Enteromorpha flexuosa Improves Insulin Sensitivity and Metabolic Control in Fructose-Induced Diabetic Rats

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Abstract
Type 2 Diabetes mellitus (T2DM) is the most common form of the disease and its complications constitute a major public health problem. Currently, there is much interest in the usefulness of seaweeds for the treatment of T2DM. We have attempted to investigate the anti-hyperglycemic, anti-hyperlipidemic and insulin sensitizing effects of Enteromorpha flexuosa in an established model of T2DM characterized by insulin resistance. Rats were fed 30% fructose solution in drinking water for 4 weeks. Animals exhibited hyperglycemia and hyperinsulinemia were selected. Diabetic and control rats were orally supplemented with 50 mg/kg body weight E. flexuosa extract for 4 weeks. At the end of 8 weeks, serum glucose, insulin, cholesterol, triglyceride and cardiovascular risk indices, as well as insulin resistance were significantly increased in fructose-fed rats. Treatment of the fructose-fed rats with E. flexuosa significantly improved this metabolic profile. Fructose supplementation produced a significant increase in serum pro-inflammatory cytokines and decreased adiponectin levels. In addition, fructose-fed rats showed significantly increased lipid peroxidation levels in liver, kidney and heart accompanied with declined glutathione content and activity of the antioxidant enzymes. Supplementation of E. flexuosa markedly alleviated these alterations. Our study demonstrates that E. flexuosa is effective in improving insulin sensitivity while attenuating metabolic disturbances, inflammation and oxidative stress in fructose-fed rats. Therefore, E. flexuosa seems to have a promising value for the development of an effective phytomedicine for the treatment of T2DM.

INTRODUCTION
Type 2 Diabetes mellitus (T2DM) is a metabolic disease characterized by the presence of chronic hyperglycemia that results from defective or deficient insulin [1,2]. It is the most common form of the disease, which accounts for more than 90% of all diabetic patients [3]. The incidence of diabetes is increasing worldwide and T2DM and its complications constitute a major public health problem [4]. It is predicted that T2DM will continue to increase in developing countries with the majority of patients being 45-64 years old [5]. According to the International Diabetes Federation, the number of patients with diabetes mellitus in 2013 was estimated to be 382 million, and is expected to increase to 592 million by 2035. In addition, health spending on diabetes accounted for 10.8% of the total health expenditure worldwide and the disease caused 5.1 million deaths in 2013 [6].

A wide variety of lifestyle factors, such as sedentary lifestyle [7], physical inactivity [8], smoking [9], and alcohol consumption [10], are of great importance to the development of T2DM. Also, diet is considered as a modifiable risk factor for T2DM. Because of an increase in using sucrose and high fructose syrup, the consumption of fructose has been increased markedly in the last few centuries [11]. It has been reported that high fructose intake over long periods is hazardous for human beings as well as animals [12,13]. In addition, studies have demonstrated that consumption of high fructose diets results in fatty liver, hyperlipidemia, and insulin resistance [14,15]. Further, Tappy and Le [16], revealed that fructose is almost completely metabolized in the liver and increases de novo lipogenesis. More recently, Wang et al., [17], demonstrated that fructose induce adipose tissue insulin resistance in rats.

Adipose tissue is now recognized as a secretory organ that plays important role in insulin sensitivity and energy expenditure [18], and dysfunction in adipocytes is associated...
with insulin resistance and type 2 diabetes [19]. Adipocytes are understood to secrete diverse pro-inflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α, as well as anti-inflammatory cytokines such as adiponectin [20]. Increased levels of TNF-α and IL-6, and reduced level of adiponectin can exacerbate insulin resistance in adipose tissue [19].

Currently, there is much interest in the usefulness of seaweeds for the treatment of diabetes. Seaweeds were traditionally used in Japanese, Korean and Chinese diet since ancient times [21]. Epidemiological evidence suggests that regular seaweed consumption may protect against a range of diseases of modernity [22]. Seaweeds are a natural source of a variety of biologically active components [23], with a broad range of biological activities, such as antibacterial, anti-inflammatory, anticoagulant, hepatoprotective, antiviral and renoprotective [24-28]. Seaweeds are potentially good sources of vitamins, proteins, fiber contents, polysaccharides, polyphenols, mineral, and essential fatty acids [29]. Recently, the green algae Enteromorpha flexuosa (Wulfen) has been found to exhibit antiviral activity [30], and to protect against the hepatotoxic effects of diethylnitrosamine [31]. To the best of our knowledge, the anti-diabetic effect of E. flexuosa against fructose-induced diabetes has not previously been assessed. Therefore, the current study was designed to investigate the anti-hyperglycemic, anti-hyperlipidemic and insulin sensitizing effects of E. flexuosa in an established model of T2DM characterized by insulin resistance. This investigation could promote an understanding of its antidiabetic mechanism, especially to modulation of adiponectin, pro-inflammatory cytokines and oxidative stress.

**MATERIALS AND METHODS**

**Preparation and preliminary phytochemical screening of E. flexuosa extract**

E. flexuosa was collected from the intertidal region of the Red Sea shores between Quseir and Marsa-Alam (Egypt). The samples were authenticated and a voucher sample has been deposited in the Herbarium of the Faculty. The collected samples were then cleaned, washed thoroughly with sea water followed by distilled water, air-dried and ground to a fine powder then extracted by 80% aqueous ethanol [26,28]. Following filtration, the filtrate was concentrated under reduced pressure in a rotary evaporator and was stored at -20°C. The included 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 normal control rats.
- **Group 2 (Control + E. flexuosa):** received 50 mg/kg b.wt. E. flexuosa extract suspended in 1% CMC and served as drug control.
- **Group 3 (Diabetic):** received 30% fructose in tap water.
- **Group 4 (Diabetic + E. flexuosa):** received 30% fructose in tap water and 50 mg/kg b.wt. E. flexuosa extract suspended in 1% CMC.

E. flexuosa extract has been administered by oral gavages for 4 weeks. The doses were balanced consistently as indicated by any change in body weight to keep up comparable dosage for every kg body weight over the entire period of study. 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 (3g/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 [33], using reagent kit purchased from Spinreact (Spain).

**Determination of serum insulin, adiponectin, TNF-α and IL-6:** Serum levels of insulin, adiponectin, TNF-α and IL-6 were determined using specific ELISA kits (R&D systems) following the manufacturer’s instructions. The concentrations of assayed parameters were measured spectrophotometrically at 450 nm. Standard curves were constructed by using standard cytokines 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) [34], as follows:

\[
HOMA-IR = \frac{Fasting \text{ insulin (μU/ml)} \times Fasting \text{ blood glucose (mmol/L)}}{225}
\]
Determination of lipid profile and cardiovascular risk indices: Serum total cholesterol [35], triglycerides [36], and HDL-cholesterol [37], were assayed using commercial diagnostic kits (Spinreact, Spain). Serum vLDL-cholesterol concentration was calculated according to the following formula [38]: vLDL-cholesterol = triglycerides/5. Serum LDL-cholesterol level was calculated from the formula [39]: LDL-cholesterol = Total cholesterol - [(Triglycerides/5) + HDL-cholesterol]. Cardiovascular risk indices were calculated according to Ross [40], 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 [41]: AAI = HDL-cholesterol x 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 [42], Teitz and Andresen [43] and Kachmar and Moss [44], 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 [45]. Reduced glutathione (GSH) content and, activity of the antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured according to the methods of Beutler et al. [46], Marklund and Marklund [47] and Matkovics et al. [48], respectively.

Statistical analysis: Data were analysed using GraphPad 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 (SEM) and a P value <0.05 was considered significant.

RESULTS

Qualitative phytochemical screening revealed the presence of sterols, carbohydrates and/or glycosides, tannins, flavonoids, saponins and triterpenes in the tested seaweed (Table 1).

OGTT of fructose-induced diabetic rats showed significantly (P<0.001) elevated fasting glucose levels and at 30, 60, 90 and 120 minutes after oral glucose loading when compared with the normal control rats (Figure 1). Oral supplementation of *E. flexuosa* extract to fructose-induced diabetic rats significantly alleviated the blood glucose levels at all points of the OGTT. The OGTT areas under curve (AUCs) of the glucose response in control and diabetic rats are shown in (Figure 2). There was non-significant (P>0.05) difference between the AUCs of normal and *E.flexuosa* supplemented normal rats. On the other hand, fructose-induced diabetic rats exhibited a significant (P<0.01) increase in AUCs when compared with the normal control rats. Interestingly, treatment of the diabetic rats with *E. flexuosa* potentially (P<0.01) decreased OGTT AUC when compared with the diabetic control rats.

Serum insulin level was significantly (P<0.001) increased in fructose fed rats compared with the normal rats as depicted in (Table 2). Oral treatment of the fructose-induced diabetic rats with *E. flexuosa* markedly ameliorated serum insulin levels. Similarly, diabetic rats exhibited a significant (P<0.001) increase in HOMA-IR when compared with either normal or *E. flexuosa* supplemented normal rats (Table 2). Oral administration of *E. flexuosa* to fructose-induced diabetic rats significantly decreased HOMA-IR index.

Conversely, serum adiponectin showed a significant (P<0.01)
decrease in diabetic rats as compared to the normal control ones as represented in (Table 2). Treatment of the diabetic rats with E. flexuosa extract potentially (P<0.01) alleviated serum adiponectin levels. The effect of E. flexuosa on serum pro-inflammatory cytokines of normal and fructose-induced diabetic rats showed a significantly increased levels of TNF-α (P<0.01) and IL-6 (P<0.001) in diabetic rats and potential alleviation following treatment with E. flexuosa extract (Table 1). However non-significant, E. flexuosa supplementation to normal rats decreased serum levels of TNF-α when compared with the corresponding normal control rats. Data represented in (Figure 3) show the effect of E. flexuosa on lipid profile, cardiovascular risk indices and antiatherogenic index of normal and diabetic rats. Compared to the normal control group, rats supplemented with E. flexuosa exhibited non-significant (P>0.05) changes in lipid profile parameters. In contrast, 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 their corresponding normal rats. HDL-cholesterol showed non-significant (P>0.05) variation between all studied groups. In addition, diabetic rats showed markedly (P<0.001) elevated HDL-cholesterol/T. cholesterol and LDL-cholesterol/HDL-cholesterol. Moreover, the antiatherogenic index was significantly (P<0.01) declined in diabetic rats. By comparison, the oral supplementation of E. flexuosa extract to diabetic rats potentially alleviated the altered serum lipid profile as well as cardiovascular risk indices (Figure 3).

After 8 weeks, serum AST, CK-MB and LDH activities were significantly increased in the diabetic group as compared to the normal control rats (Table 3). Treatment of the diabetic rats with E. flexuosa significantly ameliorated serum activities of AST (P<0.01), CK-MB (P<0.05) and LDH (P<0.001). More interestingly, oral administration of E. flexuosa to normal rats significantly (P<0.05) decreased serum LDH activity when compared with normal control rats.

Concerning lipid peroxidation, diabetic rats exhibited significantly increased MDA levels in liver (P<0.001), kidney (P<0.05) and heart (P<0.01) as compared to their respective normal controls (Figure 4). Treatment of the fructose-induced diabetic rats with E. flexuosa extract markedly alleviated liver (P<0.05), kidney (P<0.01) and heart (P<0.05) lipid peroxidation levels. Oral supplementation of E. flexuosa to normal rats exerted a non-significant (P>0.05) change in lipid peroxidation levels.

On the contrary, fructose supplementation significantly decreased liver (P<0.01), kidney (P<0.05) and heart (P<0.01) GSH content when compared with the normal control group (Figure 5). GPx activity showed a similar pattern where it was significantly declined in the liver (P<0.05), kidney (P<0.05) and heart (P<0.01) of fructose-induced diabetic rats as represented in (Figure 6). Similarly, SOD activity was significantly decreased in the liver (P<0.001) and heart (P<0.01) of diabetic rats when compared with the normal ones (Figure 7). Although declined, renal SOD activity of the diabetic rats showed a non-significant difference as compared to the normal control rats. On the other hand, treatment of the fructose-induced diabetic rats with E. flexuosa potentially ameliorated GSH content as well as activities of SOD and GPx in the liver, kidney and heart.

**Table 2:** Effect of E. flexuosa on serum insulin, HOMA-IR, adiponectin, TNF-α and IL-6 of control and fructose-fed rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Control + E. flexuosa</th>
<th>Diabetic</th>
<th>Diabetic + E. flexuosa</th>
<th>F-prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (µU/ml)</td>
<td>30.77 ± 1.82</td>
<td>22.98 ± 1.26</td>
<td>61.52 ± 3.82***</td>
<td>40.41 ± 2.09**</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>7.32 ± 0.62</td>
<td>4.95 ± 0.17</td>
<td>29.98 ± 2.69***</td>
<td>16.51 ± 0.14***</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>19.99 ± 1.68</td>
<td>20.28 ± 1.60</td>
<td>10.07 ± 0.34**</td>
<td>19.36 ± 1.57**</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>23.18 ± 2.43</td>
<td>13.43 ± 1.56</td>
<td>56.05 ± 4.57**</td>
<td>32.80 ± 4.34**</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>21.02 ± 2.72</td>
<td>23.43 ± 3.59</td>
<td>77.32 ± 6.37***</td>
<td>45.13 ± 5.81*</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

Results are mean ± SEM (N = 6). *P<0.05, **P<0.01 and ***P<0.001 vs Control, and *P<0.05, **P<0.01 and ***P<0.001 vs Diabetic group. HOMA-IR, Homeostasis model assessment estimate of insulin resistance; TNF, Tumor necrosis factor; IL, Interleukin.

**Table 3:** Effect of E. flexuosa on serum AST, CK-MB and LDH of control and fructose-fed rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Control + E. flexuosa</th>
<th>Diabetic</th>
<th>Diabetic + E. flexuosa</th>
<th>F-prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td>19.40 ± 2.98</td>
<td>17.07 ± 1.94</td>
<td>48.30 ± 6.41***</td>
<td>18.80 ± 1.28**</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>CK-MB (U/dl)</td>
<td>18.57 ± 1.84</td>
<td>16.63 ± 1.51</td>
<td>29.00 ± 1.80***</td>
<td>21.47 ± 1.98*</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>44.36 ± 7.26</td>
<td>10.90 ± 0.97†</td>
<td>138.50 ± 10.54***</td>
<td>20.89 ± 3.32***</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

Results are mean ± SEM (N = 6). *P<0.05, †P<0.01 and ‡P<0.001 vs Control, and *P<0.05, **P<0.01 and ***P<0.001 vs Diabetic group. AST, Aspartate aminotransferase; LDH, Lactate dehydrogenase; CK-MB, Creatine kinase MB.
Figure 3 Effect of *E. flexuosa* on lipid profile, cardiovascular risk indices and antiatherogenic index (AAI) of control and fructose-fed rats. Results are mean ± SEM (N = 6). *P<0.05, **P<0.01 and ***P<0.001 vs Control, and #P<0.05, ##P<0.01 and ###P<0.001 vs Diabetic group. T. Cholesterol, Total cholesterol; HDL, High density lipoprotein; LDL, Low density lipoprotein; vLDL, Very low density lipoprotein.
DISCUSSION

Type 2 Diabetes mellitus is a metabolic disorder characterized by the presence of chronic hyperglycemia, which results from resistance to insulin actions on peripheral tissues [1]. Insulin resistance both precedes and predicts T2DM; therefore, the development of drugs targeted to reverse it is of paramount importance [49]. Several studies have pointed to the deleterious effects of fructose on glucose metabolism and insulin sensitivity. In the current study, fructose-fed rats exhibited significantly impaired glucose tolerance accompanied with elevated insulin and HOMA-IR. Hence, 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 testing [2]. Previous studies have demonstrated that long term fructose feeding induces diabetes associated with insulin resistance in experimental animals [50-52]. In addition, a high-fructose diet increased fasting glycemia [53] and led to hepatic insulin resistance in healthy men [54].

The fructose-induced insulin resistance may be linked to alteration of insulin signaling. In this concern, Eiffert et al., [55], reported that a high-fructose diet decreased insulin-induced insulin receptor and insulin receptor substrate (IRS)-1 phosphorylation in rat skeletal muscles. Also, fructose-induced hyperlipidemia [56] and fat deposition [57], may generate lipid-derived metabolites which lead to a higher serine/threonine phosphorylation of IRS-1 and reduced insulin signaling [58]. Treatment of the diabetic rats with *E. flexuosa* markedly reduced blood glucose and improved insulin sensitivity. In agreement to our findings, a recent study conducted by Abouzid et al., [59], reported the antihyperglycemic effect of *E. intestinalis* in streptozotocin/nicotinamide diabetic mice.

Insulin resistance in T2DM is also associated with hyperlipidemia and atherosclerosis [60]. Fructose-fed rats in the present study exhibited hypercholesterolemia and hypertriglyceridemia. The fructose-induced dyslipidemia may be attributed to the increased de novo hepatic lipogenesis through providing large amounts of hepatic triose-phosphate.
for fatty acid synthesis [16]. In addition, studies reported that fructose increases the expression of key lipogenic enzymes and induces the expression of sterol regulatory element binding protein (SREBP)-1c which is the principal inducer of hepatic lipogenesis [61,62]. Moreover, fructose has been shown to activate carbohydrate-responsive element binding protein (ChREBP), leading to upregulated expression of hepatic fatty acid synthase and acetyl-CoA carboxylase [63]. Activation of ChREBP may be attributed to the fructose-induced expression of glucose-6-phosphate dehydrogenase and intermediary substrates of the hexose-monophosphate shunt [64].

Insulin resistance along with the compensatory hyperinsulinemia increases the excretion of triglycerides by the liver and elevates serum LDL-cholesterol [65]. The elevated triglycerides and LDL-cholesterol levels and decreased HDL-cholesterol in the fructose-induced diabetic rats represent atherogenic lipid profile, which leads to the development of coronary heart diseases [66]. 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 E. flexuosa extract significantly alleviated the altered lipid profile parameters and atherogenic indices. Reduction of these indices in treated diabetic rats strongly supported the notion that dietary supplementation with E. flexuosa may reduce the risk of developing heart diseases. These findings were further supported by the significantly declined serum activities of the cardiac markers, CK-MB, AST and LDH, in E. flexuosa treated diabetic rats. Enteromorpha sp. is known to contain sulfated polysaccharides which have been reported to have antihyperlipidemic properties [67]. Tang et al., [68], demonstrated the hypolipidemic effect of a polysaccharide fraction from E. prolifera in high-fat diet-induced mice.

The anti-diabetic effect of E. flexuosa in fructose-induced diabetic rats might be explained, at least in part, through its ability to produce a pronounced increase in serum adiponectin levels. Serum levels of adiponectin were reported to be in agreement with insulin sensitivity and its reduced levels are associated with insulin resistance and T2DM [69]. Adiponectin regulates glucose metabolism through stimulation of adenine monophosphate-activated protein kinase (AMPK) [70], and increases muscle fat oxidation and glucose transport mediated through inhibition of acetyl-CoA carboxylase [71]. Also, adiponectin has been found to decrease the expression of phosphoenolpyruvate carboxylase and glucose-6-phosphatase, leading to inhibition of hepatic gluconeogenesis [70]. Further, activation of peroxisome proliferator activated receptor (PPAR)-α leading to decreased triglyceride content in skeletal muscles and liver, is one of the adiponectin functions [72].

Concerning pro-inflammatory cytokines, the present findings showed significantly increased circulatory levels of TNF-α and IL-6, which is strongly, correlated with insulin resistance in the diabetic control rats. Previous studies demonstrated that elevated IL-6 reduces IRS-1 tyrosine phosphorylation leading to decreased association between the PI-3 kinase and IRS-1 and an inhibition of insulin-dependent activation of Akt [73]. Also, Greenberg et al., [74] showed that IL-6 reduces lipoprotein lipase activity in vivo as well as in vitro. In addition, TNF-α impairs the ability of insulin to stimulate peripheral glucose uptake and to suppress hepatic glucose production [75]. Furthermore, TNF-α has been shown to increase circulating free fatty acids (FFAs) and thus contributes to the pathogenesis of insulin resistance [76]. Therefore, due to the link between inflammation and insulin resistance, therapeutic strategies that reduce levels of inflammatory cytokines and limit inflammation may be a promising tool [77]. Interestingly, treatment of the fructose-induced diabetic rats with E. flexuosa markedly decreased serum levels of TNF-α and IL-6, confirming its anti-inflammatory efficacy. The potent anti-inflammatory effect of E. flexuosa might be attributed to the contained compounds. Phytochemical analysis of E. flexuosa extract revealed the presence of phenols, tannins, flavonoids, sterols, saponins and terpenoids which coincides well with the work of Abirami and Kowsalya [78]. These compounds have been reported to exert hypoglycemic effect by several authors [79]. It has been shown that polyphenols including flavonoids decreased the release of inflammatory cytokines in insulin resistant diabetic rats [2] and ammonium chloride-induced hyperammonemic rats [80]. In addition, pheophytin, a chlorophyll-related compound, derived from Enteromorpha sp. showed potent in vivo and in vitro anti-inflammatory effect [81]. Sulfated polysaccharides isolated from Enteromorpha sp. are also known to have immunomodulating effects [82]. Moreover, the extract of Enteromorpha sp. has been demonstrated to inhibit the production of pro-inflammatory cytokines in RAW 264.7 cells [83]. We assume that suppression of the release of pro-inflammatory cytokines following E. flexuosa administration could be a direct result of increased serum adiponectin levels. It is well evidenced that adiponectininhibits the expression of the pro-inflammatory cytokines TNF-α and IL-6 in various tissues [84].

High fructose administration leads to insulin resistance and type 2 diabetes in rats through inducing oxidative stress [15]. The formation of reactive oxygen species (ROS) in oxidative stress can cause oxidation and damage to many cellular components such as DNA, lipids and proteins [85]. ROS could react with polyunsaturated fatty acids which lead to lipid peroxidation [86]. 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 [87]. Hence, it was recommended by Mahmoud et al., [2] that therapy with antioxidants may represent a useful pharmacologic overture to the management of insulin resistance and diabetes. In the present study, fructose-administered rats showed marked elevation in lipid peroxidation levels in liver, kidney and heart. Treatment of the fructose-induced diabetic rats with E. flexuosa extract potentially alleviated lipid peroxidation levels, which in turn reflects its radical scavenging property.

GSH and the antioxidant enzymatic defenses showed a simultaneous decline in the liver, kidney and heart of diabetic rats. The enzymatic and non-enzymatic antioxidant levels are known to decrease under hyperglycemia [88] and oxidative stress [89]. On the other hand, treatment of diabetic rats by E. flexuosa significantly alleviated levels of GSH and activity of the antioxidant enzymes SOD and GPx. GSH is an important...
antioxidant that protects cellular constituents against oxidative stress by reacting with oxidants or as a substrate for GPx [90]. Similarly, SOD and GPx provide a defense system against ROS-induced damage [91]. The antioxidant effect of Enteromorpha Sp. and their constituents has been previously reported. Ahmed et al., [31] reported that E. flexuosa extract protected against the deleterious effects of diethylnitrosamine through potentiating the antioxidant defense system. The contained sullated polysaccharides are known to have antioxidant effect [92]. In addition, seaweed extracts are considered to be a rich source of phenolic compounds [93], with antioxidant activity. These findings suggest that E. flexuosa protects against fructose-induced oxidative damage by attenuating lipid peroxidation and by enhancing the antioxidant defenses.

CONCLUSION

The current study provides new information on the antidiabetic mechanism of E. flexuosa in fructose-fed rats. High fructose feeding induces diabetes along with insulin resistance, inflammation and oxidative stress. Oral administration of E. flexuosa improves insulin sensitivity and serum adiponectin, and attenuates metabolic complications along with oxidative stress and inflammation in diabetic rats. Our findings suggest that E. flexuosa could be used as a dietary supplement in diabetes treatment, pending further studies to determine its exact mechanistic pathways.

REFERENCES


