

Antidiabetic Effects of *Otostegia persica* Root in Alloxan-induced Diabetic Rats

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Abstract

Otostegia persica has been used in the Iranian traditional medicine for the treatment of diabetes, because of it, the antidiabetic effect of aqueous extract of *Otostegia persica* root was investigated in alloxan-induced diabetic rats. In the present study oral administration of the aqueous extract of *Otostegia persica* root (200, 300 and 400 mg/kg body wt.) were investigated for 28 days. The level of serum glucose, total cholesterol, triglyceride (TG), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), aspartate amino transferase (AST) and alanine amino transferase (ALT) were evaluated in diabetic rats. The aqueous extract of *Otostegia persica* roots in all doses significantly decreased serum glucose and HDL levels when compared with diabetic control rats. In the dose of 300 mg/kg body wt, ALT and TG levels were significantly decreased. A comparison was made between the action of *Otostegia persica* root extract and metformin (50 mg/kg body wt.), the known antidiabetic drug. The antidiabetic effect of the extract was the same as the metformin. It is concluded that this part of plant might be considered as excellent candidate for future study on diabetes mellitus.

Keywords: *Otostegia persica* root, Diabetes, Rat

1. Introduction

The world prevalence of diabetes among adults (aged 20–79 years) was estimated 6.4 %. Affecting 285 million adults, in 2010, and will increase to 7.7, and 439 million adults by 2030 (Shaw et al., 2010).

Several medicinal plants have been investigated for their beneficial use in different types of diabetes. The effects of these plants may delay the development of diabetic complications and correct the metabolic abnormalities using variety of mechanisms. A considerable number of plants were subjected to clinical trials and were found effective. Moreover, during the past few years many phytoconstituents responsible for Antidiabetic effects have been isolated from hypoglycaemic plants (Edwin et al., 2008).

The genus *Otostegia* belongs to family Labiate, which is an almost cosmopolitan family of about 180 genera and over 3500 species (Nasir and Ali, 1990).

Otostegia persica or "Goldar" is an endemic plant that can be found in southern provinces of Iran (Ghahraman, 1996). In Iranian traditional medicine the aqueous extract of aerial part of *Otostegia persica* is used as an antihistamin, antispasmodic and antidiabetic (Asghari et al., 2006). Recent studies on aerial parts of this plants suggested *Otostegia persica* could be considered as medicinal herbal candidate for treatment of diabetes (Akbarzadeh et al., 2012). The present study was investigated to study the antidiabetic and antihyperlipidemic effect of aqueous extract of *Otostegia persica* root in diabetic rats.

2. Material and methods

2.1. Collection of plants

The roots of *Otostegia persica* were collected during December 2011 from south Fars province, Iran. The plant and roots were identified by Ferdowsi university herbarium A voucher number specimen (23332) has been kept in herbarium in the department of Pharmacology, Ferdowsi University of Mashhad, Iran.

2.2. Preparation of extracts

Otostegia persica roots were cleaned and cut into small pieces and were allowed to dry in shade. Dried roots powdered by grinder (Moulinex AR1043-UK). Extraction was carried out by maceration of 500 g of dried powdered plant in 2500 ml of distilled water (48h) in magnetic shaker at the room temperature. After maceration, the resulting extract

was filtered and concentrated in rotavapour under reduced pressure. The concentrated extract was lyophilized to get a powder (yield 2%, w/w).

2.3. Phytochemical screening

In order to determine the presence of alkaloids, glycoside, flavones, saponins and tannins a preliminary phytochemical study (colour reactions) with various plant extracts were performed (Tiwari et al., 2011). Polyphenol content was also determined spectrophotometrically by using Folin-Ciocalteu's method as described by Zivkovic et al (Zivkovich et al., 2009). Gallic acid was used as standard to measure the total polyphenol content in 3 extracts (petroleum ether, aqueous, ethanolic).

2.4. Experimental Animals

Male albino wistar rats of body weight 180-220 g were obtained from Pastor Institute, Tehran, Iran. Animal were housed in poly ethylene cages at temperature 21-25 degrees Celsius, 12 h light /12h dark cycle and relative air humidity 40-45%. Rats had continuous access to Standard commercial food (Javaneh co, Mashhad, Iran) and tap water. Permission for the study was obtained from ethical committee for animal research works of Birjand University of Medical Sciences.

2.5. Induction of diabetes in rats

Diabetes was induced by a single intrapritoneal injection of freshly prepared. Alloxan monohydrate (Sigma-USA) by the dose of 150 mg/kg to overnight fasted rats. After 7 days of alloxan administration rats by FBS levels upon 300 mg/dl were allocated as severe diabetic. (Kumar Sing et al., 2007).

2.6. Estimation

Fasted blood glucose level estimated using portable glucometer (ACCU CHEK Active-Germany) in days 1st, 14th and 28th. Total cholesterol triglyceride (TG), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), aspartate amino transferase (AST) and alanine amino transferase (ALT) estimated by using standard kits (Pars Azmon Iran) and autoanalyzer (Liasys Random Access AMS-Italy).

2.7. Drug administration

Otostegia persica extract was suspended in normal saline and administrated orally through orogastric tube at doses of 200, 300 and 400 mg/kg body wt. Same volume (1

ml) administered in all groups. Metformin (glucophage –Merck, co, France) was suspended in normal saline and preperated freshly before administration by dose 50 mg/kg body wt.

2.8. Experimental design

In the present study, 48 rats (40 diabetic and 8 normal rats) were used. The rats were divided randomly into 6 equal groups, 8 rats in each group.

Group 1: Normal control rats were administered 1 ml of normal saline.

Group 2: Diabetic control rats were administered 1 ml of normal saline.

Group 3: Diabetic rats were administered metformin (glucophage–Merck co, France) in aqueous solution in the dose of 50 mg/kg orally by orogasteric tube.

Group 4-6: Diabetic were administrated *Otostegia persica* (200, 300 and 400 mg/kg body wt). All groups were administrated orally once per day for 28 days.

2.9. Biochemical assay

In the first day, before treatment, 14th and 28th days, fasted blood glucose were measured by glucometer. After 28 days of treatment blood samples were collected from heart of overnight fasted rats. Total cholesterol, TG, (HDL-c), (LDL-c), (AST) and (ALT) levels were determined.

3. Statistical Analysis

All the data were expressed as mean \pm S.E.M. Data were analyzed by using ANOVA and tukey post hoc test. Values were considered significantly different at $P < 0.05$.

4. Results

4.1. Preliminary phytochemical screening and total polyphenol content

The results of preliminary phytochemical screening of *Otostegia persica* root extracts showed (table 1) that all the root extracts (except petroleum ether) contain flavonoids, Tannins and saponins. However alkaloid was absent in all extracts. The results of total polyphenol content (table 2) were different between each extracts. total polyphenol contents were highest in aqueous extract of *otostegia persica* root.

4.2. Hypoglycemic Effect of *Otostegia persica* root extract

The results of this study clearly indicated that the aqueous extracts of *Otostegia persica* root in all doses exhibit significant hypoglycemic activity in alloxan–diabetic

rats, whilst there was no significant effect observed in glucose level of group 4 in 14th days, however at the end of 28th days of treatment there was a significant decrease ($P<0.001$) of serum glucose levels in this group (table 3). The standard drug metformin also significantly decreased serum glucose levels ($P<0.001$) compared with diabetic control group.

4.3. Effect of the aqueous extract of Otostegia persica root on plasma aspartate aminotransferase (AST) and alanine amino transferase (ALT) levels in diabetic rats

4.4. Effect of Otostegia persica on plasma lipid profile

The concentration of plasma lipids are presented in table-4 the concentration of total cholesterol were decreased significantly in the group 6 compared to the diabetic group on the 28th days of study. Plasma triglyceride concentration was decreased significantly in group 5 compared to diabetic control. Plasma HDL levels was also significantly decreased in all *Otostegia persica* root extract groups.

5. Discussion

Otostegia persica commonly known as "Goldar" which is an endemic plant that grows in the southern parts of Iran, especially in Fars, Kerman, Sistan and Baluchestan and Hormozgan provinces. There is a strong belief in antidiabetic effects of this plant in some regions of Iran (Ghahraman. 1996).

No pervious investigation on the antidiabetic effect of plant's root has been reported, but other studies such as: antioxidant activity (Sharififar et al., 2003), antimicrobial (Asghari et al., 2006), antidiabetic activity (Ebrahimipoor 2011, Akbarzadeh 2012) were found. All of these studies related aerial parts of *Otostegia persica* and present study is the first investigation of *Otostegia persica* root extract in diabetic rats.

The present data indicated that the aqueous extract of *Otostegia persica* root significantly decreased serum glucose and HDL in all doses, *Otostegia persica* in doses of 300 mg/kg body wt., decreased ALT and TG significantly. in agreement, two other studies that investigated aerial parts of *Otostegia persica* in diabeticrats, have shown the hypoglycemic effect and decreasing total cholesterol without change in HDL (Akbarzade 2012, Ebrahimi 2011). Most of the drugs that decrease total cholesterol also decrease HDL. (Wilson, P.W.F., 1990).

Phytochemical investigation of *Otostegia persica* root revealed the presence Flavonoids that are known to be bioactive for management of diabetes (Shammas, 1987; Rhemann and Zaman, 1989) and it is well known that certain flavonoids exhibit hypoglycemic activity (Pathak et al., 1991; Ahmed et al., 2000). Also Flavonoids are able to regeneration beta cell of pancreas (Chakravarti et al., 1981). Thus, the significant antidiabetic effect of aqueous extract of *Otostegia persica* root may be due to

the presence of more than one antihyperglycemic principle and their synergistic properties.

From this study we can conclusively state that *Otostegia persica* root aqueous extract has shown significant effect on blood glucose level in diabetic rats. Further pharmacological and biochemical investigations are necessary to elucidate the mechanism of the antidiabetic of *Otostegia persica*.

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References

- Ahmed, M., Akhtar, M.S., Malik ,T., Gilani, A.H, 2000. Hypoglycemic action of flavonoids fraction pf *Cuminum nigrum* seeds. *Phytotherapy Research*. 14, 103-106.
- Akbarzadeh, S., Bazzi, P., Daneshi, A., Nabipour, I., Purkhalili, K., Mohebbi, G., et al., 2010. Anti-diabetic effect of *Otostegia persica* extract on diabetic rats. *J. med. Plants res.* 6, 3176-3180.
- Asghari, G., Nourallahi, H., Havaie, S.A., Isa, L., 2006. Antimicrobial activity of *otostegia persica* Boiss extracts. *Res. Pharm. Sci.* 1, 53-58.
- Chakravarti, B.K., Gupta, S., Gambir, S.S., Gode, K.D., 1981. Pancreatic betacell regeneration in rats by epicatechin. *The Lancet*. 2, 759-760.
- Ebrahimpoor, M.R., Khaksar, Z., 2011. Anti_diabetic effect of orally administered *Otostegia persica* extract on streptozotocin diabetic rats. *Comp. Clin. Pathol.* 20, 523-525.
- Edwin, J., Siddaheswar Balakrishnan, J., Dharam Chadra, J., 2008. Diabetes and Herbal Medicines. *I. J. P. T.* 7, 97-106.
- Ghahraman, A., 1996. Color atlas of Irania flora. Research Inistitue of Forests Rangelands Publishing, Tehran, pp. 1072.
- Kumar Singh, S., Kesari, A.N, Kumar Gupta, R., Jaiswal, D., Watal, G., 2007. Assessment of antidiabetic potential of *Cynodon dactylon* extract in streptozotocin diabetic rats. 114, 174-179.
- Nasir, E., Ali, S.I., 1990. *Fora of west Pakistan* .Fakhri printing press, Karachi.
- Pathak, D., Pathak, K., Singla, A.K., 1991. Flavonoids as medicinal agents-recent advances. *Fitoterapia*. 62, 371-389.
- Rhemann, A.U., Zaman, K. 1989. Medicinal plants with hypoglycemic activity. *J. Ethnopharm.* 26, 1-55.
- Shammas, G., 1987. Flavonoid heterosides of *Teucrium pollium* L. *Planta medical.* 21, 144-148.

- Sharififar, f., Yassa, N., Shafiee, A., 2003. Antioxidant activity of *Otostegia persica* (Labiatae) and its constituent. Iranian. J. Pharm. Res. 2, 235-239.
- Shaw, J.E., Sicree, R.A., Zimmet, P.Z., 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res. Clin. Pract. 87, 4-14.
- Tiwari, P., Kumar, B., Kaur, G., Kaur, H., 2011. Phytochemical screening and extraction: A review. Internationale pharmaceutica sciencia. 1, 98-106.
- Wilson, P.W.F., 1990. High density lipoprotein, low density lipoprotein and coronary heart disease. Americ. J. Cardi. 66, 7A-10A.
- Zivkovic, J., Mujic, I., Zekovich, Z., Nikolic, G., Vidovic, S., Muji, A., 2009. Extraction and analysis of condensed tannins in *Castanea Sativa*. J. Cent. Eur. Agric. 10, 283-288.

Table 1. Phytochemical screening of *Otostegia persica* root extracts

Compounds	Extracts			
	Petroleum Ether	Ethanol	Aqueous	Methanol
Flavonoids	-	+	+	+
Tannins	-	+	+	+
Saponins	-	++	+++	++
Alkaloids	-	-	-	-

Table 2. Content of total phenolic compounds in investigated root aqueous, petroleum ether and ethanolic extracts, was expressed as mg of gallic acid equivalent per each gram of dry extract sample

Extracts	Content of total phenolic compounds (mg/g)
Aqueous	2.28 ± 0.005*
Ethanol	1.036 ± 0.040*
Petroleum Ether	0.006 ± 0.0020*

Data are shown as Mean ± S.E.M of 6 parallel replicates. Values with significant differences from each other type of *Otostegia persica* root extract (ANOVA followed tukey post hoc test)

Table 3. Effect of *Otostegia persica* root on serum glucose level

(n=8)	Fasting blood glucose concentration (mg/dl)					
	First day		14 th day		28 th day	
Normal group	92.00	21.33 [†]	97.3 3	10.80 [†]	90.8 3	26.46 [†]
Diabetic control	415.4 2	77.68*	405. 14	77.37*	413. 71	35.60*
Diabetic+ 50 mg/kg of metformin (metformin)	468.7 1	99.94*	195. 14	74.28 [†]	114. 71	35.60 [†]
Diabetic+ 200 mg/kg of aqueous extract (200 O.P)	442.6 2	70.85*	439. 14	123.48*	126. 00	98.65 [†]
Diabetic+ 300 mg/kg of aqueous extract (300 O.P)	440.5 0	54.74*	129. 00	51.58 [†]	67.0 0	32.43 [†]
Diabetic+ 400 mg/kg of aqueous extract (400 O.P)	478.3 3	135.22*	138. 00	42.24 [†]	138. 66	23.95 [†]

* P<0.001 compared with normal control group

† P<0.001 compared with diabetic control group

Table 4

Lipid profile concentration

Group(n=8)	Lipid profile concentration mg/dl (Mean ±S.E.M)			
	Total cholesterol	Triglyceride	HDL-c	LDL-c
Normal group	84.57 ± 9.76	73.85 ± 6.51	32.42 ± 4.68	30.00 ± 8.56
Diabetic control	98.85 ± 8.09	86.14 ± 13.20	39.85 ± 3.02	32.42 ± 7.54
Diabetic+ 50 mg/kg of metformin (metformin)	110.50 ± 10.54	113.00 ± 36.39*	41.50 ± 6.28	34.16 ± 4.35
Diabetic+ 200 mg/kg of aqueous extract (200 OP)	61.16 ± 25.12	61.66 ± 23.88	24.00 ± 8.48 [†]	25.00 ± 7.97
Diabetic+ 300 mg/kg of aqueous extract (300 OP)	80.12 ± 40.12	51.00 ± 16.69 [†]	21.12 ± 4.99 ^{†*}	29.25 ± 6.56
Diabetic+ 400 mg/kg of aqueous extract (400 OP)	54.33 ± 22.33 [†]	59.33 ± 27.22	28.66 ± 4.80 [†]	22.50 ± 3.01

* P<0.05 compared with normal control group

† P<0.05 compared with diabetic control group

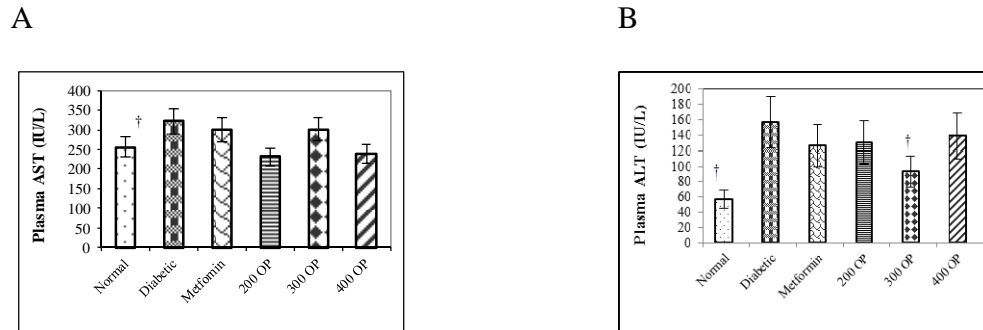


Fig. 1. Effect of aqueous extract of *Otostegia persica* root on Plasma AST (A) and ALT (B). AST: aspartate amino transferase; ALT: alanine amino transferase. Values are given as mean \pm S.E.M. for 8 rats in each group. † $P < 0.05$ compared with diabetic control group.

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