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Purslane Seeds Fixed Oil as a Functional Food in Treatment of Obesity Induced by High Fat Diet in Obese Diabetic Mice

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Abstract

The GC-MS analyses of purslane seeds fixed oil revealed the presence of 7 components. 9,12,15-octadecatrienoic acid methyl ester was the major constituent of purslane seeds fixed oil representing (41.18%) followed by, 9, 12-octadecadienoic acid methyl ester (27.23%). The aim of the present article is to investigate the effects of purslane fixed oil (150 and 300g/kg of diet) in obese diabetic mice for 8 consecutive weeks prior. The results showed that purslane fixed oil has antidiabetic and antioxidant actions in experimental diabetic mice. Indeed, purslane fixed oil was significantly prevented the increase in the levels of body weight, blood glucose, triglycerides, total cholesterol, LDL-C, atherogenic index and thiobarbituric acid reactive substances (TBARS) in the liver tissue and improved the insulin resistance index as well as liver reduced glutathione (GSH) and superoxide dismutase (SOD) when compared to the high fat diet (HFD) control mice. The results clearly suggest that purslane fixed oil containing 9,12, 5-octadecatrienoic acid methyl ester and 9,2-octadecadienoic acid methyl ester produced a higher anti-obesity, anti-atherogenic, anti-diabetic and antioxidant activities on experimental obese diabetic mice. Taken together, purslane seeds fixed oil has potential as a preventive agent for type 2 diabetes mellitus (and possibly obesity) and deserves clinical trial in the near future.

Keywords: Purslane seeds fixed oil; Antidiabetic; Anti-obesity; Antioxidant and lipid profile

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 [1]. Obesity and insulin resistance are conditions frequently associated with several complications such as hyperlipidemia [1], fatty liver [2], type II diabetes mellitus [3] and cardiovascular diseases [4]. Medicinal plants play an important role in the management of diabetes mellitus with both 2 types [5,6]. Several medicinal plants may be used to reduce or control the body weight and obesity. One of such plants, purslane, in Arabic 'Rejlah', (Portulaca oleraceae L.) occurs in the Arabian has been examined in some animal studies and clinical studies for its hepato-protective [7] and hypolipidemic effects [6]. Purslane is reported to be rich in α-linolenic acid and ß-carotene and used as a health food for patients with cardiovascular diseases [8]. It contains several types of vitamins and minerals [9], fatty acids [10], glutathione, glutamic acid, aspartic acid dopamine, dopa, coumarins, flavonoids, alkaloids, saponins, and anthocyanin [11]. Plant-derived oils are rich sources of volatile terpenoids and phenolic compounds [12]. The essential oil and lipid-soluble compounds are known to have potential to prevent obesity and have been used in aromatherapy for obese middle-aged women. Some volatile compounds extracted from plants may have antioxidant activity that could mitigate obesity-related complications, including atherosclerosis and some cancers [12-15]. Not surprisingly, plants such as purslane contain high levels of unsaturated fatty acids and poly-phenols [8,12], which are excellent scavengers of reactive oxygen species and represent a promising anti-obesity effects. In vivo tests have been conducted with purslane leaves to determine for example, its hepatoprotective [7], hypolipidemic, hypoglycemic and antioxidant activity [6]. But there are no reports about anti-obesity and antidiabetic activity of purslane seeds oil. The present study was

undertaken to investigate fixed oil composition of purslane seeds as well as evaluate anti-obesity and antidiabetic effects of purslane fixed oil in obese diabetic mice fed a high-fat diet.

Materials and Methods

Plant material

Plant materials of purslane (Portulaca oleraceae L) were collected from Horbit Village, El-Sharkyea Governorate, Egypt in May 2013. The plant material was identified, authenticated by Dr. Samir Osman, lecturer of Pharmacognosy Faculty of Pharmacy, October 6 University, 6th of October city, Egypt. Voucher specimen Po#0513 was kept in the department of Pharmacognosy, Faculty of Pharmacy, October 6 University. The seeds were cleaned, and subjected to extraction precess.

Extraction of fixed oil

After being cleaned by hand carefully to remove the foreign materials such as other seeds, stones and small stalks, purslane seed (500 g) were dried at 50°C for 12 h in an oven, and then crushed into powder in a grinder with a size range of 0.55-1.0 mm. The resulted powder was kept in a vacuum dryer until use. Purslane ground samples were mixed with hexane (1:10, m/V) at (60-80°C) using a Soxhlet apparatus. This process of extraction was repeated for 6 h, the hexane

distilled out by distillation assembly, then concentrated by hot plate drying and air-drying at temperature of 40 ± 2°C.

Materials

Boron fluoride, methanol, pentane and hexane were obtained from Sigma-Aldrich, USA.

Preparation of BF$_3$- methanol reagent

One liter of reagent grade methanol, in a 2-liter flask, is cooled in an ice bath. With the flask still in the bath, 125 grams of BF$_3$ is bubbled through a glass tube into the methanol in fume hood, and the gas should not flow so fast that white fumes emerge from the flask (The BF$_3$ must be flowing through the glass tube before it is placed in and until it is removed from the methanol or the liquid may be drawn into the gas cylinder valve system) This reagent has an excellent shelf life and has been used up to 4 months after preparation [16].

Preparation of FAME (Fatty Acid Methyl Ester)

Seeds (40 g) were dried overnight at 50°C and ground into powder with a mortar and pastle, after which 0.6 ml of dichloromethane and 4.0 ml of 0.5N sodium methoxide were added. Acidic catalysed esterification using the boron triflouride-methanol complex (14%w/v) was added according to the method described by [17,18]. The tube was shaken and heated for 30 min. at 50°C. The reaction was stopped by adding 5.0 ml of water containing 0.2 ml of glacial acetic acid. The esterified fatty acids were extracted with 3.0 ml petroleum ether (40-60°C). The clear fraction was kept at -20°C until further analysis.

Separation condition of fatty acids on GC/MS

Instrument: HP 6890 Series Gas Chromatograph System with an HP 5973 Mass Selective Detector.

The FAME in hexane (1 μL) was injected into the column with a split ratio of 100:1. The injector and detector temperature were set at 200 and 250°C, respectively. Helium was used as the carrier gas at a flow rate of 1.5 mL/min. Separation was carried out on a TR-FAME (Thermo 260 M142 P) (30 mm × 0.25 mm ID) with a film thickness of 0.25 ᴜm film) (70% Cyanopropyl –Polysilphphenylene siloxane) capillary column. The column temperature was programmed from 100 to 160°C at 2°C/min and then to 250°C at 4°C/min and finally held at 250° C for 20 min. The weights of the individual FAME were calculated on the basis of their relative peak area compared with that of internal standard, and then they were corrected using the corresponding GC response factors for each fatty acid.

Mice

This experiment was conducted in accordance with guidelines established by the Animal Care and use Committee of October 6 University. Adult mice weighing around 30 ± 3 gms were purchased from Faculty of Veterinary Medicine, Cairo University. They were housed in cages (5 per cage) in an air-conditioned room with a temperature of 22 ± 2°C, a relative humidity of 60%, and an 8:00 to 20:00 light cycle. During the acclimatization period, each animal was raised on a regular diet *adlibitum*.

The animals were randomly divided into five groups 8 mice in each, two controls groups and three treatment groups.

Normal group: (was received a regular diet for 8-week period).

Control positive: (was received a high fat diet for 8-week period).

Group III: Was fed a high-fat diet with purslane fixed oil (15%) for an 8- week period.

Group IV: Was fed a high-fat diet with purslane fixed oil (30%) for an 8-week period.

Group V: Was fed a high-fat diet with metformin (500 mg/kg bw/ml saline) orally in a single daily dose for an 8-week period [19].

Supplemented diets were prepared by combining HFD meal (850 and 700 g) with purslane seed oil 150 and 300 g, respectively, in a mechanical mixer. Diets were stored in airtight containers at 4ºC in a refrigerator. Peroxide content of the diets did not change during the storage period [20].

The regular diet consists of wheat flour 22.5%, soybeen powder 25%, Essential Fatty Acids 0.6%, Vitamins (A 0.6 mg/kg of diet, D 1000IU/kg of diet, E 35 mg/kg of diet, Niacin 20 mg/kg of diet, pantothenic acid 8 mg/kg, riboflavin 0.8 mg/1000 kcal of diet, thiamin 4 mg/kg of diet, B6 50 mcg/kg of diet and B12 7 mg/kg of diet) and Minerals (calcium 5 g/kg of diet, Phosphorus 4 g/kg of diet, fluoride 1 mg/kg of diet, iodine 0.15 mg/kg of diet, chloride 5 mg/kg of diet, iron 35 mg/kg of diet, copper 5 mg/kg of diet, magnesium 800 mg/kg of diet, potassium 35 mg/kg of diet, manganese 50 mg/kg of diet and sulfur 3 mg/kg of diet) [21]. The nutrition contents of the high fat diet were similar to those of the regular diet except for the addition of 200 g of fat/kg and 1% (w/w) cholesterol [22]. 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, insulin resistance index, atherogenic index and lipid profile levels as well as liver antioxidant and lipid peroxidation parameters were determined.

Blood sampling and biochemical assays

Blood was collected, centrifuged, and plasma was used freshly for estimation of glucose level [23]. Plasma insulin levels were measured with an ultra-sensitive rat insulin ELISA kit (Alpco Diagnostics) [24]. The insulin resistance index, calculated by insulin (mU/ml) × glucose (mM)/22.5 [25]. Plasma triglyceride, total cholesterol and HDL- cholesterol were determined using commercially available kits (Asan and Youngdong Pharmaceutical Co., Korea) [26-28]. Plasma LDL-cholesterol level was calculated according to Falholt et al. [29] formula (LDL-cholesterol = total cholesterol – triglycerides/5 – HDL-cholesterol). The Friedewald formula [30] was used to calculate plasma low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (vLDL-C) values and atherogenic index as follows: LDL-C = TC-(HDL-C + TG/5); vLDL-C = [Triglycerides/5] and atherogenic index = (TC-HDL-C)/HDL-C. Liver was blotted, weighed, homogenized with methanol (3 volumes) and used for TBARs estimation [31]. Another portion of the tissues was homogenized with phosphate buffer saline to estimate GSH [32] and SOD [33].

Statistical analysis

The data were analyzed using the one-way analysis of variance (ANOVA) [34] followed by least significant difference (LSD) test to compare various groups with each other. Results were expressed as mean ± standard error (SE) and values of $P>0.05$ were considered non-significantly different, while those of $P<0.05$ and $P<0.01$ were considered significant and extremely significant, respectively. Values are given as mean ± SD for groups of eight animals each. High-fat diet control rats were compared with regular diet control rats. Experimental groups were compared with the high-fat diet control rats.

Results

Chemical composition

Purslane seeds yielded (20% v/w) of a yellow colored fixed oil. The GC-MS analysis of the oil revealed the presence of 7 components and the major constituents were 9,12,15-octadecatrienoic acid methyl ester and 9,12-octadecadienoic acid methyl ester representing 41.18 % and 27.23%, respectively. The results are compiled in Table 1 and Figure 1.

Body weight was determined once every 2 weeks. The body weight of the regular fed mice (normal group) gradually increased as the mice grew during the 8-week trial (Table 2). During the 8 weeks of treatment, the weight of the HFD group increased significantly from the first week until the end of treatment when compared with the control group. Mice receiving purslane fixed oil (150 and 300 mg/kg. diet) underwent a 40.9% and 45.19%, respectively loss in weight relative to HFD group of mice after 8 weeks ($p<0.01$). However, metformin 500 mg/kg bw. showed a significant decrease in body weight by 38.25% when compared to the high fat diet control group ($p<0.01$).

Plasma glucose, insulin and Insulin Resistance Index (IRI)

Plasma insulin, glucose and insulin resistance index (IRI) was determined after 8 weeks and was compared between groups in Table 3. The HFD increased insulin, glucose and insulin resistance index (IRI) levels by 1.5, 1.2 and 1.8-fold, respectively when compared with group of mice feed regular diet. Mice receiving *purslane* fixed oil (150 and 300 mg/kg. diet) and metformin 500 mg/kg bw. showed significant decreased insulin, glucose and insulin resistance index (IRI) levels relative to HFD group of mice after 8 weeks ($p<0.01$).

Plasma lipid levels

Plasma lipid levels in high fat diet fed mice were dramatically increased with compared to the levels in regular diet fed mice except for the HDL-cholesterol level (Table 4). In the high fat diet control group, plasma triacylglycerols (TG), total cholesterol, LDL-cholesterol and vLDL-cholesterol were increased as compared to the regular diet group ($p<0.01$). The HFD increased triglyceride levels 2.2-fold, total cholesterol 2.09-fold, LDL-C 3.9-fold and vLDL-C 2.2-fold. But HDL-C was decreased by 0.9-fold as compared to the regular diet group ($p<0.05$). *Purslane* fixed oil (150 and 300 mg/kg. diet) and metformin 500 mg/kg bw. decreased TG, TC, LDL-c, vLDL-c as well as atherogenic index and increased HDL-c levels as compared to the HFD group ($p<0.01$) .

### Liver oxidative stress biomarkers

Liver TBARS level was significantly higher and GSH and SOD were significantly lower for the high fat diet fed group when compared to the regular fed group ($p<0.01$). For all high fat diet/ *purslane* fixed oil (150 and 300 mg/kg. diet) and metformin 500 mg/kg bw. TBARS accumulation was significantly suppressed and Liver GSH and SOD levels were markedly increased when compared to the high fat diet control group ($p<0.01$) Table 5.

Body weight of mice consuming regular diet, high fat diet, high fat diet plus purslane fixed oil or fed with high fat diet plus 500 mg/kg of metformin during the 8-week period. Values are given as mean ± SD for groups of eight animals each. High-fat diet control mice were compared with regular diet control mice. Experimental groups were compared with the high-fat diet control mice.

Discussion

Purslane seeds yielded (20% v/w) of a yellow colored essential oil.

The GC-MS analysis of the oil revealed the presence of 7 components and the major constituents were 9,12, 15-octadecatrienoic acid methyl ester and 9,12-octadecadienoic acid methyl ester representing 41.18% and 27.23%, respectively. The results are compiled in Table 1 and Figure 1.
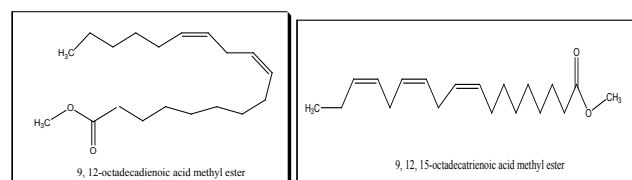
Obesity is the most common nutritional disorder in the developed world and it is considered a risk factor associated with the development of major human diseases, including cardiovascular disease, diabetes, and cancer. Increased intake of high caloric (energy and fat) food promotes body fat storage and greater body weight and adiposity in humans and animals [35]. Over-the-counter remedies based on nutritional supplements are extremely popular, especially with respect to obesity and body composition. Inhibition of the digestion and absorption of dietary fat has been used as targets in obesity treatment [36]. The anti-obesity effects of purslane seeds fixed oil was investigated using obese diabetic rats fed high-fat diet as a model of obese type-II diabetes. When fed a high-fat diet, rats develop obesity and type-II diabetes by 8-weeks old and these rats are thus widely used for research in obesity and diabetes [6].

Purslane seeds fixed oil was found to significantly suppress increases in body weight, showing anti-obesity actions (Table 2). Plasma glucose and insulin levels were significantly higher for the high-fat diet group than for the regular diet group. Purslane seeds oil supplementation suppressed these increases in plasma glucose and insulin levels. The insulin resistance index, a simpler method to measure insulin sensitivity usually used in clinical and animal studies (Table 3).

HOMA-IR has been suggested as a biomarker to assess insulin resistance and secretion and is a useful clinical index for insulin sensitivity and pancreatic β-cell functions in epidemiological studies. Although HOMA-IR has several limitations in terms of accuracy and reliability, it expresses essentially hepatic insulin resistance as confirmed by [37]. We also observed purslane seed oil significantly improved the IRI within high-fat diet group. The present results due to presence of polyunsaturated fatty acids 9,12,15-octadecatrienoic acid methyl ester and 9, 12-octadecadienoic acid methyl ester representing 41.18 % and 27.23%, respectively.

The major sources of ALA are vegetable oils (such as canola and

Peak No.	Compound	Relative Retention time (min)	Percentage (%)
1	N,N'-Trimethylurea	21.06	0.92
2	Hexadecanoic acid methyl ester	23.95	10.03
3	Methyl stearate	28.75	5.02
4	9-Octadecenoic acid, methyl ester	29.35	13.90
5	9,12-Octadecadienoic acid methyl ester	30.73	27.23
6	9,12,15-Octadecatrienoic acid methyl ester	32.38	41.18
7	9,12,15-Octadecatrienoic acid ethyl ester	32.93	1.72

Table 1: GC-MS analysis of the fixed oil constituents of *purslane* seeds.

Figure 1: Total Ion Chromatogram of the GC-MS analysis of the fixed oil constituents of *purslane* seeds.
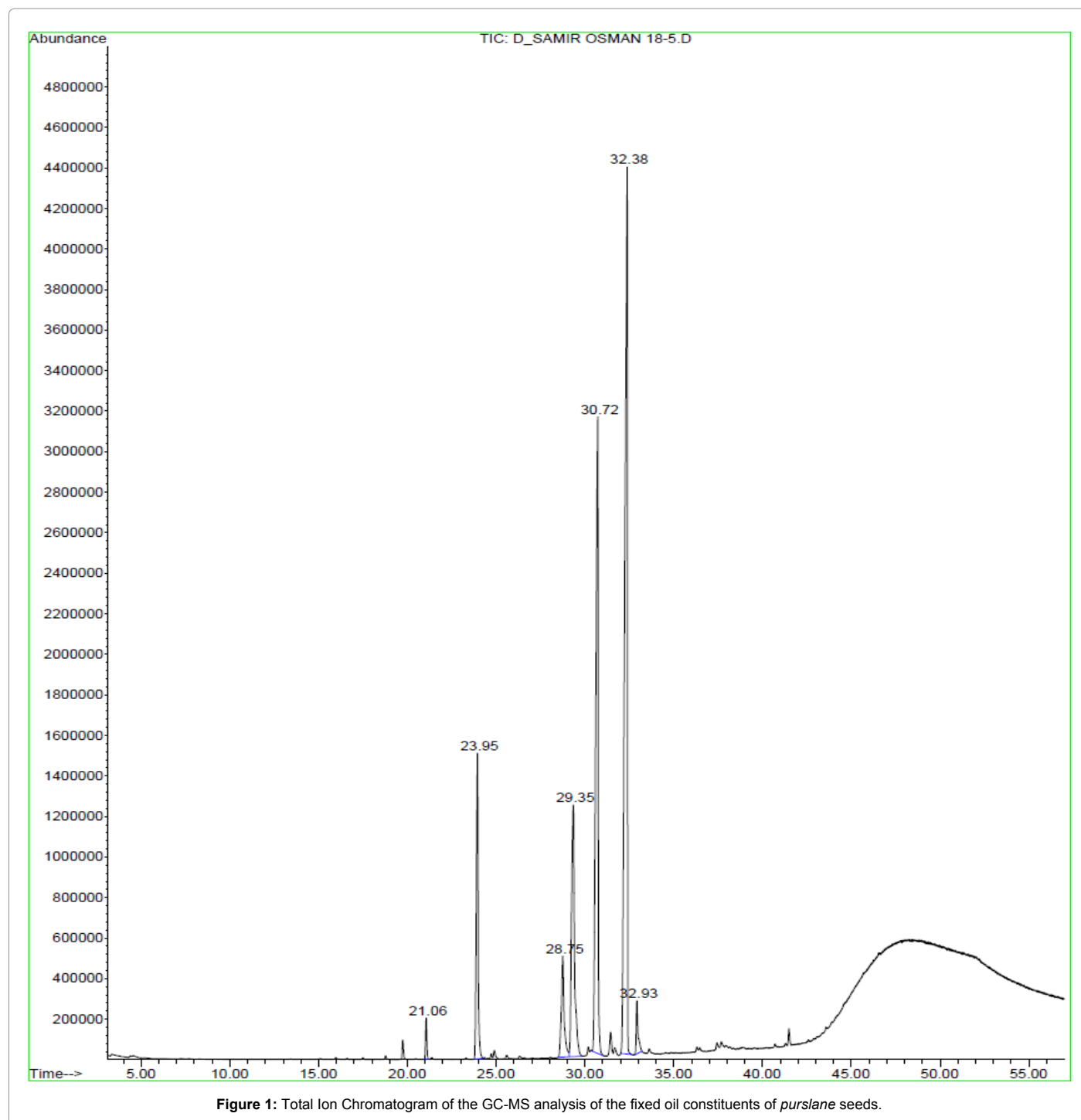
soybean) with other sources being purslane seed oil. The results of GC-MS showed that purslane is alternative to plant source of omega-3 fatty acid. It is one of the richest plant sources of omega-3 fatty acid alpha-linolenic (ALA, C18:3n-3); 9,12,15-octadecatrienoic acid methyl ester (41.18%). An in-creased intake of omega-3 fatty acids might reduce the risk of developing diabetes has been tested in mice, where supplementation with fish oil inhibited hyperglycemia and pancreatic insulitis in streptoztocin-induced diabetes. Also omega-3 fatty acid can reduce insulin resistance in skeletal muscle [38]. ALA is considered to be nutritionally essential because of its specific function as precursor of

the long-chain n-3 PUFA [39]. The higher consumption of ALA was associated with higher plasma insulin and improves glucose used and efficiency [40].

Purslane seed oil was found to significantly suppress increases in plasma lipids content, showing anti-obesity actions (Table 4). Also, lowered fat accumulation, clarifying that the tested compounds suppresses TG, TC, HDL-C, LDL-C and vLDL-C (Table 4). Most of the reduction in plasma cholesterol occurred in the fraction of LDL, because of apo-B containing lipoprotein fractions are through to be responsible

No.	Groups	Number of weeks Body weight of mice(g) 0 2 4 6 8				
(I)	Normal Regular diet (RD)	30.2 ± 2.1	32.4 ± 1.9	33.6 ± 2.3	31.1 ± 1.6	34.2 ± 1.4
(II)	Control positive High-fat diet (HFD)	29.8 ± 1.6	34.2 ± 2.3	39.5* ± 1.8	42.3** ± 2.5	44.7** ± 1.7
(III)	HFD+ Purslane fixed oil 150 g/kg of diet	31.3 ± 2.5	30.4* ± 2.6	28.1** ± 3.2	27.0** ± 1.5	26.4** ± 1.9
(IV)	HFD+ Purslane fixed oil 300 g/kg of diet	30.7 ± 1.5	28.2** ± 1.4	27.4** ± 2.2	26.1** ± 2.7	24.5** ± 3.1
(V)	HFD+ Metformin 500 mg/kg bw	32.1 ± 2.3	30.5* ± 1.9	29.8** ± 1.6	28.4** ± 2.1	27.6** ± 1.3

· *Significantly different from control group at *p<0.05*.
· **Significantly different from control group at *p<0.01*.

Table 2: Effect of the purslane fixed oil administration on decrease the daily body weight in mice.

No.	Groups	Plasma insulin (nmol/L)	Plasma glucose (mmol/L)	insulin resistance index (IRI)
(I)	Normal Regular diet (RD)	1.2 ± 0.1	4.8 ± 1.2	0.256
(II)	Control positive High-fat diet (HFD)	1.8** ± 0.07	5.9* ± 1.5	0.472**
(III)	HFD+ Purslane fixed oil 150 g/kg of diet	1.7* ± 0.3	7.7 * ± 1.08	0.581**
(IV)	HFD+ Purslane fixed oil 300 g/kg of diet	1.5** ± 0.4	6.9 ** ± 1.3	0.64**
(V)	HFD+ Metformin 500 mg/kg bw	1.6** ± 0.2	6.3** ± 0.95	0.448**

Values represent the mean ± SE (n=8). High-fat diet (HFD) control mice were compared with regular diet (RD) control mice. Experimental groups were compared with the high-fat diet control mice.
Insulin Resistance Index = Insulin (nmol/L) × glucose(mmol/L)/22.5.
· *Significantly different from control group at *p<0.05*.
· **Significantly different from control group at *p<0.01*.

Table 3: Effect of *purslane* fixed oil on plasma insulin, plasma glucose and insulin resistance index (IRI).

No.	Groups	TG (mmol/L)	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	vLDL-C (mmol/L)	Atherogenic index
(I)	Normal Regular diet (RD)	0.86 ± 0.41	17.6 ± 2.1	10.5 ± 2.09	6.92 ± 1.2	0.172 ± 0.041	0.676 ± 0.11
(II)	Control positive High-fat diet (HFD)	1.9** ± 0.30	36.8** ± 5.3	9.4* ± 1.3	27.02** ± 3.7	0.38** ± 0.065	2.914** ± 0.54
(III)	HFD+ Purslane fixed oil 150 g/kg of diet	1.2* ± 0.19	30.2* ± 4.1	12.3* ± 1.9	17.66** ± 2.9	0.24* ± 0.043	1.455* ± 0.32
(IV)	HFD+ Purslane fixed oil 300 g/kg of diet	0.94 ** ± 0.15	25.4** ± 3.6	16.1** ± 3.1	9.112** ± 1.4	0.188** ± 0.03	0.577** ± 0.68
(V)	HFD+ Metformin 500 mg/kg bw	1.4* ± 0.35	33.9** ± 3.2	13.3* ± 2.4	20.32* ± 4.1	0.28** ± 0.05	1.548** ± 0.25

Values represent the mean ± SE (n=8). High-fat diet (HFD) control mice were compared with regular diet (RD) control mice. Experimental groups were compared with the high-fat diet control mice.
LDL-C (mmol/L) = TC-HDL-[TG / 5]
vLDL-C(mmol/L) = [Triglycerides/5]
Atherogenic index = (TC-HDL-C)/HDL-C.
· *Significantly different from control group at *p<0.05*.
· **Significantly different from control group at *p<0.01*.

Table 4: Effect of *purslane* fixed oil on plasma triglycerides (TG), total Cholesterol (TC), HDL-cholesterol (HDL-C), LDL- cholesterol (LDLC), vLDL- cholesterol (vLDLC) and atherogenic index.

No.	Groups	TBARS (n mol of MDA formed/mg protein)	GSH (mg/g tissue)	SOD (U/g tissue)
(I)	Normal Regular diet (RD)	0.15 ± 0.04	3.11 ± 0.70	131.40 ± 9.5
(II)	Control positive High-fat diet (HFD)	0.22* ± 0.06	2.03** ± 0.60	105.20 ** ± 11.7
(III)	HFD+ Purslane fixed oil 150 g/kg of diet	0.09* ± 0.02	2.90* ± 0.23	115.36* ± 8.30
(IV)	HFD+ Purslane fixed oil 300 g/kg of diet	0.05** ± 0.01	3.25** ± 0.40	145.17** ± 12.30
(V)	HFD+ Metformin 500 mg/kg bw	0.10** ± 0.02	2.80* ± 0.65	127.18 ± 10.15*

Values represent the mean ± SE (n=8). High-fat diet (HFD) control mice were compared with regular diet (RD) control mice. Experimental groups were compared with the high-fat diet control mice.
· Activity of SOD is expressed as 50% of inhibition of epinephrine autooxidation per min.
· *Significantly different from control group at *p< 0.05*.
· **Significantly different from control group at *p< 0.01*.

Table 5: Effect of *purslane* fixed oil on liver thiobarbituric acid reactive substances (TBARS), reduced Glutathione (GSH) and super oxide dismutase (SOD).

for cholesterol deposition in atherosclerotic plaques [41] this change could be attributed to a reduction in LDL would be advantageous clinically extract had an improving effect on the hypercholesterolemia induced by a high fat diet. Moreover, dietary supplementation of purslane seed oil decreased the plasma cholesterol, triacylglycerols and LDL levels in rats and increase in HDL-C [6]. Furthermore, feeding the

purslane seed fixed oil containing high level of polyunsaturated fatty acids act as hypolipidamics exert prophylactic effects on cardiovascular disease, protect against insulin resistance and obesity in rodent fed high diet and insulin response to glucose in healthy human [42,43]. This results approved by Caterina et al., [44] reported that n-3 fatty acids reduced plasma TG. N-3 fatty acid suppressed hepatic lipogemesis and

reduced circulating triglyceride levels. The main effect of dietary n-3 fatty acids on plasma lipids and lipoproteins in general is reduction in plasma triglycerides in healthy subjects and even more in subjects with hypertriglyceridemia, including diabetic patients. Morise et al., [45] observed that a higher ALA intake decrease cholesterol content in liver, however, increase in the feces. The reduced hepatic cholesterol content was accounted for higher cholesterol secretion into bile thus leading to a depletion of into intrahepatic pool of cholesterol [46].

In the present study, liver TBARS level was significantly higher in the high fat diet control group, whereas GSH and SOD levels were lower. In the present study, hyperglycemia induced by high fat diet may be led to overproduction of reactive oxygen species (ROS). ROS contribute to organ injury in systems such as the heart and liver, and oxidative damage is generally increased in diabetes [47]. These results may be due contained acids 9,12,15-octadecatrienoic acid methyl ester and 9, 12-octadecadienoic acid methyl ester with high concentration. Similar results were obtained by with Garrel et al., [48] who reported that feeding the adequate omega-3 fatty acid alpha-linolenic (ALA, C18:3n-3) diet increased enzyme antioxidant.

Thus, the present study, which examined the effect of feeding purslane seeds fixed oil along with a high-fat diet on insulin resistance, lipids profile and oxidative stress biomarkers, has revealed that a high-fat diet compromises the endogenous antioxidant defense mechanisms. Dietary purslane fixed oil, which brought about significant anti-obesity influence, were also found to effectively reduce this oxidant stress in hyperolipidemic animals as indicated by countering of the depleted antioxidant molecules and decreased activity of antioxidant enzymes. Anti-obesity effect of purslane seeds fixed oil in obese diabetic rats fed high-fat diet has not been reported earlier to our knowledge and this study is might be the first observation of that kind.

In conclusion, the present study showed that that the purslane seeds fixed oil administration produced a higher anti-obesity, anti-atherogenic, anti-diabetic and antioxidant activities on obese diabetic mice. The presence of omega-3 fatty acid especially Long chain polyunsaturated fatty acid (9,12,15-octadecatrienoic acid methyl ester) in purslane fixed oil has antidiabetic effect. Purslane fixed oil was more effective of reduction glucose, insulin resistance, TC, TG, LDL, vLDL and atherogenic index more than metformin in diabetic rats. 9,12,15-octadecatrienoic acid methyl ester and 9,12-octadecadienoic acid methyl ester have potential effect to protect pancreas from up normality changes which induced in diabetic disease. The data suggested that, purslane fixed oil may be beneficial in diabetic disease.

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