Dawley rats. J Nutr 1991;121:1081-9.
43. Bantle JP, Laine DC, Thomas JW. Metabolic effects of dietary fructose and sucrose in Types 1 and 2 diabetic subjects. JAMA 1986;256:3241-6.
44. Crapo PA, Kolterman OG. The metabolic effects of 2-week fructose feeding in normal subjects. Am J Clin Nutr 1984;39:525-34.
45. Shulman GI. Cellular mechanisms of insulin resistance. J Clin Invest 2000;106:171-6.
46. Mani S, Iyer UM, Subramanian S. Studies on the effect of *Spirulina* supplementation in control of diabetes mellitus. In: Subramanian G, editor. Cyanobacterial Biotechnology. USA: Science Publishers Inc.; 1998. p. 301-4.
47. Layam A, Chandra L, Reddy K. Antidiabetic property of *Spirulina*. Diabetol Croat 2006;35:29-33.
48. Samuels R, Mani UV, Iyer UM, Nayak US. Hypocholesterolemic effect of *Spirulina* in patients with hyperlipidemic nephrotic syndrome. J Med Food 2002;5:91-6.
49. Mani UV, Desai S, Iyer UM. Studies on long term effect of *Spirulina* supplementation on serum lipid profile and glycated proteins in NIDDM patients. J Nutraceut 2000;2:25-32.
50. Prashantha Kumar BR, Praveen TK, Nanjan MJ, Karvekar MD, Suresh B. Serum glucose and triglycerides lowering activity of some novel glitazones against dexamethasone-induced hyperlipidemia and insulin resistance. Indian J Pharmacol 2007;39:299-302.
51. Shimomura I, Bashmakov Y, Horton JD. Increased levels of nuclear SREBP-1c associated with fatty livers in two mouse models of diabetes mellitus. J Biol Chem 1999;274:30028-32.
52. Matsuzaka T, Shimano H, Yahagi N, Amemiya-Kudo M, Okazaki H, Tamura Y, *et al.* Insulin-independent induction of sterol regulatory element-binding protein-1c expression in the livers of streptozotocin-treated mice. Diabetes 2004;53:560-9.
53. Denechaud PD, Dentin R, Girard J, Postic C. Role of ChREBP in hepatic steatosis and insulin resistance. FEBS Lett 2008;582:68-73.
54. Koo HY, Wallig MA, Chung BH, Nara TY, Cho BH, Nakamura MT. Dietary fructose induces a wide range of genes with distinct shift in

carbohydrate and lipid metabolism in fed and fasted rat liver. Biochim Biophys Acta 2008;1782:341-8.
55. Iwata K, Inayama T, Kato T. Effects of *Spirulina platensis* on plasma lipoprotein lipase activity in fructose-induced hyperlipidemic rats. J Nutr Sci Vitaminol (Tokyo) 1990;36:165-71.
56. Torres-Durán PV, Miranda-Zamora R, Paredes-Carbajal MC, Mascher D, Blé-Castillo J, Díaz-Zagoya JC, *et al.* Studies on the preventive effect of *Spirulina* maxima on fatty liver development induced by carbon tetrachloride, in the rat. J Ethnopharmacol 1999;64:141-7.
57. Parikh P, Mani U, Iyer U. Role of *Spirulina* in the control of glycemia and lipidemia in Type 2 diabetes mellitus. J Med Food 2001;4:193-199.
58. Lee EH, Park JE, Choi YJ, Huh KB, Kim WY. A randomized study to establish the effects of *Spirulina* in Type 2 diabetes mellitus patients. Nutr Res Pract 2008;2:295-300.
59. Kamalpreet K, Rajbir S, Kiran G. Effect of supplementation of *Spirulina* on blood glucose and lipid profile of the non-insulin dependent diabetic male subjects. J Dairy Foods Home Sci 2008;27:3-4.
60. Statnick MA, Beavers LS, Conner LJ, Corominola H, Johnson D, Hammond CD, *et al*. Decreased expression of apM1 in Omental and subcutaneous adipose tissue of humans with Type 2 diabetes. Int J Exp Diabetes Res 2000;1:81-8.
61. Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, *et al.* Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 2002;8:1288-95.
62. Tomas E, Tsao TS, Saha AK, Murrey HE, Zhang CC, Itani SI, *et al.* Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: Acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation. Proc Natl Acad Sci U S A 2002;99:16309-13.
63. MacDougald OA, Burant CF. The rapidly expanding family of adipokines. Cell Metab 2007;6:159-61.
64. Tsatsanis C, Zacharioudaki V, Androulidaki A, Dermitzaki E, Charalampopoulos I, Minas V, *et al.* Adiponectin induces TNF-alpha and IL-6 in macrophages and promotes tolerance to itself and other pro-inflammatory stimuli. Biochem Biophys Res Commun 2005;335:1254-63.
65. Bastard JP, Piéroni L, Hainque B. Relationship between plasma plasminogen activator inhibitor 1 and insulin resistance. Diabetes Metab Res Rev 2000;16:192-201.
66. Lang CH, Dobrescu C, Bagby GJ. Tumor necrosis factor impairs insulin action on peripheral glucose disposal and hepatic glucose output. Endocrinology 1992;130:43-52.
67. Ryden M, Dicker A, van Harmelen V, Hauner H, Brunnberg M, Perbeck L, *et al.* Mapping of early signaling events in tumor necrosis factor-alpha -mediated lipolysis in human fat cells. J Biol Chem 2002;277:1085-91.
68. Jones DP. Radical-free biology of oxidative stress. Am J Physiol Cell Physiol 2008;295:C849-68.
69. Karthikesan K, Pari L, Menon VP. Combined treatment of tetrahydrocurcumin and chlorogenic acid exerts potential antihyperglycemic effect on streptozotocin-nicotinamide-induced diabetic rats. Gen Physiol Biophys 2010;29:23-30.
70. Whiteman M, Gooding KM, Whatmore JL, Ball CI, Mawson D, Skinner K, *et al.* Adiposity is a major determinant of plasma levels of the novel vasodilator hydrogen sulphide. Diabetologia 2010;53:1722-6.
71. Aragno M, Mastrocola R, Catalano MG, Brignardello E, Danni O, Boccuzzi G. Oxidative stress impairs skeletal muscle repair in diabetic rats. Diabetes 2004;53:1082-8.
72. Maritim AC, Sanders RA, Watkins JB 3rd. Diabetes, oxidative stress, and antioxidants: A review. J Biochem Mol Toxicol 2003;17:24-38.
73. Viswanatha Swamy AH, Kulkarni RV, Thippeswamy AH, Koti BC, Gore A. Evaluation of hepatoprotective activity of *Cissus quadrangularis* stem extract against isoniazid-induced liver damage in rats. Indian J Pharmacol 2010;42:397-400.
74. Ahmed OM, Ashour BM, Fahim HE, Mahmoud AM, Ahmed NA. Preventive effect of *Spirulina versicolor* and *Enteromorpha flexuosa* ethanolic extracts against diethylnitrosamine/benzo(a)pyrene-induced hepatocarcinogenecity in rats. J Int Acad Res Multidiscipl 2014;2:633-50.
75. Huang Z, Guo BJ, Wong RN, Jiang Y. Characterization and antioxidant activity of selenium containing phycocyanin isolated from *Spirulina platensis*. Food Chem 2007;100:1137-43.
76. Patel A, Mishra S, Ghosh PK. Antioxidant potential of C-phycocyanin isolated from cyanobacterial species *Lyngbya*, *Phormidium* and *Spirulina* spp. Indian J Biochem Biophys 2006;43:25-31.
77. Riss J, Décordé K, Sutra T, Delage M, Baccou JC, Jouy N, *et al.* Phycobiliprotein C-phycocyanin from *Spirulina platensis* is powerfully responsible for reducing oxidative stress and NADPH oxidase expression induced by an atherogenic diet in hamsters. J Agric Food Chem 2007;55:7962-7.
78. Cherng SC, Cheng SN, Tarn A, Chou TC. Anti-inflammatory activity of c-phycocyanin in lipopolysaccharide-stimulated RAW 264.7 macrophages. Life Sci 2007;81:1431-5.
79. Bai SK, Lee SJ, Na HJ, Ha KS, Han JA, Lee H, *et al.* Beta-carotene inhibits inflammatory gene expression in lipopolysaccharide-stimulated macrophages by suppressing redox-based NF-kappaB activation. Exp Mol Med 2005;37:323-34.
80. Katsuura S, Imamura T, Bando N, Yamanishi R. beta-Carotene and beta-cryptoxanthin but not lutein evoke redox and immune changes in RAW264 murine macrophages. Mol Nutr Food Res 2009;53:1396-405.

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