Original Article

The effect of hydroalcoholic extract from the leaves of *Moringa peregrina* (Forssk.) Fiori. on blood pressure and oxidative status in dexamethasone-induced hypertensive rats

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Abstract

Background: *Moringa peregrina* (Forssk.) Fiori. is a tropical tree growing in southeast of Iran. All parts of this plant have nutritional uses and pharmacological activities. The present study was designed to evaluate the effect of hydroalcoholic extract from the leaves of *M. peregrina* in dexamethasone (Dex)-induced hypertension in rats.

Materials and Methods: Male Wistar rats received Dex (30 μg/kg, subcutaneously; s.c.) or saline (as vehicle, 1 ml/kg, s.c.) for 14 days. In a prevention study, the rats received *M. peregrina* extract (100, 200 and 400 mg/kg, orally) for 4 days, followed by Dex for 14 days. In a reversal study, the animals received *M. peregrina* extract orally from day 8 to 14. The systolic blood pressure (SBP) was measured using tail-cuff method. The hydrogen peroxide (H_2O_2) concentration and ferric reducing antioxidant power (FRAP) were assessed in plasma samples.

Results: Dex significantly increased the SBP and the plasma H_2O_2 and decreased the plasma FRAP value ($P < 0.001$). *M. peregrina* extract at a dose of 400 mg/kg prevented ($P < 0.01$) but did not reverse Dex-induced hypertension in rats. It also dose-dependently reduced the plasma H_2O_2 concentration and improved the FRAP value upon Dex administration.

Conclusions: The findings of the present study indicated the antioxidant and partially antihypertensive effects of the hydroalcoholic extract from the leaves of *M. peregrina* in Dex-induced hypertension. Further experiments on other fractions of the leaves and also other parts of this plant are suggested for better evaluation of its antihypertensive effect and finding its mechanisms of action.

Key Words: Antioxidant status, dexamethasone *Moringa peregrine* (Forssk.) Fiori, hypertension

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INTRODUCTION

Hypertension, or raised blood pressure, is a prevalent disorder which affects nearly one billion individuals worldwide. It is a well-defined risk factor for cardiovascular diseases and contributors to millions of premature death and disability every year.[1] The sustained high blood pressure is considered responsible for functional and structural abnormalities

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that damage the major end-organs. Many serious cardiovascular events such as coronary heart disease, heart failure, stroke, peripheral vascular disease, blindness and renal impairment occur because of failure to achieve the targets on blood pressure management.[2] Treatment of hypertension through lifestyle changes or pharmacological interventions is associated with a decrease in the risk of cardiovascular events. Therefore the prevention and management of hypertension is one of the most important public health challenges.[3] Unfortunately, hypertension remains inadequately managed in spite of availability of various antihypertensive drugs and only 30% of patients achieve target blood pressure.[4] Recent efforts have focused towards natural products and herbal medicines as one of the potential sources for prevention and treatment of hypertension.[5] The protective effects of fruits, vegetables and antioxidant vitamins against cardiovascular diseases have been demonstrated in epidemiological studies.[6] The blood pressure lowering effect of some herbal such as *Punica granatum*, *Berberis vulgaris*, *Hibiscus sabdariffa* and *Olea europaea* has also been shown in several investigations.[7-10]

Moringa peregrina (Forssk.) Fiori. is a small desert tree belongs to the monogenic family, Moringaceae. This tree is growing in southeast of Iran (Sistan-Baluchistan and Hormozgan provinces) with common name of "Gas-e-rowghan" or "Gaz Rokh".[11,12] The young and mature seeds of *M. peregrina* are eaten as a food plant. *M. peregrine* is also used as a medicinal plant for treatment of fever, headache, malaria, constipation, abdominal pains, muscle pains, hypertension, asthma, diabetes and burns in folk medicine.[13] Pharmacological studies have shown antioxidant, analgesic, anti-inflammatory, anticancer, anti-hyperglycemic, anti-hyperlipidemic, antibacterial and antifungal activities for *M. peregrina*.[13-18] Phytochemical analysis of the aerial parts of *M. peregrine* have shown the presence of biologically active constituents including lupeol, α- and β-amyrin, β-sitosterol, apigenin, rhamnetin, neochlorogenic acid and quercetin.[16] The endothelium-dependent vasorelaxant activity and blood pressure lowering effect of some of these active compounds have been established in pharmacological studies.[19-21] Other species of this genus such as *Moringa oleifera* have also been used in Ayurvedic medicine for treatment of hypertension.[22]

According to the traditional usage and reported compounds of *M. peregrina*, in this study an attempt has been made to evaluate the effects of hydroalcoholic extract of the leaves of this plant in dexamethasone (Dex)-induced hypertensive rats.

MATERIALS AND METHODS

Animals

Male Wistar rats with an average weight of 200-250 g were obtained from the animal house of the School of Pharmacy and Pharmaceutical Sciences (Isfahan, Iran). The animals were housed under standard laboratory conditions with a 12 h light/12 h dark cycle and were given free access to standard rat chow and tap water *ad libitum*. All procedures were performed according to the local guidelines for laboratory animal use and care and approved by the Research Committee of Isfahan University of Medical Sciences.

Chemicals

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

Plant material and preparation of extract

The leaves of *M. peregrina* were collected from Nikshahr, Sistan-Baluchistan province in southeast of Iran in September 2013. After authentication of the plant, a voucher specimen (No. 2025) was deposited in the Herbarium of the School of Pharmacy and Pharmaceutical Sciences, Isfahan, Iran.

For preparation of hydroalcoholic extract, the air-dried finely powdered leaves of *M. peregrina* were powdered and extracted with ethanol (96%), using percolation method at room temperature for 48 h. The solvent was removed by a rotary evaporator to yield a semi-solid and viscous residue. The yield of the plant extract was 36% (w/w).

Determination of total phenolic content

The total phenolic content as a quality and nutritional value of the plant extract was measured using the Folin-Ciocalteu method.[23] In brief, the plant sample was dissolved in Na_2CO_3 (20%) and mixed with diluted Folin-Ciocalteu's phenol reagent. Stock solution of tanic acid (as a standard phenolic compound) was diluted in water for preparing 0, 50, 100, 150, 250 and 500 mg/L concentrations. The absorbance of sample and the reference solutions was recorded at 765 nm using a UV spectrophotometer. The total phenolic content was reported as tanic acid equivalents (TAE) per g of the plant.

Experimental protocol

For induction of hypertension, Dex (30 μg/kg/day) was injected subcutaneously (s.c.) for 14 days.[24] The saline control group received daily injection of saline (1 ml/kg, s.c.). In a prevention study, *M. peregrina* extract (100, 200 and 400 mg/kg),[25] or captopril (40 mg/kg, as an antihypertensive positive control) was administered orally using an intragastric tube from 4 days before Dex administration and during the test period (Days 1-18). In a reversal study, rats received *M. peregrina* extract or captopril from day 8 to 14.

Six rats were used in each control and experimental groups. All rats were weighed on alternate days between 10 AM and 12 noon. At the end of the experiment, animals were sacrificed under ether anesthesia and the thymus gland was removed and weighed. Blood samples were collected into heparinized tubes. The plasma was separated and stored at -80ºC for additional experiments.

Measurement of systolic blood pressure

Systolic blood pressure (SBP) was recorded by non-invasive tail-cuff method (AD Instrument PowerLab Data Acquisition System, Sydney, Australia) at the first day and the last day of the experiment between 10 AM and 12 noon. Rats were trained with blood pressure measuring equipment for one week before initiation of the experiment and were conscious during the recording. The animals were restrained in heated chambers at 38 ± 1°C for 10 minutes before the measurement. At least 3 blood pressures were recorded for each rat and the average of them was reported as the SBP.

Measurement of thymus weight

Thymus gland weight, as a marker of glucocorticoid activity was measured and expressed relative to the body weight (mg/100g of body weight).[26]

Measurement of plasma hydrogen peroxide concentration

The plasma hydrogen peroxide (H_2O_2) concentration was measured using commercially available kit based on the ferrous ion oxidation by xylenol orange reagent in aqueous medium with sorbitol (FOX1).[27] Briefly, FOX1 reagent containing ammonium ferric sulfate was prepared in aqueous medium with sorbitol according to the manufacturer's protocol and was added to plasma samples. After incubation for 30 min in 37ºC, the absorbance of solutions was read at 540 nm using a microplate reader/spectrophotometer (Bio-Tek, PowerWave XS, Wincoski, USA). The H_2O_2 concentration of plasma samples was assessed using a standard curve generated from different concentrations of H_2O_2.

Measurement of plasma ferric reducing antioxidant power

The total antioxidant capacity of plasma samples was evaluated using ferric reducing antioxidant power (FRAP) assay based on the reduction of ferric-tripyridyltriazine complex to ferrous form.[28] Briefly, the FRAP reagent containing tripyridyltriazine/ferric chloride/acetate buffer prepared according to the manufacturer's protocol was added to plasma samples. After incubation for 40 min in 40°C, the absorbance of colored solutions was read at 570 nm using a microplate reader/spectrophotometer. The FRAP value of samples was assessed against the standard curve of FeSO4x7H2O concentration and expressed as micromole of FeII equivalents per liter.

Statistical analysis

Results were reported as the mean ± standard error of mean (SEM). Data analysis was performed by one-way analysis of variance followed by Tukey *post-hoc* test using SPSS software version 16.0. $P < 0.05$ was considered to be statistically significant.

RESULTS

Total phenolic content

The total phenolic content assay showed 2.3 ± 0.1 mg TAE/g of the dried leaves of *M. peregrina* extract.

Effect of *M. peregrina* extract on blood pressure

The effect of pretreatment with *M. peregrina* extract (100, 200 and 400 mg/kg) and captopril (40 mg/kg) on SBP in Dex-induced hypertension was shown in Figure 1. The Dex-induced hypertensive rats showed significant increase in SBP from 116.5 ± 2.5 to 149.6 ± 4.2 mmHg on day 18 ($P < 0.001$) compared with saline control group (112.6 ± 3.4 mmHg). Administration of captopril significantly prevented and reduced the

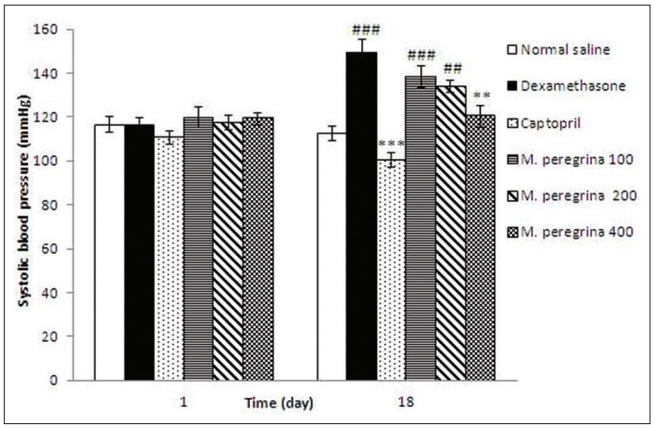

Figure 1: Effects of *M. peregrina* extract (100-400 mg/kg) and captopril (40 mg/kg) on systolic blood pressure in Dex-induced hypertension in prevention groups. Values are means ± SEM for six rats. $^{##}P < 0.01$ and $^{###}P < 0.001$ versus saline control group, $^{**}P < 0.01$ and $^{***}P < 0.001$ versus Dex control group

Dex-induced hypertension ($P < 0.001$). *M. peregrina* extract also prevented the raise in SBP at a dose of 400 mg/kg in prevention study ($P < 0.01$) [Figure 1]. However, administration of *M. peregrina* extract could not lower the SBP in Dex-induced hypertensive rats in reversal study [Figure 2].

Effect of *M. peregrina* extract on thymus weight

Dex administration caused significant decrease in the thymus gland weight in hypertensive rats ($P < 0.001$). Oral administration of *M. peregrina* extract or captopril had no significant effect on thymus weight loss [Figure 3].

Effect of *M. peregrina* extract on body weight

Injection of Dex significantly decreased the body weight in hypertensive rats when compared with the saline control group ($P < 0.01$). Oral administration of *M. peregrina* extract could not prevent body weight changes in rats and also augmented the body weight loss more than that of dexamethasone group ($P < 0.001$) [Figure 4].

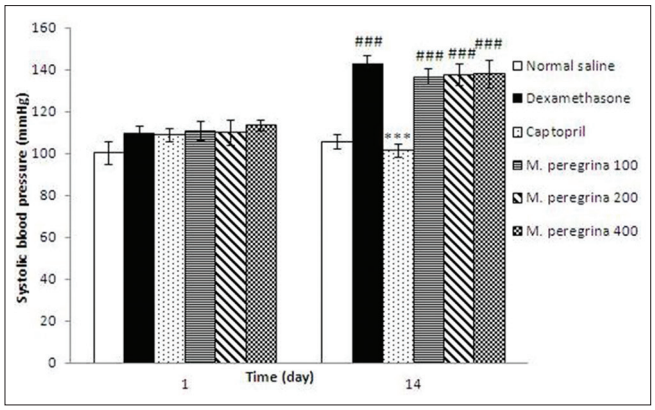

Figure 2: Effects of *M. peregrina* extract (100-400 mg/kg) and captopril (40 mg/kg) on systolic blood pressure in Dex-induced hypertension in reversal groups. Values are means ± SEM for six rats. $^{###}P < 0.001$ versus saline control group, $^{***}P < 0.001$ versus Dex control group

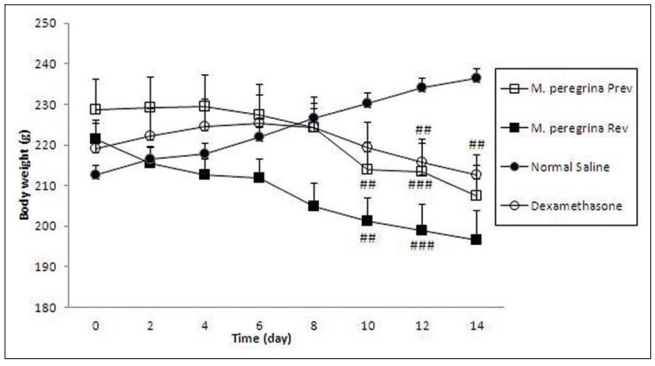

Figure 4: Effects of *M. peregrina* extract (400 mg/kg) on body weight in Dex-induced hypertension in prevention (Prev) and reversal (Rev) groups. Values are means ± SEM for six rats. $^{##}P < 0.01$ and $^{###}P < 0.001$ versus saline control group

Effect of *M. peregrina* extract on plasma H_2O_2 concentration

The level of plasma H_2O_2 showed a significant increase upon Dex administration compared to the saline control group ($P < 0.001$). Pretreatment with *M. peregrina* extract at doses of 200 and 400 mg/kg prevented the rise in H_2O_2 concentration in prevention study ($P < 0.001$) and reduced the elevated plasma H_2O_2 concentration at a dose of 400 mg/kg in reversal study ($P < 0.001$). Administration of captopril also significantly prevented and reduced the rise in plasma H_2O_2 concentration [Figure 5].

Effect of *M. peregrina* extract on plasma FRAP value

The plasma FRAP value showed a significant decrease upon Dex administration compared to the

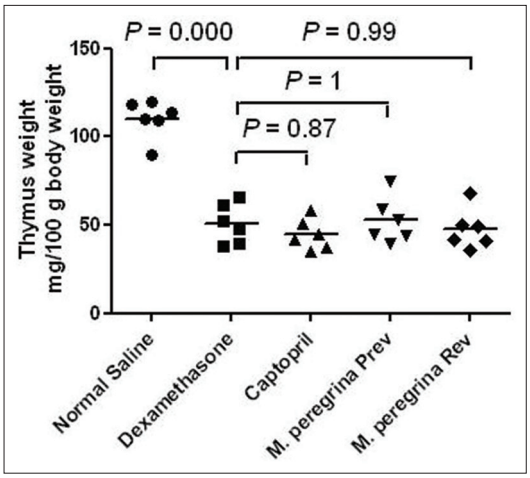

Figure 3: Effects of administration of *M. peregrina* (400 mg/kg) and captopril (40 mg/kg) on thymus weight in Dex-induced hypertension in prevention (Prev) and reversal (Rev) groups. Values are means for six rats. $P < 0.05$ is considered to be statistically significant versus Dex control group

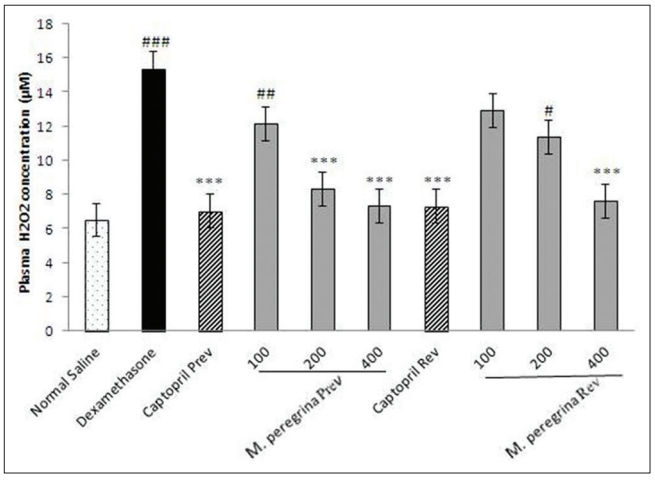

Figure 5: Effects of *M. peregrina* extract (100-400 mg/kg) and captopril (40 mg/kg) on plasma H_2O_2 concentration on Dex-induced hypertension in prevention (Prev) and reversal (Rev) groups. Values are means ± SEM for six rats. $^{#}P < 0.05$, $^{##}P < 0.01$ and $^{###}P < 0.001$ versus saline control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

saline control group ($P < 0.001$). Administration of *M. peregrina* extract at a dose of 400 mg/kg significantly increased the FRAP value in prevention and reversal groups [Figure 6].

DISCUSSION

The findings of the present study indicated that *M. peregrina* extract at a dose of 400 mg/kg prevented but did not reverse Dex-induced hypertension in rats. It also dose-dependently reduced the plasma H_2O_2 concentration and improved the FRAP value upon Dex administration.

Dexamethasone as the most potent synthetic glucocorticoid results in hypertension during chronic uses at supra-physiologic doses. Various mechanisms have been proposed as contributing to Dex-induced hypertension such as deficiency of vasodilator hormones, increased vascular pressor responsiveness, increased activity of vasoconstrictor hormones and oxidative stress.[29] There is increasing evidence implicating the role of enhanced oxidative stress in glucocorticoid-induced hypertension. Production of large amounts of reactive oxygen species (ROS) occurs in many forms of hypertension.[30] Overproduction of ROS results in nitric oxide (NO) deficiency through interaction of superoxide with NO to form peroxynitrite and also in vascular structural changes and endothelial dysfunction.[31] Several antioxidant agents and some herbal medicine have recently shown to prevent and attenuate hypertension in experimental and human studies.[5,31,32]

M. peregrina is a tropical tree with nutritional uses and pharmacological activities. Valuable nutrients for human diet have been identified in seeds and leaves of *M. peregrina*. The leaves of this plant is a good source of high iron and amino acids especially sulfur-containing amino acid cysteine, and low fat diet.[33] Phytochemical analysis of *M. peregrina* extract has shown the presence of flavanoids, tannins, sterols/triterpenes, saponins and isothiocyanates.[34,35] Although some biologically active constituents with antihypertensive and vasodilator activity such as lupeol, apigenin and quercetin have been isolated from M. peregrina,[19-21] but regarding the total phenolic assay, it seems that low amount of these phenolic compounds may be contributed to the low antihypertensive activity of *M. peregrina* extract in this study. Moreover El-Alfy and coworkers found the above active constituents in the plant collected in the spring[16] while our samples were collected in the summer and this difference in the time of collection of the plant may be another cause for our findings.

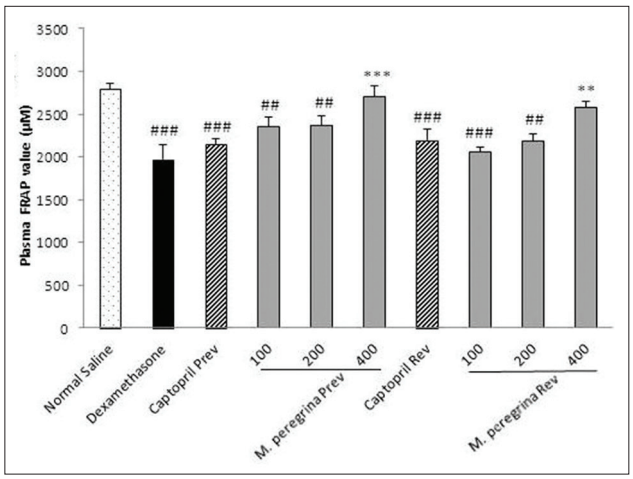

Figure 6: Effects of *M. peregrina* extract (100-400 mg/kg) and captopril (40 mg/kg) on plasma FRAP value on Dex-induced hypertension in prevention (Prev) and reversal (Rev) groups. Values are means ± SEM for six rats. $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ versus saline control group, $^{**}P <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

In the present study, *M. peregrina* extract reduced the plasma H_2O_2 concentration and improved the total antioxidant capacity of the plasma. Dehshahri *et al.* examined the antioxidant activities of M. peregrina methanolic leaf extract. Their results revealed the scavenging activity on DPPH (2,2-diphenyl-1-pycrylhydrazyl) and superoxide anion radicals in vitro. They also suggested that flavonoid glycoside, rutin, may be one of the compounds responsible for antioxidant activities of this plant.[14] Our findings also indicated the antioxidant effect of captopril. Captopril is an angiotensin-converting enzyme inhibitor with ability to scavenge the hydroxyl radical and to protect erythrocyte membranes from lipid peroxidation.[36]

The results of investigations have shown the effect of supra-physiological doses of glucocorticoids on lowering the body weight set point in several animal studies. Some evidence has confirmed the role of hypothalamus–pituitary–adrenal axis in the regulation of body weight. Administration of high doses of glucocorticoids increases the lipoprotein lipase activity and a rise in lipolysis may be participated in the animal body weight loss. Another mechanism involved in this effect may be due to the interaction of glucocorticoids with leptin. Leptin is a signal which reflects the status of fat stores into the central nervous system and its production and secretion is stimulated by high levels of glucocorticoids.[37,38] In the present study, administration of *M. peregrina* extract could not prevent the effect of Dex on body weight loss in rats. Rouhi-Broujeni *et al.* also reported the lipid lowering effect of *M. peregrina*. In their study, the hydroalcoholic extract of *M. peregrina* seeds could decrease the fasting blood sugar, cholesterol, triglyceride and also the mean

body weight in rats.[17] The result of investigation has also showed the hypolipidemic effect of *M. oleifera* extract through depressant effect on HMG CO-A reductase activity and increasing the excretion of fecal cholesterol.[39]

CONCLUSION

In conclusion, this study showed the antioxidant and partially antihypertensive effects of hydroalcoholic extract of *M. peregrina* leaves in Dex-induced hypertension. Additional experiments on other fractions of the leaves and also other parts of this plant are suggested for better elucidation of its antihypertensive effect.

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