

Acute and Chronic Effects of *Ferula persica* on Blood Pressure of Hypertensive Rats and Its Possible Mechanism of Action

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Abstract

Background: *Ferula persica* has been used in traditional medicine for treatment of high blood pressure. In this study acute and chronic effect of aqueous *F. persica* extract on BP of hypertensive rats and its possible mechanism of action have been investigated.

Methods: Eighty two male Wistar rats were divided into 12 experimental groups. Hypertension was induced by Goldblatt method in the anesthetized rats. Aqueous extract of *F. persica* (15 or 30 or 60 mg/kg, iv) or it's vehicle were administered in treatments or control groups to evaluate their effects on BP and heart rate. To assess the mechanism of *F. persica* action on BP, L-NAME (5 mg/kg), Atropine (1 mg/kg) or Indomethacin (5 mg/kg) were injected intraperitoneally followed by intravenous administration of *F. persica* (30 mg/kg) in the different groups of hypertensive rats. Chronic effect of *F. persica* (30 mg/kg) on BP was evaluated by the aqueous extract administration in drinking water for a month.

Results: Intravenous administration of *F. persica* reduced BP of hypertensive rats ($p < 0.001$). There is no significant different between three doses of *F. persica*. Intraperitoneal injection of L-NAME, Atropine or Indomethacin has no significant effect on basal BP, but L-NAME eliminated and Atropine reduced hypotensive effect of *F. persica* extract on BP. Chronic administration of *F. persica* has no effect on BP.

Conclusion: Our findings showed the hypotensive effect of *F. persica* in hypertensive rats may be mediated by muscarinic receptors and NO release.

Keywords: *Ferula persica*, Goldblatt hypertension, Rat, L - NAME

Introduction

Hypertension is a serious disease which affects 25% of the adults and increases progressively [1]. Although a lot of money spent for the treatment of cardiovascular disease, current conventional treatments (diuretics, angiotensin converting enzyme inhibitors, beta-blockers and calcium-channel blockers) cannot reduce the rising number of patients with hypertension [2]. Alternative medicine such as diet & herbs suggest an effective way to reduce number of people with high blood pressure (BP) [2]. *Ferula persica* (*F. persica*) has been used in traditional medicine as the lowering of BP, antispasmodic, carminative, laxative and expectorant [3]. New researches also revealed therapeutics effects of *F. persica* including antibacterial [4, 5], anticonvulsant [6], hypoglycemic [7] and anti cancer effects [8, 9]. Moreover *F. persica* modulate withdrawal syndrome sign in morphine-dependent mice [18]. Also a study showed antispasmodic and hypotensive effects of *F. asafoetida* [10]. As far as the literature is concerned, *F. persica* effect on BP is not clear. So, present study was performed to evaluate acute and chronic effects of aqueous extract of *F. persica* on BP in a rat model of hypertension and its possible mechanism of action.

Materials and Methods

All procedures were performed in according to the National Institutes of Health Guide for Care and Use of Laboratory Animals. Male Wistar rats were obtained from

breeding colony of Semnan University of Medical Sciences, Semnan- Iran. Animals were housed in individual cages in a 12-h light/dark cycle at 22 – 24 °C, with food and water ad libitum.

Drugs include Ketamine and xylocine prepared from Woerdn-Holland. Also pentobarbital sodium, atropine, indomethacin and NG-nitro-L-arginine methyl ester (L-NAME) were obtained from Sigma and heparin from TRITTAU-Germany. Drugs and aqueous extract were dissolved in saline.

Experimental procedure

Eighty two male Wistar rats (250 - 300 gr) randomly were divided to 12 experimental groups including: 1-sham operated, 2-control, 3-6-treatments [*F. persica*, 15 or 30 or 60 mg/kg, or it's vehicle], 7-10- mechanism evaluating [L-NAME (5mg/kg) or atropine (1mg/kg) or indomethacin (5 mg/kg) or saline were injected intraperitoneally followed by *F. persica* (30 mg/kg, iv) administration] and 11-12- chronic groups [*F. persica* (30 mg/kg) or it's vehicle administrated in drinking water for a month].

Hypertension was induced by Goldblatt method (Two-kidney One-clip) as following. The animals were anesthetized with ketamine - xylocine (60 & 8 mg/kg, ip), the left kidney was exposed and a silver clip with internal diameter of 0.2 mm was placed on the kidney's artery and sutured the surgery site. One month later, hypertensive rats were anesthetized with pentobarbital sodium (80 mg/kg, ip) and were placed on the rat temperature unit (Narco Bio-system, USA) to maintain a constant rectal

temperature of 36.5 ± 0.5 °C. Femoral vein was cannulated for extract injection. Femoral artery also was cannulated with a heparinized cannula that connected to a pressure transducer (P-1000B, Narco Bio-system, USA) for continuous measurements of arterial BP and heart rate. After stabilizing BP, extract was injected intravenously. The mean of arterial blood pressure (MABP) was calculated as the %60 of diastolic pressure added to 40% of systolic pressure [11]. The effects of aqueous *F. persica* extract (15, 30 or 60 mg/kg i.v.) or vehicle on MABP and heart rate were evaluated in four hypertensive groups of rats (n=7). To assess the mechanism of *F. persica* action on BP in another four hypertensive groups (n=7), L-NAME (5 mg/kg), atropine (1 mg/kg), indomethacin (5 mg/kg) or their vehicle (saline) was administrated intraperitoneally and about 20 minutes later *F. persica* extract (30 mg/kg) was injected intravenously. In chronic groups (n=7 *2), one month after induction of hypertension, BP was recorded noninvasively by cuff methods (Power lab- Australia). Then *F. persica* (30 mg/kg) administrated orally for a month and finally BP was recorded noninvasively again. The amounts of BP recorded by noninvasive method which was consistent with invasive method.

Plant and extract

F. persica was collected from hills around Shahmirzad city (north of Semnan Province, Iran) in the spring of 2010 and identified by botanists in Organization of Promotion, Education and Agricultural Research-Semnan. Aerial part of the plant dried in shadow,

grinded and then 50 gr of its powder was added to 500 ml absolute water. Aqueous extract was prepared by soxhlet apparatus via repeated distillation methods during 12 hours. The solution was then filtered and dried by oven at 40°C. The dried extract was dissolved in the saline to make different doses.

Statistical analysis

The results were presented as mean \pm SEM and a value of $p < 0.05$ was accepted as statistically significant different. One-way analysis of variance (ANOVA) was used for comparison between groups that followed by Holm-Sidak method. When normality test failed, ANOVA on ranks (Kruskal-Wallis) followed by Dunn's Method were used for multiple comparison.

Results

Effect of hypertension induction on MABP and kidney weight

Hypertension induction by Goldblatt method increased BP (45 mmHg) significantly in hypertensive rats compare to normal and sham operated groups ($p < 0.001$). Also hypertensive rats showed reduction in left kidney weight (31%) and increase in right one (39%) compare to sham operated rats ($p < 0.05$).

Acute effects of *F. persica* on MABP and heart rate of hypertensive rats

Aqueous extract of *F. persica* (15 or 30 or 60 mg/kg, iv) reduced significantly MABP of hypertensive rats from 142, 146 and 151 mmHg to 97, 87 and 88 mmHg respectively

($p < 0.001$). There is not formal significant different between three doses of extract (Fig. 1). Also *F. persica* has no significant effect on the heart rate.

Effects of L-NAME, atropine and indomethacin on hypotensive effect of *F. persica*

Intraperitoneal injection of saline or L-NAME (5 mg/kg) or atropine (1 mg/kg) or indomethacin (5 mg/kg) in 4 different groups had no significant effect on basal BP of hypertensive rats, but L-NAME abolished and atropine reduced hypotensive effect of *F. persica* (30 mg/kg, iv) on MABP compare to control group ($*p < 0.001$) (Fig. 2). On the other hand, Indomethacin pretreatment has no effect on hypotensive action of *F. persica* ($p < 0.001$), (Fig. 2).

Chronic effect of *F. persica* on systolic BP of hypertensive rats

Chronic administration of aqueous extract of *F. persica* (30 mg/kg) in drinking water for a month has no significant effect on systolic BP of hypertensive rats.

Discussion

Our results showed that aqueous extract of *F. persica* reduced MABP of hypertensive rats, but had no significant effect on heart rate. Repeated saline injection intravenously had no effect on BP, so probability of physical effect of extract injection on BP is rejected. Although there is no report about *F. persica* effect on BP, our results are comparable with hypotensive effect of *F. asafetida* on BP of normal rat [10].

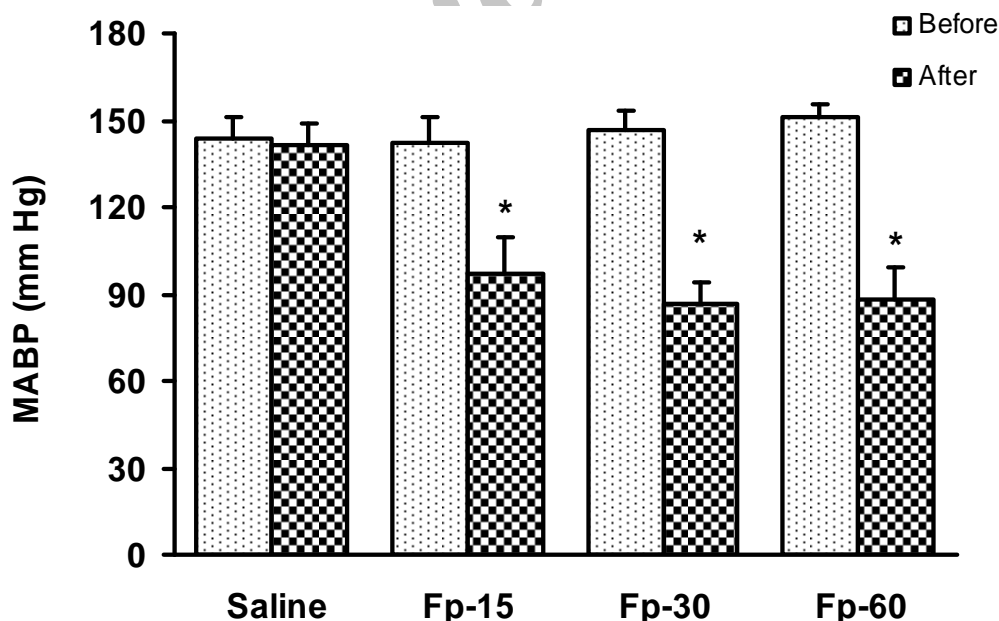


Figure 1- Effects of *F. persica* extract on MABP of hypertensive rats ($* p < 0.001$). There is no significant difference between three doses. Fp-15, Fp-30 and Fp-60 are *F. persica* doses of 15, 30 and 60 mg/kg respectively

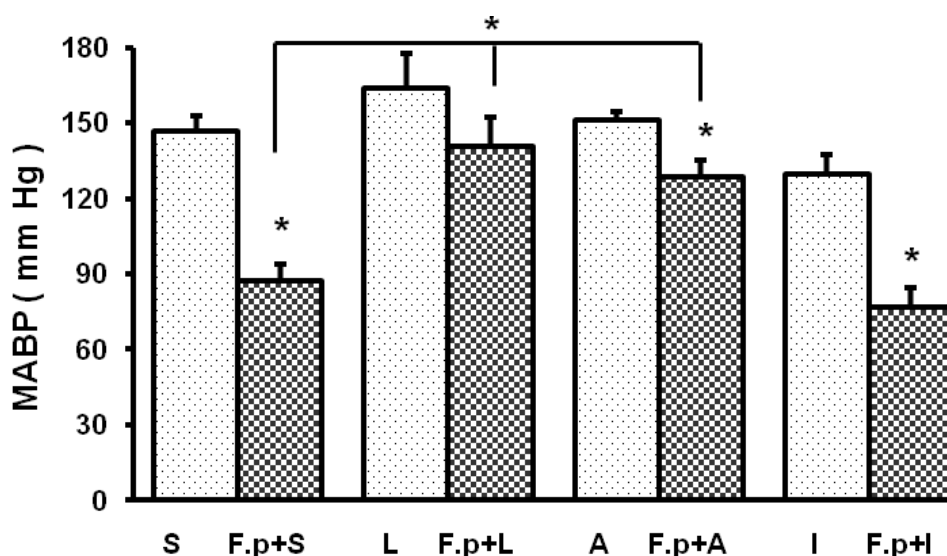


Figure 2- Effects of L-NAME, atropine or indomethacin on hypotensive effect of *F. persica*. L-NAME and atropine have no significant effect on MABP but L-NAME inhibited and atropine reduced hypotensive effect of *F. persica*. Indomethacin has no significant effect on MABP and hypotensive effect of *F. persica*. Fp: *F. persica* S: saline, Fp: *F. persica* 30 mg/kg, L: L-NAME, A: atropine, I: Indomethacin. * $p < 0.001$

The endothelial cells play a central role in the vascular tone regulation by the synthesis and release of vasoactive substances such as nitric oxide (NO), prostaglandin (PG) I_2 as well as vasoconstrictor factors [12, 13]. Regarding rapid action of *F. persica* on BP without affecting the heart rate, we proposed hypotensive effect of *F. persica* might be mediated by vascular endothelium. Following L-NAME administration, *F. persica* injection had no significant effect on BP. Therefore it seems that NO mediates hypotensive effect of *F. persica* as it is abolished by L-NAME pretreatment. Atropine administration in hypertensive rats had no effect on basal BP but it reduced hypotensive effect of *F. persica*. It appears that muscarinics receptors participate in hypotensive effect of *F. persica*. Muscarinics M3 receptors activation on the

endothelium increase intracellular calcium concentration that activates NO synthase and NO release [14]. Possibly NO synthase is the common target of the L-NAME and atropine in this effect.

Endothelial cells produce PGI_2 that is inhibited by indomethacin. Administration of indomethacin had no effect on basal BP and hypotensive effect of *F. persica*. Perhaps hypotensive effect of *F. persica* is independent of $PG I_2$.

Other possibilities of hypotensive effect of *F. persica* are as following. The essential oil of wild *F. persica* has sixty-one components including safranal [15]. Previous research has been shown intravenous injection of safranal reduced strongly BP of normotensive and hypertensive rats [16]. We do not know the exact amount of safranal in our extract, but

hypotensive effect of *F. persica* may be related to this component. Various coumarins and flavonoids have been isolated from *Ferula* genus [4]. It also has been reported angiotensin converting enzyme is inhibited by flavonoids [17]. So hypotensive effect of *F. persica* maybe related to the flavonoids of the extract.

The genus *Ferula* has both useful (*F. asafoetida*, *F. gummosa* Boiss) and toxic (*F. communis* L) plants [9]. Also in our study, intravenous injection of *F. persica* (60 mg/kg) had been killed the rats suddenly a few time. So it must be used with caution. We didn't know the toxic dose of *F. persica* and this is another pitfall of this study.

We also evaluated chronic feeding effect of *F. persica* administration on systolic BP of hypertensive rats and it had no significant effect on BP. This inconsistency of acute and chronic effects of *F. persica* maybe related to low dose of extract, procedures of digestion and assimilation in gastrointestinal tract or

something else. This remains for more clarification.

In summary, the present study provides evidence that aqueous extract of *F. persica* has hypotensive property which appears to be attributable, partly, to Muscarinic activity and NO release. Further research is required to clarify the mechanism of this effect.

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