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Original Article

Anti-diabetic Effect of *Portulaca oleracea* (Purslane) Seeds in Alloxan-induced Diabetic Rats

Reza Ghahramani[1] M.Sc., Maryam Eidi[2*] Ph.D., Hossein Ahmadian[3] M.Sc.
Mostafa Hamidi Nomani[3] M.Sc., Roya Abbasi[2] B.Sc.
Marzieh Alipour[4] M.Sc., Ali Anissian[5] Ph.D.

[1]Young Researchers and Elite Club, Islamshahr Branch, Islamic Azad University, Islamshahr, Iran.
[2]Department of Biology, College of Biological Sciences, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran.
[3]Department of Biology, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran.
[4]Department of Biochemistry, Semnan University of Medical Sciences, Semnan, Iran.
[5]Department of Veterinary, Abhar Branch, Islamic Azad University, Abhar, Iran.

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Background and Aims: *Portulaca oleracea* (purslane) herb has renewed an interest in medicinal plants because its aerial parts usage generally does not elicit any side effects. In the present study, we aimed to evaluate the anti-diabetic effect of hydro-ethanolic extract of *P. oleracea* seeds in alloxan- induced diabetic rats.

Materials and Methods: In the test group, diabetic rats received hydroethanolic extract at doses of 50, 100 and 200 mg/kg, intraperitoneally for 14 days while in the control group, they received just saline vehicle. Then, biochemical analysis was performed to evaluate serum profiles in diabetic treated rats. After that, liver sections were prepared for histopathological analysis.

Results: In comparison to the control group, the serum profiles of the test group exhibited significant changes. In the control diabetic group, the serum levels of glucose, cholesterol, triglyceride, low-density lipoprotein, urea and uric acid were 494, 122, 220, 163, 492 and 94 mg/dL and aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase were 3215, 3394 and 1527 UI/l reduced respectively to 165, 18, 120, 63, 36 and 52 mg/dL and 1219, 1229 and 1717 UI/l in the test groups. Also, the administration of the purslane extract significantly increased high-density lipoproteins and total protein levels and attenuated hispathological damage in liver tissue in alloxan- induced diabetic rats.

Conclusions: The present data indicated that hydro-ethanolic extract of *P. oleracea* seeds has anti-diabetic effect in diabetic animals. So, this plant should be considered in future therapeutic researches.

*Corresponding Author: Department of Biology, College of Biological Sciences, Varamin- Pishva Branch, Islamic Azad University, Varamin, Iran, **Tel:** +989122074832, **Email:** eidi@iauvaramin.ac.ir, maryameidi@gmail.com

Introduction

Portulaca oleracea (p.oleracea) commonly known as purslane, a well-known traditional Chinese medicine in the Chinese Pharmacopoeia, it is used as a diuretic, febrifuge, antiseptic, antispasmodic and vermifuge [1]. It has a wide range of pharmacological effects, such as antibacterial [2], hypolipidemic, anti-aging, anti-inflammatory [3], antioxidative [4], analgesic, and wound healing activities [5]. Many studies have also shown that the major bioactive components of *P.oleracea* are polysaccharides, flavonoids, coumarins, monoterpene glycoside and alkaloids [6-8]. *P.oleracea* comprises more nutritive values than other vegetables due to its ω-3 fatty acid, α-tocopherol, ascorbic acid, β-carotene and glutathione-rich shoots [9]. Until now, the majority of studies on this herb have has focused on chemical compositions and bioactivities of small molecules. Since the plant is recommended in Iranian folk medicine for treatment of diabetes mellitus, in the present study, we intend to examine the antidiabetic activities of *P.oleracea* seeds extract on serum biochemical parameters, hepatic enzymes and liver tissue to elucidate the mechanism behind it.

Materials and methods

Plant material

Fresh *P.oleracea* seeds were collected from the Varamin area (July, 2014) and authenticated in department of biology, college of biological sciences, Islamic Azad University (Voucher specimen 7450). The plant seeds were dried under shade and powdered using Ultra-Torax. The powder (60 g) was extracted with 300 ml aqueous 80% ethanol in a Soxhlet apparatus for 72 hours. The extract was filtered and concentrated to dryness under reduced pressure in a rotary evaporator at 40-50ºC which its yielding was 16.5% (w/w) plant extract. The obtained *P.oleracea* hydro-ethanolic extract was stored at -20°C until the usage. Plant extract was suspended in saline (doses of 50, 100 and 200 mg/kg body weight) prior to intraperitoneally administration to the experimental animals.

Experimental animals and induction of diabetes

Male Wistar rats initially weighing 200 to 250 g purchased from the Pasteur Institute (Karaj, Iran) were used in the experiments. The diet was purchased from Pars-Dam food service, Tehran, Iran. The animal room was maintained at 22°C±2°C with timed lighting on from 7 AM to 19 PM and relative air humidity of 40-60%. Each animal was used once only. The animal protocol was approved by the Ethics Committee of Islamic Azad University, Tehran, Iran and conforms to the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals, Iran, as also international guidelines. Male rats were made diabetic by injection of alloxan [90 mg/kg, intraperitoneal (i.p)]. Seven days after alloxan injection, the rats with fasting blood glucose higher than 180 mg/dl were used for

the experiments. Experimental groups were as follow: Group 1: as normal control group (n=6); Group 2: as control diabetic rats (both control group received 0.5 ml saline (i.p.), daily for 14 days (n=6); Groups 3, 4 and 5: Included diabetic rats which were treated with the herb extract at doses of 50, 100 and 200 mg/kg/day (i. p.), for 14 days (n=6).

Blood and liver sampling

Samples were drawn from the heart under light ether anesthesia. The animals were removed after blood collection and liver sampling.

Biochemical measurements

Fasting serum glucose, cholesterol, triglycerides, low-density lipoprotein (LDL), high-density lipoproteins (HDL), urea, uric acid, creatinine, total protein, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) levels were determined by kit (Parsazmoon Company, Iran).

Liver histopathology

For qualitative analysis of liver histology, the tissue samples were fixed for 48 h in 10% formalin-saline and dehydrated by passing successfully in different mixtures of ethyl alcohol-water, cleaned in xylene and embedded in paraffin. Sections of the tissue were prepared by using a rotary microtome and stained with haematoxylin and eosin dye, which was mounted in a neutral deparaffinated xylene medium for microscopic observations. Each damage is given one score and final score of each specimen is sum of damage's scores.

Statistical Analysis

Statistical analysis was performed using one-way analysis of variance followed by Tukey post hoc test. The criterion for statistical significance was $p<0.05$. Data were expressed as mean±SEM.

Results

Alloxan-induced diabetic condition in rats

The administration of alloxan a dose of 90 mg/kg, for 7 days to the rats of groups 2 to 5 induced stable diabetic condition in the most of the experimental rats. Alloxan-induced diabetic condition increased the serum levels of glucose from 101±6 to 494±86 mg/dL ($p<0.001$), LDL from 83±19 to 163±9 mg/dL ($p<0.05$), cholesterol from 91±7 to 122±6 mg/dL ($p<0.05$), triglyceride from 136±9 to 220±15 mg/dL ($p<0.05$), urea from 22±2 to 492±58 mg/dL ($p<0.001$), uric acid from 24±3 to 94±5 mg/dL ($p<0.001$), AST from 1541±6 to 3215±365 UI/L ($p<0.001$), ALT from 1556±6 to 3394±360 UI/L ($p<0.001$), ALP from 991±29 to 1527±101 UI/L ($p<0.001$), while decreased the serum levels of HDL from 158±14 to 39±10 mg/dL ($p<0.05$) and total protein from 5±2 to 2±0.4 mg/dL ($p<0.01$). The results are shown in table 1.

Table 1. The administration of alloxan a dose of 90 mg/kg, for 7 days to the rats

Parameters	Before Alloxan-induced	After Alloxan-induced	p value
Glucose (mg/dl)	101±6	494± 86	p<0.001
Triglycerides (mg/dl)	136± 9	220± 15	p<0.05
Cholesterol (mg/dl)	91± 7	122± 6	p<0.05
LDL (mg/dl)	83± 19	163± 9	p<0.05
HDL (mg/dl)	158± 14	39± 10	p<0.05
AST (UI/l)	1541± 6	3215± 365	p<0.001
ALT (UI/l)	1556± 6	3394± 360	p<0.001
ALP (UI/l)	991± 29	1527± 101	p<0.001
Urea (mg/dl)	22± 2	492± 58	p<0.001
Uric acid (mg/dl)	24± 3	94± 5	p<0.001
Creatinine (mg/dl)	1.3± 0.7	1.28± 0.3	
Total protein (mg/dl)	5± 2	2± 0.4	p<0.01

Data presented as mean±SEM

Anti-diabetic effect of *P. oleracea* seed hydro-ethanolic extract in rats

The results showed that intraperitoneal administration of extract doses of 50, 100 and 200 mg/kg respectively reduced the serum levels of glucose from 494±86 mg/d/L (in control diabetic group) to 196±50, 152±34 and 165±29 mg/dL (in test diabetic groups treated respectively with 50, 100 and 200 mg/kg) (p<0.001), triglycerides from 220±15 mg/dL (in control diabetic group) to 130±18, 121±7 and 120±7 mg/dL (in test diabetic groups treated respectively with 50, 100 and 200 mg/kg) (p<0.05), cholesterol from 122±6 mg/dL (in control diabetic group) to 21±3, 20±6 and 18±5 mg/dL (in test diabetic groups treated respectively with 50, 100 and 200 mg/kg) (p<0.001), LDL from 163±9 mg/dL (in control diabetic group) to 73±19, 63±16 and 71±10 mg/dL (in test diabetic groups treated respectively with 50, 100 and 200 mg/kg) (p<0.01), AST from 3215±365 UI/l (in control diabetic group) to 1232±3, 1318±11 and 1219±2 UI/l (in test diabetic groups treated respectively with 50, 100 and 200 mg/kg) (p<0.001), ALT from 3394±360 UI/l (in control diabetic group) to 1229±6, 1239±4 and 1239±3 UI/l (in test diabetic groups treated respectively with 50, 100 and 200 mg/kg) (p<0.001), ALP from 1527±101 UI/l (in control diabetic group) to 1823±118, 1717±88 and 1815±71 UI/l (in test diabetic groups treated respectively with 50, 100 and 200 mg/kg), urea from 492±58 mg/dL (in control diabetic group) to 36±15, 45±12 and 67±24 mg/dL (in test diabetic groups treated respectively with 50, 100 and 200 mg/kg) (p<0.001), uric acid from 94±5 mg/dL (in

control diabetic group) to 61±2, 57±4 and 52±2 mg/dL (in test diabetic groups treated respectively with 50, 100 and 200 mg/kg) ($p<0.001$), while elevated the serum levels of HDL from 39±10 mg/d/L (in control diabetic group) to 77±11, 120±22 and 144±47 mg/dL (in test diabetic groups treated respectively with 50, 100 and (200 mg/kg) ($p<0.05$) and total protein from 2±0.4 mg/d/L (in control diabetic group) to 3±0.2, 4±0.6 and 6±0.4 mg/dL (in test diabetic groups treated respectively with 50, 100 and 200 mg/kg) ($p<0.001$). The results are summarized in table 2.

Histopathological-protective effects of *P. oleracea* in diabetic rats

Administration of alloxan increased histopathological damages ($p<0.05$) in diabetic rats in comparison with control normal rats. Treatment of plant extract improved liver histopathological damages in treated diabetic rats in comparison to control diabetic rats significantly ($p<0.05$) (Fig. 1).

Table 2. Effect of Intraperitoneal administration of *P. oleracea* doses of 50, 100 and 200 mg/kg on serum parameters and liver damages in diabetic rats

Parameters	Alloxan-induced	Extract (mg/kg)		
		50	100	200
Glucose (mg/dl)	494± 86	196± 50 **	152±34 ***	165±29 ***
Triglycerides (mg/dl)	220± 15	130± 18 *	121± 7 *	120± 7 *
Cholesterol (mg/dl)	122± 6	21± 3 ***	20± 6 **	18± 5 ***
LDL (mg/dl)	163± 9	73± 19 *	63± 16 **	71± 10 **
HDL (mg/dl)	39± 10	77± 11	120± 22	144± 47 *
AST (UI/l)	3215± 365	1232± 3 ***	1318± 11 ***	1219± 2 ***
ALT (UI/l)	3394± 360	1229± 6 ***	1239± 4 ***	1239± 3 ***
ALP (UI/l)	1527± 101	1823± 118	1717± 88	1815± 71
Urea (mg/dl)	492± 58	36± 15 ***	45± 12 ***	67± 24 ***
Uric acid (mg/dl)	94± 5	61± 2	57± 4	52± 2
Creatinine (mg/dl)	1.28± 0.3	1.64± 0.5	0.31± 0.1	0.54± 0.1
Total protein (mg/dl)	2± 0.4	3± 0.2	4± 0.6	6± 0.4 ***
Score damage	1.3± 0.3	0.63± 0.3	0.5± 0.3	0.5± 0.2 *

Data presented as mean±SEM
*p<0.05, **p<0.01, ***p<0.001 different from Alloxan-induced diabetic group.

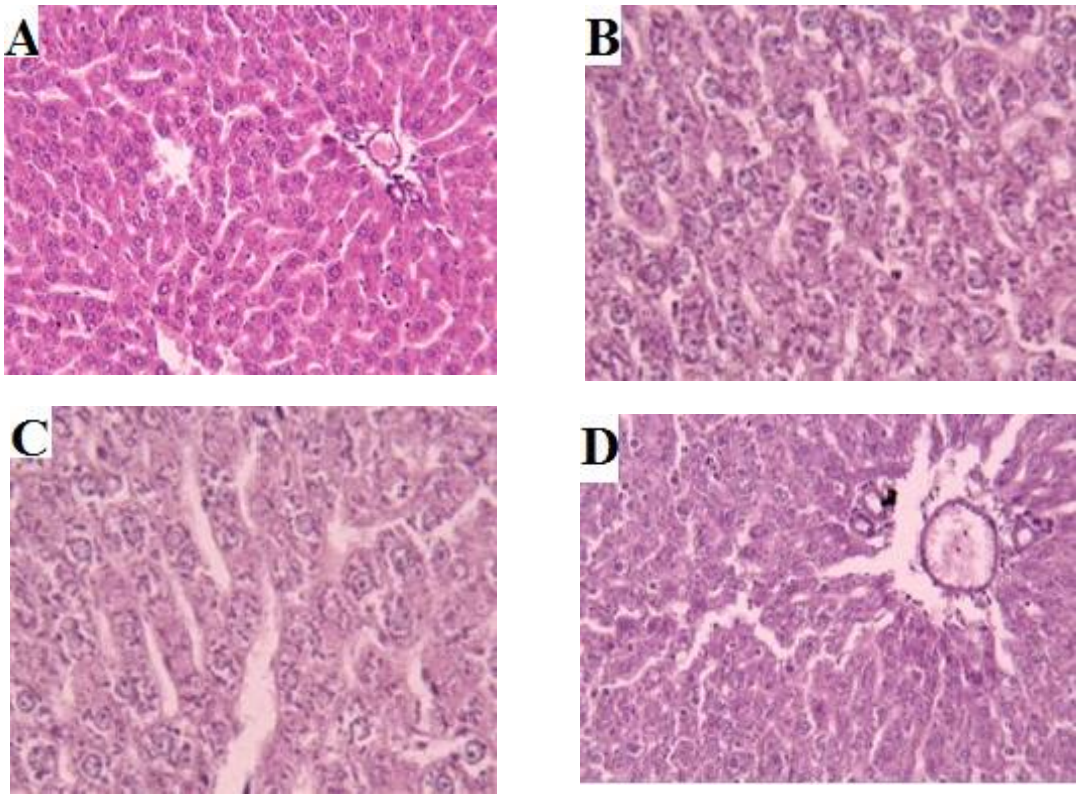

Fig. 1. Histopathology of liver tissue in diabetic rats treated with different concentrations of *P. oleracea* (i.p) for 14 days (staining with hematoxylin-eosin). (A) Normal liver tissue (×200), (B) Dilation of sinusoid (×400), (C) Mild fatty change (×400), (D) Portal tract (×200).

Discussion

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, abnormal lipid and protein metabolism along with specific long-term complications affecting the retina, kidney, liver and nervous system [10]. Synthetic hypoglycaemic agents can produce serious side effects and in addition, they are not suitable for use during pregnancy. For a long time, diabetics have been treated with several medicinal plants or their extracts based on the folklore medicine [11]. In Iranian folk medicine, some traditional and edible plants have been utilized to decrease symptoms of diabetes. Among these, purslane is a plant whose root, leaves and seeds can be used in many ways. Various pharmacological actions of *P.oleracea* such as antioxidant [12], antiviral [13], immunomodulatory [14] and antitumor activities [15] are reported.

The present study showed that the treatment of purslane extract has hypoglycaemic and hypolipidemic effects in alloxan-induced diabetic rat in 28 days. The mechanism of plant is unknown but it is reported that purslane treatment could increase concentration of serum insulin in alloxan induced diabetic mice [16].

Diabetes is also associated with hyperlipidemia. The serum cholesterol, triglycerides and LDL levels decreased significantly in diabetic rats after purslane treatment. These effects may be

due to low activity of cholesterol biosynthesis enzymes or low level of lipolysis which are under the control of insulin [17]. The purslane supplementation also results in the significant attenuation in the level of HDL in serum toward the control level which again strengthens the hypolipidemic effect of the purslane extract. Alloxan could damage pancreatic beta cell, resulting in a decrease in endogenous insulin secretion, which decreased utilization of glucose by the tissues consequently. It is reported purslane increased the concentration of serum insulin in alloxan induced diabetic mice. So, the possible mechanism of action of plant could be correlated with promoting insulin secretion [18]. Administration of purslane extract improved liver function. The present study showed the plant increased serum total protein level and decreased urea, uric acid, hepatic enzymes including ALT and AST levels in diabetic rats, significantly. Also, the purslane attenuated liver tissue damages in alloxan-induced diabetic rats. This effect may be related to antioxidant activity of purslane seeds. Purslane has anti-oxidative effect by increasing the activity hepatic enzymes [19]. The constituents of *P.oleracea* such as flavonoids (quercetin), omega-3, ascorbic acid, β-carotene and glutathione have antioxidant activity [20], so this plant may inhibit lipid peroxidation by scavenging free radicals and increasing intracellular concentration of glutathione, and thereby decrease oxidized LDL and improve insulin receptor activity. Since, an increase of HDL levels resulted in a decrease of total cholesterol and improves liver functions. Moreover, this herb is chiefly valued in scurvy and liver diseases [21]. In China, it is used for the treatment of viral hepatitis and in diabetes management [22]. Purslane improves liver functions of diabetic subjects by decreasing ALT, AST, gamma-glutamyl transferase, total and direct bilirubin close to normal levels and increasing albumin synthesis by the liver [23].

Conclusion

Our research has indicated that purslane extract possesses antidiabetic activities in rats. Therefore, the plant should be considered as a candidate for future studies on diabetes. The further studies are in progress to elucidate the molecular and cellular mechanism of purslane.

Conflict of Interest

The authors declare that there are no conflict of interest.

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