Purslane extract in the form of ethanolic formulation is rich in polyphenols, flavonoids and anthocyanin, w-3 fatty acids and melatonin. The present study was designed to investigate the anti-obesity and anti-diabetic effects of purslane using obese diabetic rats. The rats received either regular diet, high-fat diet or high-fat diet with additional purslane (150 and 300 mg/kg body weight) for 8 weeks. Purslane, co-administered with a high fat diet, significantly inhibited body weight gain, blood glucose, triglyceride, total cholesterol, LDL-C, HDL-C, free fatty acids and the atherogenic index levels in a dose dependent manner. Purslane-treated rats at doses of 150 and 300 mg/kg body weight improved the insulin resistance index when compared to high fat diet control. In conclusion, purslane ethanolic extract showed effects indicative of potential anti-obesity and anti-diabetic actions in rats fed a high fat obesity-induced diet.

**Keywords**: Atherogenic index, insulin resistance index, purslane

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**INTRODUCTION**

Insulin resistance, a term used to describe the conditions of diminished response to insulin action, is associated with a number of multifactorial diseases including obesity and Type-2 - diabetes mellitus (T2DM). The latter two diseases have long been presumed to be related, even though the link between them has not been identified (Procopiou & Philippe, 2005). In recent years, polyphenols in plant products have been reported to possess various pharmacological actions, including anti-obesity (Ohta et al., 2006) and anti-diabetic activities (Klein et al., 2007; Tomonori, Tadashi & Ichiro, 2007).

The plant purslane, in Arabic ’Rejlah’, (*Portulaca oleracea* L.) occurs in the Arabian Peninsula and adjacent areas including, United Arab Emirates and Oman. Purslane is also consumed as a vegetable in some provinces of China (Chan et al., 2000; Hu, Xu & Wang, 2003). It is also used as an antibacterial and anti-viral agent, as well as for the treatment of viral hepatitis and diabetes management in China (Meng & Wu, 2008).

Purslane is reported to be rich in α-linolenic acid and β-carotene and used as a health food for patients with cardiovascular diseases (Liu et al., 2000). It contains several types of vitamins and minerals (Mohammad, Mohammad & Farhad, 2004), fatty acids (Xin et al., 2008), glutathione, glutamic acid, and aspartic acid. Other constituents include a mucilage composed of a neutral fraction with structure determined, dopamine and dopa.
coumarins, flavonoids, alkaloids, saponins, and anthocyanin (Peksel, Arisan & Yanardag, 2006). Recently, Hussein & Abdel-Gawad (2010) studied the potential hepato-protective effect of ethanolic and aqueous extracts of air-dried leaves of purslane against paracetamol-induced hepato-toxicity and showed that the ethanolic and aqueous extracts of purslane leaves can generate antioxidants. The effect was more pronounced in ethanolic extract compared to aqueous extract. Large amounts of phenolic compounds (coumarins, flavonoids, alkaloids, and saponins) in ethanolic extract may contribute towards the antioxidant properties (Sakai et al., 1996). Purslane has been described as a 'power food' of the future because of its high nutritive and anti-oxidant properties (Al-Howiriny, 2008).

In continuation of my research on therapeutic evaluation of plants of medical importance (Hussein & Abdel-Gawad, 2010), I report herein a facile route to explain the mechanism of anti-obesity and anti-diabetic effects of purslane ethanolic extract in obese diabetic rats fed a high-fat diet.

MATERIALS AND METHODS

Fresh leaves of purslane were collected from the Horbite farms in El-sharkia, Egypt. The leaves (1.5kg) were air-dried and crushed to coarse powder and extracted exhaustively in a Soxhlet with 95% ethanol. The extract was concentrated under reduced pressure to yield a viscous mass. The ethanolic extract was kept in airtight containers at 40 °C until further use.

Adult albino rats weighing around 150 ± 5 gms were purchased from the Faculty of Veterinary Medicine, Cairo University. The animals were housed in individual cages with free access to water in a temperature-controlled facility with a 12:12-hour light-dark cycle, and were weighed periodically. During the acclimatisation period, each animal was raised on a regular diet (Dyets Inc., Bethlehem, PA) *ad libitum*.

Experimental set up

This experiment was carried out to examine the anti-obesity and anti-diabetic affects of purslane ethanolic extract in obese diabetic rats fed a high-fat diet. Ethanolic extract of purslane leaves was given repeatedly for an 8-week period *in vivo*. A suspended solution of 3 g % was prepared for intragastric intubation of rats. The animals were randomly divided into five groups of 6 rats in each, two control groups and three treatment groups.

Control Group-I received a regular diet + 1 ml tween 80 for an 8-week period, while Control Group-II received a high-fat diet + 1ml tween 80 for an 8-week period. Group III was fed a high-fat diet with purslane ethanolic extract (150 mg/kg body weight/ml tween 80) suspended in tween 80 orally in a single daily dose for an 8-week period (Al-Howiriny, 2008). Group IV was fed a high-fat diet with purslane ethanolic extract (300 mg/kg bw/ml tween 80) suspended in tween 80 orally in a single daily dose for an 8-week period (Al-Howiriny, 2008). Group V was given a high-fat diet with metformin (500 mg/kg body weight/ml tween 80) suspended in tween 80 orally in a single daily dose for an 8-week period (Lee & Morley, 1998).

Hyperglycemia induction diet was purchased from Dyets Inc. (AIN-76 diet #101772, Bethlehem, PA, USA). The nutrition contents of the high fat diet were similar to those of the regular diet except for the addition of beef tallow (Table I) (Assinewe et al., 2003). Body weights were measured weekly, and every other week, blood was collected for blood glucose analysis. At the end of the study, blood was also collected for the determination of plasma insulin and lipid levels, after which the animals were sacrificed.
Blood sampling and plasma assay

Blood was drawn from the orbital venous plexus every other week using a heparinised capillary tube without anesthesia. The blood samples were placed on ice, centrifuged, and the plasma was stored at -20°C until assayed. The plasma glucose concentration was determined using the glucose oxidase method (Youngdong Pharmaceutical Co, Korea). The plasma insulin concentration was measured according to the protocol described by the manufacturer of the mouse insulin ELISA kit (Shibayagi Co., Japan). The insulin resistance index, a simple method to measure insulin sensitivity usually used in clinical and animal studies (Sasaki et al., 2009), was calculated by insulin (mU/ml) X glucose (mM)/22.5 (Matthew et al., 1985).

Plasma glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) concentrations were quantified using Transaminase CII Test (Wako Pure Chemical Industries).

Measurement of liver triglyceride and cholesterol contents

Liver triglyceride and cholesterol contents were measured as described in Park, Ko & Chung (2005). Briefly, a portion (100 mg) of liver tissue was homogenised in phosphate buffer saline (pH 7.4, 1 ml). The homogenate (0.2 ml) was extracted with isopropyl alcohol (1 ml), and the extract was analysed using a Triglyceride E-Test (Wako Pure Chemical Industries) to determine liver triglyceride content. The homogenate (0.2 ml) was extracted with chloroform-methanol (2: 1, 1 ml), and the extract was concentrated under a nitrogen stream. The residue was dissolved in isopropyl alcohol and analysed using a Cholesterol E-Test (Wako Pure Chemical Industries).

Table 1. Composition of the diets fed to the rats

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Regular diet (g/kg diet)</th>
<th>High fat diet (g/kg diet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>3.0</td>
<td>3</td>
</tr>
<tr>
<td>Corn Starch</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Sucrose</td>
<td>500</td>
<td>150</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>Corn oil</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>Beef tallow</td>
<td>-</td>
<td>400</td>
</tr>
<tr>
<td>Mineral mixturea</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin mixturea</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Energy, kJ/g</td>
<td>0.9</td>
<td>1.30</td>
</tr>
<tr>
<td>Protein, % kcal/kg</td>
<td>13.3</td>
<td>13.3</td>
</tr>
<tr>
<td>Carbohydrate, % kcal/kg</td>
<td>47.4</td>
<td>19.8</td>
</tr>
<tr>
<td>Fat, % kcal/kg</td>
<td>8.0</td>
<td>65.7</td>
</tr>
<tr>
<td>Fibre, % kcal/kg</td>
<td>8.0</td>
<td>-</td>
</tr>
<tr>
<td>Other</td>
<td>23.3</td>
<td>1.3</td>
</tr>
</tbody>
</table>

aAIN 76A Rodent Purified Diet (Assinewe et al., 2003)
Abdalla Hussein M

Statistical analysis

All the grouped data were statistically evaluated with SPSS version 7.5. One way analysis of variance (ANOVA) followed by least significant difference (LSD) test was used to test treatment differences among the groups. *P* value of less than 0.05 was considered to indicate statistical significance. All the results were expressed as mean ± SD for six separate determinations.

RESULTS

Body weight and food intake were determined once every 2 weeks. The body weight of the normal rats in the regular diet group gradually increased as the rats grew during the 8-week trial. By contrast, the body weight of animals on the high fat diet showed rapid increases during the course of the trial (Table 2; Figure. 1). Weight gains in the regular diet and high fat diet control groups during the 8-week period were 17.7±2.4g and 56.3±4.1g, respectively. Animals fed the high fat diet and purslane ethanolic extract showed a gradual increase in body weight, but the increase was significantly less than that detected for the high fat diet control group in spite of continued and prolonged access to the high fat diet (Table 2; Figure. 1). Purslane 150 and 300 mg/kg body weight seemed to inhibit rapid weight gain, compared to the body weight gain shown by the high fat diet control group, by 11.45% and 35.41% respectively.

Feed efficiency, as calculated by weight gain divided by total food intake, during the 8-week period was compared in order to figure out the relationship between food intake and weight gain. As shown in Table 2, weight gain of the high fat diet control rats was actually due to the increased food intake. However, the body weights of the purslane extract fed rats were significantly reduced, despite a larger increase in food intake compared to the high fat diet control rats. Feed efficiency of the purslane ethanolic extract (300 mg/kg) fed group was 2.3, which is lower than that for the high fat diet

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial</th>
<th>Final</th>
<th>Weight gain (g/8 wk)</th>
<th>Food intake (g/8 wk)</th>
<th>Feed efficiency (× 10⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group-I Regular diet (RD)</td>
<td>150.7±4.2</td>
<td>168.4±9.4</td>
<td>17.7 ±2.4</td>
<td>9567</td>
<td>1.9</td>
</tr>
<tr>
<td>Control group-II High-fat diet (HFD)</td>
<td>150.6±3.9</td>
<td>206.9±5.81*</td>
<td>56.8±4.1*</td>
<td>10989*</td>
<td>5.12*</td>
</tr>
<tr>
<td>HFD+ Purslane extract 150 mg/kg bw</td>
<td>150.4±4.8</td>
<td>200.7±7.2*</td>
<td>50.31±4.5*</td>
<td>11149*</td>
<td>4.5*</td>
</tr>
<tr>
<td>HFD+ Purslane extract 300 mg/kg bw</td>
<td>150.8± 5.6</td>
<td>187.1±4.6*</td>
<td>36.70±3.2*</td>
<td>11062*</td>
<td>3.2*</td>
</tr>
<tr>
<td>HFD+ Metformin 500 mg/kg bw</td>
<td>150.2±7.2</td>
<td>176.2±5.2*</td>
<td>26.0±2.9*</td>
<td>9320*</td>
<td>2.7*</td>
</tr>
</tbody>
</table>

Values represent the mean ± SE (n=6). High-fat diet (HFD) control rats were compared with regular diet (RD) control rats. Experimental groups were compared with the high-fat diet control rats.

• *Significantly different from normal group at *p* < 0.05.
• @ Significantly different from control group at *p* < 0.01.

Feed efficiency = [weight gain (g/8 wk)]/[food intake (g/8 wk)]

Table 2. Effect of purslane ethanolic extract on weight gain, food intake, and feed efficiency
control group, indicating that purslane extract has the potential to control body weight gain despite increased food intake. On the other hand, body weight of the metformin fed group was similar to that of the regular diet fed group, due to a significant reduction in food intake compared to the high fat diet control group.

**Insulin resistance index**

Plasma glucose was determined every other week and compared among groups. Plasma glucose levels were barely increased in the regular diet fed control group, while a marked increase after 8 weeks was observed for rats fed with the high fat diet (Table 3). Purslane ethanolic extract fed rats, however, showed a significant decrease in blood glucose levels in a dose dependent manner when compared to the high fat diet control group. Plasma glucose levels in purslane 150 and 300 mg/kg body weight treated groups were also markedly decreased by 28.4% and 36.4%, respectively when compared to the high fat diet control group.

The insulin resistance index, calculated by insulin (mU/ml) X glucose (mM)/22.5, of the high fat diet control group was 3.5 times higher than that of the regular diet group, while the insulin resistance indices of purslane 150 and 300 mg/kg body weight were significantly reduced by 55.5% and 62%, respectively, when compared to the high fat diet control group (Table 3). Plasma insulin levels in purslane 150 and 300 mg/kg body weight treated groups were also markedly decreased by 36.4% and 40.3%, respectively when compared to the high fat diet control group. Improvement of insulin resistance in the purslane ethanolic extract (300 mg/kg body weight) fed group was significant when compared to the metformin (500 mg/kg bw) fed group. These results suggest that the purslane extract was able to lower the blood glucose level partially due to the improvement in insulin resistance.
Liver triglyceride and cholesterol contents

At the end of administration, liver triglyceride and cholesterol levels were significantly higher for the high fat diet fed group when compared to the regular fed group (Table 4). For both high fat diet/purslane 150 and 300 mg/kg body weight groups, triglyceride and cholesterol accumulation were significantly suppressed. Liver triglyceride levels in
Table 5. Effect of purslane ethanolic extract on plasma triglyceride (TG), total Cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), nonesterified fatty acid (NEFA and atherogenic index.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TG (mg/dl)</th>
<th>TC (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>NEFA (mEq/dl)</th>
<th>Atherogenic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group-I Regular diet (RD)</td>
<td>115.62±5.1</td>
<td>151.64±6.71</td>
<td>92.63±6.19</td>
<td>35.97±3.88</td>
<td>723.6±23.09</td>
<td>0.096±0.008</td>
</tr>
<tr>
<td>Control group-II High-fat diet (HFD)</td>
<td>195.3±9.34</td>
<td>325.42±21.7</td>
<td>42.22±5.76@</td>
<td>244.14±17.5@</td>
<td>1406±31.68@</td>
<td>0.665±0.021@</td>
</tr>
<tr>
<td>HFD+ Purslane extract 150 mg/kg bw</td>
<td>170.72±6.11</td>
<td>240.1±17.4*</td>
<td>65.12±4.33@</td>
<td>140.84±10.2@</td>
<td>1088±16.44@</td>
<td>0.419±0.021@</td>
</tr>
<tr>
<td>HFD + Purslane extract 300 mg/kg bw</td>
<td>146.20±11.9</td>
<td>190.43±13.2@</td>
<td>71.3±6.09@</td>
<td>89.89±7.22@</td>
<td>997±15.81@</td>
<td>0.312±0.017@</td>
</tr>
<tr>
<td>HFD+ Metformin 500 mg/kg bw</td>
<td>122.4±9.44</td>
<td>180.71±10.43@</td>
<td>80.43±5.66@</td>
<td>75.8±5.37@</td>
<td>816±13.47@</td>
<td>0.182±0.014@</td>
</tr>
</tbody>
</table>

Values represent the mean ± SE (n=6). High-fat diet (HFD) control rats were compared with regular diet (RD) control rats. Experimental groups were compared with the high-fat diet control rats.

- *Significantly different from normal group at p< 0.05.
- @ Significantly different from control group at p< 0.01.

LDL-C (mg/dl) = TC-HDL-[TG/5]

Atherogenic index = log (TG/HDL-C)
purslane 150 and 300 mg/kg body weight treated groups were markedly decreased by 42.8% and 60.1%, respectively when compared to the high fat diet control group. Liver total cholesterol levels in purslane 150 and 300 mg/kg body weight treated groups were also markedly decreased by 20.4% and 43.3%, respectively when compared to the high fat diet control group.

On the other hand, plasma GOT and GPT activities were significantly higher for the high fat diet fed group than for the regular diet fed group by 3.85-fold and 2.66-fold. Purslane ethanolic extract administration significantly suppressed increases in GOT and GPT. Plasma GOT activity in purslane 150 and 300 mg/kg body weight treated groups was markedly decreased by 48.6% and 56.6%, respectively when compared to the high fat diet control group. Plasma GPT activity in purslane 150 and 300 mg/kg body weight treated groups was also markedly decreased by 67.04% and 76.12%, respectively when compared to the high fat diet control group.

Plasma lipid levels

The effects of purslane ethanolic extract on plasma lipid levels were examined at the end of the treatment. The plasma lipid levels in the high fat diet fed rats were substantially increased compared to the levels in the regular diet fed rats, except for the HDL-cholesterol (HDL-C) level (Table 5). In the high fat diet control group, plasma triglyceride (TG) was increased by 1.7 times (115 to 195 mg/dl), total cholesterol by 2.2-fold, LDL-cholesterol by 6.8-fold, free fatty acid by 1.9-fold, and total cholesterol (TC) increased as reflected in the increase in LDL-cholesterol (LDL-C) concentrations, compared to the respective values in the regular diet group. Purslane 150 and 300 mg/kg body weight treated groups, however, showed considerably reduced levels of TG, TC, LDL-C, and free fatty acid (25.1%, 41.48%, 63.18%, and 29.08% inhibition in the purslane 300 mg/kg body weight fed group), while they showed an increased level of HDL-C compared to that in high fat diet fed control group (68.88% in purslane 300 mg/kg body weight fed group) in a dose dependent manner.

The atherogenic index, calculated by log (TG/HDL-C), of the high fat diet control group, was 6.9 times higher than that of the regular diet group, while the atherogenic index of purslane 150 and 300 mg/kg body weight was significantly reduced by 37.4% and 53.1%, respectively, when compared to the high fat diet control group (Table 5). Metformin markedly improved the high fat diet induced dyslipidemia, and all lipid related plasma parameters in the metformin fed rats were comparable to those in the regular diet fed rats.

DISCUSSION

The anti-obesity effects of purslane ethanolic extract were investigated using obese diabetic rats fed a high-fat diet as a model of obese type-II diabetes. When fed a high-fat diet, these rats developed obesity and type-II diabetes by 12-weeks old (Hayashi & Ito, 2002), and are thus widely used for research in obesity and diabetes (Tsuchida et al., 2005). In the present study, a high-fat diet was administered to 8-week-old rats to induce severe obesity and diabetes and the effects of purslane ethanolic extract were evaluated. A high-fat diet is widely used in studies on obesity and diabetes (Hildebrandt, Kelly-Sulliwan & Black, 2003).

Purslane was found to significantly suppress increases in body weight, showing potential for anti-obesity actions. Plasma glucose and insulin levels were significantly higher for the high-fat diet group than for the regular diet group, and when severe type II diabetes was induced. Purslane suppressed these increases in plasma glucose and insulin levels. The insulin index was significantly decreased in the purslane-treated groups compared to the high-fat diet group, indicating the probable effect of
Purslane Extract Effects on Obesity-Induced Diabetic Rats Fed a High-Fat Diet

Purslane in reducing hyperglycemia and hyperinsulinemia.

Purslane ethanolic extract was found to significantly suppress increases in liver triglyceride and cholesterol content, showing apparent anti-obesity actions. The high-fat diet also increased liver fat accumulation and induced fatty liver, but purslane administration lowered fat accumulation, indicating that purslane ethanolic extract suppressed TG, TC, LDL-C, and free fatty acid while showing an increased level of HDL-C compared to the high-fat diet fed control group. The elevation of TG, TC, LDL-C, and free fatty acids values on high fat diet feeding is in agreement with other studies (Ahmed, Alierza & Mahboobeh, 2007). The substantial reduction in the LDL fraction offers a clinical benefit of the purslane extract.

The melatonin concentration in purslane was found to exceed that reported in a number of other fruits and vegetables (Simopouloes et al., 2005). Melatonin has a variety of important functions including direct free radical scavenging and anti-inflammatory properties (Rodriguez et al., 2004). Hayos et al. (2000) showed that increases in total cholesterol and LDL-C induced by a high fat diet was reduced significantly by melatonin administration. Melatonin in purslane extract may play a role in the observed anti-obesity and anti-diabetic effects.

Consumption of plant foods is associated with a lowered risk of major chronic diseases including diabetes, cardiovascular diseases and cancer (Crozier, Jaganath & Clifford, 2009). Among these plant resources, purslane contains numerous common nutrients including having the highest concentration of ω-3 fatty acids among leafy vegetables (Xin et al., 2008). Other bioactives found in purslane are dopamine, dopa, coumarins, alkaloids and saponins (Sakai et al., 1996), polyphenols, flavonoids and anthocyanin (Peksel et al., 2006). These compounds may influence glucose metabolism by several mechanisms, such as inhibition of carbohydrate digestion and glucose absorption in the intestine, stimulation of insulin secretion from the pancreatic β-cell, modulation of glucose release from liver, activation of insulin receptors and glucose uptake in the insulin-sensitive tissues, and modulation of hepatic glucose output. This study suggests that purslane ethanolic extract containing polyphenols and ω-3 fatty acids may act on the liver to increase energy expenditure of related fatty liver degradation. Furthermore, purslane ethanolic extract may decrease mRNA expression of acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS), the rate-limiting enzymes of fatty acid synthesis in the liver, and mRNA expression of sterol regulatory element-binding protein (SREBP)-1c (Kim et al., 2001), which controls the expression of these enzymes (Guillou, Martin & Pineau, 2008).

The study findings indicate that the anti-obesity actions of purslane ethanolic extract may be due to increased energy expenditure-related fatty liver degradation and decreased fatty acid synthesis and fat intake in the liver. On the other hand, insulin resistance can be generated by decreased adiponectin secretion (Kadowaki et al., 2006). The purslane used in the present study acted on fatty liver and was shown to possess anti-obesity and anti-diabetic actions. While many studies have described plant extracts exhibiting hypolipidemic and anti-diabetic actions, to the best of my knowledge, none have demonstrated anti-obesity and anti-diabetic actions via reduction of insulin resistance and atherogenic index. Preliminary phytochemical screening of purslane revealed the presence of flavonoids or bioflavonoids which are natural products capable of modulating insulin resistance and atherogenic index.

CONCLUSION

Purslane ethanolic extract in this study showed effects indicative of potential anti-obesity and anti-diabetic actions in rats fed...
a high fat obesity-induced diet. High content of flavonoids, phenolic compounds, melatonin and omega-3 fatty acids found in ethanolic extract may be responsible for these effects. Further studies are in progress to determine the anti-hyperglycemic and hypoglycemic effects of different fractions of the purslane extract.

REFERENCES


