Ethanolic extract of *Otostegia persica* ameliorates bone loss in diabetic rats irrespective to its glucose lowering effect

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**Abstract** The present study was conducted to investigate the effect of ethanolic extract of *Otostegia persica* on bone loss in streptozotocin (STZ)-treated diabetic rats. Forty male Wistar rats were randomly divided into five equal groups and treated as follows: group 1 (control); group 2 (STZ group), received STZ 50 mg/kg by a single IP injection; groups 3, 4, and 5 treated with STZ as mentioned above + 200 mg/kg, 300 mg/kg, and 450 mg/kg of *O. persica* extract per day by oral gavage, respectively. On day 29, sera harvested and left femoral and tibiofibular bones were dissected for histomorphometric study, while right femoral and tibiofibular bones as well as L4 vertebrate were removed for determination of ash weight. Obvious hyperglycemia was seen in the STZ group as compared to the control. Administration of *O. persica* extract at the dosage of 300 mg/kg reversed the hyperglycemia. Alkaline phosphatase activity was markedly increased in all experimental groups as compared to control. Epiphyseal and metaphyseal trabecular width as well as epiphyseal bone area/tissue area significantly decreased in STZ group. *O. persica* extract at the dosage of 200 mg/kg reversed all these parameters to the control level. Marrow area/cortical area in rats treated with 450 mg/kg *O. persica* extract were highest among groups. No significant difference observed in osteoid thickness among different groups. Although the ash weights of both compact and cancellous bones in STZ group had no significant difference with control, ash weight of L4 vertebrate in rats treated with 300 and 450 mg/kg of extract was significantly lower than other groups. In conclusion, ethanolic extract of *O. persica* has bone protective effects in STZ-treated rats irrespective to its glucose lowering properties.

**Keywords** *Otostegia persica* · Diabetes mellitus · Bone loss · Rats

**Introduction**

The prevalence of diabetes mellitus (DM) for all age groups worldwide was 2.8 % in 2000 and is estimated to be increased to 4.4 % in 2030 (Wild et al. 2004). Chronic elevated blood glucose in DM initiates a battery of pathophysiologic conditions which many of them are directly or indirectly related to endothelial and vascular dysfunction (Stehouwer et al. 1997), including coronary heart disease (Avogaro et al. 2007), diabetic nephropathy (Meier et al. 2005; Najafian and Maurer 2009), diabetic retinopathy (Porta and Bandello 2002; Alghadyan 2011), and diabetic foot ulcer (Jeffcoate and Harding 2003). In severe conditions, diabetic ketoacidosis may threat patient life (Laffel 1999).

On the other hand, patients with DM have various skeletal disorders including osteopenia or osteoporosis (Schwartz 2003). It has been revealed that women with type 1 DM are 12 times more likely to report hip fractures than women without this condition (Nicodemu and Folsom 2001). Unfortunately, there are few optimal therapies for this disorder and the mechanisms behind it have not been completely clarified yet (Hamada et al. 2009).

Recently, the tendency for using medicinal herbs and drugs with natural origin has been substantially increased. In this regard, many plants with antidiabetic effects have been recognized and considered as candidates for medicinal use (Eddouks et al. 2004; Franklin et al. 2006; Eliza et al. 2009; Kaushik et al. 2010). *Otostegia persica* is a plant with antihyperglycemic and insulin secretory activities (Hedayati and Pouraboli 2012; Manzari-Tavakoli et al. 2013) which grows in
the south and south east of Iran. Regarding the importance of deteriorative effects of DM on bone and the need for finding agents that can target this complication of DM, the present study was conducted to evaluate the possible effect of *O. persica* on bone histomorphometric parameters of streptozotocin (STZ)-induced diabetic rats.

**Materials and methods**

**Preparation of extract**

Aerial parts of plant were collected in May 2011, from Kerman Province. The plant was positively identified by a botanist in the Department of Biology, Faculty of Science, Shahid Bahonar University of Kerman, air-dried, and finely grounded. Ethanolic extract was prepared by cold percolation method. Briefly, the powder was macerated for about 48 h in 96 % ethanol at room temperature. After that, ethanol was evaporated in a rotary evaporator at 40–50 °C and the extract was dried using a freeze-dryer at −50 °C. A mixture of dimethyl sulfoxide/distilled water in a ratio of 3:1 was used as a vehicle for administration of the extract.

**Animals and experimental design**

Forty male Wistar rats with about 3 months of age and a mean body weight of 244 g were used. After a week of adaptation, rats were randomly divided into five equal groups (*n* = 8 each) and treated as follows: group 1 (control), received vehicle daily by oral gavage; group 2 (STZ group), were given STZ (Sigma Chemical Co., Germany) 50 mg/kg by a single IP injection + vehicle as mentioned above; groups 3, 4, and 5, received a single injection of STZ +200 mg/kg, 300 mg/kg, and 450 mg/kg of *O. persica* extract per day by oral gavage, respectively.

Blood samples were obtained by tail clipping and assayed for fasting blood glucose level prior to injection of STZ, 72 h later (for confirmation of induction of diabetes), and at the end of the experiment. The criterion for including STZ-treated rats in the study was a blood glucose levels >250 mg/dl and all animals treated with STZ gained this criterion.

Daily oral dosing was continued for 28 days in diabetic rats. During the experimental period, animals were maintained on a 12-h light/dark cycle at 20±2 °C and had free access to tap water and a pelleted standard rat chow diet, provided by Pars Animal Feed Company. Animals were treated ethically in compliance with the local regulations of University of Tehran, Faculty of Veterinary Medicine.

**Sampling of blood and bones**

On day 29, over night-fasted rats were anesthetized by chloroform and blood samples obtained by cardiac puncture. Sera were harvested within an hour after sampling and stored in −20 °C until use. After sacrificing all animals under deep anesthesia, left femoral and tibiofibular bones were dissected for histomorphometric study as described by Shomali et al. (2009), while right femoral and tibiofibular bones as well as L4 vertebrae were removed for determination of ash weight.

**Determination of serum parameters**

Serum calcium and creatinine were determined by cresolphthalein complexone method and Jaffe method, respectively. Serum phosphorus and alkaline phosphatase levels were assayed by the photometric method. All kits and reagents were prepared by Pars Azmun Company, Ltd., Iran. Blood glucose determination was performed by using a digital glucometer (Accu-Chek®, Roche Diagnostics, India).

**Histomorphometric study**

Left tibiofibular and femoral bones were fixed in 4 % formaldehyde solution and decalcified using formic acid–sodium citrate method (Kiernan 1999). From the tibiofibular bone, transverse 6 μm cross sections were made perpendicularly to the long axis, in a region where the fibula attaches to the tibia. From the femoral bone, 6 μm longitudinal sections of the distal epiphysis and metaphysis were made in the median plate. All the sections were stained using Masson’s trichrome method (Kiernan 1999).

Histomorphometric parameters were determined by a digital photo microscope connected to a personal computer with Zieiss Axio Vision LE software. Epiphyseal and metaphyseal trabecular width (Tb.Wi), epiphyseal bone area/tissue area (B.Ar/T.Ar), and metaphyseal osteoid thickness (O.Th) of the trabeculae were determined. The region of the cancellous bone marked for the measurements was the central zone of cancellous tissue, 2–3 mm below (epiphysis) or above (secondary spongiosa of metaphysis) the margins of the growth plate in the distal epiphysis and metaphysis of the femoral bone. Marrow area/cortical area (Ma.Ar/Ct.Ar) was measured in the cross sections of tibial diaphysis where fibula attaches to it. The nomenclature of parameters is in compliance with ASBMR histomorphometry nomenclature committee (Parfitt et al. 1987).

**Ashing of bones**

L4 vertebrae as well as right tibiofibular and femoral bones were completely cleaned of soft tissues. Bone samples were