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Original research

Effect of *Otostegia persica* extraction on renal injury induced by hindlimb ischemia-reperfusion: A rat model

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HIGHLIGHTS

- Limb ischemia-reperfusion injury is one of the most common types of injuries that occur in a variety of conditions.
- The aerial part of *Otostegia persica* is reported to have high antioxidant activity.
- These findings may encourage the use of planets to reduce ischemia-reperfusion injury.

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ABSTRACT

Introduction: It is known that ischemia-reperfusion causes remote organ injury as well as local injury. In traditional systems of medicine, many plants have been documented to be useful for the treatment of various disorders including oxidative esters. This study was designed to investigate whether *Otostegia persica* extraction pretreatment has a protective effect against renal injury induced by hindlimb ischemia-reperfusion. **Methods**: Forty male Wistar rats were allocated into five groups as follows: Control, Sham, *Otostegia persica*, ischemia-reperfusion and ischemia-reperfusion + *Otostegia persica* groups. Rats in *Otostegia persica* and ischemia-reperfusion + *Otostegia persica* groups received *Otostegia persica* extraction (300 mg/kg) orally 2 days prior to operation. Hindlimb ischemia was induced by clamping the femoral artery for 2 h. After 24 h of reperfusion, blood and urine samples were obtained for kidney function tests and the kidneys were removed for histological analysis and oxidative stress measurement. **Results**: The decrease in glomerular filtration rate induced by reperfusion was significantly improved by *Otostegia persica* extraction administration ($P < 0.05$), which resulted in the decrease in serum urea and creatinine concentrations. Urinary creatinine significantly decreased in ischemia-reperfusion group compared to the other groups ($P < 0.05$). Urinary excretion rate, water intake and the ratio of kidney/body weight significantly increased in animals with reperfusion injury as compared with other groups ($P < 0.05$). On histological examination, rats pretreated with *Otostegia persica* extraction had nearly normal morphology. Skeletal muscle ischemia-reperfusion produced a significant increase in renal tissue malondialdehyde level, while pretreatment with *Otostegia persica* extraction was associated with a significantly lower malondialdehyde level ($P < 0.05$). Renal tissue catalase and superoxide dismutase activity and glutathione level were significantly ($P < 0.05$) decreased by hindlimb ischemia-reperfusion. The increases in these parameters were decreased by pretreatment with *Otostegia persica* extraction. **Conclusions**: The results of this study showed that *Otostegia persica* extraction pretreatment significantly protected the renal injury from skeletal muscle ischemia-reperfusion.

1. Introduction

Skeletal muscle ischemia-reperfusion injury is common in several clinical practice including trauma, disease and orthopedic

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surgery [1]. Reperfusion injury of the extremities is characterized by metabolic acidosis, increased serum creatine kinase, myoglobinury, hyperkalemia by the loss of intracellular potassium and free radicals release [2]. Numerous free radicals are brought along when oxygenated blood flow re-enters ischemic tissues during reperfusion, leading to additional tissue injury [3].

The development of remote organ dysfunction was observed only following reperfusion, which implies that cellular mediators produced locally in the limb were responsible for mediating remote organ injury [4,5]. Histological and biochemical evidences of lung, liver and kidney dysfunction indicates that multiple organ dysfunctions following hindlimb ischemia-reperfusion occur as a central systemic event rather than sequential failure of individual organs [5–7].

Multiple pharmacological agents such as iloprost [8], vitamin C [8], pentoxifylline [9], L-alanyl-glutamin [10], N-acetylcysteine [11] and erythropoietin [12] are proposed to be useful against renal remote injury after hindlimb ischemia-reperfusion.

Otostegia persica (Burm.) Boiss., that locally known as "Golder" is an endemic medicinal plant growing in southern and also southeastern of Iran [13]. The extract of the *Otostegia persica* is used in Iranian traditional medicine as anti-histaminic, anti-spasmodic, anti-arthritis, anti-pyretic and analgesic in toothache [14,15]. There are evidences that *Otostegia persica* extraction also is an effective treatment for saving the burn site [16]. The aerial part of *Otostegia persica* is reported to have high antioxidant activity which is related to the flavonoids [17]. Additional biological screening of the methanolic extract has revealed strong antioxidant as well as antibacterial activities against various strains of Gram-negative and Gram-positive bacteria [17,18]. According to experiments, it is determined that methanolic extract of the *Otostegia persica* has antioxidant properties [13]. Despite many uses of *Otostegia persica*, no data can be found about the antioxidant role of the plant in reperfusion syndrome. This prompted us to carry out an investigation for effects of *Otostegia persica* on renal remote organ injury after hindlimb ischemia-reperfusion in rat model.

2. Material and methods

All experimental procedures were performed according to the guidelines for the ethical treatment of experimental animals and approved by Islamic Azad University School of Veterinary Science, Animal Care and Use Local Ethics Committee.

2.1. Experimental protocol

The sample size is calculated using the following formula:

$$n = \frac{2(Z_{\alpha} + Z_{1-\beta})^{2\sigma 2}}{\Delta^2}$$

where n is the required sample size. For Z_{α}, Z is a constant (set by convention according to the accepted α error and whether it is a one-sided or two-sided effect) as shown below:

α-error	5%	1%	0.1%
2-sided	1.96	2.5758	3.2905
1-sided	1.65	2.33	

For $Z_{1-\beta}$, Z is a constant set by convention according to power of the study as shown below:

Power	80%	85%	90%	95%
Value	0.8416	1.0364	1.2816	1.6449

In the above-mentioned formula σ is the standard deviation (estimated) and Δ the difference in effect of two interventions which is required (estimated effect size).

Forty male Wistar rats weighing 300 ± 35 g were used in this study. All rats were maintained under constant room temperature of 25 ± 2 °C, 12 h/12 h light/dark cycle with access to water and commercial food *ad libitum* and placed in individual cages. Animals were randomly allocated into five experimental groups of eight rats each:

Group I – Control group, with no operation and no treatment.

Group II – Sham group with no ischemia-reperfusion. The animals were subjected to all operative procedures (isolation and exposure of the femoral artery for 2 h) except arterial occlusion and reperfusion.

Group III (*Otostegia persica group*) – which only received orally *Otostegia persica* extraction (300 mg/kg) [19] by using gavage technique, 2 days prior to operation.

Group IV (*ischemia-reperfusion group*) – The animals were subjected to ischemia-reperfusion.

Group V (*ischemia-reperfusion + Otostegia persica group*) – *Otostegia persica* extraction, 300 mg/kg, was orally administered 2 days prior to induction of ischemia-reperfusion.

Anesthesia was induced using intramuscular ketamine hydrochloride 10% (50 mg/kg) plus xylazine hydrochloride 2% (10 mg/kg). After the induction of anesthesia, 250 IU heparin was administered via the jugular vein to prevent clotting. Then, a skin incision (by using a sterile technique) was made on the medial surface of the left hindlimb. After the isolation of the femoral artery and vein from the surrounding tissues, the femoral artery was exposed. Hindlimb ischemia was induced by clamping the femoral artery. Rats were maintained in dorsal recumbency and kept anesthetized (additional doses were given as necessary) throughout the duration of the ischemic period. Body temperature was maintained with a heating pad and monitored using a rectal thermometer. After 2 h ischemia, the vascular forceps was removed and surgical site was routinely closed. After surgery, fluid losses were replaced by administration of 5 mL of warm (37 °C) isotonic saline i.p., and rats were kept in metabolic cages for 24 h to collect urine and also to measure water consumption.

2.2. Plant material

The plant *Otostegia persica* was collected from Kerman province, Iran. The plant was identified by the Department of Botany of the Islamic Azad University. A voucher specimen has been deposited at the Herbarium of Department of Botany. Aerial parts of *Otostegia persica* were finely powdered in a mill. 500 g of sample was selected and raised the volume to 1 L by ethanol (96%). The solution percolated after 48 h, then the solvent was removed under reduced pressure at low temperature and finally about 10 g concentrated extract was prepared. Doses of the extract were prepared using normal saline [14,20].

2.3. Biochemical analysis

After reperfusion period, the blood samples were collected from jugular vein and were allowed to clot for 10 min at room temperature. Clots were centrifuged at 2500 rpm for 10 min to separate the serum and submitted for evaluation of serum creatinine and urea