

Effect of Hydroalcoholic Extract of *Ribes khorasanicum* on Acute Hypertension Induced by L-NAME in Rat

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Key Words

Ribes khorasanicum, L-NAME, Blood pressure, heart rate, Hypertension

Abstract

Objectives: The aim of this study was to evaluate the effect of *Ribes khorasanicum* (*R. khorasanicum*); a plant growing in north Khorasan of Iran; on cardiovascular and stress oxidative in acute hypertension induced by N-nitro-L-arginine methyl ester (L-NAME), anitric oxide synthase inhibitor.

Methods: Rats were divided into Control, L-NAME (10 mg/kg), Sodium Nitroprusside (SNP) (50 mg/kg) + L-NAME and three treated groups with *R. khorasanicum* (4, 12 and 24 mg/kg) groups + L-NAME. L-NAME and SNP were injected intravenously and extract intraperitoneal. In *R. khorasanicum* groups, L-NAME was injected 30 min after injection of the extract. Systolic blood pressure (SBP), mean arterial pressure (MAP) and heart rate (HR) were recorded continuously using power lab software. At the end of study oxidative stress parameters including of total thiol content (SH), malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) in heart and aorta of all groups were also measured.

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Results: In groups 4 and 24 mg/kg extract +L-NAME, there was a non-significant decrease in SBP and MAP compared to L-NAME group but dose 12 mg/kg significantly attenuate the effect of L-NAME ($P < 0.05$). In L-NAME group the heart and aorta tissues antioxidant enzymes levels decreased, while in treated rats these enzymes significantly increased.

Conclusion: The extract of *R. khorasanicum* in dose 12 mg/kg show anti-hypertensive effect that is mediated by an effect on NO system or antioxidant parameters.

1. Introduction

Cardiovascular diseases are one of the most common causes of death in Iran and the world [1]. Each year, more than 51 million people die from the disease. Although significant progress has been made in patient care and decreases mortality rate in recent years, it is still a major factor in the reduction of life expectancy and disability [2]. Hypertension is systolic blood pressure above 140 mmHg and diastolic blood pressure equal to or greater than 90 mmHg and mean arterial pressure above 110 mmHg [3]. Race, male, gender, smoking, air pollution, age, and hereditary conditions can also affect the development of hypertension [4]. Based on the results, it can be said that the contractile effects of vessels or factors that cause vascular contraction or stimulants that produce direct structural changes in the vascular wall that increase the peripheral resistance are the primary cause of hypertension [5].

One of the most important factors that synthesized in

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the endothelium is nitric oxide (NO), which has numerous biological properties on vascular including modulation of vascular tone, cell growth regulation, and vascular protection against platelets and cells in the circulation. NO is an endogenous vasodilator with a short half-life of 6 to 14 seconds, made up of a group of enzymes called nitric oxide synthase (NOS). These enzymes convert arginine into citrulline, which is synthesized in this NO process. Oxygen and NADPH are required as a cofactor [6]. The two nNOS and eNOS enzymes are expressed primarily in mammalian cells and synthesize NO in response to increased levels of intracellular calcium [6]. When the heart is exposed to oxidative stress, NO is easily combined with reactive oxygen species, thereby forming nitrogen-reactive species [7]. NO also has harmful effects that are related to its oxidation products [8].

Ribes khorasanicum (*R. khorasanicum*) is a shrub belonging to the Grossulariaceae family [9]. This plant is endemic and native of Khorasan Razavi province of Iran and has not been reported from anywhere else in the country and the world. Few studies have been done about the properties of *R. khorasanicum*. In one study reported that *R. khorasanicum* contain components such as alkaloids, flavonoids, saponins, and tannins flavonoids content and anthocyanin also determined by Yazdi et al and reported that [10]. Total phenolic and anthocyanin in higher than other compounds [11]. Another material such as protein and propose such as hypertension, gastric and intestinal infections, and constipation [12]. The previous study also indicated that *R. khorasanicum* has anti-oxidant, antibacterial and antifungal effects.

Because this plant has flavonoid especially anthocyanin has a beneficial effect on the cardiovascular system and *R. khorasanicum* is contained of these compound we suggest that this plant has beneficial on the cardiovascular system. Therefore, in the present study we investigating the effect of hydroalcoholic extract of *R. khorasanicum* on cardiovascular responses in acute hypertension induced by N-nitro-L-arginine methyl ester (L-NAME, a non-selective NOS inhibitor).

2. Material and methods

2.1. Plant and extract preparation

R. khorasanicum was collected from Dargaz (northeast of Iran) and identified by botanists in the herbarium of Ferdowsi University of Mashhad (herbarium number:3242).

The hydroalcoholic extract was prepared by adding of 100 g of dried powder of *R. khorasanicum* fruit to 1800 ml of ethanol 70% (540 ml distilled water and 1260 ml ethanol) using the macerating method for 72 h with occasional shaking. The solvent was removed under decreased pressure.

2.2. Animals and surgery

The experiment was performed with 60 male Wistar rats (220-250 gr). The animals were anesthetized with urethane (1.4 g/kg, intraperitoneal (i.p)) [13]. The animal temperature was kept at 37°C by a heating lamp. The left femoral artery was cannulated with a polyethylene catheter (PE-

50) filled with heparinized saline, then catheter connected to a blood pressure transducer and blood pressure (BP) and heart rate (HR) continuously recorded by a power lab system (ID instrument, Australia) [14]. Stabilization time before injection of any drug was 20 min.

2.3. Drug

The drugs including urethane, L-NAME, and SNP provided from Sigma Chemical Company(USA). All drugs dissolved in saline

2.4. Experimental Protocol and animal groups

To induce acute hypertension, L-NAME (10 mg / kg) injected intravenously (i.v) via femoral artery [15]. In L-NAME +Sodium Nitroprusside (SNP) group firstly, SNP (50 µg/kg, i.v) [15]. injected and after 5 min L-NAME was injected and blood pressure was recorded. The treated groups received 3 doses of *R. khorasanicum* (4, 12 and 24 mg/kg, i.p) and after 30 min, L- NAME was injected. The SBP, MAP, and HR recorded throughout the experiment and those changes (Δ) were calculated and compared with control and L-NAME groups.

Sixty male Wistar rats were divided into 6 groups as following order (n=10 in each group) 1- Control group; received saline (i.v)

2- L-NAME group; received L-NAME (10 mg/kg, i.v)

3- SNP group; received SNP (50 mg/kg, i.v) 5 min before L-NAME injection

4- *R. khorasanicum* 4 + L-NAME group; received 4 mg/kg of *R. khorasanicum* extract (i.p) 30 min before L-NAME injection

5- *R. khorasanicum* 12 + L-NAME group; received 12 mg/kg of *R. khorasanicum* extract (i.p) 30 min before L-NAME injection

6- *R. khorasanicum* 24 + L-NAME group; received 24 mg/kg of *R. khorasanicum* extract (i.p) 30 min before L-NAME injection

2.5. Oxidative stress assessment

Heart and aorta tissues were removed after sacrificing the animals. The samples homogenized and oxidative stress markers including total thiol content (SH), malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) were measured. The stress oxidative kits were purchased from Sigma Company.

2. 6. Data analysis

The data were expressed as mean \pm SEM. Statistical analysis was done using the One-way ANOVA followed by the Tukey's post hoc test. A value of P < 0.05 was used to indicate statistical significance.

3. Result

3.1. Effects of L-NAME and SNP on cardiovascular responses

Injection of L-NAME significantly increased SBP and MAP. Changes (Δ) of SBP and MAP after injections of L-NAME are shown in Figure 1a, b. Maximal changes of SBP and MAP significantly increased compared to the control group (Δ SBP: 43.8 ± 7.5 mmHg and Δ MAP: 40.5 ± 6.7 mmHg; $P < 0.001$). HR decreased after injection of L-NAME but this reduction was not significant than the control group (Δ HR: 46.2 ± 11.6 beats/min; $P > 0.05$, Fig 1 c). In SNP + L-NAME group pretreatment of SNP significantly attenuate effect of L-NAME on SBP and MAP (Δ SBP: 15.5 ± 3.0 mmHg and Δ MAP: 16.0 ± 3.0 ; $P < 0.05$; Fig 1 a, b). SNP also ameliorate HR induced by L-NAME. However, this effect was not significant than L-NAME alone (Δ HR: -27.9 ± 27.7 beats / min, Fig 1c)

3.2. Effect of pretreatment with three doses of R. khorasanicum hydroalcoholic extract on the cardiovascular responses induced by L-NAME

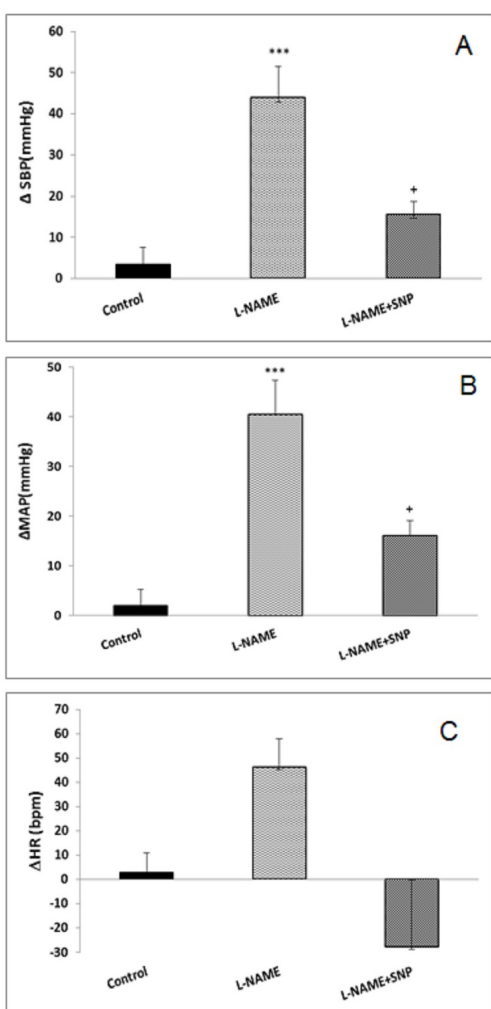


Figure 1 Effects of L-NAME on SBP(A), MAP(B) and HR (C) in anesthetized rats. Data were expressed as mean \pm SEM. one-way ANOVA followed by the Tukey's post hoc test used for Statistical analysis. (n=10) ***p < 0.001 compared to control, +p < 0.05 compare to L-NAME group

To determine the effect of R. khorasanicum on hypertension induced by L-NAME, Rats pretreated with three doses of extract (4, 12, and 24 mg/kg). After 30 min, L-NAME was injected and cardiovascular responses were evaluated. In dose 4 mg/kg, R. khorasanicum extract decreased both SBP and MAP compared to L-NAME but these effects were not significant (Δ SBP: 29.8 ± 2.9 mmHg and Δ MAP: 29.0 ± 2.5 ; $P > 0.05$, Fig 2a, b). In dose 12 mg/kg extract, MAP and bradycardia induced by L-NAME significantly attenuated in comparison to L-NAME group (Δ MAP: 14.0 ± 1.2 and Δ HR: 59.1 ± 11.5 mmHg; $P < 0.05$, Fig 2a, b).

In dose 24 mg/kg R. khorasanicum extract, increased SBP and MAP induced by L-NAME did not significantly attenuate by extract (Δ SBP: 38.5 ± 11.0 and Δ MAP: 34.1 ± 11.4 mmHg; $P > 0.05$, Fig 2a, b). However, this dose of extract in addition to reduced bradycardia induced by L-NAME also could significantly increase HR (Δ HR: 114.1 ± 22.1 beats/min, $P < 0.001$, Fig 2 c).

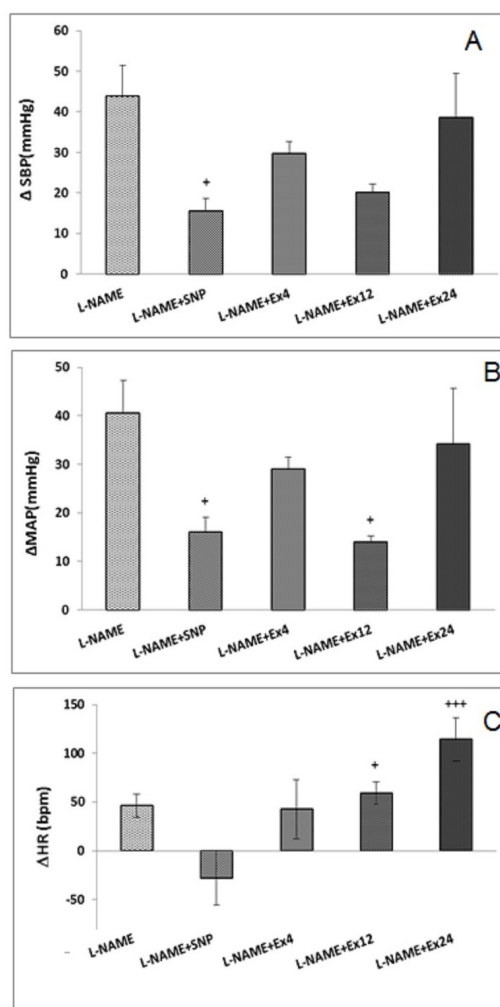


Figure 2 Effects of hydroalcoholic extract of R. khorasanicum on the SBP (A), MAP (B) and HR(C) in anesthetized rats. Data were expressed as mean \pm SEM. one-way ANOVA followed by the Tukey's post hoc test is used for Statistical analysis. (n=10) +p < 0.05, +++p < 0.001 compare to L-NAME group

3.3. Oxidative stress assessment in the heart and aorta tissues

Malondialdehyde (MDA) level in heart and aorta in the L-NAME group significantly increased compared to the control group ($P < 0.001$). Extract with dose 4 and 12 mg / kg significantly decreased the heart MDA level ($P < 0.01$ and $P < 0.001$, respectively) while in the aorta, extract with three doses significantly decreased the MDA level ($P < 0.001$) compared to L-NAME group (Fig 3.).

Also, in L-NAME group total thiol content in heart and aorta significantly decreased ($P < 0.001$) compared to control group. Extract with doses 12 and 24 mg / kg significantly increased the total thiol content in the heart ($P < 0.01 - P < 0.001$) while in the aorta, extract with three doses significantly increased the total thiol content than L-NAME group ($P < 0.01$ to $P < 0.001$) (Fig 4.).

Activity of superoxide dismutase enzyme (SOD) in the heart and aorta in the L-NAME group significantly reduced than the control group ($P < 0.001$) and extract with dose 24 mg / kg significantly increased this amount in heart ($P < 0.001$) while extract with three doses in aorta significantly increased compared to L-NAME group ($P < 0.05$ to $P < 0.001$) (Fig 5.).

Finally, the activity of catalase enzyme (CAT) in the heart and aorta in the L-NAME group significantly reduced than the control group ($P < 0.001$). Extract with doses 12 and 24 mg/kg significantly increased this amount in the heart and aorta ($P < 0.001$) (Fig 6.).

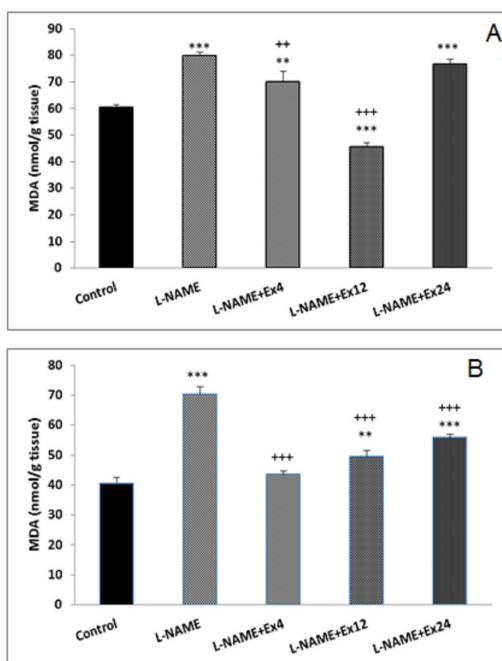


Figure 3 Effect of hydroalcoholic extract of *Ribes khorasanicum* on MDA concentration in the heart (A) and aorta (B) tissues of L-NAME hypertensive rats. Data are presented as Mean \pm SEM (n=10 in each group). ** $P < 0.01$, *** $P < 0.001$ compared to control group. ++ $p < 0.01$, +++ $p < 0.001$ compare to L-NAME group

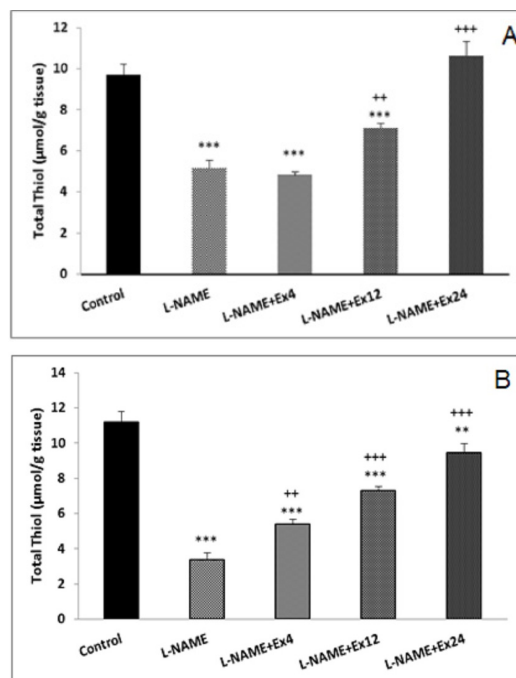


Figure 4 Effect of hydroalcoholic extract of *Ribes khorasanicum* on Total thiol content in the heart (A) and aorta (B) tissues of L-NAME. hypertensive rats.

Data are presented as Mean \pm SEM (n=10 in each group). ** $P < 0.01$, *** $P < 0.001$ compared to control group. ++ $p < 0.01$, +++ $p < 0.001$ compare to L-NAME group

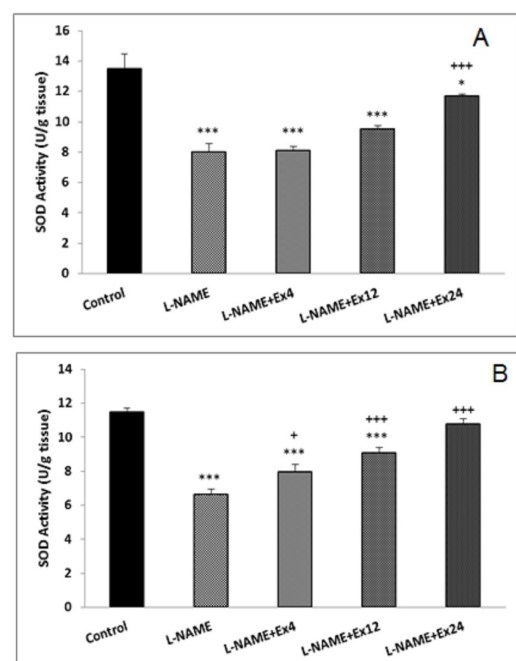


Figure 5 Effect of hydroalcoholic extract of *Ribes khorasanicum* on SOD activity in the heart (A) and aorta (B) tissues of L-NAME. hypertensive rats. Data are presented as Mean \pm SEM (n=10 in each group). * $P < 0.05$, *** $P < 0.001$ compared to control group. + $p < 0.05$, +++ $p < 0.001$ compare to L-NAME group

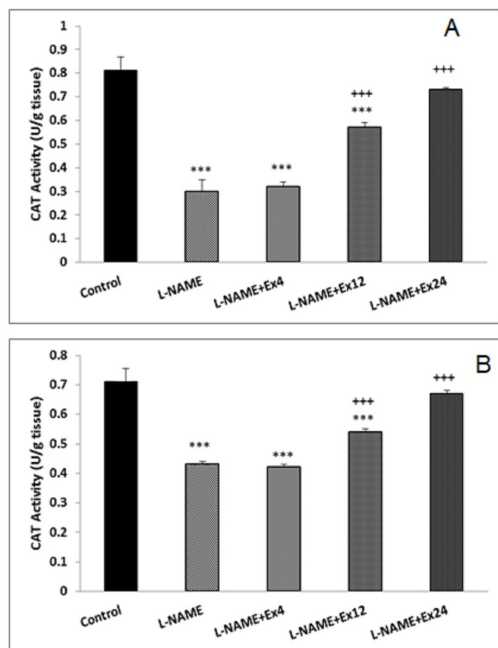


Figure 6 Effect of hydroalcoholic extract of *Ribes khorasanicum* on CAT activity in the heart (A) and aorta (B) tissues of L-NAME hypertensive rats. Data are presented as Mean \pm SEM (n=10 in each group). ***P < 0.001 compared to control group. +++p < 0.001 compared to L-NAME group
CAT: Catalase

4. Discussion

Our results indicated that L-NAME significantly increased SBP and MAP with no significant impact on HR and this effect was attenuated by SNP pretreatment. The dose 12 mg/kg of extract could significantly ameliorate MAP and HR changes induced by L-NAME that compares able with SNP. In a higher dose (24 mg/kg) extract did not affect SBP and MAP but significantly increased HR. In addition, in heart and aorta tissues of L-NAME group MDA increased and total thiol, catalase, and SOD activity decreased that ameliorate by the extract.

The NO is an important agent in the peripheral cardiovascular system that by relaxation of vessels reduce blood pressure [6].

Using of L-NAME as a nitric oxide synthase inhibitor and inhibition of nitric oxide production is an appropriate method for the evaluation of the role of NO in controlling blood pressure. Inhibition of NO production can increase blood pressure, which is accompanied by increased free radicals and oxidative stress [16, 17]. In line with our results, previous studies have shown that the antioxidant activity of superoxide dismutase, catalase, and glutathione peroxidase has decreased in the L-NAME administrated rats [17].

SNP is an antihypertensive drug that by an increased in the release of nitric oxide, relaxed smooth muscle of vessels and reduces blood pressure [18, 19]. In this study pretreatment of SNP significantly attenuate the effect of L-NAME on SBP and MAP. Three doses of hydroalcoholic extract of

R. khorasanicum also ameliorate the cardiovascular effect of L-NAME and this effect in dose 12 mg/kg was comparable with L-NAME+SNP. Based on these results we suggest that *R. khorasanicum* by the effect on NO production has a beneficial effect on hypertension. This effect of extract could be attributed to the stimulating effect of the extract on eNOS enzyme that increased NO bioavailability and causes vasodilation.

In this study, the effect of *R. khorasanicum* extract on oxidative stress parameters induced by L-NAME in cardiac and aortic tissues was also investigated. Injection of L-NAME induces oxidative stress that is in line with previous studies. Pretreatment with *R. khorasanicum* extract significantly reduced stress oxidative markers that confirm the antioxidant effect of this plant. Antioxidant effect of this plant attributed to compounds in *R. khorasanicum*. Ejtehadi et al. have been shown the presence of flavonoid compounds in *R. khorasanicum* [10], Flavonoids play an important role in NO release. For example, in a study has shown that flavonoids, by increasing the activity of the enzyme eNOS in the vasodilatation and increasing the production of NO, cause vasodilator effect and lower blood pressure [20]. In addition, it was reported that *R. khorasanicum* contain a high level of anthocyanin [11], a member of the flavonoid family. Anthocyanin has several beneficial effects such as increased NO production, anti-inflammatory, and antioxidant properties [21, 22]. Therefore, the cardiovascular effect of this plant may be mediated by this compound. There is evidence that in the L-NAME hypertension sympathetic nerve activity evoked [23] and by increased vascular tone improved hypertension [23]. It is possible that the *R. khorasanicum* by the effect on the sympathetic system exerts its antihypertensive effect. Since in this study the extract decreases oxidant parameter and increased antioxidant agents, it seems that *R. khorasanicum* is a potent antioxidant fruit that through the influence on NO improve hypertension.

In this experiment medium dose (12 mg/kg) has greatest effect in hypertension induced by L-NAME. so it could significantly attenuate MAP and SBP induced by L-NAME and increased HR. mechanism(s) of tachycardia induced by extract is unknown but it is suggested baroreflex activity are involved. In this reflex decreased of blood pressure increased HR. In present time the cardiovascular effect of *R. khorasanicum* isn't determined and more studies are needed to elucidate the mechanism(s) antihypertensive effect of this plant.

5. Conclusion

This study was showed that *R. khorasanicum* extract has a protective effect on hypertension. Because extract attenuates the cardiovascular effect of L-NAME and improved oxidative stress it is suggested that anti-hypertensive effects of *R. khorasanicum* partly mediated by an effect on NO and stress oxidative parameters.

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