

Full Length Research Paper

Evaluating the effects of freeze-dried supplements of purslane (*Portulaca oleracea*) on blood lipids in hypercholesterolemic adults

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This study was designed to evaluate the efficacy of the freeze-dried supplements of purslane in reducing blood lipids in hypercholesterolemic adults. Fresh purslane leaves were freeze-dried and the fatty acids content analyzed. Eleven (11) hypercholesterolemic subjects (5 females and 6 males) volunteered to participate in the study. The subjects consumed step I diet during a 2-week acclimation period and switched to step I diet supplemented with freeze-dried purslane leaves (6 g/day) for 4 weeks. Subjects were instructed to incorporate the freeze-dried supplements into their meals at lunch (3 g) and dinner (3 g) during the 4 weeks experimental period. Fasting blood samples were collected at the end of the acclimation period and at 2-weeks interval during the experimental period for analysis of plasma cholesterol, LDL-cholesterol, HDL-cholesterol and triacylglycerol concentrations. Data were subject to analysis of variance and means separation was conducted using the Duncan multiple range test (DMRT). Consumption of purslane for 4 weeks reduced ($P < 0.05$) plasma total cholesterol and LDL-cholesterol. HDL-cholesterol levels were increased ($P < 0.05$). Plasma triacylglycerol concentrations were not affected by the consumption of purslane supplements. Results suggest that purslane supplements have the potential to alter blood lipid metabolism in hypercholesterolemic subjects and can lower the risk of heart disease. In addition, nutrient analysis confirmed that purslane is a rich source of polyunsaturated fatty acids, crude protein, vitamins and minerals.

Key words: Purslane, hypercholesterolemic, subjects, risks factors, heart disease.

INTRODUCTION

Coronary heart disease (CHD) continues to be a leading cause of mortality and morbidity in the United States (Starfield, 2000; Smith, 2000; Jacobson, 2001; D'Agostino et al., 2000). It has been well established that hypercholesterolemia is a major risk factor for coronary atherosclerosis and atherosclerosis. Hyperlipidemia, particularly elevated serum cholesterol and Low-Density Lipoprotein (LDL) levels, is the risk factor in the development of atherosclerotic heart disease (Romerol-Corral

et al., 2006). Although several studies have reported that cholesterol lowering drugs like statins have been quite effective in lowering total cholesterol, low-density lipoprotein cholesterol, and prevent incidence of coronary heart disease, and of ischaemic stroke (Law et al., 2003; Wanner et al., 2005; Colivicchi et al., 2007).

However, some of these drugs have been associated with side effects like elevated liver enzymes, muscle pain and joint aches, nausea, diarrhea, constipation (FDA MEDWATCH Reporting System, 2003; Chung et al., 2001; Scheen, 2001; Silva et al., 2006). As a result of concern of potential side effect from cholesterol-lowering drugs, there is a growing consumer/patients' demand for non-traditional and/or diet related approaches that could

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Figure 1. Purslane (*Portulaca oleracea*) plant.

lower blood lipid and cholesterol, and prevent cardiovascular disease and stroke. Thus, there is a renewed effort from researchers (Movahedian et al., 2006; Prasad, 2005) to identify natural sources, such as nutraceuticals and phytochemicals with hypolipidemic and hypocholesterolemic properties. It has been reported that medicinal herbs like purslane (*Portulaca oleracea*) might provide therapeutic benefits to patients with cardiovascular disease risk factors (Movahedian et al., 2007). Purslane (*P. oleracea*, Figure 1) is found growing in the wild or cultivated in many parts of the world (Minaiyan et al., 2005) and the leaves of purslane are commonly eaten extensively in soups and salads in the Mediterranean countries (Chan et al., 2000). *P. oleracea* is widely distributed in tropical and subtropical areas of the world, where it is consumed as a nutritious vegetable, and used for its pharmacological properties (Ezekwe et al., 2004).

However, purslane is regarded as a weed in the United States. Purslane leaves have been used in Iranian folk medicine to treat several disorders such as hyperlipidemia, pain and inflammatory disorders, some urinary and tropical diseases (Minaiyan et al., 2005; Chan et al., 2000). Several studies have confirmed the high content of omega-3 fatty acids in purslane (Liu et al., 2002; Simopoulos et al., 2005). Compared to other known vegetables that are widely eaten, purslane is believed to be the richest plant source for w-3 fatty acids (FA) yet examined. Subsequent reports confirmed that high levels of w-3 FA and even traces of 22:5 w-3 and 22:6 w-3 FA are present (Omara-Alwala et al., 1991; Simopoulos et al., 1992).

In addition, similar studies have revealed that purslane is a rich source of nutrients like flavonoids, vitamins A, C and E, beta-carotene, and minerals (Xu et al., 2006; Xiang et al., 2005; Lim and Quah, 2007). Compositional

analysis of purslane accessions from various geographical locations around the world indicate high concentration of total lipids, crude protein, polyunsaturated FA and other essential nutrients compared to commercialized leafy vegetables in the United States (Table 1) (Ezekwe et al., 1999).

Pharmacological tests have revealed that purslane has anti-inflammatory, and anti-fungal effects, and antioxidant properties (Karimi et al., 2004; Malek et al., 2004). It was reported that dietary supplementation of purslane was effective in reducing plasma total cholesterol and triacylglycerol in rats (Ezekwe et al., 1995). It was reported that healthcare cost for the treatment of patients with cardiovascular disease was \$ 448.5 billion in 2000 (American Heart Association, 2008). Based on this report, the objective of the present study was to evaluate the efficacy of purslane leaf supplements in reducing plasma lipids in free-living human subjects with elevated cholesterol levels, cardiovascular disease risk factors.

MATERIALS AND METHODS

Subjects

Hypercholesterolemic men and women aged 25 to 60 years volunteered to participate in the dietary purslane intervention study. Subjects were recruited following health-screening activities organized at Alcorn State University, Claiborne County Hospital and Claiborne County Family Healthy Center to screen people for cardiovascular risk factors. Free-living participants, showing plasma total cholesterol concentration of ≥ 200 mg/dl were selected for the study. People who were willing to participate in the study were given a detailed description of the study. A detailed protocol and procedure for maintaining confidentiality was explained to all participants prior to being enrolled. A written informed consent was obtained from all participants. A screening test that consisted of measurements of body weight, height, blood pressure, plasma total, LDL and HDL cholesterol concentrations, plasma triglycerides, plasma glucose and hematocrit were conducted on twenty five subjects.

In addition, all subjects completed a general and medical history. After the screening, 15 hypercholesterolemic subject (7 men and 8 women) were selected for the study according to the following inclusion criteria: systolic blood pressure <120 mmHg and diastolic blood pressure <85 mmHg, total plasma cholesterol >200 mg/dl, BMI <25 kg/m², not diabetic, not anemic, no use of medication or prescription diet that can affect lipid or glucose metabolism, no history of coronary heart disease, cancer, kidney disease or liver disease, no abuse of drugs and/or alcohol. Exclusion criteria for the study included: pregnancy, or breast feeding, subjects on cholesterol lowering drugs, smoking, blood donation or participation in another feeding trial. All participants underwent a physical examination and completed a lifestyle questionnaire. Questions regarding current medication use and activity patterns of each subject were asked weekly.

After the first week of the study, two subjects (1 man and 1 woman) withdrew from the study. The other eleven (11) volunteers completed the study. All participants were African-Americans. The mean age of the men was 45 ± 9 years, body weight averaged 87 ± 15.9 kg and BMI was 24.5 kg/m². For women the mean age was 42 ± 7 , body weight was 64 ± 10 kg and BMI was 22.4 kg/m². Prior to the intervention study, baseline lipid concentration (Table 2) were performed and used to observe any changes overtime based on

Table 1. Chemical composition (DM) of purslane used in the study.

Items	Concentrations
Crude protein, %	22.9
Crude fiber, %	2.17
Ash, %	27.0
Total lipids, %	6.9
Pectin, %	19.6
Vitamin C, ppm [§]	68.3
Vitamin E, mg/100 g [§]	17.9
B-carotene, IU/kg [§]	53,842.3
Saturated fat, g/100 g [¶]	0.407
Polyunsaturated fat, g/100 g [¶]	1.25
Monounsaturated fat, g/100 g [¶]	0.147
Linoleic acid (w-6), g/100 g [¶]	0.33
Alpha linolenic acid (w-3), g/100 g [¶]	0.95

[§]Analysis performed by Ralston Analytical Lab, St Louis, MO. [¶]Analysis performed by Midwest Laboratories, Inc., Omaha, Nebraska.

purslane supplement. Participants who successfully completed the study were offered an incentive of \$ 60.00 at the end of the feeding period. The study was approved by Alcorn State University's Research Ethics Committee.

Diet and experimental design

Fresh leaves from purslane plant (Figure 1) grown at Alcorn State University Agriculture Experimental Station, were washed, freeze-dried and packaged in 3 g packs and provided to participants as purslane supplements. Freeze-dried purslane supplements were added to participant's meal to increase the dietary content of omega-3 fatty acids and pectin. The subjects were trained by a registered dietician to consume meals in line with recommendations of the American Heart Association Step 1 diet for two weeks prior to purslane supplementation.

At the end of the 2-weeks acclimation period, fasting blood samples were collected from all participants that served as baseline data. Subjects were then asked to continue on the AHA step 1 styled diets, supplemented with 6 g of freeze-dried purslane leaves per day, split between lunch and dinner, for a further 4 week period. Six grams of freeze dried purslane leaves were considered adequate to elicit a response in human subjects based on the results from previous studies with laboratory animals that showed that 20% freeze dried purslane was more effective in reducing plasma lipids than 10% (Ezekwe et al., 1995). All lunch sessions during week days took place at supervised settings at Alcorn State University campus and at the local county hospital cafeteria. The subjects consumed the remaining 3 g of purslane leaves with their dinner meals at home. Pre-weighed and packaged purslane packs were provided to the participants for consumption during weekends or holidays. Although subjects were discouraged from dining away from home during the experimental period, they were educated about suitable choices, if they chose to eat away from home. Each subject in the study served as their own control. All subjects were encouraged to maintain their usual physical activity levels and lifestyles, and were asked to record their dietary intakes on 2 weekdays and 1 weekend day of every week during the trial.

Subjects were instructed to fast for 12 h and to avoid alcohol consumption for 24 h prior to collection of blood. Individual body

weights were recorded at the end of the acclimation period and during the experimental period on day 14 and 28. Fasting venous blood samples collected after a 2-week acclimation period (baseline values) and during the experimental period on day 14 and 28 were analyzed for plasma glucose, total cholesterol, LDL-cholesterol, HDL-cholesterol and triacylglycerol, and blood hematocrit.

Individual body weights were recorded at the end of the acclimation period and during the experimental period on day 14 and 28.

Analytical methods

Nutrient analysis was performed on freeze-dried purslane used in the study (Table 1). Proximate analysis for the determination of proteins in purslane leaves was performed using the Buchi Kjeldahl Line Digestion Unit (Buchi B324, Switzerland) for digestion of samples, and the Buchi Distillation Unit (Buchi B324, Switzerland) for the distillation and titration of digested samples. Pectin concentration of purslane was determined using the method of Phatak et al. (1988). Crude fiber in purslane was determined using the Ankom® Fibre analyzer. Total fat content in purslane leaves was determined using the method described by Sukhija and Palmquist (1988). Fatty acid methyl esters were separated and quantified with Shimadzu gas chromatography equipped with J & W DB 23-column (30 m x 0.32 mm ID, 0.25 µ film) with the following conditions: initial and final oven temperature at 75 and 210°C, respectively, helium as carrier gas (2 ml/min, set at 225°C), detector temperature at 280°C, injection temperature at 240°C and split ratio of X200, and with an FID detector. Plasma total cholesterol was determined using the *In Vitro* Enzymatic Colorimetric assay kit (Wako, cat.# 439-17501) and HDL-cholesterol using enzymatic colorimetric assay kit (Wako, cat. # 278-67409), plasma triacylglycerol was determined using colorimetric assay kit (Sigma, cat. # 343). Percentage of hematocrit in whole blood was measured (Henry, 1984) immediately after samples were collected from participants. Plasma glucose was measured using colorimetric assay kit (Sigma, St Louis MO). Plasma albumin and minerals were analyzed using ATAC ISE buffer reagent kit (Clinical Data Inc. product #547-008).

Statistical analysis

General linear models and the PROC mean functions were used to analyze the data. A One-Way analysis of variance was conducted to determine treatment response over time. Means and standard error of the mean were calculated using the PROC mean function (SAS, 1999). Mean separations were conducted using the Duncan's multiple range test. Charts were created using excel program.

RESULTS

Nutrient analysis confirmed that purslane is a rich source of polyunsaturated fatty acids, crude protein, vitamins and minerals. In the intervention study, all eleven subjects that participated in the feeding trial completed the study and followed the meal plan as instructed prior to the beginning of the study. Baseline and experimental values of body weight, plasma glucose and blood hematocrit are shown in Table 2. Plasma total cholesterol, LDL, HDL and triacylglycerol are shown in Figure 2. The baseline values for each subject were used

Table 2. Effect of purslane supplementation on plasma lipids and blood hematocrit.

Items	Baseline	Day 14	%Δ	Day 28	%Δ
Total cholesterol, mg/dl	266.46 ^a	240.37 ^b	-9.8	225.26 ^{bc}	-15.5
LDL-Cholesterol, mg/dl	182.45 ^a	153.69 ^b	-15.8	132.09 ^{bc}	-27.6
HDL-Cholesterol, mg/dl	49.46 ^{bc}	46.06 ^c	-6.9	54.06 ^a	+9.3
Triglycerides, mg/dl	151.41	150.01	-0.97	158.51	+4.7
Hematocrit	41.55 ^b	41.73 ^b	+0.4	48.09 ^a	+15.7

^{abc}Means with different superscript differ at $P < 0.05$. %Δ = percent change compared to baseline value; Sign (-) = decrease in percent change compared to baseline value; Sign (+) = increase in percent change compared to baseline value.

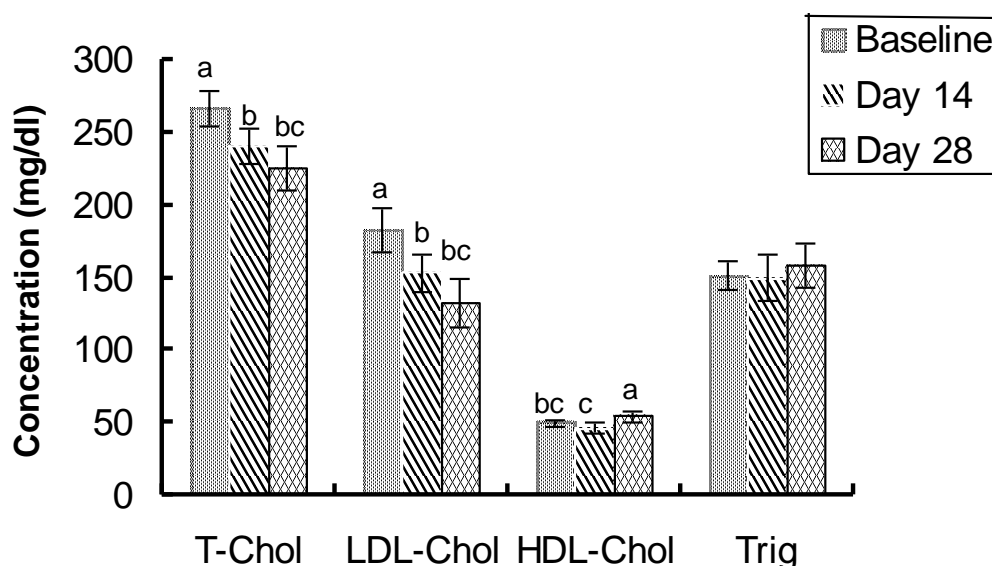


Figure 2. Effect of purslane supplementation on lipid metabolism. Variables (T-chol = total cholesterol, Trig = triacylglycerol) show treatment response over time. ^{abc}Means with different superscript differ significantly at $P \leq 0.05$ (Duncan multiple range test).

to determine changes over time. There were no significant changes in body weight among treatments (Table 3). No significant changes ($P > 0.05$) were observed in plasma glucose concentration; however, blood hematocrit concentrations significantly increased ($P \leq 0.05$) by day 28 (Table 2). Blood hematocrit did not differ ($P > 0.05$) between baseline values and those of day 14, but was higher ($P < 0.05$) by 15.7% on day 28. Relative to baseline values, plasma total cholesterol significantly decreased ($P \leq 0.05$) by 9.8 and 15% on day 14 and 28, respectively (Figure 2, Table 2). LDL-cholesterol concentrations significantly decreased ($P \leq 0.05$) by 15.8 and 27% on day 14 and 28, respectively (Figure 2, Table 2). There were no differences ($P > 0.05$) in HDL-cholesterol concentrations, when baseline values were compared with those of day 14, but HDL increased ($P > 0.05$) by 9% by day-28. Plasma triacylglycerol concentration did not change ($P > 0.05$) over time, when

baseline values were compared with those obtained on day 14 and 28. Baseline and experimental values for plasma albumin, potassium, magnesium, and iron are summarized in Table 4. On the whole, plasma albumin, potassium, magnesium, and iron concentrations were not different ($P \leq 0.05$) by day-28, indicating that purslane supplementation may not affect minerals or albumin status of hypercholesterolemic subjects.

DISCUSSION

The main objective of this feeding trial was to determine the feasibility of using a novel food product rich in omega-3 fatty acids, pectin and other essential nutrients (Table 1) to alter lipid metabolism in hypercholesterolemic subjects. In our study, consumption of purslane in the diet produced a marked improvement in the lipid profile,

Table 3. Effect of purslane supplementation on body weight, and blood glucose and hematocrit.

Items	Baseline	Day-14	Day-28
Body weight, kg	87.0±15.9	87.5±16.3	87.0±14.2
Glucose, mg/dl	92.7±4.9	97.1±4.1	84.0±5.4
Hematocrit, %	41.6±1.2 ^b	41.7±1.4 ^b	48.1±1.8 ^a

^{ab}Means in the same row with different superscript differ significantly at $P < 0.05$ (Duncan multiple range test).

Table 4. Effect of purslane supplementation on albumin and minerals in plasma.

Items	Baseline	Day-28
Albumin, g/dl	4.042±0.1	4.120±4.1
Magnesium, mEq/L	1.758±0.1	1.680±1.7
Iron, mg/dl	78.33±11.5	86.60±7.7
Potassium, Eq/L	4.217±0.1	3.970±0.1

There were no differences within rows in any variable ($P > 0.05$).

lowering plasma total cholesterol and LDL-cholesterol concentrations, and induced higher HDL-cholesterol concentrations without affecting triacylglycerol. These observations are in agreement with previous studies that reported cholesterol lowering effect of purslane (Ezekwe et al., 1995; Ezekwe et al., 1995). A recent study with cholesterol fed rabbits demonstrated serum total cholesterol was significantly reduced in rabbits fed purslane extract (Movahedian et al., 2007). The therapeutic effect of purslane has been demonstrated mostly in animal studies. Ezekwe et al. (1995) previously showed that rats fed 10 or 20% purslane for 6-weeks had a significant reduction of plasma total cholesterol by 23.9 and 15.7%, respectively. Plasma triacylglycerol concentrations were reduced by 28.4% for rats fed 10 and by 17% in rats fed 20% purslane (Ezekwe et al., 1995).

In a similar study, Ezekwe et al. (2004) made similar observations in pigs fed purslane showing a decreased serum total cholesterol (26.8%), LDL-cholesterol (53.4%) and triacylglycerol (16.2%). Our data also seem to suggest that purslane has a positive effect on HDL-cholesterol. The potential effect of purslane on HDL-cholesterol may be attributed to polyunsaturated fatty acids content in purslane. Previous studies have shown that consumption of omega-3 fatty acids derived from fish oil significantly lowered plasma triacylglycerol concentrations and decreased LDL-cholesterol significantly by 11% after 28 days (Laidlaw and Holub, 2003). Experimental data from human subjects suggest an association between dietary intake of polyunsaturated fatty acids, specifically omega-3 fatty acids and improved plasma HDL-cholesterol and cause a reduction in LDL-cholesterol (Okuda et al., 2005). The cholesterol lowering

effect of purslane may be attributed to the combined effect of omega-3 fatty acids and pectin since purslane is richer in w-3 fatty and pectin than most commercial vegetables (Table 1). Several animal and human studies have shown the hypolipidemic effect of pectin. In a human feeding study, Kay and Truswell (1977) reported a reduction of plasma total cholesterol by 13% in subjects who consumed 15 g/day of pectin. Aprikian et al. (2003) reported a significant reduction in liver cholesterol and triglycerides when rats were fed a pectin containing diet. In addition, Terpstra et al. (2002) reported a significant reduction in plasma total cholesterol in hamsters fed pectin diet.

Based on these results, it appears that supplementation of purslane in the diet of subjects with elevated plasma cholesterol may have a time effect on lipid metabolism. Plasma triacylglycerol concentrations were not affected by consumption of purslane, indicating that purslane may not have an effect when subjects' plasma triacylglycerol is within the physiological level of <160 mg/dl. This result is consistent with those of Ezekwe et al. (2004) that has shown that purslane had no effect, if triacylglycerol concentrations were at physiological levels. Our study is not consistent with other studies that have reported a reduction in plasma triglyceride with dietary supplementation of n-3 polyunsaturated fatty acids (Weber and Raederstorff, 2000; Balk et al., 2006; Block et al., 2008). Inconsistency observed in our results may imply that a 4-week experimental period with 6 g of freeze-dry purslane may be too short a period to alter triglyceride levels in blood. We concluded that the amount of omega-3 fatty acids in the diet was not sufficient to increase oxidation or reduce synthesis of triglycerides. Blood hematocrit concentrations significantly increased by day 28, but within normal levels, thus indicating that freeze-dried purslane may potentially improve hematocrit concentrations in human subjects.

Conclusion

The decrease in plasma total and LDL-cholesterol observed in this study suggest that supplementation of freeze-dried purslane plant in the diet of hyperlipidemic subject may alter blood lipid metabolism. The decrease in

plasma cholesterol observed in this study may be attributed to the combined effect of high concentration of soluble fiber (pectin) and omega-3 fatty acids that are found in purslane plant.

The study also revealed that purslane plant is a good source of β -carotene, vitamins E and C, pectin, and crude protein; and could help to maintain an individual's iron status. However, additional studies with a much longer experimental period (~3 months) and a larger sample size are needed to observe the impact of purslane on lipid metabolism and to establish the appropriate dose level required to maintain a consistent efficacy and response. At the conclusion of the study, participants with plasma total cholesterol concentration greater than 200 mg/dl were advised to meet with their physicians and dietitians for treatment.

LIMITATION

Lack of a suitable vegetable to serve as a control group and a small sample size (11 subjects) was the major limitation for a conclusive interpretation of our data.

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