Original Article

The Effect of Hydroalcoholic Extract of Olive Leaves on Blood Pressure in Rat Model of Two-Kidney, One-Clip Goldblatt Hypertension

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Background: In Iran's traditional medicine, the leaves of olive tree are of value for the treatment of hypertension. This study was designed to examine the effects of hydroalcoholic extract of olive leaves in rat model of two-kidney, one-clip hypertension and to further explore whether its hypotensive activity was mediated by enhancing the basal release of endothelium-derived nitric oxide.

Methods: Animals were divided into two main groups including sham-operated and renal artery-clipped ones. The latter was further divided into 5 groups of untreated rats, vehicle-treated rats, which received daily oral administrations of one ml distilled water, and extract-treated rats receiving olive leaves extract at 50, 150 or 500 mg/kg in the same volume of vehicle starting the next day after the operation. Four weeks later, mean blood pressure and heart rate were measured under anesthesia before and after the administration of N^G-nitro-L-arginine methyl ester (L-NAME).

Results: Mean arterial pressures, and right kidney and heart weights of untreated and vehicle-treated renal artery-clipped rats were significantly higher but left kidney weights were significantly lower than those of sham-operated animals. However, there was no significant difference between the heart rates of these groups. Compared to vehicle-treated renal artery-clipped rats, treatment with hydroalcoholic extracts of olive leaves at 50, 150 or 500 mg/kg/day was associated with significantly lower mean arterial pressure, right kidney and heart weights but did not affect heart rate or left kidney weights. The intravenous administration of L-NAME resulted in a significant increase in mean arterial pressure in sham-operated and extract-treated rats whereas there was no change in renal artery clipped or vehicle-treated groups.

Conclusion: The findings of the study show that hydroalcoholic extract of olive leaves prevents the clip-induced increase in mean arterial pressure, which might be partly mediated by enhancing the basal release of nitric oxide.

Keywords: Olive Leaf, Hydroalcoholic Extract, Goldblatt Hypertension, N^G-nitro-L-arginine Methyl Ester

Manuscript received: January 05, 2011; Accepted: February 25, 2011
Iran Cardiovasc Res J 2011;5(1):1-6

Introduction

According to Iran's traditional medicine, the leaves of olive tree are of value for the treatment of hypertension.[1-3] A number of experimental studies reported on the hypotensive effects of olive leaf extract by different solvent and methods, ranging from hot water extracts[4-6] glycerol/ethanol extracts[7] and ethanol extract.[8] Moreover, the administration of triterpenoids isolated from olive leaves for 6 weeks prevented the development of severe hypertension and atherosclerosis in Dahl salt-sensitive, insulin-resistant rat model of genetic hypertension.[9] Also, the consumption of aqueous extract of olive leaves for 3 months led to the reduction of blood pressure in hypertensive patients.[10]

There are different suggestions about the mechanisms of hypotensive activity of olive leaves. Zarzuelo and colleagues demonstrated that lyophilized decoction of olive leaves reduced blood pressure in spontaneous hypertensive rats, and relaxaed isolated aortic rings from the same rats in an endothelium-independent manner.[11] However, a more recent study showed that oleuropein, the bitter prin-

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ciple of olive leaves, potentiated the production of nitric oxide (NO) by mouse macrophages during endotoxin challenge.[12] Wether or not such a finding can be extrapolated to the hypotensive property of olive leaves needs further investigations.

To our knowledge the putative hypotensive effect of olive leaf extract has not been examined in rat model of two-kidney one-clip hypertension. Considering the varied pathophysiological mechanisms of human hypertension, and the variations in animal models developed to represent different aspects of the disease, this study was designed to examine the effects of hydroalcoholic extract of olive leaves on rat model of two-kidney, one-clip hypertension and to further explore whether its hypotensive activity was mediated by enhancing the basal release of endothelium-derived NO.

Patients and Methods

The following drugs and chemicals were used: Ketamine (Trittau; Germany); Xylazine (Alfasan; The Netherland); Sodium Thiopental (Biochem GmbH, Vienna; Australia); Diazepam (Chemistry drug; Iran); N^G-nitro-L-arginine methyl ester (L-NAME) (Fluka; Switzerland); Heparin (Leo; Denmark); Penicillin G (Jaber Ebne Hayyan; Iran).

Olive leaves were collected from an Olive garden near the city of Shiraz, Fars, Iran in November. The exact species of the plant was determined by an herbal specialist from the Biology Department, Shiraz University. A sample of the plant deposited in Herbarium Leaves was dried in the shade, and then ground to powder. Hydroalcoholic (50% ethanol and 50% distilled water v/v) extract of the leaves was prepared using percolation method. The yield was about 30-35%.

Male Sprague-Dawley rats (200-250 g), obtained from Animal Breeding Center of Shiraz University of Medical Sciences, Shiraz, Iran were subjected to sham operation or placement of a Plexiglass clip around left renal arteries. Briefly, under Ketamine (60 mg/kg) and Xylazine (8 mg/kg) anesthesia, a left flank incision was made, and left renal artery was exposed. The arteries were then dissected from surrounding connective tissues. Afterwards, solid Plexiglass clips were placed around them. Penicillin G powder (Jaber Ebne Hayyan, Tehran, Iran) was then applied to the site of incision, and the abdominal wall and the skin were sutured using cat gut (3/0) and silk (3/0) suture materials, respectively. The same procedure was applied to sham-operated rats, but no clip was placed around renal arteries. The animals were then recovered from anesthesia, and kept for 4 weeks under standard conditions (temperature; 22 ± 2 ºC, relative humidity; 50% and 12 hours light/dark cycle) with food and water ad libitum.

Animals were divided into two main groups including sham-operated (n=11) and renal artery-clipped ones (n=56). The latter was further divided into 5 groups including untreated rats (n=11), vehicle-treated rats (n=8), which were assigned to receive a daily oral administration of one ml distilled water, and extract-treated rats which received olive leaves extract at 50 (n=10), 150 (n=9) or 500 (n=9) mg/kg in the same volume of vehicle starting the next day after the operation.

Four weeks later, animals were anesthetized with diazepam (3 mg/kg) and sodium thiopental (70 mg/kg, I.P), and were then tracheotomized to facilitate breathing during the experiment. The right jugular vein was cannulated using PE-50 polyethylene catheters (Polyethylene Tubing, Becton Dickinson; USA) for administration of L-NAME (2 mg/kg) or supply of anesthetic as needed. The right carotid artery was then cannulated and connected to a Gould Statham pressure transducer (model P23D) of Grass Polygraph (model 7P1D) for the measurement of arterial blood pressure and heart rate (HR). After a resting period of 30 min to allow recovery from surgical stress, a baseline measurement of arterial blood pressure and heart rate was performed. The animals then received an intravenous injection of L-NAME. Arterial blood pressure and HR were recorded at the plateau of increase in arterial pressure (10-15 minutes after the L-NAME injection). At the end of the experiment, animals sacrificed and heart, kidneys and lungs weights were determined.

Mean arterial pressure (MAP) was calculated as diastolic blood pressure plus one third of the pulse pressure. Heart rate was counted from the arterial pressure upstroke. The weights of heart and right and left kidneys were calculated as a percentage of body weight.

Statistical Analysis

Data, presented as Mean±SEM, were analyzed using one-way analysis of variance (ANOVA) or paired t tests. Baseline values of MAP and HR as well as weights of the animals, hearts and kidneys were analyzed using one-way ANOVA. Where a significant difference was found with ANOVA, the source of difference was located using Tukey test. The MAPs or HRs before and after administration of L-NAME were analyzed using paired t test. A

Table 1. The values (mean ± SEM) of heart weight (HW, g/kg body weight), right kidney weight (RKW, g/kg BW), left kidney weight (LKW, g/kg BW), heart rate (beats/min), body weight at the onset of the study (BW1, g) and body weight at the conclusion of the study (BW2) of sham-operated, untreated renal artery clipped (RAC) rats, and RAC rats treated with vehicle (one ml distilled water/day) or olive leaves extract (OLE) at 50, 150 or 500 mg/kg/day in the same amount of vehicle.

	Sham-operated	RAC-Untreated	RAC-Vehicle	RAC-OLE50	RAC- OLE150	RAC-OLE500
HW	0.34±0.01	0.47±0.02*	0.48±0.01*	0.34±0.01	0.36±0.01	0.34±0.01
RKW	0.37±0.01	0.52±0.01*	0.56±0.02*	0.45±0.01	0.45±0.01	0.45±0.02
LKW	0.35±0.01	0.16±0.01*	0.18±0.01*	0.17±0.01	0.16±0.02	0.16±0.02
HR	410±14	443±13	448±10	454±14	441±12	434±15
BW1	212±6	217±6	224±7	204±8	202±7	218±8
BW2	255±7	255±10	262±10	235±9	234±10	251±8

* Denotes significant difference from sham-operated rats

P value of ≤0.05 was considered statistically significant. The statistical analysis was performed using Sigmastat 3.0 statistical software.

Results

Mean arterial pressures of untreated and vehicle-treated renal artery-clipped rats were significantly higher than that of sham-operated rats (Figure 1). However, there was no significant difference between the HRs of these groups (Table 1). The heart and right kidney weights of untreated and vehicle-treated renal artery clipped rats were significantly higher than that of the sham-operated rats. The left kidney weights of untreated and vehicle-treated renal artery clipped rats were significantly lower than that of the sham-operated rats (Table 1). Compared to vehicle-treated renal artery-clipped rats, treatment with hydroalcoholic extract of olive leaves at 50, 150 or 500 mg/kg/day was associated with significantly lower MAP, which was not significantly different from that of sham-operated rats (Figure 1). Moreover, treatment with the olive leaves extract resulted in a significantly lower heart and right kidney weights compared to those of vehicle-treated renal artery-clipped rats (Table 1). However, treatment with the extract did not change HR or the left kidney weights relative to those of vehicle-treated rats. There was no significant difference between the weights of sham-operated and renal artery-clipped rats either at the onset or at the

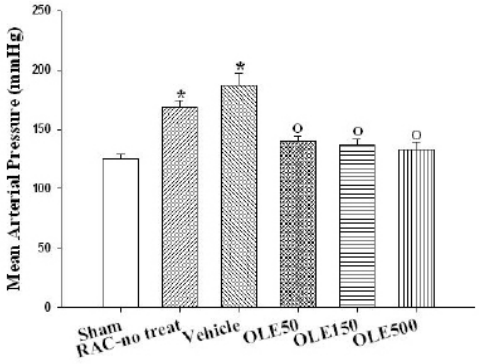

Figure 1. Baseline mean arterial pressure (mean ± SEM) of sham-operated and untreated renal artery clipped (RAC) rats, and RAC rats treated with vehicle (1 ml distilled water/day) or olive leaves extract (OLE) at 50, 150 or 500 mg/kg/day in the same amount of vehicle. *: Indicates significant difference from sham-operated group. O:Indicates significant difference from renal artery-clipped rats treated with vehicle.

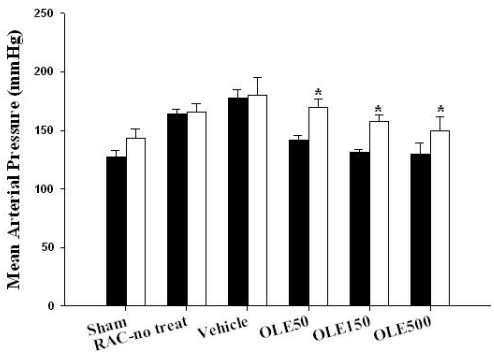

Figure 2. The mean arterial pressure (mean ± SEM) of sham-operated and untreated renal artery clipped (RAC) rats, and RAC rats treated with vehicle (one ml distilled water/day), or olive leaves extract (OLE) at 50, 150 or 500 mg/kg/day in the same amount of vehicle before and after receiving intravenous L-NAME (2 mg/kg). *: Indicates significant difference from sham-operated group.

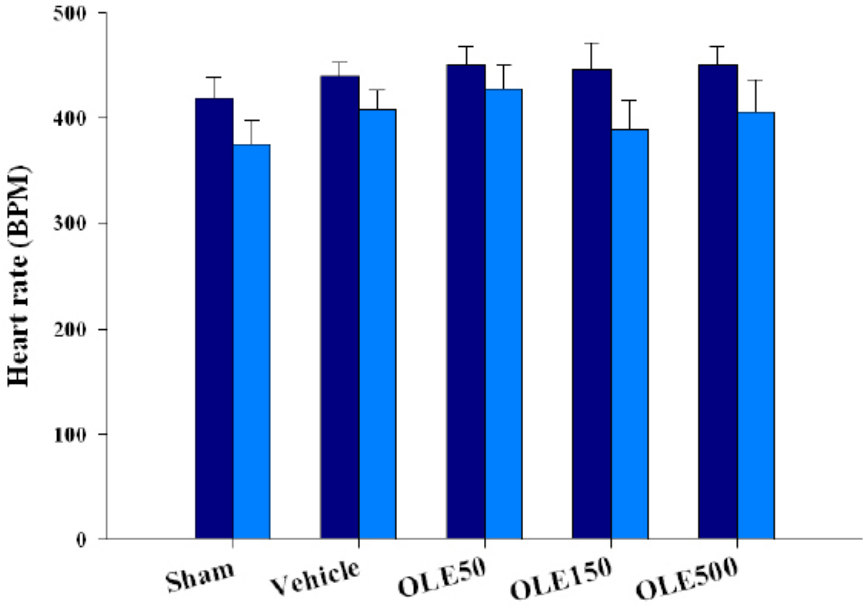

Figure 3. The heart rate (mean ± SEM) of sham-operated and untreated renal artery clipped (RAC) rats, and RAC rats treated with vehicle (1 ml distilled water/day) or olive leaves extract (OLE) at 50, 150 or 500 mg/kg/day in the same amount of vehicle before and after receiving intravenous L-NAME (2 mg/kg).

end of the experiments (Table 1).

The intravenous administration of L-NAME resulted in a significant increase in MAP in sham-operated rats, whereas the L-NAME-induced increase in MAP did not reach statistical significance in untreated or vehicle-treated renal artery clipped rats. Moreover, the administration of L-NAME was associated with significant increase in MAP in the extract-treated rats (Figure 2). The administration of L-NAME did not result in significant change in the HR of sham-operated or untreated, vehicle-treated or extract-treated renal artery-clipped rats (Figure 3).

Discussion

The findings of the present study demonstrated that placement of Plexiglass clips on left renal arteries did result in hypertension characterized by increased MAP as well as increased heart and right kidney weights and decreased left kidney weight. It also showed that hydroalcoholic extract of olive leaves prevented the clip-induced increase in blood pressure. Moreover, it showed that the administration of L-NAME in renal artery-clipped rats was associated with a significant increase in blood pressure.

In agreement with previous reports,[13,14] the placement of Plexiglass clip led to the induction of renal hypertension. The values of blood pressure in the present study are similar to those produced by the placement of silver clips.[15-18] Moreover, the values of heart and left and right kidney weights in the present study are comparable to those of earlier reports using either Plexiglass[13] or silver[19-23] clips to induce renal hypertension. The olive leaves hydroalcoholic extract at the doses used did prevent the increase in blood pressure without changing the heart rate. Such a finding is similar to those of previous reports using olive leaves extract or compound isolated from the leaves extract.[6,8,11] The injection of olive leaves decoction in normotensive rats exhibited hypotensive activity.[11] The administration of aqueous extract of olive leaves for 60 days resulted in the decrease of blood pressure level in normotensive rats.[24] Moreover, the administration of olive leaves aqueous extract to L-NAME induced hypertensive rats for 8 weeks was also associated with significant reduction in blood pressure.[8]

The findings in regards to the possible ingredient(s) responsible for the preventive or hypotensive activity of olive leaves extract are varied. There are several reports on the antihypertensive activity of oleuropein and its derivatives such as tyrosol and hydroxytyrosol. Petkov and Manolov reported that the observed hypotensive activity of Olea europaea could likely be attributed to oleuropein.[25] Moreover, it was shown that two triterpenoids of Olea europaea including oleanolic acid and

ursolic acid, could prevent the rising blood pressure in salt-sensitive, insulin resistant hypertension in genetic Dahl model of hypertension.[9] Such a finding prompted the authors to suggest that in addition to oleuropein and oleacein, triterpenoid derivatives might contribute to the potent overall antihypertensive effect of olive leave extract [9]. In addition, hydroxytyrosol, the esterifying alcoholic component of the iridoid glycoside oleuropein, was shown to exert vasorelaxant effects on isolated aortic rings.[26]

The present study showed that L-NAME-induced increase in blood pressure in renal artery clipped rats receiving no treatment or vehicle was lower than that in sham-operated rats. The attenuated response to L-NAME has been suggested to be an indicative of reduced NO involvement in the regulation of vascular tone.[27,28] Therefore, it might be concluded that the present model of renal hypertension was associated with reduced release of basal NO. Renovascular hypertension leads to the stimulation of rennin-angiotensin system and increased production of angiotensin II. The increased levels of angiotensin II may have caused excessive oxidative stress and may have strongly affected endothelial function.[29,30]

The study also showed that L-NAME-induced increase in blood pressure was restored by treatment of renal hypertensive rats with olive leaves extract at foregoing doses. This might suggest that treatment with the extract restored the renal hypertension-induced attenuation of basal release of NO. Consequently, it might be possible to conclude that the hypotensive effects of olive leaves extract is partly mediated by the enhanced release of basal NO. Such a conclusion receives support from a previous report showing that olive leaves extract did reduce blood pressure in rats with L-NAME-induced hypertension due to several mechanisms such as endothelium-dependent and endothelium-independent pathways.[8] It has also been reported that other mechanisms are also involved in the hypotensive activity of olive leaves extract. Olive leaves decoction was shown to exert vasorelaxant activity in isolated thoracic aorta via endothelium-independent mechanisms.[11] Moreover, Hansen showed that olive leaves might have angiotensin converting enzyme (ACE) inhibiting activity.[31] Also, olive leaves extract was shown to block L-type calcium channel directly and reversibly in isolated rabbit hearts.[32] It needs to be investigated whether or not this mechanism is operative in the present study.

Whether or not other mechanisms are involved in the hypotensive activity of olive leaves extract is not clear. Also, whether olive fruit imparts hypotensive or vasorelaxant activity remains unclear. The hypotensive or vasorelaxant property of olive fruit was shown to be mediated by calcium channel antagonism,[26,33] ACE inhibition [34] and endothelial-dependent relaxation.[35,36]

In Conclusion, The findings of the present study suggested that placement of Plexiglass clips on left renal artery resulted in hypertension. Moreover, they showed that hydroalcoholic extract of olive leaves blunted the increase of blood pressure in such a model, a finding that is in agreement with the view from Iran's traditional medicine that olive leaves have hypotensive activities. They also indicated that the preventive effects of the extract on blood pressure might be partly mediated by enhancing the basal release of NO.

Acknowledgement

The authors would like to thank the Office of Vice Chancellor for Research of Shiraz University of Medical Sciences for financial support. The authors declare that they have no conflicts of interest.

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