

The Effect of Methanolic Extract of *Otostegia persica* on Serum Glucose Level and Renal Function Indicators in Streptozotocin Induced Diabetic Rats

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Article information	Abstract
<p>Article history: Received: 8 July 2011 Accepted: 1 Aug 2011 Available online: 13 Feb 2012</p>	<p>Background: Regarding the antioxidant property of <i>Otostegia persica</i> extract and the role of antioxidants in Diabetes mellitus treatment, in this study the effect of extract on serum glucose level and renal function indicators was determined in diabetic male rats.</p> <p>Materials and Methods: Diabetes mellitus (type I) was induced in male rats using intraperitoneal injection of streptozotocin (STZ) (65 mg/kg). To determine blood glucose, urea, and creatinine serum levels; fasting blood samples were collected twice (before STZ injection and 5 days later). The rats with their serum glucose level exceeding 250 mg/dl were considered diabetic and divided into 10 groups separately received <i>Otostegia persica</i> alcoholic extract (100, 200, and 300 mg/kg/day doses), glibenclamide with 600 µg/kg dose and 0.5 ml distilled water for 3 and 6 days using gavage. After 3 and 6 days, blood samples were collected again and glucose, urea, and creatinine serum levels were assessed using spectrophotometry technique by respective kits.</p> <p>Results: Treating diabetic rats by <i>Otostegia persica</i> extract (100, 200, and 300 mg/kg/day doses) for 6 days results in a significant decrease of glucose and creatinine, yet an increase of serum urea with 200 mg/kg dose. Also, administration of the extract for 3 days (300 mg/kg) reduced glucose, and (in various doses) urea and creatinine serum levels.</p> <p>Conclusion: <i>Otostegia persica</i> extract has hypoglycemic effect and administering it in diabetes mellitus not only had no undesirable renal side effects, but also improved renal function to some extent.</p>
<p>Keywords: Diabetes Mellitus <i>Otostegia persica</i> Kidney</p>	
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Introduction

Diabetes mellitus (type I) is a metabolic disease in which insulin secretion by pancreas is impaired and as a result hyperglycemia is induced because of glucose intake reduction in cells or excessive production of glucose [1]. Diabetic nephropathy is among the other significant reasons for diabetic patients' mortality. 30% of the patients suffering from diabetes usually become infected to the disease after 10 or 20 years of having diabetes [2].

Kidneys excrete metabolic wastes including urea, uric acid, creatinine and ions, so chemical structure of the body liquids remains at optimum level, however, concentration of the metabolites increases in uncontrolled renal impairments resulting from diabetes mellitus [3]. Diabetes is recognized as a situation and disease where reactive oxygen species (ROS) production increases so that the resulting hyperglycemia induces antioxidant systems destruction, autoxidative glycosylation of cell membranes, lipid peroxidation and finally tissue injuries [4]. Accordingly, controlling glucose [5] and balancing antioxidant status in different tissues improve the conditions in diabetes mellitus [6].

Glibenclamide is of sulphonylureas. It reduces blood glucose through stimulating cells and increasing insulin secretion, yet the use of it is limited because of its

pharmacokinetic properties and side effects [7]. Over 800 plants with hypoglycemic effects are widely used, nevertheless just hypoglycemic effects of a small proportion of them are experimentally determined [8]. *Otostegia persica* from Lamiaceae family grows in the eastern Asia [9].

The plant grows in southern Iran (between Shiraz and Jahrom, Fars province) and also southeastern Iran (Sistan- Baluchistan province) [10].

It is used in Iran traditional medicine to treat malaria, fever and diabetes [11]. Aqueous extract of *Otostegia persica* aerial parts has antihistamine, antispasmodic, and antiarthritis effects [12]. Furthermore, hydroalcoholic extract of *Otostegia persica* improves morphine withdrawal syndrome [13]. Also, another study demonstrated that *Otostegia persica* various extracts (methanolic, hexanoic, and chloroformic) show antimicrobial effects against Gram-positive bacteria [10].

According to the experiments, it is determined that methanolic extract of the plant has antioxidant properties [14] and regarding the role of antioxidants in healing diabetes mellitus, in this study the effect of methanolic extract of *Otostegia persica* on serum glucose level and renal function indicators (urea and creatinine) was determined in diabetic male rats.

Materials and Methods

Sixty Male wistar rats (200-250 g) were used in this study. They were kept in animal house of Biology department (in 12:12-h light: dark cycle, suitable temperature, and free access to water and diet) and principles of laboratory animal care were followed.

The plant was collected from Jiroft zone located in southern Kerman and approved by botanists of Biology Department of Shahid Bahonar University of Kerman. Aerial parts of the plant was ground by electrical grinder. The powder was macerated in methanol for 48 h and extraction was carried out using Soxhlet device. The extract solvent was removed by Rotary Evaporator and dried at -50 °C using Freeze dryer. Extraction efficiency was 10%. The extract was prepared in distilled water in desirable doses and administered orally in the rats using gavage technique.

Diabetes mellitus type I was induced by intraperitoneal injection of streptozotocin (STZ) (65 mg/kg) and blood samples were collected from cavernous sinus of the animal's eye [15] before and 5 days later to determine glucose, urea and creatinine serum levels [16].

In addition, blood glucose level was immediately assessed using Glucometer (Accu-check model, Roch Co., Germany) and rats with blood glucose levels exceeding 250 mg/dl were considered diabetic [17] and divided into 10 groups: 1. Diabetic rats orally administered 0.5 ml extract solvent (distilled water) for 3 and 6 days (Sham groups). 2. Diabetic rats orally administered glibenclamide (600 µg/kg) for 3 and 6 days (two positive control groups). 3. Diabetic rats orally and separately administered methanolic extract of *Otostegia persica* (100, 200, and 300 mg/kg/day doses) for 3 and 6 days (6 treatment groups).

After 3 and 6 days, anaesthetized fasted animals sacrificed by decapitation and blood immediately collected into tubes centrifuged and serum samples were stored at -20°C until utilized for measurements.

Glucose, urea, and creatinine serum levels were assessed using spectrophotometry technique by respective kits.

Mean data in each group was compared to paired t-test before and after diabetes induction and after receiving glibenclamide, extract or its solvent (distilled water). The comparison between different groups receiving the extract was conducted using one-way ANOVA followed by the Tukey post hoc test. All the data were expressed as mean ± S.E.M. The criterion for statistical significance was $p < 0.05$.

Results

The comparison of glucose, urea, and creatinine serum levels in intact rats (before diabetes induction) to diabetic ones indicates a significant increase but after diabetes induction mean fasting glucose serum level in diabetic rats receiving various doses of the extract and glibenclamide for 6 days ($p < 0.01$) and the extract (300

mg/kg) for 3 days ($p < 0.05$) was significantly decreased comparing to the diabetic rats (Fig. 1 & 2).

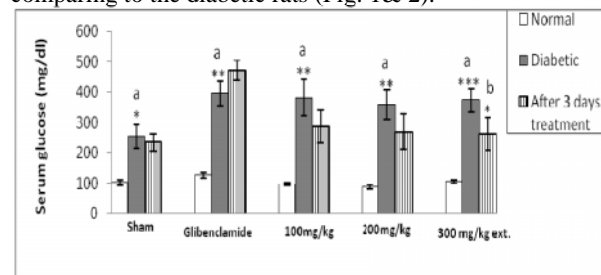


Figure 1. Effect of oral administration of *Otostegia persica* methanolic extract at doses 100, 200 and 300 mg/kg body wt. and glibenclamide (600 µg/kg) for 3 days on serum glucose in diabetic rats. Each column represents mean±S.E.M for 6 rats. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. a: vs. Normal group, b: vs. Diabetic group.

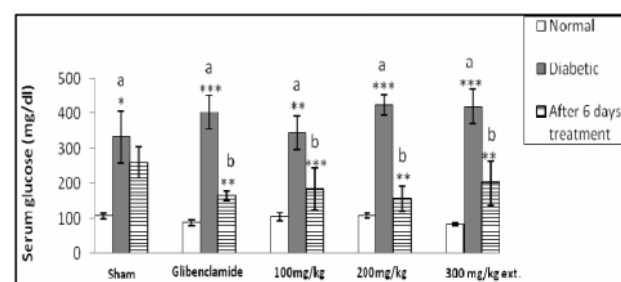


Figure 2. Effect of oral administration of *Otostegia persica* methanolic extract at doses 100, 200 and 300 mg/kg body wt. and glibenclamide (600 µg/kg) for 6 days on serum glucose in diabetic rats. Each column represents mean±S.E.M for 6 rats. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. a: vs. Normal group, b: vs. Diabetic group.

Administering various doses of the extract in diabetic rats for 3 and 6 days led to a significant reduction ($p < 0.05$) of creatinine serum level (Table 1). *Otostegia persica* extract (all doses) and glibenclamide for 6 days reduced ($p < 0.05$) urea serum level, but administering the extract (200 mg/kg dose) for 3 days increased this indicator ($p < 0.01$) (Table 1 & 2).

Comparing different variables between the groups receiving various doses of the extract for 3 or 6 days presented no significant difference, and administering the extract solvent had no significant effect on glucose, urea and creatinine serum levels in the diabetic rats.

Table 1. Mean values of serum urea and creatinine in different groups received distilled water (sham), glibenclamide or extract (100, 200, 300 mg/kg, doses) daily for 3 days

Groups	Metabolites	Creatinine (mg/dl)	Urea (mg/dl)
Sham	N	0.5 ± 0.04	41.75 ± 6.75
	D	1.2 ± 0.09 ^{a*}	86.75 ± 4.98 ^{***}
	D±distilled water	1.5 ± 0.24	93.5 ± 14.47
Control	N	0.77 ± 0.07	44.57 ± 2.03
	D	1.22 ± 0.08 ^{a**}	108 ± 5.2 ^{a*}
	D±glibenclamide	0.7 ± 0.18 ^{b***}	114 ± 3.76
Treatment	N	0.51 ± 0.04	43 ± 1.3
	D	0.81 ± 0.09 ^{a**}	97 ± 10.37 ^{a*}
	D±Extract (100 mg / kg)	0.63 ± 0.05 ^{b*}	98.83 ± 9.91
Treatment	N	0.62 ± 0.07	39.42 ± 2.24
	D	0.84 ± 0.10 ^{a*}	85.42 ± 12.04 ^{a*}
	D±Extract (200 mg/kg)	0.61 ± 0.05 ^{b*}	140 ± 11.78 ^{b**}
Treatment	N	0.61 ± 0.06	45.83 ± 3.2
	D	0.88 ± 0.07 ^{a***}	102.83 ± 8.26 ^{a*}
	D±Extract (300 mg/kg)	0.66 ± 0.07 ^{b***}	124.66 ± 15.2

$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. a: vs. normal (N) rats, b: vs. Diabetic (D) rats. Values are Mean±S.E.M for 6 rats

Table 2. Mean values of serum urea and creatinine in different groups received distilled water (sham), glibenclamide or extract (100, 200, 300 mg/kg, doses) daily for 6 days.

Groups	Metabolites	Creatinine (mg/dl)	Urea (mg/dl)
Sham	N	0.67 ± 0.02	33 ± 2.3
	D	1.1 ± 0.1 ^{a**}	72 ± 9.07 ^{a*}
Control	D±distilled water	1 ± 0.1	70 ± 18
	N	0.6 ± 0.03	35 ± 3.7
	D	1.2 ± 0.2 ^{a**}	94 ± 8.4 ^{a***}
Treatment	D±glibenclamide	1.6 ± 0.3	62 ± 10.66 ^{b*}
	N	0.7 ± 0.03	42 ± 1.8
	D	0.9 ± 0.03 ^{a*}	119 ± 9 ^{a***}
Treatment	D±Extract (100 mg/kg)	0.66 ± 0.06 ^{b*}	81 ± 10 ^{b*}
	N	0.53 ± 0.08	39 ± 3
	D	0.8 ± 0.08 ^{a*}	92 ± 11 ^{a***}
Treatment	D±Extract (200 mg/kg)	0.6 ± 0.05 ^{b*}	70 ± 5 ^{b*}
	N	0.5 ± 0.04	46 ± 2
	D	0.8 ± 0.07 ^{a*}	116 ± 17 ^{a**}
Treatment	D±Extract (300 mg/kg)	0.5 ± 0.04 ^{b**}	82 ± 13.9 ^{b*}

*p<0.05, **p<0.01, ***p<0.001. a: vs. normal (N) rats, b: vs. Diabetic (D) rats. Values are Mean±S.E.M for 6 rats.

Discussion

Results showed that administering *Otostegia persica* extract for 3 and 6 days decreases glucose, creatinine, and even urea serum levels in the diabetic rats. Streptozotocin induces diabetes in the rats [1]. It destructs pancreas β -cells by inducing inflammation and consequently production of cytokines and reactive oxygen species, results in insulin production decrease and diabetes mellitus type I disease [18]. When insulin is not available, proteins' catabolism increases and amino acids decomposition leads to urea concentration increase in blood. The increase in uric acid, creatinine and urea levels in blood indicates renal impairment [3]. The administration of ethanolic extract of *Otostegia persica* (200, 350, 500 mg/kg) for 3 weeks also resulted in a significant decrease in glucose serum level in diabetic rats [19]. The antioxidant effects are reported in some plants containing compounds such as flavonoid, phenol, ascorbic acid, and tocopherol [6]. The antioxidant

activity of methnolic extract of *Otostegia persica* is because of Morin and Quercetin compounds in it and its antioxidant activity is higher than *Ginkgo biloba* and partially as a par with *Camellia sinensis* [12]. Generally, Morin as a flavonoid contains antioxidant, anti-allergic, anti-inflammatory, anti-mutation and anti-cancer effects [20]. Also, it has the capability to scavenge reactive hydroxyl and superoxide species. Quercetin is a strong antioxidant leading to reactive xanthine superoxide and xanthine oxide species removal. Long term treatments of Quercetin in the diabetic rats resulted in the reduction of oxidative stress [21].

Monoterpene derivatives in the flowers of *Otostegia persica* are other important compounds capable of removing reactive hydroxyl and superoxide species [1]. Some of the hypoglycemic plants decrease or block carbohydrates absorption from the intestine by inhibiting enteric α -glycosidase enzyme and consequently blood glucose reduction, however, *Otostegia persica* cannot produce so strong inhibitory effects [22]. The antioxidant factors in methanolic extract of *Otostegia persica* might rehabilitate the islets of Langerhans, increase insulin secretion and, as a result, reduce glucose serum level [23]. The extract is also capable of healing kidney directly or indirectly via decreasing blood glucose. Regarding the effects of the plant in reducing urea and creatinine serum levels, it can be said that using this plant in diabetic patients not only has renal side effects but also prevents from the renal impairments resulted from diabetes to some extent.

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