International Journal of Biology Research
ISSN: 2455-6548; Impact Factor: RJIF 5.22
Received: 23-12-2018; Accepted: 24-01-2019
www.biologyjournal.in
Volume 4; Issue 1; January 2019; Page No. 46-54

Egyptian Purslane (*Portulaca oleracea* L.) efficacy on amelioration of BMI, PON1 and Irisin in obese rats

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Abstract
Obesity is a chronic metabolic disorder that is raised by multiple biological and environmental factors. The objective of this study was to determine the thermogenesis potential of Egyptian purslane seeds (PS) against obesity disorder. Adult male Wistar albino rats (120-150g) were randomly divided into four groups (10 animals each) as follows: group (1) healthy rats fed standard diet and served as control, group (2) animals fed PS-standard diet (20%), group (3) obese rats fed high-fat diet and group (4) obese animals fed PS-high-fat diet (20%). After six weeks of feeding, the results revealed that feeding of obese animals on PS (20% of the high fat diet) succeeded to decline the obesity-induced disorder and manage the weight; this was evidenced by the significant reduction of serum of ALAT, ASAT, urea, creatinine, total cholesterol, triglycerides, LDL, LDH, CK, PON1, TNF-α, leptin and glucose as well as cardio-hepatic MDA and nitric oxide levels coupled with marked improvement in serum HDL and Irisin as well as cardio-hepatic GSH, SOD and catalase. Moreover, the histopathological findings showed marked regenerated. In conclusion, PS, as food supplement, could play a beneficial role in management of obesity and its disorders; this could be exhibited through its bioactive components with multiple pathways.

Keywords: obesity, Irisin, purslane, paraoxonase-1, rats

Introduction
Obesity is defined as a disease process characterized by excessive body fat accumulation with multiple organ-specific consequences; its prevalence is increasing to epidemic proportions globally [1]. Also, it is described as a chronic metabolic disorder that is raised by multiple biological and environmental factors, a sedentary lifestyle, and a genetic predisposition; it is associated with a multitude of adverse health effects. Central or visceral fat in obesity pours out free fatty acids and increases insulin resistance. The adipose cells secrete multiple hormones that are known as 'adipokines', and markers of inflammation. Unfortunately, it is associated with a higher risk of diabetes, hypertriglyceridemia, decreased high-density lipoprotein (HDL) cholesterol, hypertension, stroke, proteinuria, gallstones, fatty change in the liver, nonalcoholic steatohepatitis, pancreatitis, venous thrombosis, hypoventilation syndrome, atherosclerosis, and osteoarthritis [2, 3]. Other conditions for which obesity poses an increased risk include sleep apnea, asthma, stress incontinence, depression, and several types of cancer [4].
Numerous population studies have linked elevated concentration of total cholesterol or LDL– cholesterol in plasma with increased incidence of atherosclerotic events. It has further been shown that the clinical complications of atherosclerosis could be diminished and life prolonged when plasma lipids are lowered by hypocholesterolemic agents. Many drugs with proven hypocholesterolemic activity are available clinically to ameliorate cases of individuals with premature atherosclerosis and those with other risk factors, such as hypertension or diabetes mellitus.
In many cultures of the world, herbal remedies are increasingly being employed in an attempt to achieve the same purpose. *Portulaca oleracea* L. (subsp. oleracea; herbaceous member of family *Portulacaceae*), a weed spread in the Egyptian fields, has been used as a nutritious vegetable for human nutrition. It has been mentioned in Egyptian texts from the time of the Pharaohs; and it has been listed in the World Health Organization as one of the most used medicinal plants. The taste of the green plant is slightly acidic and salty. In folk medicine, it has been used for remediation dysentery, boils and sores, eczema, erysipelas, checking cough, dispelling phlegm and snake and insect bite, diuretic, febrifuge, antiseptic, antispasmodic and vermifuge [5]. There are several species of genus *Portulaca*, which are distributed in tropical, subtropical and temperate regions throughout the world [6]. The preliminary screening of the *Portulaca oleracea* plant revealed the presence of protein, soluble carbohydrate, inorganic acids, alkaloids, flavonoids, coumarins, cardiac glycosides, anthraquinone glycosides, saponin and tannins. The leaves are reported to have high amount of iron, omega-3 fatty acids, and α-linolenic acid [7]. The most interesting metabolites, from the therapeutic point of view, ω-3 fatty acid from different parts of *Portulaca oleracea* plays a major role in the regulation of inflammation controlling gene products. The stem, leaves and the whole plant have been employed for the treatment of scorpion sting and also used as anti-helmintic, cooling or moistening agent for fever, etc. Favorable pharmacological results have been demonstrated that the aerial parts of this plant exhibit a wide range of properties such as hepatoprotective, antioxidant, and neuroprotective, etc. Moreover, presence of secondary metabolites makes this plant medically more important to be exploited by clinicians and scientists to gain more insight into its biological and medicinal properties [8]; other

pharmacological effects were documented as a consequence of the use of this plant like anti-oxidation, anti-bacteria, anti-virus, anti-ulcerogenic, anti-inflammatory, skeletal muscle-relaxant, wound-healing and hypoxic nerve tissue protective effect. The seeds or its extracts; infusion, 70% alcoholic and petroleum ether have hypolipidemic and hypoglycemic activity in hyperlipidemic rats [9].

Evidence suggests that a clustering of sources of oxidative stress exists in obesity; hyperglycemia, increased tissue lipid levels, inadequate antioxidant defenses, increased rates of free radical formation, and chronic inflammation [10]. Obesity affected many organs in the body such as liver, heart and kidney. Fatty liver and nephropathy are common complication of obesity [11]. Arthrosclerosis and cardiac complications are more common among obese individuals [12, 13]. Therefore, the objective of present study was to explore the weight gain management, thermogenic, anti-atherosclerotic potential of Egyptian *Portulaca oleracea* seeds, and to evaluate the effect of that seeds on oxidative stress voltage and antioxidant battery in both the liver and the heart of obese rats to focus the light on herbal management of obesity and its complication.

Materials and Methods

The seeds of Egyptian purslane (*Portulaca oleracea*) were obtained from a local supplier (Abd El-Rahman Harraz, Bab El-Khalk zone, Cairo, Egypt), identified and authenticated by scientific botanists at Botany Department, Faculty of Science Al-Azhar University and was found carrying taxonomic serial number (TSN) 20422.

Determination of total phenolic content

Phenolic content of the powdered Egyptian purslane seeds was performed in a 1:20 w/v of acidified (1% acetic acid) methanol solution (80%) at room temperature with fixed stirring for 180 minutes. The mixture was then centrifuged at 4000 RPM for 15 minutes at 24°C in a Thermo Sorval centrifuge (Legend XT Series, Fisher Scientific, Nepean, Ontario, CA). The supernatant was passed through a filter (Nylon, 0.25 - 0.45 µm). Different concentrations of the extracts were prepared in redistilled H_2O with serial dilution at two-fold, four-fold and eight-fold. Gallic acid standards were prepared in redistilled H_2O at concentrations of 0.75, 0.5, 0.25, 0.125, 0.0625, 0.03125 mg/mL. Then 475 µL (10 times diluted Folin-Ciocalteu reagent) was added to all standards and samples, except the blank (700 µL diluted Folin-Ciocalteu reagent); samples and standard were vortexed and left to stand for five minutes; then, 475 µL of sodium carbonate solution (6 g/dl) was added, with exception of the blank where 700 µL was added; incubation in the dark for 2 hours and the absorbance was measured spectrophotometrically at 725 nm [14].

Radical scavenging activity

The capacity of antioxidants in the seeds powder to quench DPPH radical was determined using the method of Nogala-Kalucka *et al*, [15] In this method gallic acid was used as standards in 80% methanol solution at concentrations of 1, 10, 20, 30, 40, 50, and 60 µg/mL. Dried powdered seed samples of PS were also soaked in 80% methanol solution. Two hundred µL of the methanol solution was used as a blank and 200 µL of 50 µM DPPH was used as a positive control. The remainder of the standards and samples were plated in the microplate at a volume of 20 µL. The same DPPH solution was added to each well at 180 µL, with the exception of the blank (200 µL methanol) and positive control, and then gently mixed, stored in the dark for 60 minutes. After incubation, the absorbance was read at 519 nm against blank (200 µL methanol). Antioxidant activity was determined by the equation below. The percent DPPH scavenging activity was graphed as a function of the different concentrations of gallic acid standards. The calculated sample values were compared to the standards, and their antioxidant capacity expressed in mg GAE/g of sample.

$$\text{DPPH Scavenging Activity (\%)} = [1 - (Asample\ Acontrol\ t{=}0)] \times 100\%$$

Determination of moisture, protein and lipid content and fatty acid composition

Moisture percentage was estimated to constant weight at 105°C as described by Russian pharmacopeia, 1990; protein content was determined using Kjeldhal method [16]. Oil content of the seeds was the reference method described in ISO 659 as it was extracted using Soxhlet apparatus, and the solvent was eliminated using rotary vacuum. After trans-methylation with 2N methanolic KOH at 50°C and purification by TLC, fatty acid composition of the oil was estimated as described in ISO 5508 (1990) using GC. The identification was in relation to the retention time of the sample with that of a standard mixture under the same conditions.

Animals and induction of obesity

Adult male Wistar albino rats (*Rattus norvegicus*) weighting 150-170g were obtained from Animal Colony, National Research Centre, Cairo, Egypt. The animals were housed in suitable plastic cages for one week for acclimation before the experimental study. Excess tap water and standard rodent food pellets [20.3% protein (20% casein and 0.3% DL-Methionine), 5% fat (corn oil), 5% fibers, 3.7% salt mixture and 1% vitamin mixture; obtained from Meladco company for animals and rodents food pellets, El-Obour City, Cairo, Egypt] were always available. All animals received human care in compliance with the standard insitiuations criteria as cited by animal ethical committee number FWA00014747, National Research Centre. After the animals being acclimatized, a number of rats were fed on a high (46%) fat diet [25.5 % corn oil and 20.5% beef tallow or lard), 24% carbohydrates (6% corn starch and 18% sucrose), 20.3% proteins (20 casein and 0.3% DL-Methionine), 5% Fiber, 3.7% salt mixture, and 1% vitamin mixture] for 16 weeks according to Noeman *et al*, [17] The weight and nose-anus length of each rat of both control and obese groups were measured at the start of the experiment and after seven weeks. BMI was determined by dividing the weight (g) by the square of the nose-anus length (cm^2). Animals with BMI greater than 0.68 g/cm^2 were considered obese as previously described by Novelli *et al*, [18] Rats that recorded BMI values below that level were excluded from the study. However, all rats of the obese group attained the target BMI and were all included.

Experimental Design

After induction of obesity, both normal and obese rats were randomly divided into four groups (10 animals each); 1) control group included healthy rats fed standard diet; 2) group included normal rats fed powdered purslane seeds mixed with the standard diet (20% powdered seeds); 3) group included

obese rats fed high fat diet; and 4) group included obese rats fed powdered-purslane seeds mixed with the same high fat diet (20% powdered seeds).

BMI and Body Weight Gain

After induction of obesity, body weights, nose-anus length, weight gain and BMI of both obese and normal rat groups were recorded at start and end of the experiment. Both BMI value and body weight gain percentage were calculated according to the formulae below.

$$BMI = \frac{weight\ (g)}{nose - anus\ length\ (cm^2)}$$

$$\text{Body weight gain (\%)} = \frac{W2-W1}{W1} * 100$$

W_1 is the animals' weight at start.
W_2 is the animals' weight at the end of the experiment.

Blood and tissue sampling

At the end of the study period (six weeks) and after recording the end weight and length of the animals, they were fasted overnight. Following diethyl ether anesthesia and using heparinized capillary tubes, blood specimens were withdrawn from the retro-orbital plexus into vacutainer collecting tubes and left 20 minutes to clot, then centrifuged at 3000 rpm for 10 minutes using cooling centrifuge (IEC centra-4R, International Equipment Co., USA). The sera were separated, divided into aliquots and stored at -80°C until biochemical measurements were carried out as soon as possible. After blood collection, the animals were rapidly sacrificed and a part of liver and whole heart of each animal was dissected out, washed with saline, dried, rolled in a piece of aluminum foil and stored at -80°C until homogenization and biochemical determinations; another part of each liver was preserved in a formalin-saline solution (10%); immediately processed, sectioned, stained and prepared for microscopic examination for histological changes.

Biochemical determinations

The activity of serum aminotransferases (ALAT and ASAT) was determined according to the kinetic method described by Schumann and Klauke [19] and using instruction manual of reagent kits purchased from Human Gesell Schaft fur Biochemical und Diagnostic mbH, Germany. Serum GGT activity was measured according to the kinetic method described by IFCC [19] using reagent kits purchased from Bio Systems S.A. Costa Brava 30, Barcelona, Spain. Serum ALP activity was assayed according Moss & Henderson [21] method according to the instruction of reagent kits purchased from DiaSys Diagnostic systems GmbH Germany. Serum total proteins and albumin concentrations were evaluated according to the photometric systems of Johnson *et al*, [22] using reagent kits purchased from DiaSys Diagnostic systems GmbH Germany. Serum total cholesterol, triglycerides, LDL and HDL levels were determined according to Artiss & Zak, Cole *et al*, Wieland & Seidel and Lopes-Virella *et al*, [23, 24, 25, 26] respectively, using reagent kits purchased from DiaSys Diagnostic System GmbH, Germany. Serum CK and LDH activities were determined according to the method described by the kinetic method described by IFCC [27] and method described by Van der heiden [28]. using reagent kits purchased from Spectrum Diagnostic System MDSS GmbH, Egypt. Serum glucose level was determined, at time of sampling, according to the method described by Young [29], using reagent kits purchased from DiaSys Diagnostic System GmbH, Germany. Urea and creatinine levels were determined according to the methods described by Young [29] using reagent kits purchased from Diamond Diagnostic, MDCS GmbH Schiffgraben, Hannover, Germany.

Paraoxonase-1 (PON-1) activity

Serum PON1 activity was determined according to the kinetic spectrophotometric chemical method described by Eckerson *et al*, [30] using a substrate buffered mixture [Paraoxon (1.0 mo L^{-1}), $CaCl_2$ (1.0 mmol L^{-1}), Glycin Buffer (50 mmol L^{-1})]. Under the above system, PONase can hydrolyze paraoxon (sigma) to ρ-nitrophenol and diethylphosphate. The rate of paraoxon hydrolysis can be measured spectrophotometrically at 405 nm and 37 ºC by monitoring the increase of absorbance at zero time and each two minutes interval for 10 minutes. All samples were run in duplicate; the average value was used for activity calculation using a molar extinction coefficient of 18, 300 M^{-1} cm^{-1} for p-nitrophenol. Results are expressed as U/L for PON1 activity (nanomole paraoxon hydrolyzed per minute).

Leptin, irisin and TNFα levels

Using ELISA (Dynatech Microplate Reader Model MR 5000, 478 Bay Street, Suite A213 Midland, ON, Canada), serum leptin, irisin and TNFα concentrations were measured using reagent kits (SG-10057, SG-10179 and SG-10127, respectively) purchased from Sino Gene Clon Biotech Co., Ltd, No.9 BoYuan Road, YuHang District 311112, Hang Zhou, China.

Tissue GSH, SOD, CAT, NO and lipid peroxidation (MDA)

Liver and heart GSH, SOD, CAT and NO levels were determined according to the methods of Beutler *et al*. [31], Aebi [31], Nishikimi *et al*. [31] and Montgomery *et al*. [34] respectively, using reagent kits obtained from Biodiagnostic Co., Dokki, and Giza, Egypt. Lipid peroxidation level (MDA) of both liver and heart homogenates was estimated chemically according to the method described by Ruiz-Larrea [35] on the base of MDA reaction with thiobarbituric acid (TBA) which forms a pink complex that can be measured photometrically. In this method 0.5 ml liver homogenate supernatant [1g Liver or heart tissue was homogenized in 10 ml phosphate buffer pH 7.4 and cool centrifuged at 5000 rpm for 10 minutes] was added to 4.5 ml working reagent [0.8 g TBA was dissolved in 100 ml percloric acid 10% and mixed with 20% trichloroacetic acid in volume ratio 1 to 3, respectively]. In a boiling and shaking water bath, the sample-reagent mixture was left for 20 minutes, then carried out to cool at room temperature, and centrifuged for 5 minutes at 3000 rpm. The absorbance of the clear pink supernatant was measured photometrically at 535 nm against reagent blank (0.5 ml distilled water + 4.5 ml working reagent). The lipid peroxidation level was calculated in nM MDA/gram liver tissue according to the following formula:

$$\text{MDA (nmol g}^{-1}) = [\{A_{535} \times 10^9 / (1.56 \times 10^5) \times 10^3\} \text{ x AD}] \times 10^{-1}$$

Where, $1.56x10^5$ $M^{-1}L^{-1}cm^{-1}$ = extinction coefficient of MDA, AD is assay dilution

Histopathology

The liver of different groups was sectioned into 5um thick paraffin sections, stained with hematoxylin and eosin [36] and investigated by light microscope.

Statistical analysis

Comparisons between means were carried out using to student T-test and one-way ANOVA test followed by post hoc test (Duncan) at level of $p \leq 0.05$ [37] using SAS program software; copyright (c) 1998 by SAS Institute Inc., Cary, NC, USA.

Results

Analysis of Egyptian purslane (*Portulaca oleracea*) seeds resulted in 13.2% moisture, 6.42% proteins and 12.8% oil contents. Composition and saturation of fatty acid of the extracted oil are illustrated in figure 1. The yield amount of three different solvents, total phenolic content and radical scavenging activity of the powdered seeds are shown in figure 2.

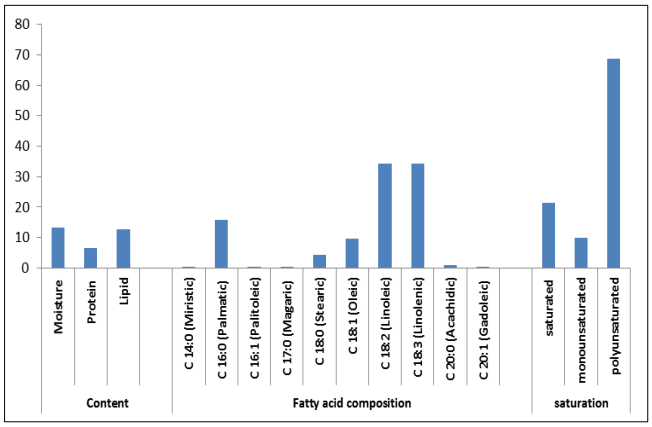

Fig 1: Shows analysis of moisture, protein and triacylglycerol content as well as fatty acid composition and saturation of *Portulaca oleracea* seeds.

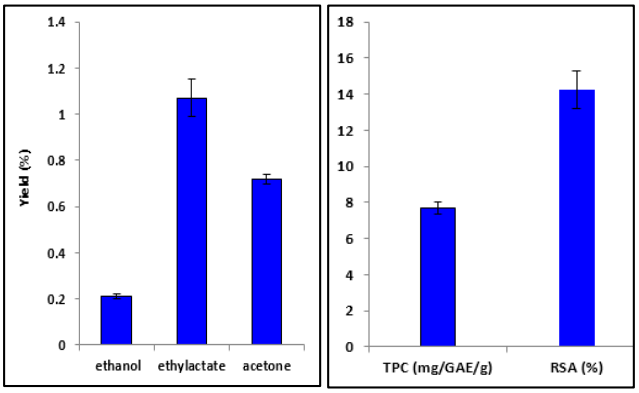

Fig 2: Yield (%), total phenolic content (mg GAE/g powder) and radical scavenging activity (%) dry powdered Egyptian purslane (*Portulaca oleracea*) seeds.

The data of the *in vivo* study revealed that obese rats recorded a significant elevation in body mass index (BMI) and body weight gain (BWG) in compare to healthy control. While administration of obese rats with PAE resulted in a significant reduction in both BMI and BWG close to that of healthy control (Figure 3&4).

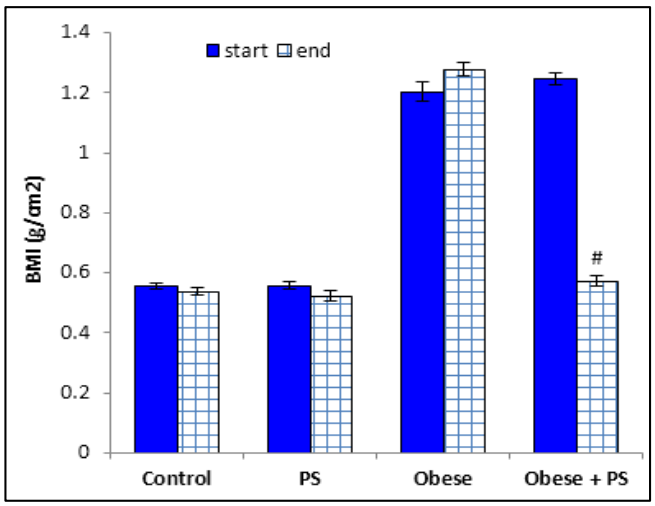

Fig 3: Body mass index (BMI) change of obese and PS-fed rats. Data were treated with ANOVA followed post hoc (Duncan) test at level p≤0.05. (*) is significance from control group and (#) is significance from obese group.

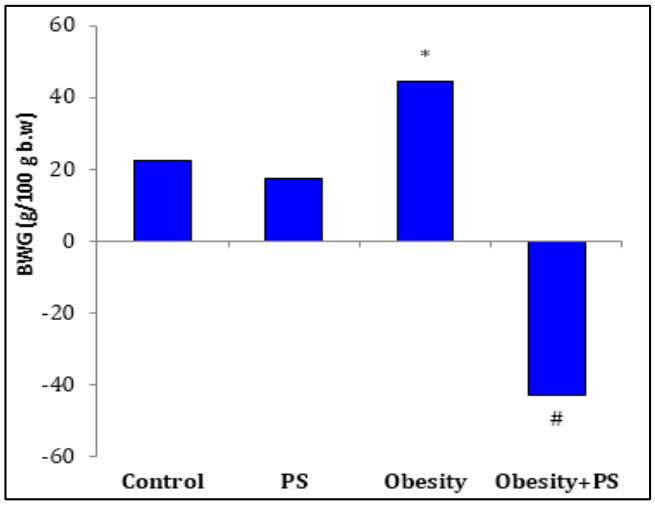

Fig 4: Body weight gain (BWG) of obese and PS-fed rats. Data were treated with ANOVA followed post hoc (Duncan) test at level p≤0.05. (*) is significance from control group and (#) is significance from obese group.

Treatment of healthy animals with PS neither deteriorate the activities of serum ALAT, ASAT, GGT and ALP nor the levels of total proteins, albumin, globulin, creatinine and urea. In contrast, obese animals group recorded a significant increase in the activity of ALAT, ASAT, GGT and ALP, but didn't deteriorate either protein profile (total protein and albumin) or kidney function (urea and creatinine) when both groups were compared with the healthy group. Fortunately, treatment of the obese-rats group with PAE resulted in significant improvements in the activity of ALAT, ASAT, GGT and ALP in compare to obesity-rats' group (Table 1).

Table 1: Activity of serum ALAT, ASAT, GGT and ALP and levels of total proteins, albumin, globulin, creatinine and urea of control, obese and PS-treated animals groups.

	Control	PS	Obese	Obese +PS
ALAT (U/L)	27.3±2.4^{B}	28±1.08^{B}	52.5±3.3^{A}	32.5±1.6^{C}
ASAT (U/L)	46.7±3.1^{C}	51.7±2.1^{C}	143.5±9.9^{A}	92.4±2.9^{B}
GGT (U/L)	52±0.41^{B}	53 ± 0.17^{B}	64±0.38^{A}	50 ± 0.45^{B}
ALP (U/L)	196.4±8.6^{B}	193.5±6.8^{B}	288±30.4^{A}	187.4±14.5^{B}
Albumin (g/dl)	4.6±0.31A^{A}	4.8±0.19^{A}	4.4±0.18^{A}	4.9±0.14^{A}
Proteins (g/dl)	6.9±0.31^{A}	7.1±0.18^{A}	6.4±0.17^{A}	6.5±0.18^{A}
Creatinine (mg/dl)	0.88±0.04^{A}	0.93±0.06^{A}	0.9±0.03^{A}	0.89±0.03^{A}
Urea (mg/dl)	44.3±2.4^{A}	44.7±3.5^{A}	40.7±5.2^{A}	43.1±5.7^{A}

Data are expressed as mean ± standard error. All data were subjected to one-way ANOVA followed by post hoc test (Duncan) at $p \leq 0.05$. Within the same row, means with different superscript letters are significantly different. (PS) is purslane seeds.

Table 2: Serum glucose, total cholesterol, triglycerides, HDL, LDL, LDH and CK levels of control, obese and PS-treated animals groups.

Groups	Control	PS	Obese	Obese + PS
Glucose (mg/dl)	71±2.4^{C}	74±8.1^{C}	106±6.6^{A}	92±6.7^{B}
Cholesterol (mg/dl)	84±6.1^{C}	79±1.6^{C}	178±6.7^{A}	114±9.3^{B}
Triglycerides (mg/dl)	75±3.2^{C}	72±3.2^{C}	160±10.8^{A}	84±4.7^{B}
HDL-c (mg/dl)	42±2.1^{A}	44±1.9^{A}	32±2.2^{B}	43 ± 3.1^{A}
LDL-c (mg/dl)	41 ± 5.2^{C}	38±3.5^{C}	138±7.2^{A}	72.5±7.3^{B}
CK (U/L)	55±1.6^{C}	57±4.1^{C}	111±2.7^{A}	77±4.8^{B}
LDH (U/L)	1596±56^{C}	1522±57^{D}	2799±131^{A}	1886±88^{B}

Data are expressed as mean ± standard error. All data were subjected to one-way ANOVA followed by post hoc test (Duncan) at $p \leq 0.05$. Within the same row, means with different superscript letters are significantly different. (PS) is purslane seeds.

Regard to Table (2) and comparing to healthy control group, administration of rats with PS didn't disturb the level glucose or lipid profile or CK activity, but significantly reduced LDH activity. Counteract, obese rats group revealed a significant elevation in serum glucose, total cholesterol, triglycerides and LDL-c levels as well as CK and LDH activity matched with a significant reduction in HDL-c level. Moreover and in compare to obese group, treatment of obese rats with PS resulted in a significant decrease in serum levels of glucose, total cholesterol, triglycerides and LDL-c, and activity of serum CK and LDH levels coupled with a marked raise in HDL-c.

Table 3: Serum irisin and TNFα levels, and activity of PON1 of control, obese and PS-treated animals groups.

Groups	Control	PS	Obese	Obese + PAE
Irisin (µg/mL)	4.2 ± 0.13^{A}	4.2 ± 0.37^{A}	2.4±0.22^{B}	3.8±0.21^{A}
PON1 (IU/l)	586± 19^{A}	597±15^{A}	242±16.5^{C}	431±1.3^{B}
TNFα (ng/ml)	36.5±3.8^{C}	35.9±1.8^{C}	79.4± 3^{A}	45 ±3.5^{B}
Leptin (ng/ml)	78±4.5^{C}	70±2.6^{C}	175±4.8^{A}	88±3.3^{B}

Data are expressed as mean ± standard error. All data were subjected to one-way ANOVA followed by post hoc test (Duncan) at $p \leq 0.05$. Within the same row, means with different superscript letters are significantly different. (PS) is Portulaca aqueous extract.

Similarly, PS-feeding never disturbs the serum irisin, TNFα and leptin nor activity of PON1; while obese group showed a significant reduction in serum irisin as well as serum PON1 activity coupled with a significant elevation in serum TNFα and leptin levels when both rats groups were compared with control group. In compare to obese group, favorably treatment of obese animals with PS-feeding significantly down-regulated the serum TNFα and leptin levels, and up regulated serum irisin level and PON1 activity (Table 3).

Table 4: Levels of NO, MDA and GSH and activity of SOD and CAT of liver tissue of control, obese and PS-treated animals groups.

Groups	Control	PS	Obese	Obese + PS
NO (µmo1/g tissue)	63.8±2.2^{C}	59.8± 4^{C}	141.3± 5^{A}	77.1±2.8^{B}
GSH (mg/g tissue)	71.5±3.9^{A}	73.5±3.6^{A}	48.6±2.5^{C}	67.3±2.1^{AB}
SOD (U/g tissue)	39459±2034^{A}	41205±2237^{A}	22901±9647^{C}	36657±1104^{B}
MDA (nmol/g tissue)	170±11.4^{C}	166 ± 11^{C}	401±15.2^{A}	219±13.5^{B}
CAT (U/g tissue)	73.6±3.2^{A}	74.1± 2.1^{A}	53.1±2.8^{C}	65.6±2.4^{B}

Data are expressed as mean ± standard error. All data were subjected to one-way ANOVA followed by post hoc test (Duncan) at $p \leq 0.05$. Within the same row, means with different superscript letters are significantly different. (PS) is purslane seeds.

Table 5: Levels of NO, MDA and GSH, and activity of SOD and CAT of heart tissue of control, obese and obese-treated animals groups.

	Control	PS	Obese	Obese + PS
NO (µmo1/g tissue)	22.9±3.3^{C}	21.1±1.1^{C}	37.9±1.8^{A}	28.8±1.7^{B}
GSH (mmol/g tissue)	28.1±1.4^{A}	27.6±1.5^{A}	17.4±0.49^{B}	2.8±1.7^{A}
MDA (nmol/g tissue)	78.3±4.5^{C}	73.3±5.7^{C}	118.4±4.3^{A}	91.3±2.7^{B}
SOD (IU/g tissue)	1425±60.1^{C}	1466±81.8^{C}	907±48.6^{A}	1116±33.6^{B}
CAT (IU/g tissue)	10.1±0.08^{A}	9.9 ± 0.13^{A}	7.6 ± 0.3^{C}	8.9 ± 0.28^{B}

Data are expressed as mean ± standard error. All data were subjected to one-way ANOVA followed by post hoc test (Duncan) at $p \leq 0.05$. Within the same row, means with different superscript letters are significantly different. (PS) is purslane seeds.

Comparing with normal rats, rats fed PS (20%) mixed with standard diet neither adverse the livers' nor the hearts' oxidative stress voltage (NO and MDA) and the antioxidant battery (GSH, SOD and CAT), while obese rats group recorded a significant reduction in the values of the antioxidant battery (GSH, SOD and CAT) lined with a significant increase in the oxidative stress voltage (NO and MDA). In addition, feeding of obese rats with PS (20%) mixed with high-fat diet resulted in a significant decrease in MDA and NO levels, matched with a significant increase in the values of the antioxidant battery (GSH as well as activity of SOD and CAT) n both liver and heart tissues when compared to the obese rats group Table (4&5).

Microscopic examination

Results of histological examination of liver sections of the study groups are described and illustrated by figures (5-9).

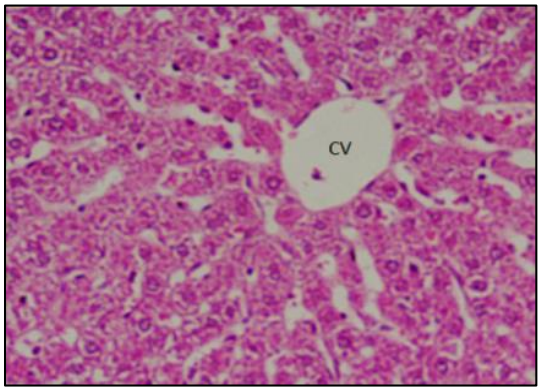

Fig 5: Section of the liver of control rats showing normal histological structure of hepatic lobules and central vein (CV). (H&E 400)

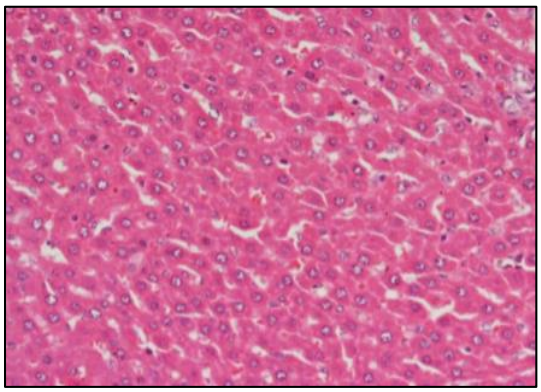

Fig 6: Section of the liver of l rats fed purslane seeds showing normal histological structure of hepatic lobules. (H&E 200)

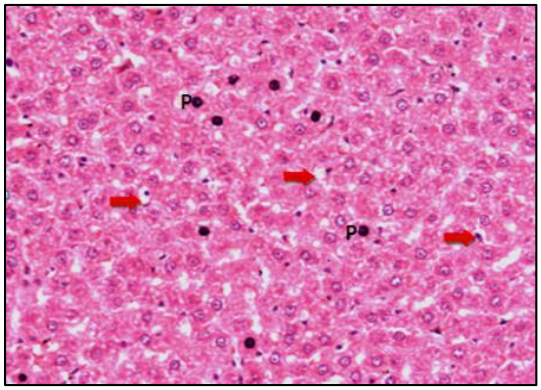

Fig 7: Section of liver of obese rats showing micro-vesicular (red arrow) steatosis and sings of degeneration in the form of pyknosis (P). (H&E 200).

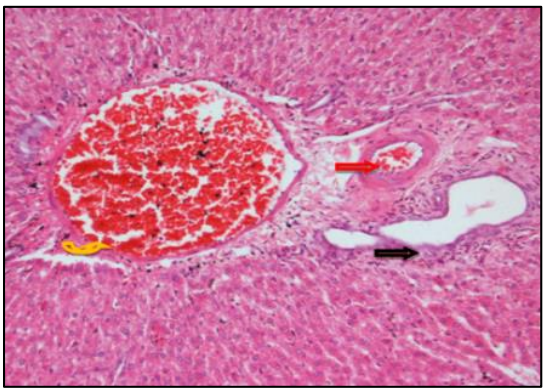

Fig 8: Another filed of obese rats liver showing dilated, congested portal vein (orange arrow), fibrosis in portal area, dilated bile duct (red arrow) and cellular infiltration around (black arrow). (H&E100)

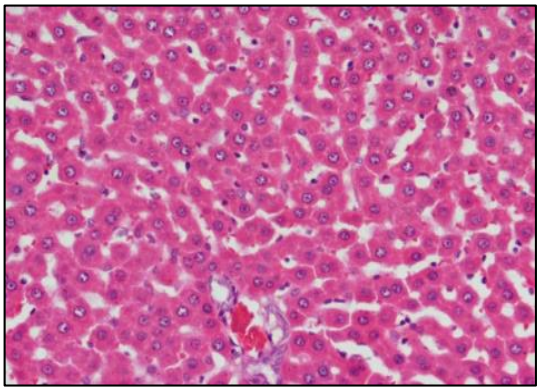

Fig 9: Section of the liver of obese rats fed portulaca seeds, more or less, showing appeared normal but congestion in few areas of blood sinusoids (black arrow). (Hx&Ex400)

Discussion

Overweight and obesity are global public health problems, especially among women in urban settings. There is evidence that the risks of coronary heart disease, ischemic stroke and type 2 diabetes mellitus increase steadily with increasing body mass index (BMI), which also is found to elevate the risk of breast, colon, prostate, endometrium, kidney and gall bladder cancers and recently it is showed that overweight and obesity are linked to 13 different cancers. There is no suitable drug for treating obesity caused by high fat; in regard of that, herbs are implicated as potential protective agents; therefore, the present study attempted to investigate the obesity controlling or thermoregulatory potential of Portulaca seeds in an obese-rat model. Herein, this study declared that feeding with Portulaca seeds mixed diet didn't disturb either hepatic or kidney functions this was monitored from the non-deteriorated serum ALAT, ASAT, GGT and ALP activities or total proteins, albumin, globulin, creatinine and urea levels as well as liver histological structures. This finding reflects the safe effect of PS and is concomitant with Ramadan *et al.* [38]. Fortunately, The present study recorded that feeding of obese rats with PS significantly reduced the rate of body weight gain and BMI values as well as serum leptin, glucose, total cholesterol, triglycerides and LDL-cholesterol (those were elevated as a consequence of obesity) coupled with significant improvement in HDL (which was decreased due to obesity) showing apparent anti-obesity potential. This finding is in agreement with many previous studies [38-41], this improvement could be due one or more mechanisms; PS may reduce fat accumulation and free fatty acid, and/or it may increase energy expenditure-related fatty liver degradation and decreased fatty acid synthesis and fat intake in the liver. Also, the melatonin concentration in PS has a variety of important functions including direct free radical scavenging and anti-inflammatory properties. Other bioactive constituents are found in PS such as dopamine, dopa, coumarins, alkaloids and saponins, polyphenols, flavonoids and anthocyanin 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 ß-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 [42, 43]. In addition, purslane was found containing polyphenols that able to inhibit digestive enzymes such as salivary amylase, intestinal sucrase and a-glucosidase, consequently reduce digestibility

action and promotes pancreatic β-cells. It was reported that purslane showed the most pronounced effect on lipid metabolism of hyperlipidemic rats [44-46].

Purslane seeds significantly improved the lipid profile as it reduced total cholesterol, triglycerides and LDL-c besides the up-regulation of HDL-c; this finding was in accordance with Xiao-xu *et al*. [47] and Abdalla [40] reflecting its efficiency for reducing the risk of cardiovascular disease. Chisolm and Steinberg [48] reported that obesity is a dominant risk factor of atherosclerosis as it can increase the cholesterol content of platelets, polymorphonuclear leukocytes and endothelial cells, so that endothelial and smooth muscle cells, neutrophils and platelets may be sources of free radicals and oxygen free radicals that have been implicated in the pathogenesis of hypercholesterolemic atherosclerosis and antioxidants suppress its development. Purslane is a rich source of omega-3 fatty acids that able to reduce dyslipidemia [49], also, it excellent antioxidant, vitamins, α-tocopherol, β-carotene, and L-norepinephrine found in PS were able to inhibit and stabilize these reactive radicals.

The significant inhibition ASAT, ALAT, GGT and ALP activities, that associated with marked improve of serum proteins, especially albumin, post-feeding of obese rats with PS agree with Xiao-xu *et al*, [47] and El-Sayed *et al*, [50] This effect might be due to the potent antioxidant property of PS contents (vitamins, a-tocopherol, ascorbic acid and 3-carotene, as well as glutathione) that act against oxidative stress, indicates its protective role against liver damage. This protective action may be also due to enhancement of hepatic steatosis and fat accumulation in liver [40, 50-52].

The potential of PS to restore SOD and CAT activities as well as GSH level, and reduce MDA and NO in hepato-cardiac tissues matched with many previous reports [52-54]. Portulaca has been suggested as a rich source of many amino acids [leucine, isoleucine, lysine, methionine, phenylalanine, cystine, tyrosine, threonine and valine] and described as a "power food of the future" because of its high nutritive and antioxidant properties.

PS performed anti-inflammatory, thermogenic and anti-atherosclerotic behaviors; it markedly reduced the inflammatory cytokine (TNF-α), elevated the thermogenic myokine (irisin) and raised the activity of the anti-atherosclerotic enzyme (PON1) in PS fed obese rats. This result is in agreement with Pennathur and Heinecke [55] and Mirza *et al*. [56]

Sridevi *et al*, [57] declared that obesity leads to increased levels of monocytes that secrete increased amounts of TNF-α throgh up-regulation of P38 MAPK, protein kinase (PKC-α and PKC-β), protein kinase (PKC-α and PKC-β), and nuclear factor (NF)-κB. Moreover, Villarroya [58] mechanisms underlying irisin, combined with the increase of brown fat, may unravel the basis of physical exercise concluded a decrement in serum irisin level in high fat diet fed animals, and he suggested that benefits on different conditions. Irisin seems to induce a brown-like phenotype in some white adipocytes.

Post-treatment activities of *Portulaca oleracea* of obese rats with PS led to significant reduction in serum TNF-α, consequently inhibited production of intracellular ROS; this reflecting a protective effect of PS constituents on damaged adipose cell induced by obese conditions.; this result goes in line with Huang and Dong [59] and Jagan *et al*, [60] who reported the anti-nociceptive and the anti-inflammatory activities in trinitrobenzenesulfonic and acid acetic acid intoxicated rats, respectively. It as have reported that blocking of TNF-α signaling pathway decrease the amounts of inflammatory mediators and amelioration of inflammatory diseases; Inhibition of TNF-α can be achieved by various approaches such as monoclonal antibodies and fusion proteins which some of them are now clinically approved for treatment of TNF-α mediated complications [61].

Moradi *et al*. [62] reported that purslane able to decrease oxLDL and ApoB, and increase PON1 activity and ApoA1 concentration; PON1 is synthesized mainly by the liver and circulates in association with apoA-1 and HDL [63]; It inactivates lipid peroxides and hydrogen peroxides; therefore offering protection against oxidative stress. There are discrepancies regarding the correlation between PON1 activity and apoA-I level; however, a recent study showed that lack of apoA-I severely reduced PON1 activity [64]. Additionally, Suehiro *et al*, [65] suggested that purslane is a rich source of polyphenols that have antioxidant potential and able to modulate gene expression of PON1 that in its turn improves PON1 activity; therefore, the increment of PON1 activity of obese rats after fed PS herein could be due to polyphenol and the other antioxidant constituents included in PS.

Conclusion

Purslane has many bioactive compounds with multiple pharmacological and medical properties; it can markedly down-regulate BMI, atherosclerotic markers as well some well-known cardiovascular risk factors, and preferably up-regulates PON1 activity and irisin level. It can presumably be considered, as food supplement, for future long-term studies on prevention and treatment of obesity, dyslipidemia and atherosclerotic disorders.

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