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Full Length Research Paper

Anti-diabetic effect of *Otostegia persica* extract on diabetic rats

Samad Akbarzadeh[1], Parviz Bazzi[2], Adel Daneshi[3], Iraj Nabipour[4], Khalil Pourkhalili[5], Gholamhossein Mohebbi[3], Kohzad Sartavi[6], Mohammadreza Abdi[3], Mostafa Mirzaei[4] and Afshar Bargahi[3]*

[1]Department of Biochemistry, School of Medicine, Bushehr University of Medical Sciences, Bushehr, Iran.
[2]Department of Anatomy, School of Medicine, Bushehr University of Medical Sciences, Bushehr, Iran.
[3]The Persian Gulf Marine Biotechnology Research Center, Bushehr University of Medical Sciences, Bushehr, Iran.
[4]Department of Endocrine and Metabolic Diseases, The Persian Gulf Tropical Medicine Research Center, Bushehr University of Medical Sciences, Bushehr, Iran.
[5]Department of Physiology, School of Medicine, Bushehr University of Medical Sciences, Bushehr, Iran.
[6]Research Center of Agricultural and Natural Resources of Bushehr, Bushehr. Iran.

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The *Otostegia persica* is traditionally used in some regions of Iran as medicinal herb for anti–diabetic properties. Thus, in this study the effect of aqueous extract of *O. persica* on the blood glucose, insulin, triglycerides, HDL-C, total cholesterol, and histopathology of pancreas of streptozotocin (STZ) induced diabetic rats were evaluated. Thirty five male Wistar rats weighing 150-200 g were randomized into five groups as follows: The control group (C); the diabetic control group (DC); and T_{100}, T_{200}, and T_{400} as diabetics groups that were treated with daily doses of *O. persica* extract. Experimental diabetes was induced by intraperitoneal injection of STZ. The fasting blood sugar, insulin, triglycerides, total cholesterol and HDL-C were assayed. Pancreatic samples were taken for light microscopy, and histopathology effects of *O. persica* extract was evaluated. During the 10th, 20th and 30th days of study, the fasting blood sugar of animals treated with all doses of *O. persica* extract (especially 400 mg/ kg b.w) was significantly decreased comparing to the DC groups ($p < 0.05$). Also, on the 30th day of experiment in the T_{400} group, the serum triglycerides, and homeostasis model assessments for insulin resistance (HOMA.IR) were decreased comparing to the DC group. Histopathological images showed the decrease in mass and number of β- cells of pancreases in the T_{400} comparing to the DC group at the end of study. Therefore *O. persica* could be considered as medicinal herbal candidate for remedy of diabetes.

Key words: Cholesterol, diabetes, HDL-C, Insulin, insulin resistance, *Otostegia persica*, triglycerides.

INTRODUCTION

Diabetes is a global chronic metabolic disorder and is currently a common problem of humans (Solati and Soleimani, 2010). Many people are suffering from diabetes and by the year 2025 this number is estimated to be reached to 300 million (Hida et al., 2005; Kralisch et al., 2007).

Diabetes mellitus type 1 is characterized by absolute or relative deficiency of insulin due to pancreatic beta cell dysfunction. Diabetes mellitus type 2 is distinguished either by decreased sensitivity of target tissues to the insulin effects or by insulin dependent disorders of carbohydrate, lipid and protein metabolism (Wadkar et al., 2008). Hyperglycemia that is caused by diabetes

*Corresponding author: E-mail: abargahi@yahoo.com. Tel: +987712541827. Fax: +987712541828.

Abbreviations: ELISA, Enzyme-linked immunosorbent assay; **FBS,** fasting blood sugar; **HOMA.IR,** homeostasis model assessments for insulin resistance; **HDL-C,** high density lipoprotein-cholesterol; **STZ,** streptozotocin.

leads to the micro vascular diseases such as retinopathy, neuropathy, nephropathy, and macro vascular diseases such as cardiovascular diseases (King, 2008).

Considering the high cost of medication and side effects of synthetic medicine and lack of full recovery of diabetic patients treated with chemical agents, has encouraged the researchers to using herbal medications (Maiti et al., 2005). Recent researches have shown that most medicinal plants such as onions, garlic and corcumin have hypoglycemic effects due to having some active terpenoids. Also herbal medicines have negligible side effects comparing to the chemical drugs.

Since large people in the world use the herbal medicinal plants, the World Health Organization (WHO) has encouraged the use of traditional medicines (Goleniowski et al., 2006). Treatment of diabetes mellitus with herbal medicines in the world has been successful and diabetic patient's tendency towards herbal therapy is growing (Wadkar et al, 2008). *Otostegia persica* of the mint family grows in southern and southeastern of Iran and is used as anti -diabetics remedy in Iranian folk medicine. In the Iranian traditional medicine the aqueous extract (decoction) of aerial parts of *O. persica* has antihistamine, antispasmodic, anti-arthritis and anti -diabetic properties (Asghari et al., 2006). The extract of aerial parts of the *O. persica* due to having of flavonoids like Quercetin and Maureen, is effective in decreasing blood sugar and healing dysfunctions of pancreatic β-cell. In the current study, the effort is to assess the effect of the *O. persica* extract on serum glucose, insulin, insulin resistance, cholesterol, HDL-C, triglycerides, and also on the histopathology of pancreas of STZ- induced diabetic rats.

MATERIALS AND METHODS

Plant material

The aerial parts of *O. persica* was collected during April 2011 from northern mountains of Bushehr province, Iran. Sample specimen was authenticated by herbarium of research center of Agriculture and Natural Resources of Bushehr Province, Iran. The voucher was deposited in the Herbarium of Bushehr University of Medical Sciences.

The samples were air-dried at room temperature. The aqueous extract of *O. persica* plant was obtained by boiling aerial parts for 30 min in distilled water at a ratio of 1:100 w/v, and incubated overnight at 40°C with slow shaking on an orbital shaker (Stuart Scientific Orbital Shaker, UK). The hydrosoluble part was centrifuged (6000 ×g, 10 min) and insoluble precipitate was discarded. The supernatant was filtered by Whatman No. 1 paper. The filtrate was concentrated under reduced pressure at 40°C using a rotary evaporator (Laborota 4000, Heildolph, Germany) and finally freez-dried to get *O. persica* extract. The resulting sample was powdered and plastic sealed for next use.

Animals

Thirty five male Wistar rats weighing 150-200 g were used. The animals were allowed to acclimatize for one week and were fed with standard pellet diet and water *ad libitum* under standard environmental conditions of temperature (25°C), humidity (65%) and 12 h light/dark cycle. All experimental protocols were performed under the approval from the Animal Care and Use Committee for Animal Investigations. After fasting for 18 h, the experimental diabetes was induced by intraperitoneal injection a single dose of 60 mg/kg of STZ (Alexis Biochemical, Lot-L24553). After 5 days of the STZ injection, for monitoring of glucose, the blood tail was measured using glucometer (Bionime Rightest GM 300, Switzerland) and animals with blood glucose value of >300 mg /dl were assumed to be diabetics (Heidarian and Soofiniya, 2011). Then animals were divided into 5 groups of 7 members. The control group (C), the diabetic control or diabetic group without *O. persica* treatments (DC). The treated groups (T_{100}, T_{200}, and T_{400}) as diabetic's rats that were fed with oral doses of 100, 200 and 400 mg/kg b.w, respectively, of *O. persica* extract daily. On the 10th and 20th days of study, only the fasting blood sugar and insulin were measured. On the 30th day of study animals were anesthetized and blood samples were centrifuged (3000 ×g, 15 min, 4°C) and biochemical parameters such as glucose, insulin, triglycerides, total cholesterol and HDL-C concentrations were determined as following: Insulin, triglycerides, total cholesterol, and HDL cholesterol were measured by auto analyzer selectra-2 (vital science, spankeren, Netherlands). Enzymatic methods of glucose oxidase, cholesterol oxidase, glycerol phosphate oxidase were used for assay of glucose, cholesterol and triglycerides respectively. Insulin was measured using ELISA (Alpco Insulin ELISA kit). Insulin resistance (HOMA.IR) of fasting blood sugar (FBS) was calculated by the following formula (Song et al, 2007).

$$\text{HOMA. IR} = \frac{\text{Insulin}\left(\frac{\mu\text{IU}}{\text{ml}}\right) \times \text{FBS}\left(\frac{\text{mmol}}{\text{ml}}\right)}{22.5}$$

Histopathology

For evaluation of histopathology changes in pancreatic β- cells of STZ-induced diabetic rats, pancreatic biopsies were taken on the 30th day of study. The biopsies were processed via histological method (paraffin embedding, cutting 3 µ section, and H and E staining) and evaluated with light microscopy.

Statistical analysis

For data analysis, the one-way analysis of variance (ANOVA) was used. All data were expressed as, mean ± SD and ($p < 0.05$) was considered significant.

RESULTS

In this study following oral administration of *O. persica* extract, the fasting blood sugar, insulin, triglycerides, total cholesterol and HDL-C were assayed. During the 10th, 20th and 30th days of study, the fasting blood sugar in all the *O. persica* treated groups decreased comparing to that of the DC group (Figure 1). On the 10th, 20th and 30th days the difference in the fasting insulin concentrations were not significant between T_{100}, T_{200} and T_{400} groups comparing to the C and DC groups. However, the HOMA.IR decreased in all of the treated groups comparing to the DC group on the 30th day (Table 1). Serum triglyceride concentrations were decreased

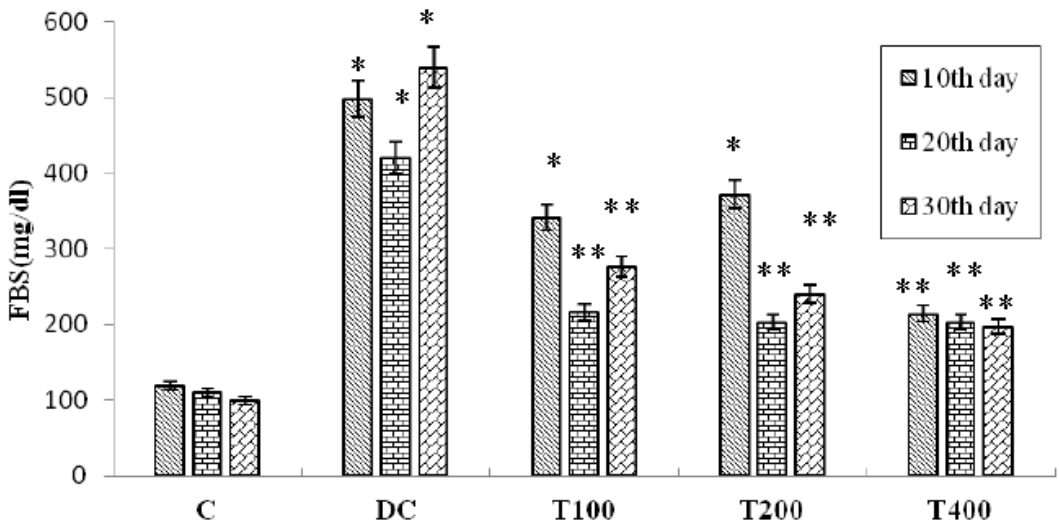

Figure 1. FBS, fasting blood sugar; C, the control group; DC, the diabetic control group; T_{100},T_{200} and T_{400} the diabetic groups that were treated with 100, 200 and 400 mg/kg of *Otostegia persica* extract, respectively (n = 7, mean ± SD, p < 0.05). *Significant differences with C group, ** Significant differences with DC group.

Table 1. Effects of *Otostegia persica* extract on the serum insulin and HOMA.IR in treatment groups comparing to the C and DC groups.

Group	10th Day		20th Day		30th Day	
	Insulin (U/L)	HOMA.IR	Insulin (U/L)	HOMA.IR	Insulin (U/L)	HOMA.IR
C	2.41±0.36	0.72±0.56	2.96 ± 0.93	0.66±0.23	3.02 ± 0.85	1.06±0.97
DC	2.49± 0.82	3.5±2.1*	2.77 ± 1.26	2.74±1.3*	3.47± 1.21	4.67±2.62*
T_{100}	3.08 ± 1.19	1.68±0.76*	2.3± 0.6	2.17±2	2.95 ± 0.91	1.63±0.59**
T_{200}	2.25± 0.48	1.86±0.43*	2.42± 0.16	1.68±1.91	2.05± 0.47	1.35±1.04**
T_{400}	2.86± 1.02	1.89±1.15*	3.11± 0.82	1.37±1.12	3.93 ± 1.09	1.26±0.81**

HOMA.IR, Homeostasis model assessments for insulin resistance; C, the control group; DC, the diabetic control group; T_{100}, T_{200} and T_{400} the diabetic groups that were treated with 100, 200 and 400 mg/kg of *Otostegia persica* extract respectively. (n = 7, mean ± SD, p < 0.05). * Significant differences with C group. ** Significant differences with DC group.

significantly in the T_{100}, T_{200} and T_{400} groups comparing to the DC group on the 30th day of study (Table 2).

The pancreatic histopathology slides of all the groups were prepared on the 30th days of study. As shown in Figure 2 the *O. persica* extract induced an increasing in the number of β- cells of pancreatic islets in all of the treated groups, (especially in the T_{400}) comparing to the C and DC group.

DISCUSSION

This study was designed to evaluate the effects of *O. persica* aqueous extract on improving the functions and structural damages of pancreas in STZ- induced diabetic rats. Our results show reducing of the insulin resistance, glucose, triglycerides concentrations and increasing of the β- cells regeneration after treatment with *O. persica*.

Herbal medicines exert anti-diabetic and anti-hyperlipidemia effects through multiple mechanisms (Ju et al., 2008). The researchers have classified active mechanism of anti-diabetic herbals into 15 groups (Ju et al., 2008). Some of these mechanisms are: stimulation of β- cells of Langerhans islets for insulin secretion and decreasing insulin resistance (Pulok et al., 2006). *O. persica* extracts contains mono/di sesquiterpen, flavonoids, steroids, and different terpenoids. Chromatographic techniques have shown that 400 mg of *O. persica* methanolic extract contains 80 mg Maureen and 67 mg Quercetin (Sharififar et al., 2003; Yassa et al., 2009). Maureen, Quercetin, Camphorol, and Isovitexin have strong antioxidant activity (Yassa et al., 2005). The *O. persica* have reported in traditional medicine to be effective in reducing blood glucose concentration. Some

Table 2. Serum concentrations of cholesterol, triglycerides and HDL-C in the *Otostegia persica* extract treated groups comparing to the C and DC groups on the 30th day of study.

Group	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-C (mg/dl)
C	67.25 ± 13.84	61.2 ± 7.94	59.44 ± 10.67
DC	94.75 ± 13.84*	202.75 ± 69.98*	60.16 ± 20.36
T_{100}	85.5 ± 22.18	114.83 ± 30.83**	54.95 ± 12.91
T_{200}	84.16 ± 10.26	118.33 ± 67.55**	51.68 ± 9.67
T_{400}	76 ± 17.64	70.75 ± 15.17**	65.02 ± 12.10

HDL-C, High density lipoprotein-cholesterol; C, control; DC, diabetic control; T_{100}, T_{200} and T_{400} diabetic groups that were treated with 100, 200 and 400 mg/kg of *Otostegia persica* extract respectively. (n = 7, mean ± SD, p <0.05). * Significant differences with C group, ** Significant differences with DC group.

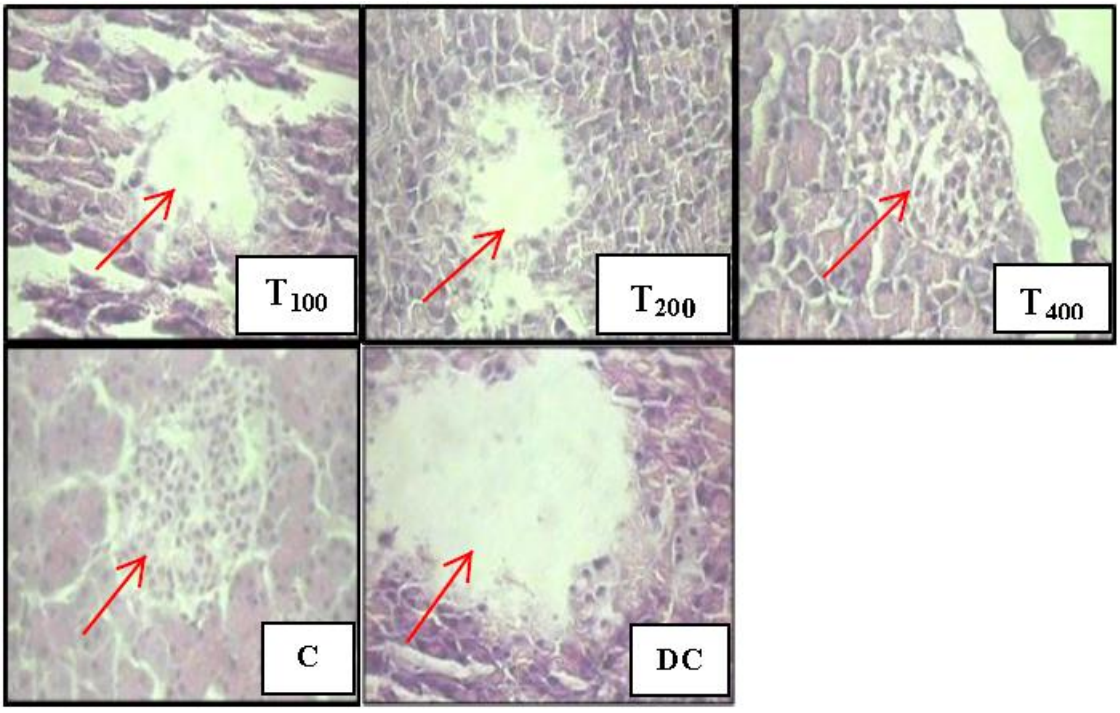

Figure 2. The Photomicrographs of pancreatic images of STZ- induced diabetic rats. C, the control group; DC, the diabetic control group. T_{100}, T_{200} and T_{400} the diabetic rats that were treated with 100, 200 and 400 mg/kg of *Otostegia persica* extract respectively (H and E staining, ×400 magnification).

studies have indicated that anti-diabetic effect of Quercetin on streptozotocin diabetic rat is related to increasing of insulin absorption and glucose uptake (Cnop et al., 2005). Quercetin in amounts of 10 to 50 mg/kg b.w. is capable of normalizing glucose levels in Alloxan diabetic rats and increasing the liver glycogen levels. Also, studies have shown that Quercetin can reduce hepatic output through its effects on the hepatic gluconeogenesis and glycogen breakdown. Quercetin has similar effect as Metformin (Kannappan and Anuradha, 2009). Primarily, diabetes in rodents is more complex than in humans. Most of the researches have shown that even in rodents with type 1 diabetes, injection of STZ leads to the decreased cell response to insulin despite the increasing in the number of insulin receptors (Ordonez et al., 2007). After a prolong insulin resistance, the number of β- cells reduces and this leads to the hyperglycemia and abnormal lipid metabolism (Wang et al., 2007). In accordance to the above finding, in this study *O. persica* reduces serum concentration of glucose and insulin resistance. Studies on the *O. persica* indicate that Maureen has a significant role on the reduction of plasma lipids such as triglycerides, fatty liver, total cholesterol and LDL-C in rats that were treated with high-fat diet. In addition, the impact of Maureen on reducing the rate of triglycerides is faster than that of the total

cholesterol but it has no effects on the serum HDL-C. On the other hand the effect of Quercetin on the total cholesterol, triglycerides and liver fat is insignificant. Quercetin also reduces the amount of HDL-C and LDL-C, respectively, in long term treatment (seventh and tenth week) (ju et al., 2005). Because of the above Maureen effects, in this study the triglyceride concentrations was reduced significantly in the *O. persica* treated groups comparing to the DC group during 30 days of the study. Therefore, the insignificant effect of *O. persica* extracts on the serum HDL-C in diabetic rats is related to the lack of impacts of Quercetin and Maureen on the HDL-C in short term (30 days) treatment. One of the anti-diabetics effects of herbal plants is increasing the size and number of pancreatic β- cells and restoration of these cells (Mohamed et al., 2006). The antioxidant effect of *O. persica* is related to the flavonoids such as Quercetin and Camphorol (Adewole et al., 2006) that are rich in this plant. Quercetin protects pancreatic tissue from abnormal compression of Langerhans islets, degeneration and degranulation, necrotic changes caused by STZ injuries (Abd El-Baky, 2011).

On the basis of these findings, in our study these protective effects of *O. persica* at different concentrations on the pancreatic tissue (especially at 400 mg/kg b.w) were observed. Therefore, after 30 days of *O. persica* treatment, degeneration, degranulation and necrotic changes caused by STZ in the damaged pancreases were reduced and returned to the normal state.

Conclusions

The *O. persica* prevents the loss of the β- cells and regulates the serum lipids and sugars in diabetic animals by reducing insulin resistance, serum glucose concentration and improving insulin functions.

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