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Original Article

Antihypertensive and antioxidant effects of a hydroalcoholic extract obtained from aerial parts of *Otostegia persica* (Burm.) Boiss.

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Abstract

Otostegia persica (Burm.) Boiss. is used for the treatment of various diseases in traditional medicine. The aim of this study was to assess the effects of hydroalcoholic extract of the aerial parts of *O. persica* in dexamethasone (Dex) induced hypertension in male Wistar rats. For induction of hypertension, Dex at 30 µg/kg/day was administered subcutaneously for 14 days. In a prevention study, animals received *O. persica* extract orally at various doses of 100, 200 and 400 mg/kg 4 days before Dex administration and during the test period lasted for 18 days. In a reversal study, rats received *O. persica* extract from day 8 to 14. Systolic blood pressure (SBP) was measured using tail-cuff method. The weight of thymus gland was measured as a marker of glucocorticoid activity. The hydrogen peroxide (H_2O_2) concentration and ferric reducing antioxidant power (FRAP) were determined in plasma samples. Dex injection significantly increased SBP and plasma H_2O_2 levels while decreased the body and thymus weights and FRAP values. Oral administration of *O. persica* extract prevented and dose-dependently reversed a rise in SBP. Pre-treatment with *O. persica* extract also reduced the plasma H_2O_2 concentration, increased the plasma FRAP levels and prevented the body weight loss upon Dex administration. These results suggest antihypertensive and antioxidant effects of *O. persica* extract in Dex-induced hypertension. However, further investigations are needed to elucidate the detailed mechanism(s) of antihypertensive effect of this traditional herbal medicine.

Keywords: *Otostegia persica*; Hypertension; Antioxidant activity

INTRODUCTION

Hypertension is one of the most critical concerns for human health that nearly influences 40% of people in the world (1). The prevalence of hypertension rises with advancing age and more than half of people aged 60 to 69 years are affected by this disease (2). Elevated arterial pressure causes pathological changes in the vasculature and is a major risk factor for life-threatening cardiovascular diseases such as myocardial infarction, stroke, heart and renal failure (3). Several mechanisms are known to participate in the pathogenesis of this disease including disruption of the autonomic nervous system, activation of the renin-angiotensin-aldosterone system, oxidative stress, inflammation, immune system disorder, endothelial dysfunction and imbalance between vasoconstrictor and dilator factors (4-7). Despite the various antihypertensive drugs and regimens, hypertension remains inadequately managed and less than 25% of treated patients achieve target blood pressure (8). The antihypertensive drugs are also associated with relative benefits and disadvantages. Many adverse drug effects may complicate the patient's therapeutic condition. Due to the limited efficacy and undesirable side effects of current drugs, development of more efficacious and better tolerated antihypertensive agents would be needed. Recent investigations have considered natural products and herbal medicines as one of the potential sources for treatment of hypertension (9).

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Genus *Otostegia* belongs to the Lamiaceae family which is a small genus containing about 33 species with important medicinal use (10). Some species of this genus are used as traditional medicine for treatment of hypertension such as *Otostegia integrifolia* (10). *O. limbata* has also been proposed as a good remedy for hypertension which is rich in iron, potassium and calcium (11).

O. persica Boiss. is an endemic plant growing in Iran with common name of "Goldar". It is distributed in south (Fars province) and southeast of Iran (Kerman and Sistan-Baluchistan province) (12). In folk medicine, *O. persica* is used for the treatment of a wide range of diseases including malaria, fever, cough, headache, arthritis, stomachache, toothache, diabetes, cardiac distress, palpitation and high blood pressure (13). *O. persica* has a strong antioxidant effect which is comparable with beta-carotene, green tea and *Ginkgo biloba* (14,15). The aerial parts of this plant have shown some pharmacological activities including antimicrobial, anti-malarial, anti-inflammatory, hypoglycemic and hepatoprotective properties and also efficacy for the treatment of morphine withdrawal syndrome (13,16-21).

Pharmacological studies to evaluate the anti-hypertensive activity of *O. persica* have not yet been conducted. In this study, therefore, an attempt has been made to determine the dose-dependent effects of chronic administration of hydroalcoholic extract of the aerial parts of *O. persica* in dexamethasone (Dex)-induced hypertensive rats.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 180 to 220 g were randomly selected from the animal house of the School of Pharmacy and Pharmaceutical Sciences (Isfahan, Iran). The animals were kept under standard laboratory conditions with a 12 h light/12 h dark cycle and free access to water and standard animal feeds. Rats were allowed to acclimatize to the laboratory condition for 1 week at the experimental site. They were weighed on alternate days. All animal experiments were approved by the Ethics Committee of Isfahan University of Medical Science and performed in accordance with National Institute of Health Guide for the Care and Use of Laboratory Animals.

Chemicals

Captopril was purchased from Tehran Darou Pharmaceutical Co. (Tehran, Iran) and Dex was obtained from Darou Pakhsh Pharmaceutical Co. (Tehran, Iran). Folin-Ciocalteu reagents were purchased from Merck Co. (Mumbai, India). The standard kits for the measurement of plasma hydroperoxides and ferric reducing antioxidant power (FRAP) assay were purchased from Hakiman Shargh Research Co. (Isfahan, Iran).

Plant material and preparation of the extract

The aerial parts of *O. persica* were collected from the Jiroft Mountains, Kerman province in Iran during June 2013. After identification of the plant by Dr. Lili Ghaemmaghami, the botanist of the Department of Biology of Isfahan University (Isfahan, Iran), a voucher specimen (No. 2835) was deposited at the Herbarium of the School of Pharmacy and Pharmaceutical Sciences of Isfahan University of Medical Sciences (Isfahan, Iran).

For preparation of hydroalcoholic extract, the powdered sample of air-dried aerial parts of the plant was extracted with ethanol (70%) percolated for 48 h, at the rate of 18 drops per min at room temperature. Then the extract was filtered under pressure and solvent was removed by a rotary evaporator (Bibby RE200, UK) at 50 ºC. The obtained viscous residue was freeze-dried and stored at -20 ºC. The yield of the plant extract was 15.93 % (w/w).

Determination of total phenolic content

The total phenolic content of *O. persica* extract was estimated spectrophotometrically using the Folin-Ciocalteu method (22). In brief, the plant sample was mixed with Na_2CO_3 (20%) and treated with diluted Folin-Ciocalteu's phenol reagent and the absorbance was measured at 765 nm using a spectrophotometer (Bio-Tek, PowerWave XS, USA). The total phenol content was assessed by comparison with a standard curve generated from different concentrations of tanic acid (50, 100, 150, 250, and 500 mg/l) and was expressed as tanic acid equivalents (TAE) per g of the plant.

Experimental protocol

Rats received subcutaneous (s.c.) injection of Dex (30 µg/kg/day) for 14 consecutive days to induce hypertension (23). The saline control group received daily injection of 1 ml/kg saline subcutaneously. In a prevention study, rats treated with oral administration of *O. persica* extract at 100, 200 and 400 mg/kg body weight (24) or captopril at 40 mg/kg, which served as antihypertensive positive control using an intragastric tube from 4 days before Dex administration and during the test period (Days 1-18). In the reversal study, animals treated with oraladministration of *O. persica* extract or captopril from day 8 to 14 following Dex administration. Six rats were used in each control and experimental groups. All animals were weighed on alternate days. At the end of the experiment, rats were sacrificed under ether anesthesia, the blood was collected, and the thymus gland was isolated. The plasma samples were used for further experiments.

Measurement of systolic blood pressure

The systolic blood pressure (SBP) was recorded by non-invasive tail-cuff method (AD Instrument PowerLab Data Acquisition System, Australia) at the first day and the last day of the experiment in conscious rats. Before the measurements, the rats were restrained in heated chambers at 38 ± 1 ºC for 10 min. A training period of one week was established before initiation of the experiment to allow the rats to become acclimated to the procedure. Three blood pressure measurements were taken for each rat and their averages were used to obtain a mean SBP.

Measurement of thymus weight

The thymus gland was weighed and expressed as milligrams per 100 g body weight. The thymus weight was used as a marker of glucocorticoid activity (23).

Measurement of plasma hydrogen peroxide concentration

The hydrogen peroxide (H_2O_2) level in the plasma was measured based on the ferrous ion oxidation by xylenol orange reagent (25). Briefly, the FOX-1 reagent containing ammonium ferric sulfate was prepared in aqueous medium with sorbitol according to the manufacturer's protocol. Then the plasma samples were mixed with reagent and after incubation for 30 min at 37 ºC, the absorbance of the solutions was measured at 540 nm using a microplate reader/spectrophotometer (Bio-Tek, PowerWave XS, USA). The H_2O_2 concentrations of plasma samples were calculated using a standard curve of H_2O_2 with several concentrations.

Measurement of plasma ferric reducing antioxidant power

The total antioxidant capacity of plasma samples was determined by the measurement of FRAP (26). FRAP values were evaluated based on the reduction of ferric tripyridyl triazine complex to ferrous form by colorimetric method. Briefly, the FRAP reagent containing tripyridyl triazine/ferric chloride/acetate buffer was prepared according to the manufacturer's protocol and was added to the plasma samples. The mixture was incubated for 40 min at 37 °C and then the absorbance of colored solutions was measured at 570 nm using a microplate reader/spectrophotometer. The FRAP values of samples were calculated using a standard curve generated from different concentrations of FeSO4x7H2O and reported as micromole of Fe (II) equivalents per liter.

Statistical analysis

All values were represented as the mean ± SEM. For statistical analysis, a one-way analysis of variance (ANOVA) followed by Tukey post-hoc test was used (SPSS software version 16.0). *P* values <0.05 were considered significant.

RESULTS

Total phenolic content

Total phenolic content of *O. persica* extract was found to be 42.41 ± 0.1 mg TAE per gram of the dried aerial parts.

Effect of Otostegia persica extract on blood pressure

Fig. 1 shows the effect of pretreatment with *O. persica* extract (100, 200 and 400 mg/kg) and captopril (40 mg/kg) on SBP examined in

Dex-induced hypertensive rats. The administration of Dex significantly increased blood pressure from 115.3 ± 4.2 to 143.3 ± 1.3 mmHg (P<0.001) compared to the saline control group (116.6 ± 2.4 mmHg). Pretreatment with *O. persica* extract at doses of 200 and 400 mg/kg significantly prevented increasing in SBP (P<0.001) (Fig. 1). In reversal study, treatment with *O. persica* extract at dose of 400 mg/kg reduced the SBP in Dex-induced hypertensive rats (P<0.001) (Fig. 2). Captopril caused an obvious decrease in SBP in prevention and reversal studies.

Effect of Otostegia persica extract on body weight

Dex administration caused significant decrease in body weight in hypertensive rats when compared to saline control group (P<0.001). Administration of *O. persica* extract at dose of 400 mg/kg could prevent weight loss in rats but captopril had no effect on weight loss induced by Dex (Fig. 3).

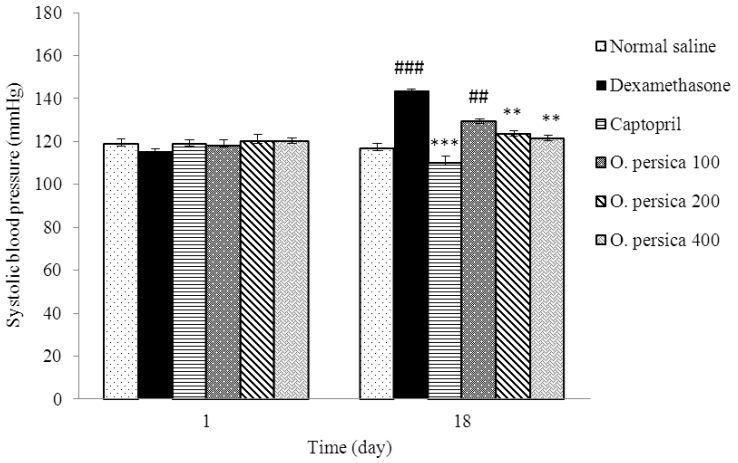

Fig. 1. Effects of *O. persica* extract (100-400 mg/kg) and captopril (40 mg/kg) on systolic blood pressure in Dex-induced hypertension in prevention groups. Values are means ± SEM for six rats. ##; P<0.01 and ###; P<0.001 versus saline control group, **; P<0.01 and ***; P < 0.001 versus Dex control group.

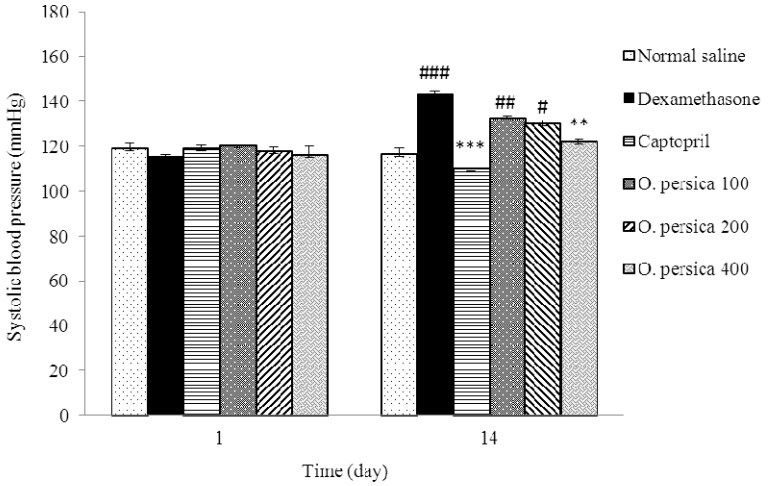

Fig. 2. Effects of *O. persica* extract (100-400 mg/kg) and captopril (40 mg/kg) on systolic blood pressure in Dex-induced hypertension in reversal groups. Values are means ± SEM for six rats. #; P<0.05, ##; P<0.01 and ###; P<0.001 versus saline control group, **; P<0.01 and ***; P<0.001 versus Dex control group.

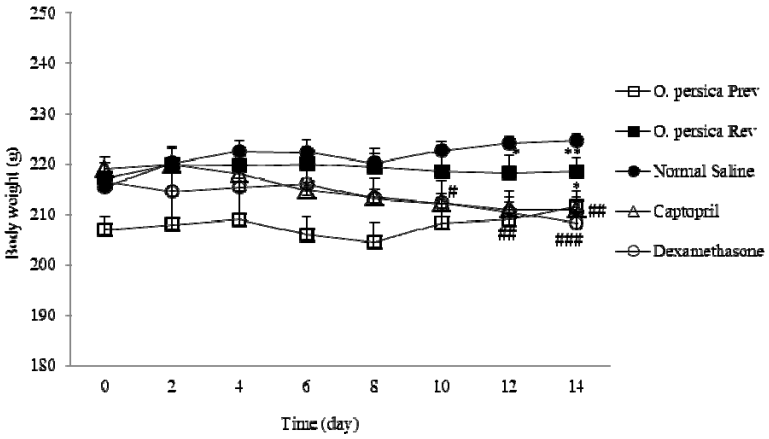

Fig. 3. Effects of *O. persica* extract (400 mg/kg) and captopril (40 mg/kg) on body weight in Dex-induced hypertension in prevention (Prev) and reversal (Rev) groups. Values are means ± SEM for six rats. $^{\#}$; $P<0.05$, $^{\#\#}$;$P<0.01$ and $^{\#\#\#}$; $P<0.001$ versus saline control group, *; $P<0.05$ and **; $P<0.01$ versus Dex control group.

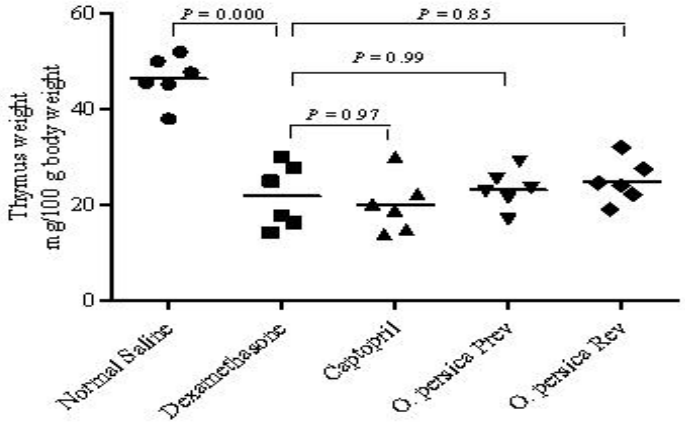

Fig. 4. Effects of administration of *O. persica* (400 mg/kg) and captopril (40 mg/kg) on thymus weight in Dex-induced hypertension in prevention (Prev) and reversal (Rev) groups. Values are weights for six rats.

Effect of Otostegia persica extract on thymus weight

Administration of Dex significantly reduced the thymus gland weight in hypertensive rats ($P<0.001$) and treatment with *O. persica* extract and captopril could not prevent the thymus weight decrease (Fig. 4).

Effect of Otostegia persica extract on plasma H_2O_2 concentration

Dex injection significantly increased the levels of plasma H_2O_2 compared to the saline control group ($P<0.001$). In the prevention study, administration of *O. persica* extract at all doses decreased H_2O_2 levels even to the concentrations lower than saline control group. In reversal study, *O. persica* at dose of 400 mg/kg reduced the elevated plasma H_2O_2 concentration ($P<0.05$). In captopril-treated rats, the levels of plasma H_2O_2 were also lower than saline control group (Fig. 5).

Effect of Otostegia persica extract on plasma FRAP value

The plasma FRAP values were significantly decreased in Dex-induced hypertensive rats compared to the saline control group ($P<0.001$). Pre-treatment with *O. persica* extract at dose of 400 mg/kg significantly increased the plasma FRAP values ($P<0.01$). Administration of *O. persica* extract had no beneficial effect on the FRAP values in the reversal study (Fig. 6).

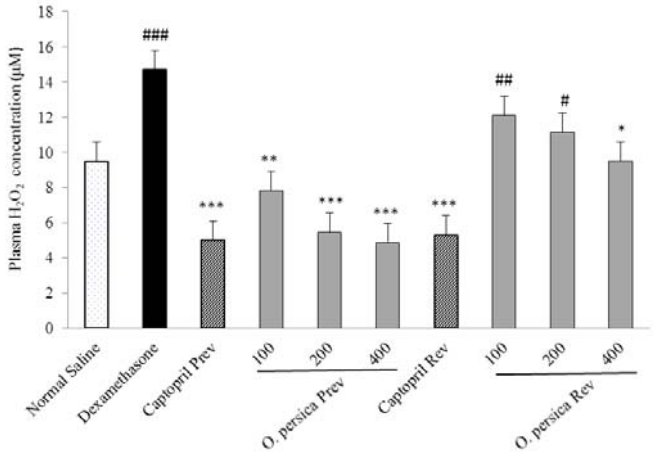

Fig. 5. Effects of *O. persica* extract (100-400 mg/kg) and captopril (40 mg/kg) on plasma H_2O_2 concentrations on Dex-induced hypertension in prevention (Prev) and reversal (Rev) groups. Values are means ± SEM for six rats. $^{\#;}$ P<0.05, $^{\#\#}$; P<0.01 and $^{\#\#\#}$; P<0.001 versus saline control group, **; P<0.01 and ***; P<0.001 versus Dex control group.

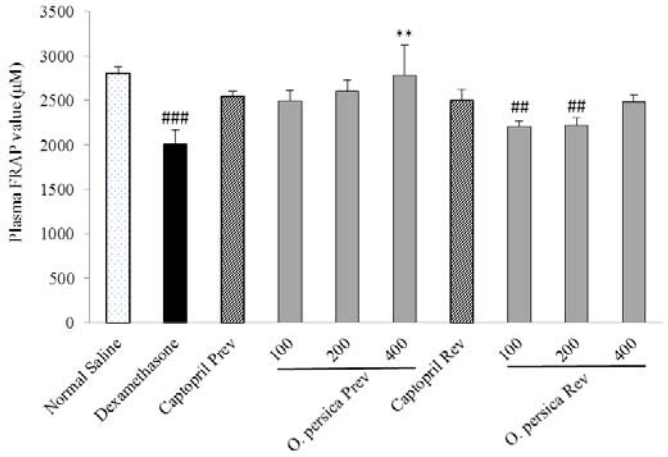

Fig. 6. Effects of *O. persica* extract (100-400 mg/kg) and captopril (40 mg/kg) on plasma FRAP values on Dex-induced hypertension in prevention (Prev) and reversal (Rev) groups. Values are means ± SEM for six rats. $^{\#\#}$; P<0.01 and $^{\#\#\#}$; P<0.001 versus saline control group, **; P<0.01 versus Dex control group.

DISCUSSION

The present study investigated the antihypertensive effect of hydroalcoholic extract of the aerial parts of *O. persica* in Dex-induced hypertension in an animal model. Our results showed that oral administration of *O. persica* extract prevented and dose-dependently reversed a rise in SBP. Pre-treatment with *O. persica* extract also reduced the plasma H_2O_2 concentration, increased the plasma FRAP levels, and prevented the body weight loss upon Dex administration. In this animal model of hypertension, increased activity of sympathetic system, renin-angiotensin-aldosterone system and other vasopressor hormones along with hemodynamic alterations and vasodilator hormones deficiency contribute to the development of elevated blood pressure (27). Oxidative stress due to production of large amounts of reactive oxygen species (ROS) is also considered as one of the most important factors involved in the pathogenesis of glucocorticoid-induced hypertension. The ROS interact with the nitric oxide and contribute to the nitric oxide deficiency, vascular structural changes, endothelial dysfunction, and vasoconstriction (28).

O. persica is a plant with various medicinal applications and pharmacological activities (14). Phytochemical analysis of *O. persica*

extract has revealed the presence of flavonols and terpenoids. Three main flavonols including quercetin, kaempferol and morin and one C-glucoflavone including isovitexin have been isolated from this plant. The presence of major terpenoids including alpha-pinene, linalool and verbenol (15,10) in the aerial parts of *O. persica* may be involved in its antioxidant and antihypertensive properties. Total phenolic content assay showed that phenolic compounds constituted 42.41 % of *O. persica* extract indicating high therapeutic potential of this plant. Various protective effects such as antioxidant, anti-inflammatory, vasorelaxant, antihypertensive and anti-atherogenic effects on the cardiovascular system have been reported for phenolic compounds especially flavonols (29). Quercetin, the prototypic flavonol has shown antihypertensive effects in various animal models of hypertension and also in hypertensive patients (30). In addition to its antioxidant effect, quercetin may affect a large number of physiological and pathological processes. Numerous helpful activities including protective effects on nitric oxide and endothelial function, endothelium-independent vasodilator effects, inhibition of LDL oxidation and platelet aggregation, reduction of adhesion molecules and other inflammatory markers, prevention of morphological and functional changes in the heart, vessels and kidney under conditions of oxidative stress and ischemia on cardiovascular system have been established for quercetin (29,30). Kaempferol and morin are other common flavonols with various biological activities. Kaempferol has shown antioxidant and protective effects against endothelial cell damage, reduction in ROS production in aortic endothelial cells, and improvement of nitric oxide production (31). Morin has also exhibited antioxidant and antihypertensive effects. In deoxycorticosterone acetate-salt hypertensive rats, morin supplementation has shown significant reduction in systolic and diastolic blood pressure and heart rate, and also prevention from renal, heart, aorta and liver hypertrophy. Morin inhibits lipid peroxidation and significantly restores the activities of free radical scavenging enzymes (32). Vasorelaxant, hypotensive and antioxidant effects of terpenoid compounds such as alpha-pinene and linalool have also been demonstrated (33).

The antioxidant effect of *O. persica* extract may also contribute to the antihypertensive effects seen in Dex-induced hypertension. In this study, administration of *O. persica* extract reduced the plasma H_2O_2 concentration and improved the total antioxidant capacity of the plasma. The high antioxidant activity has been reported for aerial parts of *O. persica* which is related to its bioactive phytochemicals specially flavonols (15). Antioxidant therapy has been widely studied in hypertension. Some valuable effects have been proposed for antioxidants in prevention and attenuation of hypertension, improvement of endothelial dysfunction and amelioration of renal and cardiac damages (32,34).

CONCLUSION

In conclusion, this study revealed the beneficial effects of hydroalcoholic extract of aerial parts of *O. persica* in the management of Dex-induced hypertension and improvement of oxidative stress. Further investigations are needed for understanding the detail mechanisms of the antihypertensive effect of this traditional herbal medicine and determining its real clinical value in human hypertension.

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