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Effects of hydroalcoholic extract of saffron petal on blood pressure and heart rate in hypertension induced by angiotensin II and L-NAME in anesthetized rats

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Abstract

The saffron petals are a by-product part of the saffron flower with a cardiovascular effect. This study evaluated the effect of the saffron petal on hypertension induced by angiotensin II (AII) and NG-nitro-L-arginine methyl ester (L-NAME, a NOS inhibitor). Rats were divided into 11 groups: 1) Control, 2) AII (50.00 ng kg^{-1}), 3) Losartan+ AII, 4) L-NAME (10.00 mg kg^{-1}), 5) sodium nitroprusside (SNP) + L-NAME, 6, 7) Saffron petals extract; 8, 9) saffron petals (100 and 200 mg kg^{-1}) + AII and 10,11) saffron petals (100 and 200 mg kg^{-1}) + L-NAME. Hypertension induced by intravenous injection of AII and L-NAME in separate groups. In treated groups, 30 min before injection of AII or L-NAME rats received two doses of extract via intraperitoneal administration. The femoral artery was cannulated and cardiovascular parameters recorded by a transducer connected to power lab apparatus. Maximal changes (Δ) of mean arterial pressure (MAP), systolic blood pressure (SBP) and heart rate (HR) from baseline were calculated and compared to with those in hypertensive and control groups. Results showed that both AII and L-NAME significantly increased SBP and MAP than control, however, HR in AII was decreased and in the L-NAME group increased. Pre-treatment with saffron petals could significantly attenuate the cardiovascular responses induced by both AII and L-NAME. However, the effect of the extract in AII hypertensive rats was more effective than L-NAME groups. The findings showed that the hydroalcoholic extract of the saffron petals had an antihypertensive effect that mainly was mediated by inhibition of AII activity.

Introduction

Saffron (*Crocus sativus*) is a member of the iridaceous family that is being cultivated in several countries, especially Iran, South Khorasan province. The saffron flower has several parts including stigma (red color), petals (purple color), stamens (yellow color) and filiform style ending (white color).[1] The valuable part of saffron is the stigma that besides use in food as a spice, bears therapeutic properties.[2] Traditionally, after separating stigma from flowers, other residues of saffron including petals, stamens and filiform styles are considered as by-products and completely discarded. It is estimated that out of 78.00 kg of saffron about 1.00 kg of dried stigma could be harvested. Total production of dried stigma in Iran is about 170 tons per year. Therefore, thousands of tons of by products are thrown away. Due to affordability and probable therapeutic effects, attention to these parts of the saffron flower has been increased.[3]

In terms of weight, the greatest amount of saffron is saffron petals. As a result, more attention has been paid to it. Saffron petals consist of several constituents such as glycosides, anthocyanin and various flavonoids (rich in flavonoids).[4] Recent pharmacological studies have demonstrated that saffron petals extract has antioxidant,[5] anti-inflammatory,[6] antidepressant, [7] and nerve protection properties.[8] Its inhibitory effect on the blood pressure also is shown by Fatehi *et al.*[4]

Nitric oxide (NO) and angiotensin II (AII) are two important factors that involve in the regulation of the cardiovascular system. The NO is a signaling molecule that has different biological functions such as regulation of

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regional blood flow, control of blood pressure, the release of neurotransmitters, platelet aggregation and immune activity.[9, 10]

The NO is produced by nitric oxide synthase (NOS). This enzyme has three isoforms: Neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS). The eNOS is mostly located in endothelium and plays acritical role in the regulation of blood pressure.[11] The NG-nitro-L-arginine methyl ester (L-NAME) is a non-specific NOS inhibitor used in animal studies to induce acute and chronic hypertension.[12]

The renin-angiotensin system (RAS) is an important system with several physiological and pharmacological functions.[13] In the cardiovascular system, the RAS plays an important role in the maintenance and regulation of blood pressure and fluid homeostasis.[14] Presence of this system in several tissues such as kidney, vascular, brain, adrenal glands, lung, heart and reproductive organs have been shown.[15,16] The AII is the final product of RAS that by vasoconstriction, excitation of sympathetic system, inhibition of NO production plays an important role in cardiovascular regulation. Overproduction of AII also could evoke hypertension and drugs that inhibit the effect of AII are widely used for the treatment of hypertension. Acute hypertension induced by AII is also an experimental method for evaluation anti-hypertensive effects of drugs and plants.[17]

Although effect of saffron petals on blood pressure in normotensive rats has been shown, however, its effects on blood pressure and heart rate (HR) in hypertension condition has not been yet evaluated. Therefore, the purpose of this study was to determine whether the hydroalcoholic extract of the saffron petals has effect on blood pressure and HR in acute hypertension induced by L-NAME and AII.

Materials and Methods

Extract preparation. Saffron flowers were collected from saffron farms of Esfeden, a city of South Khorasan province and identified by herbarium of Ferdowsi University of Mashhad (No: 143-0319-1). The saffron petals were carefully separated from other parts of the flower and dried in shadow. Hydroalcoholic extract of the saffron petals was prepared using 100 g of petals dried powder in 1000 mL ethanol 70.00% and shake for 72 hr in 37.00 ˚C, and then the mixture was filtered through different filter sizes. The solvent was evaporated by an oven at 40.00 ˚C.

Determination of chemical compositions. The polyphenols content of the saffron petals extract was evaluated based on the Folin–Ciocalteu method in which saffron petals extract had 59.60 mg gallic acid g^{-1} crude extract. The flavonoids content based on the aluminum chloride colorimetric method was 52.90 mg quercetin g^{-1} crude extract. Finally, the anthocyanin content of the saffron petals extract was evaluated based on the pH-differential method described by Rodriguez-Saona in which saffron petals extract had 0.30 mg g^{-1} crude extract.[18] The linear regression equations for standard curves of gallic acid and quercetin were $Y = 0.0669X + 0.0116$ and $Y = 0.06632X - 0.01448$, respectively.

Drug and animal groups. The drugs used in this study were urethane, AII, L-NAME, losartan (an AT1 receptor antagonist) and sodium nitroprusside (SNP; nitric oxide donor) provided from Sigma (St. Louis, USA). All drugs were dissolved in saline. Sixty-six rats used in this study were provided from animal house of Faculty of Medicine, Mashhad and kept in good health conditions (temperature: 22.00 ± 2.00 ˚C, water and food *ad libitum*, 12-hr on/12-hr off light cycle). Animals were randomly divided into 11 groups (n = 6) as follow:

1) Control group: Animals received saline intravenously (IV) instead of AII or L-NAME,

2) AII: Animals received 50.00 ng kg^{-1} AII (IV)[19] for induction of hypertension same as RAS over activity,

3) Losartan + AII group: Animals received 10.00 mg kg^{-1} losartan intraperitoneally (IP)[17] after 30 min AII injected (positive group of L-NAME),

4) L-NAME group: Animals received 10.00 mg kg^{-1} L-NAME (IV)[20] for induction hypertension same as nitric oxide deficit,

5) SNP + L-NAME: Animals received 50.00 µg kg^{-1} SNP (IP)[11] after 30 min L-NAME injected (positive group of L-NAME),

6, 7) Saffron petals extract groups: Animals received 100 and 200 mg kg^{-1} of extract (IP), separately in basal condition,

8, 9) Saffron petals extract + AII groups: 100 and 200 mg kg^{-1} of extract injected (IP) separately, after 30 min AII (50.00 ng kg^{-1}, IV) injected in each group,

10,11) Saffron petals extract +L-NAME groups: Animals received 100 and 200 mg kg^{-1} of extract (IP) separately, after 30 min L-NAME (10.00 mg kg^{-1}, IV) injected in each group.

Volume of intraperitoneal injections was 0.50 mL and in all intravenous injections was 0.40 mL administered into the femoral vein.

Experimental protocol. After anesthesia with urethane (1.50 g kg^{-1}; IP),[11] left femoral artery was cannulated with angiocatheter No. 24 filled with heparinized saline (50.00 IU mL^{-1}) and connected to the pressure transducer connected to Power Lab system (ID Instrument, Sydney, Australia). After 15 min cardio-vascular responses were continuously recorded then systolic blood pressure (SBP), mean arterial pressure (MAP) and heart rate (HR) were calculated from each group before and after drug administration, and thereafter post-drug changes (Δ) were compared among groups. Induction of acute hypertension was done by slow IV

injection of AII and L-NAME into the cannula inserted into the femoral vein. Because urethane has long effect and also minimum effect on cardiovascular parameters,[21] the animals were anesthetized with urethane in this experiment. The ethical code for animal work was IR.MUMS.MEDICAL.REC.1397.034.

Data analysis. The maximal changes (Δ) of MAP, SBP, and HR were calculated and expressed as mean ± SEM. Statistical analysis was performed by One-way ANOVA followed the Tukey's post hoc test using SPSS Software (version 16.0; IBM Corp., Armonk, USA). A value of $p < 0.05$ was considered statistically significant.

Results

Effect of hydroalcoholic extract of saffron petals on the cardiovascular responses in normotensive rats. Changes induced by both doses of extract decreased ΔSBP (doses 100 and 200: – 4.70 ± 2.30 and –6.20 ± 3.90 mm Hg *versus* Control: 2.30 ± 0.80 mm Hg), ΔMAP (doses 100 and 200: –3.00 ± 1.80 and –4.80 ± 2.50 mm Hg *versus* Control*:* 1.70 ± 0.70 mm Hg) and ΔHR (doses 100 and 200: –10.60 ± 2.80 and –13.80 ± 8.13 *versus* Control: 5.40 ± 2.40 beats per min (bpm). However, these effects were not significant compared to saline group (Table 1).

Effects of AII on cardiovascular responses. Maximal changes of SBP and MAP in AII alone group were significantly higher than control group (ΔSBP; AII: 53.40 ± 4.90 mm Hg *versus* Control: 2.30 ± 0.80 mm Hg; $p < 0.001$) and (ΔMAP; AII: 43.30 ± 3.70 mm Hg *versus* Control: 1.70 ± 0.70 mm Hg, $p < 0.001$). AII reduced HR but this effect was not significant compared to the control group (ΔHR, AII: –17.80 ± 8.60 *versus* Control: 5.40 ± 2.40 bpm). Pretreatment with losartan significantly attenuated the effect of AII on SBP and MAP ($p < 0.01$; Figs. 1A, and 2).

Effect of hydroalcoholic extract of saffron petals on the cardiovascular responses induced by AII. Administration both doses of saffron petals significantly attenuated ΔSBP (SP: 24.50 ± 3.40, 21.40 ± 2.00 mm Hg *versus* AII: 53.40 ± 4.90 mm Hg; $p < 0.01$) and ΔMAP (SP: 23.10 ± 2.20 and 19.20 ± 1.60 mm Hg *versus* AII: 43.30 ± 3.70 mm Hg) compared to AII alone (Figs. 1B and 2). ΔHR also decreased but was not significant compared to AII alone (SP: –7.30 ± 4.20 and –3.80 ± 4.30 *versus* AII: –17.80 ± 8.60 bpm).

Table 1. The cardiovascular changes (Δ) of intraperitoneal injection of saffron petal (SP) extract in anesthetized rats. The data are expressed as mean ± SEM.

Parameters	Saline	SP 100 mg kg^{-1}	SP 200 mg kg^{-1}
ΔMAP (mm Hg)	1.70 ± 0.70	–3.00 ± 1.80	–4.80 ± 2.50
ΔSBP (mm Hg)	2.30 ± 0.80	–4.70 ± 2.30	–6.20 ± 3.90
ΔHR (bpm)	–5.40 ± 2.40	10.60 ±3.80	–13.80 ± 3.50

SBP: Systolic blood pressure, MAP: Mean arterial pressure, HR: Heart rate.

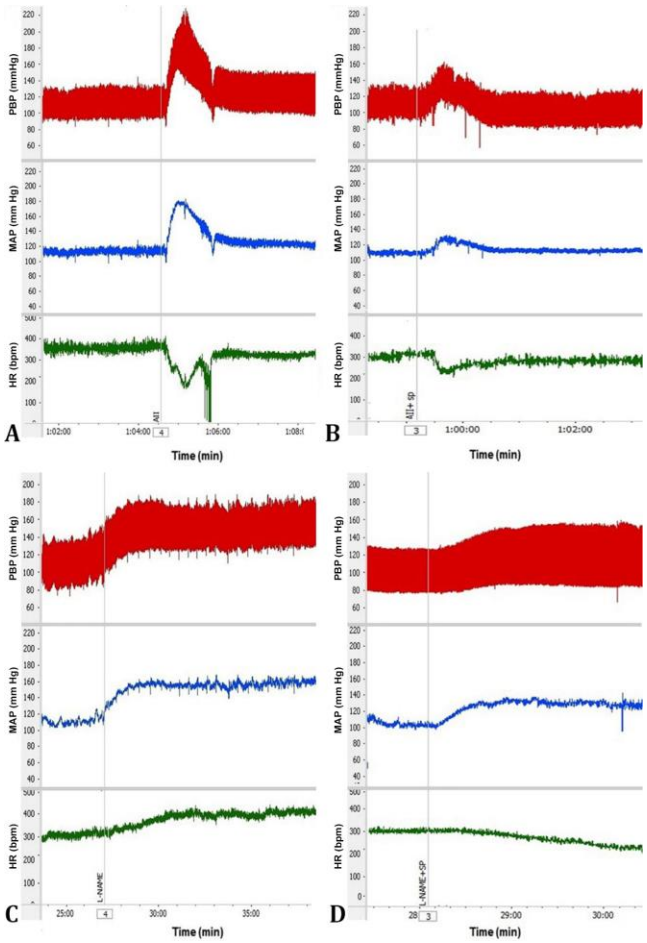

Fig. 1. A recording sample of cardiovascular responses induced by intravenous administration of **A)** AII alone, **B)** AII + saffron petal (SP) 200 mg kg^{-1}, **C)** L-NAME alone and **D)** L-NAME + saffron petal 200 mg kg^{-1}. The line indicates time of injection. PBP: Pulsatile blood pressure, MAP: Mean arterial pressure, HR: Heart rate.

Effects of L-NAME on cardiovascular responses. Injection of L-NAME (IV) significantly increased all cardiovascular parameters (Fig. 1C). Maximal changes of SBP (44.10 ± 4.00 mm Hg *versus* 2.30 ± 0.80 mm Hg, $p < 0.001$ and MAP: 40.80 ± 4.00 mm Hg *versus* 0.60 ± 1.70 mm Hg; $p < 0.001$) were significantly increased compared to the control group (Figs. 3A and 3B). Maximal ΔHR: 27.30 ± 12.00 *versus* 5.40 ± 2.40 beats per min were not significant compared to the control group ($p > 0.05$; Fig. 3A). Pretreatment with SNP significantly attenuated the effect of L-NAME on SBP and MAP ($p < 0.01$; Figs. 3B and 3C). Tachycardia induced by L-NAME was also decreased by pretreatment with SNP, however, this effect was not significant compared to L-NAME alone (Fig. 3A).

Effect of hydroalcoholic extract of the saffron petals on the cardiovascular responses induced by L-NAME. In saffron petals (100 and 200 mg kg^{-1}) + L-NAME groups, both doses of the extract significantly attenuated

ΔSBP induced by L-NAME (30.40 ± 6.50 mm Hg and 28.40 ± 5.50 mm Hg *versus* 44.10 ± 4.00 mm Hg, $p < 0.05$; Figs. 2B, 3B and 3C). The ΔMAP only in the 200 mg kg^{-1} was significantly lower than L-NAME (24.90 ± 4.60 mm Hg *versus* 40.80 ± 4.00 mm Hg, $p < 0.05$; Figs. 2B and 3B and 3C). Only 100 mg kg^{-1} significantly decreased ΔHR compared to the L-NAME group (–38.60 ± 6.80 beats per min *versus* 27.30 ± 12.00 beats per min, $p < 0.05$; Figs. 2B and 3A).

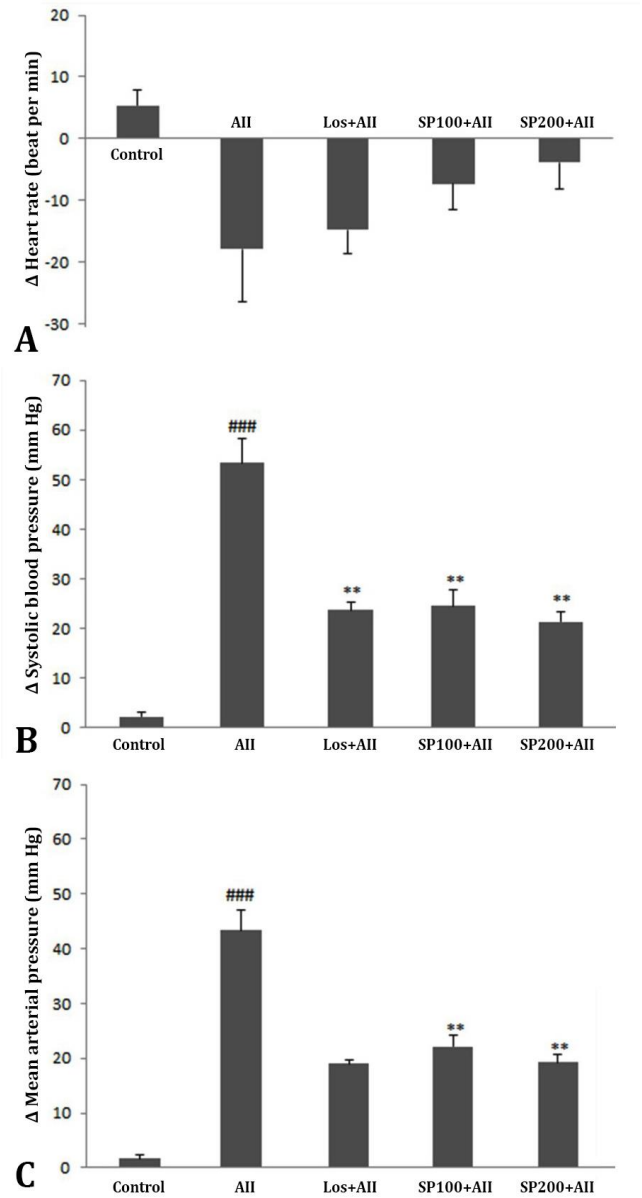

Fig. 2. Effects of two doses (100 and 200 mg kg^{-1}) of hydroalcoholic extract of saffron petal (SP) on **A)** Heart rate, **B)** Systolic blood pressure and **C)** Mean arterial pressure in angiotensin II (AII) hypertensive rats. The data are expressed as mean ± SEM. Los: Losartan.
##: $p < 0.01$, ###: $p < 0.001$ *versus* Control **: $p < 0.01$, ***: $p < 0.001$ *versus* AII.

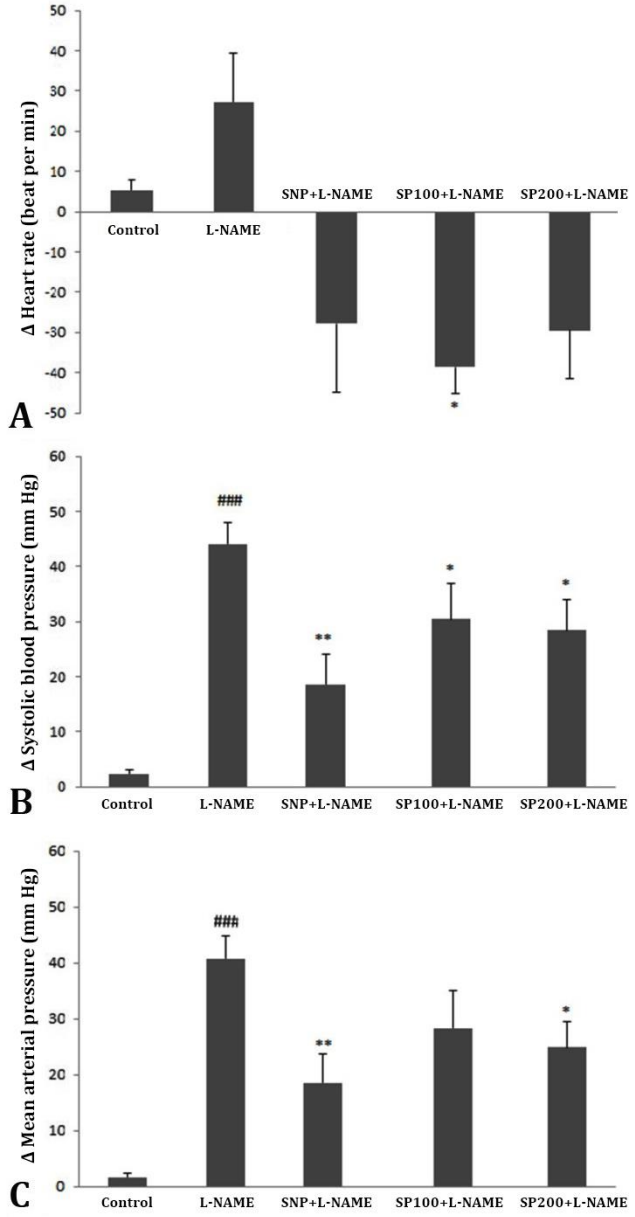

Fig. 3. Effects of two doses (100 and 200 mg kg^{-1}) of hydroalcoholic extract of saffron petal (SP) on **A)** Heart rate, **B)** Systolic blood pressure and **C)** Mean arterial pressure in L-NAME hypertensive rats. The data are expressed as mean ± SEM. SNP: Sodium nitroprusside.
###: $p < 0.001$ *versus* control *: $p < 0.05$ and **: $p < 0.01$ *versus* L-NAME.

Discussion

In the current study, the anti-hypertensive effects of hydroalcoholic extract from saffron petals in acute hypertension induced by AII and L-NAME were studied. For this purpose, 30 min before injection of AII and L-NAME were studied. For this purpose, 30 min before injection of AII and L-NAME, animals received two doses of the extract. Our results showed that both doses of extract

attenuated the hypertensive effect of L-NAME and AII and this effect in AII induced hypertension was greater. These results indicated the beneficial effects of the saffron petals on hypertension induced by RAS as well as NO system.

Inconsistent with our results, Fatehi *et al*. have shown that aqueous and ethanolic extracts of saffron petals significantly decreased the basal blood pressure in a dose-dependent manner.[4] Also, the saffron petals has a relaxant effect that may be mediated by an effect on adrenergic as reported by Fatehi *et al*.[4] In addition, previous studies have shown that saffron petals contains various compounds such as glycosides, flavonoids, crocin, polyphenols, anthocyanin, and kaempferol.[22-24] We also determined contents of polyphenols (59.60 mg gallic acid g^{-1} crude extracts), flavonoids (52.90 mg quercetin g^{-1} crude extract) and anthocyanin (0.30 mg g^{-1}) of this extract. Each of these constituents of extracts especially the flavonoids and polyphenols may take part in cardiovascular regulation.

Crocin is an ingredient of saffron petals that has several effects including the inhibitory effect on AII hypertensive rats.[25] It has been shown that flavonoids have antioxidant property. In addition, products of flavonoids[26] such as anthocyanin[27] and kaempferol[28] modulate effect on RAS and preservation of endothelial NO. Anthocyanin beside antioxidant effect also can increase the production of NO via activation and expression of the eNOS enzyme.[23] Thus, we suggested that the flavonoids of the saffron petals ameliorate hypertension via effect on AII or NO.[29] Polyphenols such as resveratrol, quercetin, and isorhamnetin present in saffron petals also could activate the eNOS promotor and via enhancement of NO production leads to anti-hypotensive effects.[30,31] In consistent with our opinion Kurin *et al*. have shown that the mixture of resveratrol, quercetin, kaempferol, and isorhamnetin has a moderate synergic effect on the eNOS promotor activation.[30] Another study showed that glycosides increased NO production via activating eNOS.[32] Hence, this extract maybe involved in regulation of hypertension by each one of its components.

In this study hypertension induced by AII was significantly decreased by administration of saffron petals extract because saffron petals extract could attenuate effect of AII. We concluded that anti- hypertensive effect of extract was mostly mediated by effect on AII. Injection of AII also decreased HR. Bradycardial effect of AII is related to baroreflex activity. In this reflex, enhancement of blood pressure decreases the HR.[33] Since in AII groups the blood pressure was lowered by the extract, we concluded that the HR was reflexively decreased. In hypertension induced by L-NAME, HR was increased and our results revealed that saffron petals decreased tachycardia induced by L-NAME. This effect could explain that this part of saffron probably had an inhibitory effect on the heart. However, future studies are needed to confirm this issue.

Our experiment also showed that the effect of saffron petals extracts on cardiovascular responses in hypertension induced by AII was more effective than L-NAME. Mechanism(s) of this effect of the extract is unknown. However, we concluded that the beneficial effects of saffron petals components on AII were higher than NO in control of hypertension. Also, AII could inhibit NO production,[25] therefore, inhibition of AII could increase NO bioavailability and this effect may justify the greater effect of the extract on hypertension induced by AII. However, future studies are needed for the identification and isolation of compounds effective on each one of the animal models of hypertension. There was no significant difference between the two doses of extract in both AII and L-NAME hypertensive rats. The mechanism of this effect was unknown, however, one of the reasons for this effect could be use of higher doses, therefore, these doses had maximal effects.

Finally, our findings provided evidence that saffron petals had an anti-hypertensive effect and this effect was mediated by an inhibitory effect on AII or increased NO production. Due to more effects of the extract on hypertension induced by AII than L-NAME, it is conceivable that the antihypertensive effect of extract was mostly mediated by inhibition of AII effect.

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Conflict of interest

There is not any conflict of interest.

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