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Original Article

Antihypertensive Effects of Hydroalcoholic Extract of *Crataegus Azarolus Subspecies Aronia* Fruit in Rats with Renovascular Hypertension: An Experimental Mechanistic Study

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What's Known

- It has been shown that various species of Hawthorn have antihypertensive, inotropic, antihyperlipidemic, antidiabetic, and antiarrhythmic effects.
- *Crataegus azarlus subspecies aronia* is one of the Hawthorn species grown in Fars province, Iran. A far as the literature is concerned the antihypertensive activity of such a species has not been studied.

What's New

- Hydroalcoholic extract of *Crataegus azarlus subspecies aronia* fruit has antihypertensive effects in rats with two-kidney, one-clip renovascular hypertension.
- The antihypertensive effects may be attributed to antioxidant and enhancement of endothelium-derived nitric oxide releasing effects.

Abstract

Background: Hawthorn species decreases blood pressure and relaxes precontracted vessels. This study aimed at examining the antihypertensive effect and related mechanisms of hydroalcoholic extract of *Crataegus azarolus subspecies aronia* fruit in rats with renovascular hypertension.

Methods: Six groups of male Sprague-Dawley rats, each containing 6 to 8 rats, were studied. The groups comprised of one sham group and 5 renal artery-clipped groups. The sham group received vehicle (distilled water 0.5 ml/day) and the renal artery-clipped groups received vehicle or the extract at 5, 10, 20 or 30 mg/kg/day. Oral vehicle or extract was administered daily for 4 weeks following sham-operation or induction of hypertension. Systolic blood pressure and heart rate were measured weekly. Isolated aorta study was performed by last week and serum superoxide dismutase and glutathione reductase were measured. The findings were analyzed using one-way analysis of variance and Duncan's multiple range tests at $P \leq 0.05$ using SigmaStat software.

Results: The data obtained after 4 weeks of treatment showed that the renal artery-clipped group receiving vehicle had significantly higher systolic blood pressure (P=0.002) and phenylephrine maximal response (P=0.01); and lower acetylcholine maximal response (P=0.01), serum superoxide dismutase (P=0.006) and serum glutathione reductase (P=0.006) than those of the sham group. The renal artery-clipped group receiving extract had significantly lower systolic blood pressure (P=0.03) and phenylephrine maximal response (P=0.01); and significantly higher acetylcholine maximal response (P=0.01), serum superoxide dismutase (P=0.015), and serum glutathione reductase (P=0.015) than those of the renal artery-clipped group receiving vehicle.

Conclusion: Our findings show that the hydroalcoholic extract of *Crataegus azarolus subspecies aronia* fruit has antihypertensive effects, which may be partly due to antioxidant and nitric oxide releasing effects.

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Keywords ● Crataegus ● Renovascular ● Nitric oxide ● Oxidative stress ● Hypertension

Introduction

Hypertension is one of the main public health challenges around the world that leads to cardiovascular and cerebrovascular disabilities. The number of people with hypertension is on the rise and predicted to increase to 1.5 billion by 2025.[1] Hypertension is mainly treated using a variety of synthetic drugs, including angiotensin-converting-enzyme inhibitors, angiotensin II receptor blockers, calcium channel blockers, and beta adrenergic receptor antagonists.[2] However, there has been an upsurge of interest in using herbal drugs to control blood pressure in hypertensive patients.[3] Various hawthorn species (Crataegus spp.) have been shown to decrease blood pressure in rats with L-NAME-induced hypertension,[4] normotensive rats,[5,6] relaxed precontracted rat aorta,[7-9] mesenteric artery,[10] porcine coronary artery,[11] and human internal mammary artery[8] rings. The blood pressure lowering or vasorelaxant effects of hawthorn species have been attributed to enhanced nitric oxide (NO) release,[4,6,8] decreased oxidative stress,[9,10] and proinflammatory cytokines.[9]

One of the hawthorn species, *Crataegus azarolus subspecies aronia* (zaalzaalak in Persian) grows natively in Fars province, Southern Iran, in an area extending from Noorabad to Dashtarzhan and Khanzenyan districts.[12] As far as literature is concerned, the antihypertensive activity of such species has not been studied using animal models. Hence, the present study was designed to examine the possible antihypertensive effects of hydroalcoholic extract of *Crataegus azarolus subspecies aronia* fruit in a rat model of two-kidney, one-clip renovascular hypertension. We also aimed at investigating whether endothelium-derived NO and antioxidative stress were involved in such effects.

Materials and Methods

Plant Collection and Extract Preparation

Crataegus azarolus subspecies aronia fruit was collected from the mountains around Noorabad (Fars province, Iran) in September 2013. The exact genus and species of the plant were characterized by a specialist at the Department of Pharmacognosy, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran. Seeds were removed from the fruit and the pulp was dried at room temperature for two weeks and then ground into powder. Hydroalcoholic (70% ethanol and 30% distilled water v/v) extract of the powder was prepared using the percolation method.[13] The yield was about 30-35% w/w.

Animals

Male Sprague-Dawley rats weighing 200-250 g were obtained from Animal Breeding Center, Shiraz University of Medical Science, Shiraz, Iran. They were kept under standard conditions (12:12 light-dark cycle, temperature 20-22 °C, and humidity 25%-35%) with rat chow and water ad libitum. Animals were cared for according to the university guidelines for laboratory animal care and use.

Experimental Design and Protocol

The animals were anesthetized with 60 mg/kg ketamine (Pan Pharama, Trittau, Schleswig-Holstein, Germany) and 8 mg/kg xylazine (Alfasan, Woerden, The Netherlands). As described by Nekooeian et al.,[14] these were administered to sham-operation or placement of plexiglass clips on the left renal artery. After shaving the left flank area, incisions were made in the skin and abdominal walls to expose the left kidney. Left renal arteries were gently dissected away from the left renal veins and surrounding tissues. Then, plexiglass clips (internal diameter of 0.20-0.22 mm) were placed on the arteries as close to aortas as possible. Penicillin antibiotic in powder form (Jaber Ebne Hayyan Pharmaceutical Company, Tehran, Iran) was then applied to the incision sites. Finally, the abdominal walls and skins were closed using absorbable (catgut) and non-absorbable (silk) suture materials, respectively. Sham-operated rats were subjected to the same procedure, but no clip was placed on the left renal arteries. The animals were then recovered from anesthesia and kept under standard conditions.[14]

A day after the operations, animals were divided into 6 groups, each containing 6 to 8 rats. The sham group (Sham-V) was assigned to receive distilled water (0.5 ml/day) as vehicle, the left renal artery-clipped group (RAC-V) was assigned to receive the vehicle, and the remaining four left renal artery-clipped groups were assigned to receive hydroalcoholic extract of *Crataegus azarolus subspecies aronia* fruit at 5 mg/kg/day (RAC-E5), 10 mg/kg/day (RAC-E10), 20 mg/kg/day (RAC-E20), or 30 mg/kg/day (RAC-E30). The vehicle and the extract were administered daily by oral gavage for the following 4 weeks. On days 7, 14, 21, and 28, systolic blood pressure (SBP) and heart rate (HR) were measured using non-invasive tail-cuff method (Chart 5.0 software, PowerLab 4/30, AD Instruments Inc., MA, Australia). Three consecutive SBP measurements, which had a difference of less than 5 mmHg, were considered as valid. The mean of the three such measurements was

noted as the SBP value for every instance. The HR was counted from SBP upstrokes. The HR for every instance was the average of the HRs derived from the three SBP measurements, which were used for the calculation of SBP.

On day 28, after SBP and HR measurements, animals were anesthetized using 60 mg/kg thiopental sodium (Biochem GmbH, Vienna, Austria). Then, the chest cavities of animals were opened, thoracic aortas were cut, and blood samples were collected from the blood pools of the animals' chest cavity. The thoracic aortas were then dissected and used to study isolated aorta. The blood samples were allowed to clot for 30 minutes, centrifuged at 3,000 rpm for 20 minutes, and their sera were separated and stored at -80 °C until analysis of the measured superoxide dismutase (SOD) and glutathione reductase (GR).

Isolated Aorta Study

The isolated aorta study was performed as described by Nekooeian et al.[15] Thoracic aortas were cleaned by removing the surrounding connective tissues, sliced into 3-4 mm length rings, and were mounted on hooks connected to force transducers, scheduler organ bath apparatus (Hugo Sachs Electronik-Harvard Apparatus GmgH, March Hugstetten, Germany). The organ baths were filled with 20 ml physiological solution containing the following composition (mmol/L): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, and D-glucose 11.1. The solution was bubbled constantly with O_2 (95%) and CO_2 (5%) at a pH of 7.4 and a temperature of 37ºC. Tensions were recorded with a four-channel polygraph (Model 705/1, Hugo Sachs Electronik, Germany). The tissues were allowed to stabilize for 60 minutes. Then, full concentration-responses to phenylephrine (Phe) (Sigma-Aldrich Chemical Co., Steinheim, Germany) were performed. After two washes and 30-minute equilibration, each ring was contracted with Phe using concentrations that caused a contraction similar to that of 50% of the maximal contraction in the Sham-V group. Dose-relaxation responses to acetylcholine (Ach) or sodium nitroprusside (SNP) (Sigma-Aldrich Chemical Co., Steinheim, Germany) were performed at the plateau of contractile response to Phe.[15]

Biochemical Measurements

Serum levels of SOD and GR were determined using Biorexfars chemical kits (Shiraz, Iran). The method to measure SOD was based on using xanthine and xanthine oxidase to generate superoxide radicals that react with 2-(4-iodophenyl)-3 (4-nitrophenol)-5-phenyl tetrazolium chloride to form a red formazan dye. Superoxide dismutase activity was measured by the degree of inhibition of this reaction. The assay to measure serum GR was based on oxidation of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) to NADP catalyzed by a limiting concentration of glutathione reductase. One GR unit is defined as the amount of enzyme catalyzing the reduction of one micromole of oxidized glutathione. One molecule of oxidized glutathione is consumed for each molecule reduction of oxidized glutathione. Therefore, the reduction of oxidized glutathione was determined indirectly by measuring the consumption of NADPH.

Calculations of Pharmacological Parameters

The EC_{50} (the concentration that causes 50% of maximal response) of Phe, and IC_{50} (the concentration that causes 50% of maximal inhibition) of Ach and SNP were calculated using Curve Expert software (version 1.34).

Statistical Analysis

The data, presented as mean±SEM, were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests for pairwise comparisons. P values ≤0.05 were considered statistically significant. Statistical analysis was performed using Sigma Stat statistical software (version 3.0) and illustrations were prepared using Sigma Plot software (version 8.0).

Results

The data obtained after one week of treatment showed that the SBP of RAC-V group was significantly (P=0.02) higher than that of the Sham-V group (table 1). Moreover, there was no significant difference between the SBP of RAC-V group and those of the groups receiving *Crataegus azarolus subspecies aronia* extract at 5 mg/kg/day (RAC-E5), 10 mg/kg/day (RAC-E10), 20 mg/kg/day (RAC-E20), or 30 mg/kg/day (RAC-E30) (table 1). At week 2, the SBP of RAC-V group was significantly (P=0.02) higher than that of the Sham-V group. There was no significant difference between the SBP of RAC-E5 and RAC-V groups, but the SBPs of other extract-receiving groups (RAC-E10, RAC-E20, and RAC-E30) were significantly (P=0.03) lower than that of the RAC-V group (table 1). At weeks 3, the SBP of RAC-V group was significantly (P=0.02) higher than that of the Sham-V group. There was no significant difference between the SBP

of RAC-E5 and RAC-V groups. Moreover, the SBPs of other extract-receiving groups (RAC-E10, RAC-E20, and RAC-E30) were significantly (P=0.01) lower than that of the RAC-V group. At weeks 4, the SBP of RAC-V group was significantly (P=0.02) higher than that of the Sham-V group. However, the SBPs of other extract-receiving groups (RAC-E10, RAC-E20, and RAC-E30) were significantly (P=0.01) lower than that of the RAC-V group (table 1).

There was no significant difference between the HRs of the Sham-V, RAC-V, RAC-E5, RAC-E10, RAC-E20, and RAC-E30 groups at week 1, 2, 3, or 4 of the treatment (table 2).

The E_{max} of contraction response to Phe in aortic rings from the RAC-V group was significantly (P=0.01) higher than that of the Sham-V group (table 3, figure 1A). The E_{max} of other extract-receiving groups (RAC-E5, RAC-E10, RAC-E20, and RAC-E30) were significantly (P=0.01) lower than that of the RAC-V group (table 3, figure 1A).

The EC_{50} of contraction response to Phe in aortic rings from the RAC-V group was significantly (P=0.001) lower than that of the Sham-V group (table 3). The EC_{50} of RAC-E5 was significantly (P=0.01) higher than that of the Sham-V group. In addition, the EC_{50} of other extract-receiving groups (RAC-E10, RAC-E20, and RAC-E30) were significantly (P=0.001) higher than that of the RAC-V group (table 3).

The E_{max} of relaxation response to Ach in aortic rings from the RAC-V group was significantly (P=0.01) lower than that of the Sham-V group (table 3, figure 1B). The E_{max} of the extract-receiving groups (RAC-E5, RAC-E10, RAC-E20, and RAC-E30) were significantly (P=0.01) higher than that of the RAC-V group (table 3, figure 1B).

The IC_{50} of relaxation response to Ach in aortic rings from the RAC-V group was significantly (P=0.001) higher than that of the Sham-V group (table 3). The IC_{50} of relaxation response to Ach in aortic rings from the RAC-E5 group was significantly (P=0.01) lower than that of the Sham-V group. In addition, the E_{max} of other extract-receiving groups (RAC-E10, RAC-E20, and RAC-E30) were significantly (P=0.001) lower than that of the RAC-V group (table 3).

There was no significant difference between the E_{max} of relaxation response to SNP of aortic rings from the Sham-V, RAC-V, RAC-E5,

Table 1: Weekly values of systolic blood pressure following treatment with vehicle or *Crataegus azarolus subspecies aronia* extract. Data obtained from all groups during weeks 1 to 4

Systolic blood pressure (mmHg)				
	Week 1	**Week 2**	**Week 3**	**Week 4**
Sham-V	123.9±0.9	119.7±1.4	124.9±1.3	120.1±1.4
RAC-V	168.5±8.7*	180.8±5.1*	202.1±12.2*	217.1±14.2*
RAC-E5	171.9±4.2	160.0±6.1	162.3±5.2	153.5±5.8$^{\Delta}$
RAC-E10	130.7±3.2	137.2±2.7$^{\Delta}$	156.3±8.9$^{\Delta\Delta}$	151.1±11.4$^{\Delta\Delta}$
RAC-E20	154.2±8.1	135.8±6.5$^{\Delta}$	140.2±1.6$^{\Delta\Delta}$	142.2±3.5$^{\Delta\Delta}$
RAC-E30	153.8±7.9	132.1±8.3$^{\Delta}$	143.5±7.6$^{\Delta\Delta}$	156.5±4.4$^{\Delta\Delta}$

The values (n=6-8) are presented as mean±SEM. Sham-V: Sham-operated group receiving vehicle (distilled water; 0.5 ml/day), RAC-V: Left renal artery-clipped group receiving the vehicle, RAC-E5: Left renal artery-clipped group receiving 5 mg/kg/day *Crataegus azarolus subspecies aronia* extract, RAC-E10: Left renal artery-clipped group receiving the extract at 10 mg/kg/day, RAC-E20: Left renal artery-clipped group receiving the extract at 20 mg/kg/day extract, RAC-E30: Left renal artery-clipped group receiving the extract at 30 mg/kg/day. *Significant difference (P=0.002) from Sham-V group; $^{\Delta}$Significant (P=0.03) difference from RAC-V group; $^{\Delta\Delta}$Significant (P=0.01) difference from RAC-V group

Table 2: Weekly values of heart rate following treatment with vehicle or *Crataegus azarolus subspecies aronia* extract. Data obtained from all groups during weeks 1 to 4

Heart rate (beats/min)				
	Week 1	**Week 2**	**Week 3**	**Week 4**
Sham-V	406.1±3.4	399.8±4.4	401.7±3.4	410.8±3.7
RAC-V	463.9±18.1	393.7±9.5	443.8±7.2	365.9±20.8
RAC-E5	359.7±8.3	362.8±14.8	342.9±6.4	339.6±13.1
RAC-E10	359.6±12.1	353.6±12.5	350.8±12.2	374.5±8.6
RAC-E20	418.8±15.2	409.9±12.3	428.7±19.5	407.8±5.2
RAC-E30	396.6±8.2	382.8±6.8	384.8±6.6	401.6±16.9

The values (n=6-8) are presented as mean±SEM. Sham-V: Sham-operated group receiving vehicle (distilled water; 0.5 ml/day), RAC-V: Left renal artery-clipped group receiving the vehicle, RAC-E5: Left renal artery-clipped group receiving 5 mg/kg/day *Crataegus azarolus subspecies aronia* extract, RAC-E10: Left renal artery-clipped group receiving the extract at 10 mg/kg/day, RAC-E20: Left renal artery-clipped group receiving the extract at 20 mg/kg/day, RAC-E30: Left renal artery-clipped group receiving the extract at 30 mg/kg/day

Table 3: The pharmacological parameters of phenylephrine and acetylcholine dose-response curves of all experimental groups after 4 weeks of treatment with vehicle or *Crataegus azarolus subspecies aronia* extract

	Phenylephrine		Acetylcholine	
	E_{max} (% contraction)	EC_{50} -Log (M)	E_{max} (%relaxation)	IC_{50} -Log (M)
Sham-V	100±0.00	-7.5±0.75	85.9±4.1	-7.5±0.20
RAC-V	160.9±6.1*	-8.3±0.32**	70.3±4.9*	-5.3±0.07**
RAC-E5	112.9±10.2$^{\Delta}$	-7.9±0.52$^{\Delta}$	84.4±3.4$^{\Delta}$	-5.9±0.01$^{\Delta}$
RAC-E10	139.8±7.1$^{\Delta}$	-7.2±0.21$^{\Delta\Delta}$	79.7±2.3$^{\Delta}$	-6.1±0.11$^{\Delta\Delta}$
RAC-E20	135.6±4.7$^{\Delta}$	-7.2±0.32$^{\Delta\Delta}$	85.3±4.7$^{\Delta}$	-6.2±0.19$^{\Delta\Delta}$
RAC-E30	136.3±7.1$^{\Delta}$	-7.2±0.14$^{\Delta\Delta}$	83.5±5.1$^{\Delta}$	-6.2±0.45$^{\Delta\Delta}$

The values (n=6-8) are presented as mean±SEM. Sham-V: Sham-operated group receiving vehicle (distilled water; 0.5 ml/day), RAC-V: Left renal artery-clipped group receiving the vehicle, RAC-E5: Left renal artery-clipped group receiving 5 mg/kg/day *Crataegus azarolus subspecies aronia* extract, RAC-E10: Left renal artery-clipped group receiving the extract at 10 mg/kg/day, RAC-E20: Left renal artery-clipped group receiving the extract at 20 mg/kg/day, RAC-E30: Left renal artery-clipped group receiving the extract at 30 mg/kg/day. *,**Significant (*P=0.01, **P=0.001) difference from Sham-V group; $^{\Delta,\Delta\Delta}$Significant ($^{\Delta}$P=0.01, $^{\Delta\Delta}$P=0.045) difference from RAC-V group

RAC-E10, RAC-E20, and RAC-E30 groups (figure 1C).

Serum concentration SOD of the RAC-V group was significantly lower (P=0.006) than that of the Sham-V group (table 4). There was no significant difference between serum SOD of the RAC-E5 and RAC-V groups. Serum SOD of the RAV-E10 group was significantly (P=0.04) higher than that of the RAC-V groups. Moreover, the serum levels of SOD levels of other extract-treated groups (RAC-E20 and RAC-E30) were significantly (P=0.015) higher than that of the RAC-V group (table 4).

Serum concentration GR of the RAC-V group was significantly lower (P=0.006) than that of the Sham-V group (table 4). Serum GR of the RAC-E5 group was significantly (P=0.04) higher than that of the RAC-V groups. Moreover, serum levels of GR for the other extract-treated groups (RAC-E10, RAC-E20, and RAC- E30) were significantly (P=0.015) higher than that of the RAC-V group.

Table 4: The values of serum biomarkers of oxidative stress following treatment with vehicle or *Crataegus azarolus subspecies aronia* extract. Data obtained from all groups in week 4

	SOD (unit/ml)	GR (unit/ml)
Sham-V	239.7±27.3	78.8±3.9
RAC-V	135.9±22.1*	38.9±1.4*
RAC-E5	138.3±13.2	64.8±4.6$^{\Delta}$
RAC-E10	173.8±18.9$^{\Delta}$	62.3±3.2$^{\Delta\Delta}$
RAC-E20	207.8±24.3$^{\Delta\Delta}$	58.6±4.7$^{\Delta\Delta}$
RAC-E30	222.5±26.6$^{\Delta\Delta}$	59.8±2.1$^{\Delta\Delta}$

The values (n=6-8) are presented as mean±SEM. SOD: Superoxide dismutase, GR: Glutathione reductase, Sham-V: Sham-operated group receiving vehicle (distilled water; 0.5 ml/day), RAC-V: Left renal artery-clipped group receiving the vehicle, RAC-E5: Left renal artery-clipped group receiving 5 mg/kg/day *Crataegus azarolus subspecies aronia* extract, RAC-E10: Left renal artery-clipped group receiving the extract at 10 mg/kg/day, RAC-E20: Left renal artery-clipped group receiving the extract at 20 mg/kg/day, RAC-E30: Left renal artery-clipped group receiving the extract at 30 mg/kg/day. *Significant (P=0.006) difference from Sham-V group; $^{\Delta,\Delta\Delta}$Significant ($^{\Delta}$P=0.04, $^{\Delta\Delta}$P=0.015) difference from RAC-V group

Discussion

The findings of the present study show that the placement of solid plexiglass clips on the left renal arteries results in renovascular hypertension associated with attenuated release of NO and increased oxidative stress. Moreover, the findings show that the administration of hydroalcoholic extract of *Crataegus azarolus subspecies aronia* attenuates the development of hypertension. The findings also show that the antihypertensive effects of the extract might be attributed to the enhancement of NO release and the attenuation of oxidative stress.

In agreement with our past investigations using plexiglass clips,[14] and those of Kaur et al.,[16] the present study shows that the placement of clips on the left renal arteries results in renovascular hypertension. Moreover, our findings show that the present model of renovascular hypertension is associated with increased contraction response to Phe.[17] The increased responsiveness to Phe may be related to decreased endothelium-derived relaxing factors such as NO[18] or increased contracting factors such as endoperoxides,[19] thromboxane A_2 and endothelin.[20]

The present model of renovascular hypertension was also associated with decreased and unchanged vasorelaxation response to Ach and SNP, respectively. Acetylcholine vasorelaxant action is mediated by muscarinic M3 receptors activation,[21] which leads to the release of NO from endothelial cells.[22] Vasorelaxation response to Ach in isolated aortic rings has been taken as an indication of the

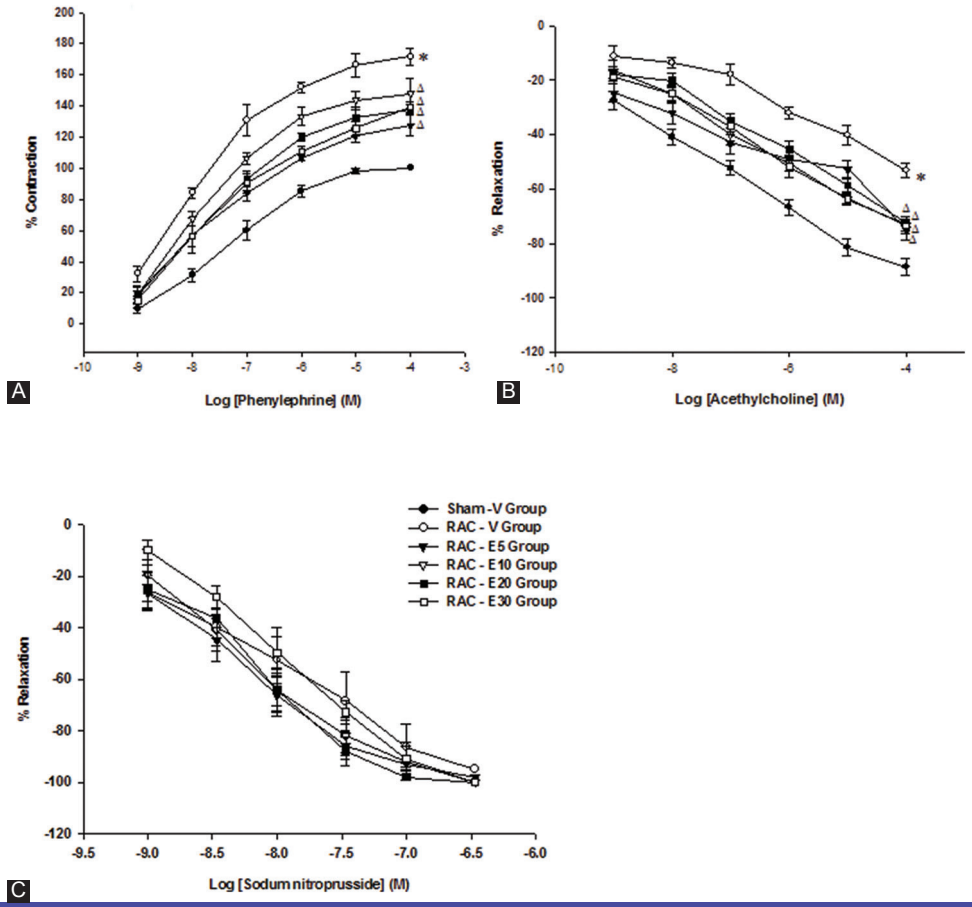

Figure 1: Concentration-response curves (phenylepherine (A), acetylcholine (B), and sodium nitroprusside (C)) in aortic rings from all groups after 4 weeks of treatment with vehicle or *Crataegus azarolus subspecies aronia* extract. The data points (n=6-8 each) are as mean±SEM. Sham-V: Sham-operated group receiving 0.5 ml/day distilled water as vehicle, RAC-V:Left renal artery-clipped group receiving the vehicle, RAC-E/: The extracts at 5 (RAC-E5), 10 (RAC-E10), 20 (RAC-E20), and 30 (RAC-E30) mg/kg/day. *Significant (P=0.002) difference from Sham-V group; *Significant (P=0.01) difference from Sham-V group; ᐞSignificant (P=0.01) difference from RAC-V group.

status of NO release and vascular endothelium-dependent relaxation,[18] whereas that of SNP has been used as an evidence of the status of endothelium-independent relaxation.[23] These findings are in agreement with our report[15] and Kaur et al.[16] that the renovascular model of hypertension and other models of hypertension such as spontaneous hypertensive rats[24] were associated with a decreased release of NO. Moreover, inhibitors of NO synthesis are associated with increased blood pressure.[10] Nitric oxide is believed to modulate blood pressure in vivo[25] by activating soluble guanylate cyclase and thereby increasing intracellular cGMP, which leads to the decrease of intracellular calcium and sensitivity of contractile proteins, and subsequent relaxation of vascular smooth muscle.[26] Therefore, the attenuated release of NO in the present model may partly contribute to the development of hypertension.

The findings also show that the present model of renovascular hypertension is associated with decreased antioxidants such as SOD and GR, indicating that the model is associated with increased levels of oxidative stress. The increased level of oxidative stress may partly justify the increase of blood pressure in the present model, as it has been previously shown that two-kidney, one-clip renovascular hypertension[15] and other models of hypertension[27] were associated with increased levels of oxidative stress. Two-kidney, one-clip renovascular hypertension is the result of increased activities of rennin-angiotensin system and subsequent increase in serum levels of angiotensin II,[28] which has been shown to increase indices of oxidative stress.[29]

The present study shows that at doses examined, the hydroalcoholic extract of *Crataegus azarolus subspecies aronia* attenuates the development of hypertension. The Crataegus species examined in the present study is native to Fars province, Southern Iran. As far as literature is concerned, to the best of our knowledge, there is no published study on its antihypertensive or vasorelaxant activity. Our finding is in agreement with those of previous

studies that demonstrated other species of Crataegus decreased the blood pressure in rats with L-NAME-induced-hypertension[4] and normotensive rats.[5,6] Moreover; our findings are similar to those studies showing that other various Crataegus extracts relaxed precontracted rat aorta,[7-9] mesenteric artery,[10] porcine coronary artery,[11] and human internal mammary artery.[8]

Our findings show that hydroalcoholic extract of *Crataegus azarolus subspecies aronia* improves the endothelium-dependent relaxation response to Ach. This finding is in agreement with previous reports demonstrating that other Crataegus species, including *Crataegus tanacetifolia,*[4] *Crataegus aronia syn. Azarolus,*[6] *Crataegus microphyhlla,*[9] and a special extract prepared from *Crataegus* oxyacantha or *Crataegus* monogyna,[8,10,11] enhanced endothelium-dependent relaxation by Ach. The improved endothelium-dependent relaxation response to Ach has been taken as a functional evidence of enhanced NO release.[30] Moreover, donors of NO and stimulants of NO release were associated with decreased blood pressure[31] or vasorelaxation.[8] Therefore, it is reasonable to suggest that the enhancement of NO release may partly explain the antihypertensive effects of *Crataegus azarolus subspecies aronia* extract. Improvement of NO release has been reported to explain the antihypertensive effects of other herbs, including *Allium eriophyllum*[32] and *Ginkgo biloba*[33] as well as synthetic drugs such as inorganic nitrates.[31]

The present study shows that the administration of *Crataegus azarolus subspecies aronia* extract to renovascular hypertensive rats reduces the level of oxidative stress characterized by increased levels of SOD and GR. Similar antioxidant effects have been reported for other species of Crataegus, including *Crataegus microphylla*[9] and a special extract prepared from *Crataegus* oxyacantha or *Crataegus* monogyna.[10,11] It has been suggested that by increasing intracellular calcium in vascular smooth muscle,[8,34] decreasing bioavailability of NO and prostacyclin,[35] and increasing central sympathetic outflow,[36] oxidative stress contributes to the pathophysiology of hypertension. Our findings show that the extract increased endothelium-derived NO, as indicated by increased endothelium-dependent relaxation to Ach. Therefore, it is reasonable to conclude that the antihypertensive effects of the extract may be partly attributed to the reduction of oxidative stress, which can result in increased bioavailability of NO.

The findings of the present study should be interpreted in the light of at least two limitations. First, improved endothelium-dependent relaxation response to Ach was taken as a functional evidence of enhanced NO release. Although this method is valid, however, the measurement of serum levels of NO metabolites (NO_3^-/NO_2^-) could give a more direct measurement of serum NO. Moreover, although the used doses of Crataegus extract did not cause any mortality, prior toxicology test could provide a range of doses over which the study could be performed.

Conclusion

The findings of the present study have shown that the placement of plexiglass clips on the left renal arteries led to renovascular hypertension associated with increased sensitivity to Phe, impaired release of NO, and increased oxidative stress. It has also shown that increased release of NO and attenuation of oxidative stress may partly explain the antihypertensive effects of *Crataegus azarolus subspecies aronia* hydroalcoholic extract. Our findings may be used as a rationale for further preclinical as well as clinical studies using various extracts and fractions of *Crataegus azarolus subspecies aronia* fruit.

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