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The Effects of Purslane and Celery on Hypercholesterolemic Mice

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Abstract: The propose of this study was to investigate the effects of purslane, purslane seeds, celery and celery seeds on serum lipids of mice fed with a hypercholesterolemic diet. 60 male albino mice were divided into ten groups six rat each group for 6 weeks: control group, hypercholesterolemic mice group (fed the basal diet containing 1%cholesterol and 16% fat) the other groups of mice fed the same previous hypercholesterolemic diet supplemented with purslane, purslane seeds, celery and celery seeds (10%, 20%). The present study showed that 1% cholesterol and 16% fat administration caused hypercholesterolemia. Induced hypercholesterolemia caused significantly increases in body weight, TG, TC (in serum and liver), LDL, Atherogenic index (AI) and decreases in HDL and HTR compared to the control group. Supplemented diet of hypercholestroemic mice with purslane and celery (fresh and seeds) lead to decrease in body weight, TC, TG (in serum and liver), LDL, AI and increase in HTR. 20% purslane and purslane seed were the most effective to reduced TC, TG, LDL, AI and increased HTR ratio. Supplemented hypocholesterolemic diet with 20% purslane fresh and seed caused significant decrease of activity of AST and ALT compared with hypercholesterolemic group. Purslane contained the highest crude fiber, while purslane seed contained the highest level of omega-3 fatty acid. The results revealed that significantly increase $P<0.05$ in liver glutathione of hypercholesterolemic mice fed with purslane and celery (fresh and seeds) when compared to the HC group. Our results suggest that the supplementation diet with purslane and celery (fresh, seeds) to reduced lipid levels in a hypercholesterolemia disease to prevent from the development the cardiovascular diseases.

Key words: *Portulaca oleracea* · *Apium graveolens* · Lipid profile · Hypercholesterolemic mice · Triglyceride · Fiber

INTRODUCTION

Hypercholesterolemia has been considered as a major risk factor for coronary heart disease and atherosclerosis. Hyperlipidemia, particularly elevated serum cholesterol and Low-Density Lipoprotein (LDL) levels, is a risk factoring in the development of atherosclerotic heart disease [1]. To aid in cholesterol reduction, there have recently been many attempts to use certain common plants that are already well-known in traditional medicine for having biological components that can be used to reduce the lipid levels in body [2-4]. Since it is more beneficial to prevent dietary diseases than to cure them and to change one's diet rather than take medicine, the diet of choice in modern societies should include food with functionality. *Portulaca oleracea* (*P. oleracea*) belonging to the family "*Portulacaceae*" is an herbaceous plant widely distributed throughout the world and is commonly called "*Rejlah*" in Egypt. It contains many biologically active compounds and is a source of many nutrients like, alkaloids, omega-3 fatty acids, coumarins, flavonoids, anthraquinone, protein, free oxalic acids [5], α-linolenic acid and β-carotene [6, 7], mono terpene glycoside, [8], N-trans-feruloyltyramine [9]. It was also found to contain vitamin C, saponins, tannins, polysaccharides, triterpenoids, α-tocopherol and glutathione [10, 11]. The high contents of a variety of phytoconstituents present in this plant were considered to be responsible for the biological activities reported for the plant like antibacterial, antifungal [12], analgesic,

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anti-inflammatory [13], anti-fertility [14], muscle relaxant [15] and wound healing properties [16]. This plant which is normally used as a vegetable to prepare curry by the native people of Andhra Pradesh is used in combination with tomato. Celery (*Apium graveolens*, Family *Apiaceae*) is an excellent source of vitamin C. It is a very good source of dietary fiber, potassium, folate, manganese and vitamin B6. Celery is also a good source of calcium, vitamin B1, vitamin B2, magnesium, vitamin A, phosphorus and iron [17]. *Apium graveolens* has been used in traditional medicine primarily as a diuretic and to treat bronchitis, asthma, liver and spleen diseases [18]. A. graveolens has been extensively studied for its biological activities. Aqueous extract of celery caused significant reduction in serum total cholesterol level in hypercholesterolemic rats [19].

The aim of the present study is to evaluate and to compare the influence of fresh purslane, purslane seeds, fresh celery and celery seeds on the change of the lipid components in serum and liver tissue of hypercholesterolemic mice.

MATERIALS AND METHODS

Materials: Fresh purslane and celery were collected in its fresh state at a local farm in, Egypt. Celery and purslane seeds were obtained from a local market, Egypt. Potassium hydroxide, potassium phosphate, chloroform, methanol and cholesterol were purchased from Sigma Chemical Co. (USA). Kits for blood analysis were purchased from Biodiagnostic Company, Egypt. All other reagents used were analytical grade.

Methods

Chemical Analysis of Purslane and Celery (fresh and seeds): Moisture content, protein content, ash, crude fiber, fat was determined according to the method described in A.O.A.C [20]. Determined of total carbohydrate was calculated by the following equation:

Total carbohydrate = 100- (protein% + fat%+ ash% + fiber).

HPLC Analysis of Phenolic Compounds in Purslane and Celery: Phenolic compounds in purslane and celery fresh and seeds were determined by HPLC according to the method of Goupy *et al*. [21] as follows: 5g of sample were mixed with methanol and centrifuged at 1000 rpm for 10 min and the supernatant was filtered through a 0.2Mm Millipore membrane filter than 1-3 ml was collected in a vial for injection into HPLC Hewllet Packard (series 1050) equipped with auto sampling injector, solvent degasser, ultraviolet (UV) detector set at 280 nm and quaternary HP pump (series 1100). Packed column Hypesil BDs-C18 4.0 x 250 nm was used to separation phenolic compound. The column temperature was maintained at 35°C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1ml/min. Phenolic acid standard from Sigma Company were dissolved in mobile phase and injected into HPLC. Retention time and peak area were used to calculation of phenolic compounds concentration by data analysis of Hewllet ware, Germany.

GC Mass Analysis of Fatty Acids in Purslane and Celery Seeds: Fatty acids in purslane and seeds were extracted according to the method by Folch [22]. Fatty acid was converted to fatty acid methyl ester according to Gibson and Kneebone [23]. Trace GC Mass ultra thermo was used to separate fatty acids on 10 m capillary column (0.1 nm IDX 0.2 Mm). The carrier gas used was helium set at a flow rate of 0.5 ml/min and split less, time 1 min. The injection temperature was 250°C and the FID detector was 350°C. Initial oven temperature was 40°C and raised 230°C and holding for 20 min. Identification of compounds was based on molecular weight of fatty acid.

Animals Experiment: 60 male Albino mice weighing ranged (26-28 g) obtained from El- Salam Farm Giza, Egypt. They were adapted for one week before the experiment. The animal housed individually in stainless steel cages under controlled condition at constant temperature (22°C) and lighting 12h light-dark cycle) and give free access to food and water at all time. Animals randomly enrolled into ten groups of six animals in each group and treated as following:

Group 1 (G1): Control mice; fed basal diet according to Reeves *et al*. [24]. It contained 14% casein, 5% cellulose, 3.5% mineral mixture, 1% vitamin mixture, 0.25% cholin, 0.3 DL- methionine, 7% oil and 68.95% starch.

Group 2(G2): Hypercholesterolemic mice (HC), fed the basal diet + 1% cholesterol+ 16% fat and 0.2% Cholic acid according to Harnafi [25].

Groups 3 and 4 (G3& G4): Hypercholesterolemic diet supplemented with 10% and 20% fresh purslane.

Groups 5 and 6(G5& G6): Hypercholesterolemic diet supplemented with10% and 20% pruslane seeds.

Groups 7 and 8 (G7& G8): Hypercholesterolemic diet supplemented with10% and 20% fresh celery.

Groups 9 and 10 (G9& G10): Hypercholesterolemic diet supplemented with10% and 20% celery seeds.

Each rat was weighted at the initial and the end of experimental and food intake also was recorded daily. At the end of experimental period (6 weeks), mice were sacrificed after overnight fasting and blood of each rat was taken from the abdominal aorta under anesthesia by diethyl ether. The serum was separated by allowing blood samples left for 15 minutes at temperature of 25°C then centrifuged at 3000 rpm for 20 minutes, then kept in plastic vials at -20°C until analysis. Liver tissues were quickly dissected, rinsed in ice-chilled normal saline, blotted on filter paper. The tissues were cut into small portions and stored at -20°C until using for measuring cholesterol and triglyceride contents.

Biochemical Analysis: Serum was analyzed for the following biochemical parameter: triacylglycerol, total cholesterol, HDL-c and LDL-c were determined by the method of (Jacobs and Vander mark [26], Richmond [27], Burstein *et al.* [28], Wieland and Sidel [29]. Serum AST and ALT were determined according to Reitman and Frankel [30]. Atherogenic index (AI) and HTR ratio calculated by an equations development by Friedewald *et al*. [31]:

$$\text{Atherogenic index (AI)} = (\text{serum total cholesterol} - \text{HDL-c})/\ \text{HDL-c}$$

$$\text{HTR ratio} = \text{HDL-c/TC} \times 100$$

Extraction and Analysis Liver Total Cholesterol, Triglycerides and Determination of Glutathione: At the end of the experiment, liver was removed, rinsed in ice chilled normal saline and blotted on filter paper and then tissues were cut into small portions and stored at -20°C before use. Extraction of liver analysis of total cholesterol and triglycerides was carried out according to the method by Hostmark [32]: 1g of liver portion from each animal was homogenized in 10 ml isopropanol. The liver homogenate was allowed to stand for 48 h at 4°C. The mixture was centrifuged 15 min at 2500 rpm and the supernatant was used for lipid analysis. Total cholesterol and triacylglycrol were quantified using enzymatic as described above. Glutathione reduced activity (GSH) of liver was measured according to the method by Beulter *et al.* [33].

Statistical Analysis: The results obtained were analyzed using SPSS program (version 17.0) and expressed as mean and standard deviations (SD). Statistical significance ($p<0.05$) among the groups were determined by one-way ANOVA followed by Duncan's multiple range test.

RESULTS AND DISCUSSION

Chemicals Composition of Purslane and Celery: The content of moisture, protein, crude fiber, ash and carbohydrate of the purslane, purslane seed, celery and celery seed are shown in Table 1. The moisture content almost the same in all treatment ranged from 5.31 of purslane to 5.62 of celery at dray weight basis. These results are in agreement with those obtained by Michael *et al*. [5], Lee *et al*. [34] and Syed and Rajeev [35] reported that the moisture content of purslane powder was 5.14% and celery (5.1-11%). While, the highest value of protein was recorded for purslane (23.71%) followed by purslane seed, celery and celery seed (22.34, 19.27 and 18.19%) respectively. This result is in agreement with previous studies reported by Lee *et al*. [34], Kabulov and Tashbekov [36], Ezekwe *et al*. [37], Obied *et al*. [38] and Besong *et al*. [39]; they found that the protein of purslane was ranged from 17.9% to 26.7%. The crude protein content of celery seed was similar with that obtained by Syed and Rajeev [35] reported the crude protein levels in celery seed was 18.1%.

The fat content of purslane seed was found to be higher (9.1%) than that of purslane (4.42%), celery (1.15%) and celery seed (3.25%). The total lipids content of purslane observed in the present study are in agreement with the findings of Michael *et al*. [5], Lee *et al*. [34] and Mangalan *et al*. [40], who reported that fat content of purslane powder was 4.9%. While, the total lipids content of celery was similar to the findings of Reem *et al*. [41], who reported that the lipid content in celery 1.36%. The results revealed that the purslane contained the highest crude fiber (21.61%), while the lowest value of crude fiber (12.12%) was observed in celery seed. These results are in line with those obtained by Obied *et al*. [38], who reported that the crude fiber content of purslane was 20.3%. The highest value of ash was recorded for celery (21.96%) followed by celery seed, purslane and purslane seed (20.83. 16.62 and 15.39%) respectively. Ash results were less than recorded by Obied *et al*. [38] mentioned that ash content of purslane was 32.5%.

Table 1: Chemical composition (g/100g dry matter) of purslane and celery

Samples	Moisture	Protein	Fat	Crude fiber	Ash	Carbohydrates
Purslane	5.31	23.71	4.42	21.61	16.62	28.33
Purslane seeds	5.933	22.34	9.1	13.37	15.39	33.87
Celery	5.62	19.27	1.15	19.32	21.96	32.68
Celery seeds	6.39	18.19	3.25	12.12	20.83	39.22

Table 2: Relative Percentage of Fatty acid Composition in Purslane and Celery (mg/100 g) by GC Mass

Items	Fatty acids	Purslane	Purslane Seeds	Celery	Celery Seeds
Saturated fatty acid	Caprylic	14.53	0.76	1.26	1.46
	Capric	1.33	0.03	8.64	1.0
	Lauric	6.72	0.12	4.60	3.51
	Myristic	2.87	0.06	1.46	0.70
	Palmatic	23.05	7.37	17.21	7.46
	Stearic	2.56	0.08	7.38	--
Total saturated fatty acid	TSFA	51.06	9.02	40.55	14.13
MUSFA	Oleic	8.15	4.61	3.74	77.74
Omega-6	Linoleic	15.83	10.82	16.72	6.36
	Arachidonic	--	0.14	0.60	0.23
Omega-3	Linolenic	24.96	76.01	38.39	1.54
Total unsaturated fatty acid	TUSFA	48.94	90.98	59.45	85.87

Table 3: Polyphenolic Compounds of Purslane and Celery by HPLC

Phenolic compounds	Purslane	Purslane seeds	Celery	Celery seeds
Gallic acid	--	2.69	--	4.49
Protocateachuic acid	--	1.85	14.07	37.17
Catechol	0.52	3.23	18.69	--
P- Coumaric acid	0.06	1.39	7.07	6.66
Catechin	--	3.21	31.28	--
Caffeic acid	0.39	0.57	4.05	--
Vanillic acid	--	1.42	3.43	0.78
Syrinigic	0.64	--	--	--
Salicylic acid	1.94	--	112.34	110.30
Ferulic acid	--	2.13	12.87	10.07
Cinnamic acid	--	--	-	0.13
Chrysin	--	--	2.43	--

Fatty acids Composition and Polyphenolic Compounds of Purslane and Celery: Table 2 shows the fatty acids composition of purslane, purslane seeds, celery and celery seeds. The results of the total fatty acids analysis revealed that linolenic acid (omega-3) was the major unsaturated fatty acid in purslane, purslane seeds and celery; it was present at 24.96%, 76.01% and 38.39%, respectively of the total fatty acids. These results are in agreement with those obtained by Michael *et al*. [5] and Omara–Alwala *et al*. [42], who reported that linolenic acid was the most abundant fatty acid in purslane, ranged from 27.7% to 39.1%. Meanwhile oleic acid was the major unsaturated fatty acid in celery seeds (77.74%). Moreover, palmatic acid was the major saturated fatty acid in purslane, purslane seeds, celery and celery seeds (23.05%, 7.37%, 17.21% and 7.46%, respectively). Our results was similar to those noted by Oliveira *et al*. [43] finding the palmatic acid of purslane ranged 19.3- 24.3%. Polyphenolic compounds of purslane, purslane seeds, celery and celery seeds (Table 3). The salicylic acid was major phenolic compound in purslane, celery and celery seeds (1.94%, 112.34% and 110.30%, respectively. Syrinigic was only identified in purslane and Chrysin in celery. Meanwhile, it is not detectable in purslane seeds. Catechol and Catechin was the major phenolic compound in purslane seeds (3.23% and 3.21%, respectively). The higher concentration of p-coumaric acid was detectable in celery (7.07 mg/100g) followed by celery seeds (6.66 mg/100g), purslane seeds (1.39 mg/100g) and purslane (0.06 mg/100g).These results are in agreement with Yao *et al*. [44] reported that major phenolic acid identified in extract of celery was caffic acid, p-coumaric and ferulic acid. Park [45] reported that the highest amount of Gallic acid were detected in dried sample of purslane and salicylic acid in fresh purslane, large amount of ferulic acid in purslane seeds. Erkan [46] reported the purslane extract contained caffic acid, chlorogenic acid, p- coumaric and ferulic acid.

Table 4: Changes of body weight, food intake and FER in the hypercholesterolemic mice administered with Purslane and Celery (g) (Mean ±SD)

Groups	IBW*	FBW•	BWG†	FI‡	FER§
G1 control	26.92±0.684^{abc}	40.38±0.485 ^{b}	13.5±0.707^{b}	9.82±2.31^{b}	1.37±0.01^{bc}
G2 (HC)	26.3±0.583^{c}	41.8±0.809^{a}	15.5±0.676^{a}	10.05±2.09^{a}	1.54±0.012^{a}
G3(HC)+10% fresh purslane	26.72±0.627^{abc}	39.02±1.389^{c}	12.3±0.66^{c}	9.35±3.62^{c}	1.31±0.019^{bcd}
G4 (HC))+20% fresh purslane	27.1±0.684^{abc}	37.9±0.865^{de}	10.8± 0.809^{de}	8.67±6.07^{ef}	1.25± 0.03^{cde}
G5 (HC) +10% purslane seeds	26.5±0.707^{bc}	38.6±0.792^{cd}	12.1±1.05^{c}	9.30±6.16^{c}	1.46±0.396^{ab}
G6 (HC) +20% purslane seeds	27.2±0.645^{ab}	37.4±0.681^{e}	10.2±0.645^{ef}	8.53±3.75^{f}	1.2±0.018^{de}
G7 (HC))+10% fresh celery	27.4±0.456^{a}	38.9±0.851^{cd}	11.5±0.707^{cd}	9.02±3.74^{d}	1.27±0.019^{cde}
G8- (HC))+20% fresh celery	26.8±0.809^{abc}	36.3±0.844^{f}	9.5±0.707^{fg}	8.31±6.12^{g}	1.14±0.03^{e}
G9 (HC))+10% celery seeds	27.6±0.738^{a}	38.7±0.772^{cd}	11.1±0.6^{de}	8.84±3.54^{e}	1.26±0.019^{cde}
G10(HC))+20% celery seeds	26.4±0.68 ^{bc}	35.4±0.708^{f}	9±0.949^{g}	7.63±5.4^{h}	1.18±0.029^{de}

*Initial body weight, •Final body weight, †Body weight gain, ‡food intake, §food efficiency ratio

Mean with the same letters in the column are not significantly different at P<0.05

Table 5: Effect of Purslane and Celery on the lipid profiles, in serum of hypercholesterolemic mice (mmol/l) Mean ±SD

Groups	TG*	TC●	HDL-C†	LDL-C‡
G1 control	1.6± 0.26 ^{d}	2.9±0.51^{e}	1.7±0.4^{a}	0.88±0.146^{f}
G2 (HC)	2.96±0.275^{a}	4.29±0.136^{a}	1.21±0.136^{c}	2.49±0.224^{a}
G3(HC)+10% fresh purslane	1.8±0.141^{d}	3.65±0.139 ^{c}	1.34±0.141^{bc}	1.92±0.281^{d}
G4 (HC))+20% fresh purslane	1.74±0.139^{d}	3.22±0.136^{d}	1.45± 0.136^{b}	1.42± 0.13^{e}
G5 (HC) +10% purslane seeds	1.82± 0.188^{d}	3.6±0.141^{c}	1.35±0.119^{bc}	1.89±0.141^{d}
G6 (HC) +20% purslane seeds	1.71±0.146^{d}	3.24±0.132^{d}	1.48±0.136^{b}	1.42±0.13^{e}
G7 (HC))+10% fresh celery	2.66±0.13^{bc}	3.95±0.136^{b}	1.26±0.13^{bc}	2.16±0.141^{bc}
G8- (HC))+20% fresh celery	2.47±0.15^{c}	3.75±0.13^{bc}	1.29±0.141^{bc}	1.97±0.13^{cd}
G9 (HC))+10% celery seeds	2.74±0.15^{b}	4±0.141^{b}	1.25±0.114^{bc}	2.2±0.141^{b}
G10(HC))+20% celery seeds	2.68±0.1^{bc}	3.94±0.132^{b}	1.27±0.104^{bc}	2.13±0.14^{bc}

*Triacylglycerol, • Total Cholesterol, †High density lipoprotein Cholesterol, ‡Low density lipoprotein Cholesterol

Mean with the same letters in the column are not significantly different at P<0.05

Body Weight Change, Food Intake and FER: As shown in Table 4 induced hypercholesterolemia caused a significant increase in body weight gain and food intake and FER in G2 as compared with healthy control G1. Previous study by Lecumberri *et al*. [47] reported that rats fed high cholesterol diet showed significant increase in body weight. Barakat [48] reported that induced hypercholesterolemia caused significant increase in body weight gain. Administration of purslane and celery fresh or purslane and celery seeds to hypercholesterolemic mice caused a significant decrease in body weight gain as compared with healthy control group. Decrease in the body weight gain was increased with concentrated increasing of purslane and celery. This result may be due to high present fiber in purslane and celery (12-21%). The dietary fiber reduces the gastric emptying rate and makes it possible for mice to feel full, while delaying the absorption and digestion of nutrients and reduced food intake which lead to decrease body weight gain Torsdottir *et al*. [49]. Our results are in agreement with the findings of Kim *et al*. [50], who reported that when 10% mulberry leaf powder was added to hypercholesterolemic rats, the decreased body weight and the reduction of the FER were dependent on the dietary fiber. In this study, the groups fed the fresh purslane and celery (10%, 20%) or purslane and celery seeds (10%, 20%) showed a significant decrease in terms of the daily food intake and FER.

Lipid Profiles Level: The results of lipid profiles are shown in Table (5). Hypercholesterolemic group exhibit significant increase p<0.05 in TG, TC, LDL-c and decrease in HDL-c compared to the control group. This result are agreement with Harnafi *et al*. [25] and Kumar *et al*. [51] reported that TC, TG and LDL-c levels in hypercholesterolemic control rats were significantly higher than the normal control group. Supplemented diet of hypercholesterolemic mice with purslane and celery (fresh and seeds) at different concentration (10% and 20%) lead to significant decrease p<0.05 in TC, TG and LDL compared to HC group. Feeding hypercholesterolemic mice on diet supplemented with 20% purslane fresh caused significant decrease p<0.05 of TG, TC and LDL-c, rate of decrease was 41.2%, 24.9% and 42.9%, respectively. Meanwhile, rate of decrease of 20% celery fresh was 49%, 12.58% and 26.39%, respectively. Also, 20% purslane seeds were more effective to decrease TG, TC and LDL-c than 20% celery seeds. From the present results it could be noticed that 20% purslane fresh

Table 6: Levels of AI, LDL/HDL Ratio and HTR of hypercholesterolemic mice fed with Purslane and Celery Mean± SD.

Groups	AI*	LDL/HDL Ratio	HTR Ratio
G1 control	0.71±0.02^{f}	0.52±0.05^{e}	58.6±0.37^{a}
G2 (HC)	2.55±0.07^{a}	2.1±0.26^{a}	28.2±0.28^{i}
G3(HC)+10% fresh purslane	1.72±0.06^{d}	1.5±0.41^{cb}	36.7±0.23^{e}
G4 (HC))+20% fresh purslane	1.22±0.06^{e}	0.98±0.05^{d}	45±0.34^{c}
G5 (HC) +10% purslane seeds	1.67±0.05^{d}	1.4±0.32^{c}	37.5±0.32^{d}
G6 (HC) +20% purslane seeds	1.19±0.03^{e}	0.96±0.04^{d}	45.7±0.28^{b}
G7 (HC))+10% fresh celery	2.13±0.02^{b}	1.7±0.46^{cb}	31.9±0.46^{g}
G8- (HC))+20% fresh celery	1.91±0.03^{c}	1.5±0.41^{cb}	34.4±0.32^{f}
G9 (HC))+10% celery seeds	2.2±0.14^{b}	1.8±0.26^{ab}	31.3±0.23^{h}
G10(HC))+20% celery seeds	2.1±0.26^{b}	1.7±0.20b^{c}	32.2±0.23^{g}

*Atherogenic index

Mean with the same letters in the column are not significantly different at P<0.05

and purslane seeds was the most effective to decrease TC, TG and LDL-c than 20% celery fresh and celery seeds. The cholesterol lowering effect of purslane may be attributed to the combined effect of omega-3 fatty acid and fiber. Since, purslane is rich in fiber and omega-3 fatty acid. Moreover, the dietary fiber could be promoting the elimination of bile, the lack of bile in body could be reproduced from dietary cholesterol and then the level of serum cholesterol could be decrease. These results are in line with the results obtained by Besong *et al*. [39], who reported that the TC levels revealed significant decrease P<0.05 when supplying 3% purslane powder in the hypercholesterol diet in comparison with the HC group and they reported that the reduction of total cholesterol level of serum is highly associated with omega-3 fatty acid of purslane. In addition to having high level of omega-3 fatty acid, purslane also has high levels of γ-linolenic acid, fiber and polyphenols [43], all of which have been shown to have a reducing effect on serum lipid levels [50, 52]. In particular, the ethanol extract of purslane also demonstrated a lowering effect of total lipid, total cholesterol and triglyceride levels in the serum of hypercholesterolemic rats [52].

Data in presented in Table 5 showed no significant difference in HDL-c of all group compared to HC group except for 20% purslane and purslane seed showed significantly increase in HDL-c.. Our results agreement with Besong *et al*. [39] observed that HDL-c level was increased of hypercholestrolemic rats fed 3% purslane for 28 days. Additionally, the reduction of LDL-C by the supplementation of purslane and purslane seeds (10% and 20%) within the hypercholesterol diet is expected to be effective for the prevention of arteriosclerosis and cardiovascular diseases, since an increase of serum LDL-C level is considered to be a stronger risk factor for the occurrence of cardiovascular diseases than the increase of the total cholesterol level. In fact, the reduction of LDL-C is emphasized more for the therapy of hyperlipidemia [53].

Data in Table 6 showed that the Levels of AI, LDL/HDL ratio and HTR ratio of hypercholesterolemic mice administered purslane, purslane seeds, celery and celery seeds. Atherogenic index increased significantly p<0.05 in the HC group (2.55) in comparison with the control group (0.71). The atherogenic index decreased significantly according to the amount of purslane (fresh, seeds) and celery (fresh, seeds) added in comparison with HC group. The lowest atherogenic index in all treatments was recorded to mice feeding 20% purslane seeds (1.19) and fresh purslane (1.22). The atherogenic index decreases due to significant reduction in LDL/HDL ratio, the reduction in this ratio is considered as an anti atherosclerotic factor. Since, there is appositive correlation between an increased LDL/HDL ratio and the development of atherosclerosis and related cardiovascular events. These results are in agreement with those obtained by Movahedian *et al*. [54], who reported that rabbits fed high cholesterol diet showed significant decrease in atherogenic index by using purslane. The atherogenic index markedly decreased causing a significant reduction in LDL/HDL ratio in all groups fed diet supplemented with purslane or celery except for 10% celery seeds.

Data in Table 6 also indicated that a significantly increase in HTR ratio in all groups was observed compared to HC group. The highest value of HTR recorded of 20% purslane and purslane seed. Reduction in HTR ratio is a major in importance predicting coronary heart disease in human being, an increase in this ratio is believed to furnish a beneficial effect. Our results are in

Table 7: Activities of AST and ALT in serum of hypercholesterolemic mice fed with Purslane and Celery (U/mL) Mean± SD.

Groups	AST*	ALT†
G1 control	92.9±0.322^{g}	40.8±0.424^{h}
G2 (HC)	111.57±0.312^{a}	59.24±0.136^{a}
G3(HC)+10% fresh purslane	93.5±0.369^{e}	51.54±0.144^{d}
G4 (HC))+20% fresh purslane	93.4±0.141^{ef}	42±0.369^{f}
G5 (HC) +10% purslane seeds	93.2±0.141^{f}	50.65±0.139^{e}
G6 (HC) +20% purslane seeds	93.2±0.141^{f}	41.7±0.141^{g}
G7 (HC))+10% fresh celery	109.4±0.141^{c}	56.2±0.141^{b}
G8- (HC))+20% fresh celery	107.4±0.141^{d}	54.4±0.141^{c}
G9 (HC))+10% celery seeds	111.4±0.141^{ab}	59.1±0.141^{a}
G10(HC))+20% celery seeds	111.3±0.141^{b}	59±0.322^{a}

Aspartate aminotransferase*, Alanine aminotransferase†

Mean with the same letters in the column are not significantly different at P<0.05

Table 8: Levels of total cholesterol, triglyceride and GSH in liver tissue of hypercholesterolemic mice fed with Purslane and Celery (mg/g, wet liver)

Groups	TG* Mean± SD.	TC● Mean± SD.	GSH† Mean± SD.
G1 control	12.42±0.133^{h}	2.45±0.138^{e}	4.67±0.494^{a}
G2 (HC)	17.82±0.13^{a}	3.39±0.141^{a}	3.18±0.519^{c}
G3(HC)+10% fresh purslane	14.13±0.134^{f}	2.92±0.136^{c}	3.98±0.623^{ab}
G4 (HC))+20% fresh purslane	13.22±0.133^{g}	2.63±0.15^{d}	4.29±0.400^{ab}
G5 (HC) +10% purslane seeds	14.24±0.126^{f}	2.88±0.141^{c}	3.99±0.623^{ab}
G6 (HC) +20% purslane seeds	13.15±0.13^{g}	2.6±0.141^{de}	4.32±0.424^{ab}
G7 (HC))+10% fresh celery	16.3±0.2^{d}	3.2±0.141^{b}	3.87±0.566^{b}
G8- (HC))+20% fresh celery	15.65±0.13^{e}	3±0.141^{c}	4.16±0.510^{ab}
G9 (HC))+10% celery seeds	16.9±0.141^{b}	3.25±0.114^{ab}	3.91±0.651^{b}
G10(HC))+20% celery seeds	16.6±0.141^{c}	3.18±0.158^{b}	4.20±0.522^{ab}

*Triglycerides, ● Total Cholesterol, †Reduced Glutathione

Mean with the same letters in the column are not significantly different at P<0.05

agreement with those obtained Makni *et al*. [55], who stated that the increase in HDL-C or HTR ratio is one of the most important criteria of anti-hypercholesterolemic agent.

AST and ALT Activities in Serum: Data in Table 7 illustrated that liver function of hypercholesterolemic mice fed on different concentrations of purslane and celery. The activities of AST and ALT increased significantly in the HC group (111.57 and 59.24U/ml) in comparison with the normal control group (92.9 and 40.8 U/ml). The activities of AST and ALT tend to increase according to the exogenous cholesterol content from diet [56]. Feeding hypercolesterolemic mice on purslane and celery (fresh and seed) caused significant decrease in ALT and AST levels except for celery seed (10% and 20%) compared to the control group. These results were on line with obtained results by Ali *et al.* [57], who reported that purslane administration significantly reduced serum levels of hepatic enzymes. It is expected that the adding of purslane (fresh and seed) and celery fresh to hypercholesterol diet will be effective for the recovery of hepatic function by the improved of lipid metabolism or delaying the hepatic disorder. Lee *et al*. [34] reported the activity of ALT and AST were decrease of hypercholesterolemic rats groups fed the purslane powder compared to HC group.

Lipid Levels of Liver Tissue: Table 8 depicts the liver lipid analysis of ten experimental groups. High cholesterol diet a statistically significant increase concentration of hepatic cholesterol (43.78%) and triacylglycerol (38.37%) as compared with normal control mice. The administration of purslane and celery (fresh and seeds) with hypercholesterolemic diet consistently reduced hepatic TC and TG except for 10% celery seeds revealed no significant difference in TC. The polyphenolic compound of plants decreases the cholesterol level for liver tissue [58]. Liver cholesterol lowering effect may be due to purslane and celery reduced absorption of cholesterol and fat or increases the fecal excretion of fat and cholesterol. Kang *et al.* [59] reported that purslane blocks the re-absorption of the bile acid and restrains the creation of endogenous cholesterol. Data in Table 8 also showed that the content of liver reduced glutathione (GSH) of hypercholesterolemic mice fed with purslane, purslane seeds, celery and celery seeds. The content of GSH decreased significantly in the HC group (G2) in

comparison with the normal group (G1), while the groups fed the purslane, purslane seeds, celery and celery seeds significantly increased comparison with HC group. Our results are accordance with data reported by Mohamed *et al*. [60], who indicated that rats which consumed aqueous juice of purslane caused a significant increase in glutathione content of liver when compared to control rats. Our results also approved with Mohammed and Soad [61], who reported that rats consumed ethanolic and aqueous extracts of purslane leaves with Paracetamol (induced hepatic damage) caused a significant increase in glutathione content of liver when compared to control rats (was given Paracetamol only). Al- Qurais *et al*. [62] reported that rats fed purslane at dose 1.5 mg/kg body weight for 12 days induced a marked improve in glutathione GSH. Our findings are in agreement with those obtained by Jovanka *et al*. [63], who reported that rats which consumed celery juice caused increase in reduced glutathione content of liver when compared to control rats. Furthermore, the present findings are in accordance with data reported by Jabbar *et al*. [64], who observed increased GSH content in liver of rats treated with n-butanol extract of celery (*Apium graveolens*) seed when compared to control and diabetic rats.

CONCLUSION

The current study proved the efficiency of using fresh purslane, purslane seeds, celery and celery seeds on hypercholesterolemia and liver function in mice fed high cholesterol diets.

REFERENCES

1. Romero-Corral, A., V.K. Somers, J. Korinek, J. Sierra-Johnson, R.J. Thomas, T.G. Allison and F. Lopez-Jimenez, 2006. Update in prevention of atherosclerotic heart disease: management of major cardiovascular risk factor. Revista de Investigations Clinica, 58: 237-244.
2. Rhee, S.J., J.M. Ahn, K.H. Ku and J.H. Choi, 2005. Effects of radish leaves powder on hepatic antioxidative system in rats fed high-cholesterol diet. J. Korean Soc Food Sci. Nutr., 34: 1157-1163.
3. Lee, J.J., M.H. Choo and M.Y. Lee, 2006. Effect of *Pimpinella brachycarpa* extract on lipid metabolism in rats fed high cholesterol diet. J Korean Soc Food Sci. Nutr., 35: 1151-1158.
4. Kim, Y.H., J.H. Lee, B.K. Koo and H.S. Lee, 2007. Isoflavone-rich bean sprouts improve hyperlipidemia. J Korean Soc Food Sci. Nutr., 36: 1248-1256.
5. Michael, O.E., R.O. Thomas R. and M. Tadesse, 1999. Nutritive characterization of purslane accessions as influenced by planting date. Plant Foods Hum Nutr., 54: 183-91.
6. Liu, L.X., P. Howe, Y.F. Zhou, Z.Q. Xu, C. Hocart and R. Zhang, 2000. Fatty acids and b-carotene in Australian purslane (*Portulaca oleracea*) varieties. J. Chromatography, 893: 207-13.
7. Barbosa–Filho, J.M., A.A. Alencar, A.C. Nunes Tomaz, J.G. Sena Filho and P.F. Athayde Filho, 2008. Sources of alpha, beta, gamma, delta and epsilon-carotenes: A twentieth century review. Rev Bras Farmacogn, 18: 135-54.
8. Sakai, N., K. Okamoto, Y. Shizuru, Y. Fukuyama and A. Portuloside, 1996. A monoterpene glucoside from *Portulaca oleracea*. Phytochemistry, 42: 1625-8.
9. Mizutani, M., 1998. Factors responsible for inhibiting the mortality of zoospores of the phytopathogenic fungus *Aphanomyces* cochlioides isolated from the non-host plant *Portulaca oleracea*. FEBS Lett., 438: 236-40.
10. Simopoulous, A.P., H.A. Norman, A. Gillaspy and J.A. Duke, 1992. Common purslane: A source of omega-3 fatty acids and anti-oxidants. J. Am. Coll Nutr., 11: 374-82.
11. Prashanth, K.L., H. Jadav, P. Thakurdesai and A.N. Nagappa, 2005. Cosmetic potential of herbal extracts. Nat Prod Radiat., 4: 351.
12. Oh, K.B., I.M. Chang, K.I. Hwang and W. Mar, 2002. Detection of anti-fungal activity in *Portulaca oleracea* by a single cell bioassay system. J. Phytother Res., 14: 329-32.
13. Chan, K., M.W. Islam, M. Kamil, R. Radhakrishna, M.N. Zakaria and M. Habibullah, 2000. The analgesic and anti-inflammatory effects of *Portulaca oleracea* Linn. J. Ethnopharmacol., 73: 445-51.
14. Verma, O.P., S. Kumar and S.N. Chatterjee, 1982. Antfertility effects of common edible *Portulaca oleracea* on the reproductive organs of male albino mice. Indian J. Med. Res., 75: 301-10.
15. Parry, O., J.A. Marks and F.K. Okwuasab, 1993. The skeletal muscle relaxant action of *Portulaca oleracea*: Role of potassium ions. J. Ethnopharmacol., 49: 187-94.
16. Rasheed, A.N., F.U. Affif and A.M. Disi, 2003. Simple evaluation of the wound healing activity of crude extracts of *Portulaca oleracea* in Muss musculus JVJ-1. J Ethnopharmacol., 68: 131-6.
17. Mitra, S.K., M.V. Venkataranganna, S. Gopumadhavn, S.D. Anturlikar and U.V. Udupa, 2001. The protective effect of HD-03 in CCl_4- induced hepatic encephalopathy in rats. Phytother Res., 15: 493-6.

18. Singh, A. and S. Handa, 1995. Hepatoprotective activity of Apium graveolens and Hygrophila auriculata against paracetamol and thioacetamide intoxication in rats. J. Ethnopharmacol., 49: 119-126.
19. Tsi, D. and B. Tan, 2000. The mechanism underlying the hypocholesterolaemic activity of aqueous celery extract, its butanol and aqueous fractions in genetically hypercholesterolaemic rats. Life Sci., 66: 755-767.
20. A.O.A.C. 2000. Official Methods of Analysis. 17th Edition of AOAC International Published by AOAC International Sut Gaithersburg, Maryland.
21. Goupy, P., M. Hugues, P. Biovin and M.J. Amiot, 1999. Antioxidant composition and activity of barley (Hordeum vulgare) and malt extracts and isolated phenolic compounds. J. Sci. Food Agric., 79: 1265-1634.
22. Folch, J., M. Lees and G. Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226: 497-509.
23. Gibson, R.A. and G.M. Kneebone, 1981. Fatty acid composition and mature milk. Am J. Clin. Nut., 34: 252-257.
24. Reeves, R.G., F.H. Nielsen and G.C. Fahey, 1993. AIN-93 Purified Diets for Laboratory Rodents. J. Nutr., 123(1): 1939-1951.
25. Harnafi, H., M. Aziz and S. Amrani, 2009. Sweet basil (*Ocimum basilicum L.*) improves lipid metabolism in hypercholesterolemic rats. Span the European Journal of Clinical Nutrition and Metabolism, 4: e181-e186.
26. Jacobs, N.J. and P.J. Vander mark, 1960. Determination of serum triacylglycerol. Arch. Biochem. Biophs., pp: 88-250.
27. Richmond, N., 1973. Preparation properties of a cholesterol oxidase from nacardia SP. enzymatic assay of total cholesterol in serum. Clin. Chem., 19: 1350-1356.
28. Burstein, M., H.R. Scholnick and R. Haarfin, 1970. Rapid method for isolation of lipoprotein from human serum by precipitation with polyamine. Lipid Research, 11: 385-395.
29. Wieland, H. and D. Seidal, 1983. A Simple Specific Method for precipitation of low density lipoprotein. J. Lipid Res., 24: 904-909.
30. Reitman, S. and S. Frankel, 1957. A calorimetric method for determination of serum AST. Am. J. Clin. Path., 18: 26.
31. Friedewald, W.T., K.T. Levy and D.S. Fredrickson, 1972. Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin. Chem., 226: 499-504.
32. Hostmark, H.A., 1987. Lipoprotein lipases, lipoprotein and tissue lipids rats fed fish oil and or coconut oil. Journal of Nutrition, 117: 1011-1017.
33. Beulter, E., O. Duran and M.B. Kelly, 1963. Determination of reduced glutathione. J. Lab. Clin. Med., 61: 882.
34. Lee, S.M., M.J. Kang, M.J. Kim, S.H. Kim and N.J. Sung, 2011. Effect of *Portulaca oleracea* powder on lipid levels of rats fed hypercholesterolemia inducing diet. J. Food Nutr., 16: 202-206.
35. Syed, S.F. and K.S. Rajeev, 2012. Review on the pharmacognostical & pharmacological characterization of *Apium graveolens* Linn. Indo Global Journal of Pharmaceutical Sciences, 2(1): 36-42.
36. Kabulov, D. and T.I. Tashbekov, 1979. Purslane. Kaftofel'i Ovoschi., 8: 45-46.
37. Ezekwe, M.O., T.R. Omara-Alwala and T. Mebrahtu, 1994. The influence of planting date on nutritive quality of purslane accessions. Faseb J., 8: 923A
38. Obied, W.A., E.N. Mohamoud and O.S.A. Mohamed, 2003. Portulaca (Purslane): nutritive composition and clinic- pathological effect on Nubian goats. Small Ruminant Research, 48: 31-36.
39. Besong, S.A., M.O. Ezekwe and E. Ezekwe, 2011. Evaluating the effects of freeze-dried supplements of purslane (*Portulaca oleracea*) on blood lipids in hypercholesterolemic adults. International Journal of Nutrition and Metabolism, 3(4): 43-49.
40. Mangalan, S., M. Daniel and S.D. Sabnis, 1989. Nutritional and phytochemical aspects of some vegetables of centrospermae. J. Econ Tax Bot., 13: 227-230.
41. Reem, T., A. Iman, S. M. Said and A. K. Taha, 2009. A diet rich fiber improves lipid profile in rats fed on high fat diet. Turkish Journal of Biochemistry, 34(2): 105-11.
42. Omara-Alwala, T.R., T. Mebrahtu and D.E. Prior Ezekwe, 1991. Omega-3 fatty acids in purslane (*Portulaca oleracea*) tissues. J. Am. Oil Chem. Soc., 68: 198-199.
43. Oliveira, I., P. Valentão, R. Lopes, P.B. Andrade, A. Bento and J. A. Pereira, 2009. Phytochemical characterization and radical scavenging activity of *Portulaca oleracea* L. leaves and stems. Microchem J., 92: 129-134.
44. Yao, Y., W. Sang, M. Zhou and G. Ren 2009. Phenolic composition and antioxidant activities of 11 celery cultivars. J. Food Sc., 75(1): 9-11.
45. Park, J.S., 1988. Identification of physiologically active compounds from purslane (*Portulace olerocea L.*). Korean Journal of Weed Science, 8(2): 169-175.

46. Erkan, N., 2012. Antioxidant activity and phenolic compounds of fractions from *Portulaca oleracea* L. Food Chemistry, 133: 775-781.
47. Lecumberri, E., L. Goya and R. Mateos, 2007. A diet rich in dietary fiber from cocoa improves lipid profile and reduced malondialdehyde in hypercholesterolemic rats. Nutrition, 23: 332-341.
48. Barakat, L.A.A., 2011. Hypolipidemic and antiatherogenic effects of dietary chitosan and wheat bran in high fat high cholesterol fel rats. Australia J. of Basic and Applies Science, 5(10): 30-37.
49. Torsdottir, I., M. Alpsten, G. Holm, A.S. Sandberg and T.J. llim, 1991. A small dose of soluble alginate-fiber affects postprandial glycemia and gastric emptying in humans with diabetes. J. Nutr., 121: 795-799.
50. Kim, A.J., S.Y. Kim, M.K. Choi, M.H. Kim, M.R. and K.S. Chung, 2005. Effects of mulberry leave powder on lipid metabolism in high cholesterol-fed rats. Korean J. Food Sci. Technol., 37: 636-641.
51. Kumar, D., V. Parcha, F. Dhulia and A. Maithani, 2011. Evaluation of anti-hyperlipidemic activity of method extract *Salvador olcoides* (Linn) leaves in Triton WR-1339 (Tyloxaoal) Induced Hyperlipidemic Rats. J. Pharmacy Res., 4: 512-513.
52. Park, B.S., 2002. Effect of dietary γ-linolenic acid on plasma lipid metabolism in rats. J. Korean Oil Chemists Soc., 19: 181-188.
53. NCEP, 2002. National Cholesterol Education Program Expert Panel. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on detection, evaluation and treatment of high blood cholesterol in adults (adult treatment panel III) final report. Circulation, 106: 3143-3421.
54. Movahedian, A., A. Ghannadi and M. Vashirnia, 2007. Hypocholesterolemic effects of purslane extract on serum lipids in rabbits fed with high cholesterol levels. International Journal of Pharmacology, 3(3): 285-28.
55. Makni, M., H. Fetoui, N. Gargouri, H. Jaber, T. Boudawara and N. Zeghal, 2008. Hypolipidemic and hepatoprotective effects of flaxseed and pumpkin seed mixture in ω-3 and ω-6 fatty acids in hypercholesterolemic rats. Food Chem. Toxicol., 46: 3714-3720.
56. Kim, A.R., J.J. Lee, Y.M. Lee, H.O. Jung and M.Y. Lee, 2010. Cholesterol-lowering and anti-obesity effects of *Polymnia sonchifolia* Poepp. & Endl. powder in rats fed a high fat-high cholesterol diet. J. Korean Soc Food Sci. Nutr., 39: 210-218.
57. Ali, S.I., M.M. Said and E.K. Hassan, 2011. Prophylactic and curative effects of purslane on bile duct ligation- induced hepatic fibrosis in albino rats. Annals of Hematology, 10: 340-346.
58. Igarashi, K. and M. Ohmuma, 1995. Effect of isorhamnetin, rhamnetin and quercetin on the concentration of cholesterol and lipoperoxide in the serum and liver and on the blood and liver antioxidative enzyme activities of rats. Biosci Biotech Biochem, 59: 595-598.
59. Kang, S.M., J.Y. Shim, S.J. Hwang, S.G. Hong, H.E. Jang and M.H. Park, 2003. Effects of Saengshik supplementation on health improvement in diet-induced hypercholesterolemic rats. J. Korean Soc Food Sci. Nutr., 32: 906-912.
60. Mohamed, A.D., E.A.M. Ahmed, A. Saleh and A.S. Reda, 2011.Antioxidant effect of purslane (*Portulaca oleracea*) and its mechanism of action. Journal of Medicinal Plants Research, 5(9): 1589-1563.
61. Mohammed Abdalla, H. and A.G. Soad Mohamed, 2010. *In vivo* Hepato-protective properties of purslane extracts on paracetamol-induced liver damage. Mal. J. Nutr., 16(1): 161-170.
62. AL-Quraishy, S., M.A. Dkhil and A.E. Abdel Mobeim, 2012. Protective effect of *Portulaca oloracea* against rotenone mediated depilation of glutathione in the striatum pf rats as an animal model of Parkinson's disease. Pesticide Biochemistry of Physiology, 103: 108-114.
63. Jovanka, K., P. Mira, M. Momir, M. Radoslav and G. Ljiljana, 2009. Protective effects of celery juice in treatments with doxorubicin. Molecules, 14: 1627-1638.
64. Jabber A.A.Al-Sa'aidi, Mohsen N.A. Alrodhan and Ahmed K. Ismael, 2012. Antioxidant activity of n-butanol extracts of celery (*Apium graveolens*) seed in streptozotocin-induced diabetic male rats. Research in Pharmaceutical Biotechnology, 4(2): 24-29.