ORIGINAL ARTICLE

The Effect of Purslane Seeds on Fasting Blood Glucose and Serum Liver Enzymes in Patients with Nonalcoholic Fatty Livers

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Abstract

Objective: Nonalcoholic Fatty Liver Disease (NAFLD) is the most common form of liver disease in the world and it is one of the liver transplantation causes. The present study aimed to investigate the effect of Purslane seeds on anthropometric parameters, fasting blood glucose (FBS), and liver enzymes in patients with nonalcoholic fatty livers.

Materials and Methods: in this clinical trial, 54 patients with fatty livers were participated and randomly divided into two groups: the 1st group received 10 grams of purslane seeds per day along with a weight loss diet and the 2nd (control) group were only engaged in a weight loss diet. At the beginning and at the end of the 8th week of intervention, anthropometric parameters, FBS, liver enzymes and liver sonography were studied in both groups.

Results: After the intervention, significant reductions in weight, waist and hip circumference (P=0.01) were observed in both groups, but only the changes in hip circumference were significant between the two groups (P=0.01). Body mass index significantly reduced only in the purslane group (P<0.05). The average intake of energy, protein, carbohydrate, and fat was reduced in both groups (P<0.05). No significant changes occurred in the fasting blood glucose before and after the study in both groups; however, FBS was significantly different after 8 weeks compared with the control group (P<0.05). Purslane consumption after 8 weeks significantly reduced alanine aminotransferase (P=0.03) and aspartate aminotransferase (P=0.03) in the relevant group; yet the changes were not significant in the control group. Diet and purslane consumption were unaffected on alkaline phosphatase (ALP) levels. In both group liver steatosis decreased but in the purslane group was more significant.

Conclusion: This study showed that consumption of purslane seeds besides the diet for 8 weeks have beneficial effects on anthropometric parameters, FBS, liver enzymes and liver steatosis. Purslane consumption significantly reduced alanine aminotransferase and aspartate aminotransferase. Liver steatosis decreased in the purslane group more than control group.

Keywords: Purslane, Non-alcoholic fatty liver, Liver enzymes.

Introduction

Non-alcoholic Fatty Liver Disease (NAFLD) is the fat accumulation in liver with the absence of excessive alcohol consumption (less than 20 g per day) and any other specific causes of hepatic steatosis such as viral hepatitis, autoimmune

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hepatitis, medications such as corticosteroids, estrogen and other factors. NAFLD includes a range of liver diseases like steatosis, which may lead to the disease progress to steatohepatitis, fibrosis, cirrhosis and liver cancer (1-3). It is the most important common form of liver diseases in the world that is a public health problem (4,5). This disease is mostly associated with Type II diabetes, obesity, and hyperlipidemia. In near future, with the growth of obesity and diabetes, NAFLD will be the first cause of liver transplantation in the world. The prevalence of NAFLD is 20-30% in the West and 15% in Asian countries (6). It is estimated that about 30% of adults in America suffer from NAFLD. However, the disease leads to steatosis with inflammation, fibrosis and necrosis in about 2-6% of adult Americans and 20% of obese people (3).

The most important hypothesis in the etiology of this disease are:

1) Insulin resistance (a key mechanism leading to steatosis and steatohepatitis)

2) Oxidative stress (which leads to inflammation and disease progress) (7).

Hence, NAFLD is known as hepatic insulin resistance or metabolic syndrome (8).

So far, there is no cure for the fatty liver. Therefore, finding an alternative therapeutic approach for existing treatments is required.

Omega-3 fatty acids and flavonoids are efficient on the improvement of fatty livers (10,9). Among the medicinal plants used to treat NAFLD, purslane is one of the most popular medicinal herbs which is a good source of biologically active compounds including Omega-3, α-tocopherol, ascorbic acid, niacin, thiamine, β-carotene (11,12), amino acids, glutathione, minerals, and phenolic compounds (13). In addition to antioxidants, flavonoids, and fatty acids, purslane contains hypocholesterolemic and hypoglycemic effects (14,15). Some studies have examined the impacts of purslane seeds on blood glucose and liver enzymes in humans, the results of which have been contradictory (15,16).

Since insulin resistance is one of the liver disease etiology and with respect to lack of clinical trials aiming to prescribe purslane seeds, especially in patients with nonalcoholic fatty livers.Also presence of conflicting results about purslane seeds effects on lowering blood sugar, we decided to study the effects of purslane seeds on blood glucose, liver enzymes, and lipid profiles in patients with fatty livers.

Materials and Methods

This study was a randomized clinical trial involving 60 patients with NAFLD, who referred to Najaf Abad Teachers Clinics. We included, people between 25-75 years old with body mass index (BMI) higher than 25, fatty liver confirmed by ultrasound who did not vitamin supplements. Participating in any other research projects, using a weight loss diet in the last 3 months, and having other types of liver, kidney, lung, and heart diseases, kidney stones, gastrointestinal bleeding diagnosis, a history of ischemic heart disease and strokes, an advanced proliferative and non-proliferative retinopathy, any changes in the dosage and type of medication to reduce lipid and blood pressure in the last 2 months, and diabetes type 1 and 2 based on global standards were excluded.

Also the patients who had a change in the pattern of physical activity or the dosage and type of medication for any reasons during the study, other diseases, unwilling to participate in the study, and pregnancy and lactation conditions were excluded. With regard to the type I error (α=0.05), test power of 85%, reference to the previous similar study (16), and the main variable of AST study, the sample size in each group was found to be 26 people. Also, considering an elimination of 15%, the number of samples were obtained 30 people in each group. At the end, 60 people were selected and then randomly divided into the two control and purslane groups after taking a written consent. During the study 6 participants left the study out of the control and purslane groups due to lack of willingness

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to participate, taking a trip, and unwillingness to attend in the study, respectively and eventually 54 people completed the study process. After the final selection of all the participants, they were divided into two groups of consuming purslane seeds and control randomly using a computerized generated random number table. Anthropometric data, fasting blood sugar (FBS), liver enzymes and sonography were measured before and after the intervention. The purslane group members were given a weight loss diet and 10 g/day of purslane for 8 weeks and the control group only took a weight loss diet. Based on the previous studies, the dosage varied 5.7-10 g/day (15,17). However, according to similar studies, a dose of 10 g/day was taken into account as an effective dose in this study.

The intervention group received 10 g of purslane seeds per day before the two meals of breakfast and dinner along with a weight loss diet for 8 weeks. The control group only received an 8 weeks weight loss diet.

To prevent a loss to follow-up and remind the patients to use purslane, they were contacted every 7 days and their process of weight loss diet and purslane consumption were questioned. Also, the participants were asked not to change their physical activities during the study. To evaluate their degrees of activity, an International Physical Activity Questionnaire (IPAQ) was used. In addition, to estimate the intakes of energy, macronutrients, and micronutrients as well as to check whether a person's eating habits have changed during the study or not, a questionnaire of 3 days food record was taken at the beginning and end of the study.

Furthermore, the patients were given a diet of 500 kcal energy less than their needs in terms of their modified weights and a ratio of 30% fat, 15% protein, and 55% carbohydrate. Their heights and weights were also measured at the beginning of the study. Their dietary follow-up visits were conducted 2 weeks and 1 month after the intervention and after the end of the study.

The patients' general information questionnaires, including age, occupation, disease duration, and type and dose of medication were completed via interviews at the beginning of the project. To assess anthropometric indices, their weights were measured using Seca digital scale, made in Germany, with an accuracy of 100 g and minimum coverage. Also, their heights were measured without shoes as 4 parts of the body were stuck to the wall using a stadiometer with an accuracy of 0.5 cm.

Liver ultrasonography was performed by a single radiologist at the beginning and end of study after 6-8 hours fasting. The radiologist was blinded to the treatment group of the patients.

The tests of liver enzymes and blood sugar were performed for all the participants at the beginning and end of the study period. The required amount of sample blood was 10 ml, which was collected after 10 to 12 hours of fasting. Measurements of blood glucose and liver enzymes were also performed at the same day. To measure glucose and liver enzymes, the kits of Pars Azmoon Co. and alpha classic device were utilized.

Our proposal was recognized morally permissible by Ethics Commission in the Research and Technology Deputy Department of Shahid Sadoughi University of Medical Sciences and Health Services, Yazd and it is registered at www.irct.ir with the code of IRCT2014090210826N14.

Ultimately, the data analysis was performed using SPPS 16 software. To compare the variables between the groups and averages of variables in each group, an independent-sample T-test and paired T-test were employed, respectively. The significance level was considered less than 0.05. Friedman Test was used to assess the effect of treatment on liver steatosis.

Results

From the 60 patients participating in the study, 54 patients completed it (Figure 1). The mean ± standard deviation (SD) of age of the

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participants in the purslane and control groups were 40.07 ± 9.52 and 8.39 ± 8.84 years, respectively. The gender distributions of 6 males and 21 females were equal in both groups.

Two groups medication for fatty liver disease were not significantly different by using chi square test. The baseline characteristics of both groups of the patients were in table 1.

Daily intake of energy and macronutrients are shown in Table 2. The receptions of energy, carbohydrate, fat, and protein have significantly reduced in both groups before and after the intervention.

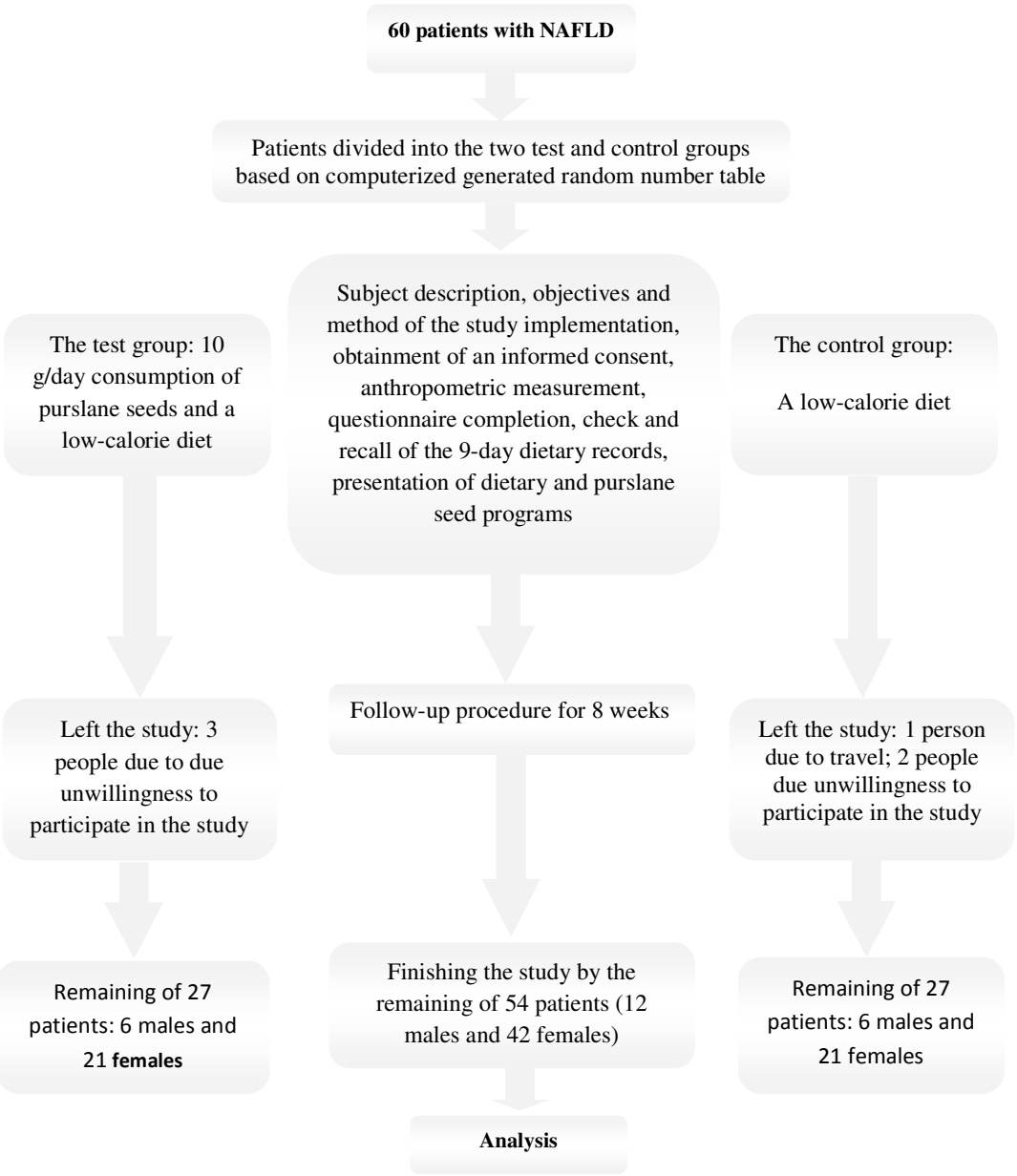

Figure 1. Schematics of the study methodology

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Table 1. The baseline characteristics of both groups before the intervention

Variable	Intervention (27 persons)	Control (27 persons)	P-value
Age (years)	540.07±9.52	39.81±8.84	0.98*
Weight (kg)	88.44±11.24	84.01±14.38	0.49*
Waist circumference (cm)	93.85±6.68	93.18±10.20	0.77*
Hip circumference (cm)	112±9.07	110.90±5.50	0.59*
BMI (kg/m^2)	32.77±3.63	31.08±3.24	0.07*
Gender			
Female (%)	6(22.2)	6(22.2)	1.00**
Male (%)	21(77.8)	21(77.8)	

*Independent t-test
**Pearson Chi- Square

Table 2. Comparison of the average daily intakes in the two groups at the beginning and end of the study

Variable	Study groups	Before	After	P-value*	The difference between before and after the intervention
Energy (kcal)	Intervention(n=27)	2358.92±420.14	2019.73±289.92	<0.005	339.19±181.90
	Control(n=27)	2365.94±576.07	2011.242±384.73	<0.005	354.52±268.98
P-value**		0.95	0.92		0.80
Carbohydrate (g)	Intervention(n=27)	323.96±61.03	274.04±40.53	<0.005	49.91±36.69
	Control(n=27)	319.03±67.10	275.79±57.22	<0.005	43.23±42.45
P-value**		0.77	0.89		0.53
Protein (g)	Intervention(n=27)	80.85±19.56	74.43±18.21	<0.05	6.41±14.81
	Control(n=27)	83.82±26.41	70.87±19.57	<0.005	12.95±19.24
P-value**		0.67	0.49		0.16
Fat (g)	Intervention(n=27)	82.18±21.73	68.3±14.71	<0.005	16.73±25.80
	Control(n=27)	85.37±32.76	78.74±14.71	<0.005	13.34±14.97
P-value**		0.67	0.95		0.55

*Paired t-test
**Independent t-test

The results of paired T-test represented that the mean of weight, waist circumference, and hip circumference significantly reduced in both groups after 8 weeks of intervention; yet, BMI showed a significant decrease only in the intervention group and no changes were observed the control group (Table 3).

No significant changes of FBS was observed in both groups before and after the study; however, its changes were significantly different in the purslane compared to the control group after 8 weeks (Table 3). Purslane consumption after 8 weeks significantly reduced the alanine aminotransferase and aspartate aminotransferase in the purslane group, while there were no significant changes in the control group. Diet and purslane seeds had no effects on alkaline phosphatase levels (Table 3).

The result of Friedman Test showed in table 4. The weight loss diet and consumumption of purslane seed were effective more than the weight loss diet alone in decreasing liver steatosis.

Represented that the mean of weight, waist circumference, and hip circumference significantly reduced in both groups after 8 weeks of intervention; yet, BMI showed a significant decrease only in the intervention group and no changes were observed in the control group

Discussion

The results of the present study revealed that a weight loss diet had an impact on waist and hip circumferences and its accompanying by purslane leads to reduced BMI in the intervention group. Based on our knowledge,

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Table 3. Comparison of anthropometric indices, mean of fasting blood glucose and serum liver enzymes s for each group at the beginning and end of the intervention

Variable	Study group	Before	After	*P*-value*	Change
Weight(kg)	Intervention(n=27) Control(n=27)	86.44±11.24 84.01±14.38	84.26±11.13 82.32±14.51	<0.05 <0.05	2.18±2.36 1.68±1.80
P*-value*		0.49	0.58		0.39
Waist circumference (cm)	Intervention(n=27) Control(n=27)	93.85±6.86 93.18±10.20	91.76±6.22 91.10±9.15	<0.05 <0.05	2.08±3.94 2.08±4.37
P*-value*		0.77	0.75		0.99
Hip circumference (cm)	Intervention(n=27) Control(n=27)	112.00±9.07 110.90±5.50	107.74±10.54 109.38±5.34	<0.05 <0.05	4.25±5.48 1.51±1.87
P*-value*		0.59	0.47		<0.05
BMI (kg/m^2)	Intervention(n=27) Control(n=27)	32.77±3.63 31.08±3.24	31.96±3.78 30.79±3.99	<0.005 0.49	0.80±0.87 0.29±2.06
P*-value*		0.07	0.27		0.23
Fasting blood glucose (mg/dl)	Intervention(n=27) Control(n=27)	98.55±8.50 96.54±13.01	95.03±10.11 99.57±11.63	0.09 0.90	3.51±10.45 -3.02±9.00
P*-value*		0.50	0.13		<0.05
Alanine aminotransferase (IU) * **	Intervention(n=27) Control(n=27)	30.88±13.97 27.70±11.16	25.97±8.11 27.31±10.18	<0.05 0.87	4.91±11.39 0.38±12.88
P*-value*		0.36	0.59		0.17
Aspartate transaminase (IU)	Intervention(n=27) Control(n=27)	25.29±6.53 24.68±9.33	22.33±5.31 21.72±5.16	<0.05 0.11	2.96±6.91 2.96±9.39
P*-value*		0.78	0.67		1
Alkaline phosphatase (IU)	Intervention(n=27) Control(n=27)	211.74±53.91 211.87±57.00	205.85±43.91 210.8±56.64	0.45 0.88	5.88±40.46 1.05±38.46
P*-value*		0.99	0.72		0.66

*Paired T-test
**Independent T-test
***To analyze alanine aminotransferase data, the data log was used due to their non-normality.

Table 4. Comparison of liver sonographyfor each group at the beginning and end of the intervention

	Intervention group				Control group			
	normal	**Grade 1**	**Grade2**	**Grade3**	**normal**	**Grade1**	**Grade 2**	**Grade3**
Before	0(0.0%)	13(48.1%)	11(40.7%)	3(11.1%)	0(0.0%)	16(59.3%)	7(25.9%)	4(14.8%)
After	11(40.7%)	10(37.0%)	5(18.5%)	1(3.7%)	3(11.1%)	17(63.0%)	4(14.8%)	3(11.1%)
***P*-value**	<0.005				0.034			

the results of all the studies measuring the impact of purslane on anthropometric data have been in line with this study (15,16,18,19). The previous studies were conducted on animal models or in patients with type II diabetes. However, the greater sample size, longer duration of the intervention, the study population (patients with NAFLD), and simultaneous prescription of a weight loss diet for the intervention and control groups have made a difference between the mentioned intervention and the previous studies.

In the current study, purslane consumption for 8 weeks accompanied with a weight loss diet caused a significant decrease in FBS compared to the control group. So far, several clinical trials have investigated the impact of various forms of purslane on animal models, the results of which were consistent with this study (19,20). In past studies, only the effects of purslane polysaccharides were investigated on animal models, while purslane seeds were used along with a weight loss diet in humans in this study since its seeds are richer than the plant itself (15). Additionally, its anti-diabetic effects were proven in other studies (21-23). However, there are contradictory results in the human studies conducted. In El-Sayed's study, the effects of purslane seeds on the reduction of blood sugar in people with type II diabetes were shown compared to metformin, which was consistent with our findings (15). The sample size of El-Sayed's study was small and pilot intervention was done on people with

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diabetes. In the study of Zakizadeh et al., purslane consumption had no effect on blood glucose levels in diabetic patients (16). The present study was designed based on a parallel clinical trial, while Zakizadeh study was a cross over one. The sample size and duration of the intervention were further besides using a weight loss diet along with purslane in the current study, the intervention of which was performed on patients with fatty livers. According to our knowledge, this is the first study examining the impacts of purslane seeds on patients with NAFLDs. In some studies, purslane role in lowering blood sugar has been attributed to the presence of unsaturated acids, flavonoids, and polysaccharides in it (24-26). By closing ATP_K+channels, exerting membrane depolarization, and stimulating Ca^{2+} penetration, purslane polysaccharides enhance insulin secretion (19).

In our study, consumption of purslane seeds decreased AST, ALT levels and liversteatosis, yet having no effect on ALP levels. In the study of Dkhil et al., aqueous extract of purslane reduced AST and ALP levels, but had no effect on ALT (27), the results of which were almost in line with the present study despite that in our study, the effects of purslane seeds on human subjects with NAFLDs were assessed along with a weight loss diet and the seeds only reduced ALT levels and no changes in ALP levels were observed. In other studies, the protective effect of purslane on liver against oxidative stress and reduction of liver fat and enzymes have been shown (26,28,29). In this respect, the results of these studies were consistent with those of our study. In Lee's study, purslane improved liver function and lessened liver fat and enzymes in mice (28) and the results were consistent with the present study with the difference that our study was conducted on human subjects. In Prabhakaran's study, the protective effect of aqueous extract of purslane against D galactosmaine on rats were proven and its consumption protect liver tissue and decrease liver enzymes (29). Also, in Chen's study, the aqueous extract of purslane reduced liver and blood lipid peroxidation of diabetic rats with fatty livers and their liver fat contents and enhanced levels of antioxidant activities (26). In this study, purslane seeds were used along with a weight loss diet, but in other studies, merely the plant extract or other parts were utilized. In the mentioned studies, laboratory samples were employed, which often examined purslane effects on oxidative stress and liver enzymes or on the complications of liver diseases, diabetes, a high fat diet, or drug-induced liver injury. In this study, fatty liver disease in humans was specifically assessed. Based on our knowledge, only one study investigated the effects of purslane seeds on liver enzymes, the results of which were in agreement with the present study. In this study, which was conducted as a pilot, the patients with Type II diabetes were employed with a small sample size (15). In addition, the study aimed to give its patients a weight loss diet besides purslane seeds and was performed only on NAFLDs.

In Chen's study, purslane led to decreased levels of liver leptin (26). Leptin causes insulin resistance and liver disease in vitro and in animal models (30). New reports suggest that serum leptin is associated with hepatic steatosis and not fibrosis (31). In patients with NFLDs, the two-phase theory of insulin resistance and oxidative stress is raised and purslane is a potential antioxidant containing omega 3 fatty acids, glutathione, alpha-Tocopherol, ascorbic acid, beta-carotene, and isoleucine, methionine, lysine, cysteine, phenylalanine, tyrosine, valine, and threonine amino acids (12,27,32,33). Therefore, the reduced enzyme activities are probably due to the effects of antioxidant present in purslane, which protect the liver against oxidative stress (27). The role of glutathione as an antioxidant has been proven in several studies (35) in a way that it can be directly absorbed by the digestive system to improve the body's antioxidant status (35). Addition of purslane improves liver function by improving lipid metabolism. Purslane stops reabsorption of bile acids and prevents the production of

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cholesterol (36). The plant flavonoid compounds reduce liver cholesterol levels, while quercetin increases HDL and decreases LDL levels (37).

Lack of placebo in the control group and open labeled study can be considered as the limitations of this study. Also, an increased duration of the study may further improve the results. Although biopsy method is the best way to diagnose fatty livers; it is invasive one. Thus, the ultrasound method was used for their detection. Perhaps, using this method was the other limitations of the present study.

Intervention on human target group can be considered as the strength of this study since most studies have been conducted on animal models. Prescription of a weight loss diet along with purslane consumption in most randomized clinical trials was of the other strong points of this intervention.

Conclusion

This study revealed that the daily consumption of 10 grams of purslane seeds accompanied with a weight loss diet for 8 weeks has beneficial effects on some anthropometric factors and improves FBS, liver enzymes and liver steatosis.

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