

## Subchronic Administration of Aqueous Garlic (*Allium Sativum* L.) Extract Attenuates $\alpha_1$ -Adrenoceptor Agonist-Induced Contraction of Isolated Aorta in Rat

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### Abstract

**Introduction:** Garlic is considered to be one of the best disease preventive foods because of its potent and widespread effects. On the other hand, some cardiovascular disorders including hypertension are accompanied with altered responsiveness of vascular alpha-adrenergic receptors. The purpose of this study was to investigate the effect of aqueous garlic extract on alpha<sub>1</sub>-adrenoceptor agonist-induced contraction of rat aorta, aorta ring from rats pretreated with aqueous garlic extract were used as a model in this study.

**Materials and Methods:** Four and eight weeks after treatment with garlic extract, aortic rings were studied for contractile and relaxation response to phenylephrine (PE) and acetylcholine, respectively.

**Results:** The results showed that the contractile response of aortic rings to PE in garlic treated rats for 4 and 8 weeks decreased ( $P < 0.05$ ), and their endothelium-dependent relaxation response increased in comparison with the controls ( $P < 0.05$ ). The relaxing activity of garlic on rat aorta was time-dependent and this effect was attenuated following endothelium removal.

**Conclusion:** These data suggest that alpha<sub>1</sub>-adrenoceptor antagonistic action of garlic on rat aorta involves at least two different mechanisms: one is direct relaxing action of garlic on smooth muscle, and the other is indirect and dependent on garlic effect on alpha<sub>1</sub>-adrenoceptors signal transduction cascade.

**Key words:** Aqueous garlic extract, Aortic rings, Alpha<sub>1</sub>-adrenoceptor agonist, Phenylephrine, Rat



## Introduction

Alteration in blood vessels reactivity to neurotransmitters and circulating hormones has been suggested to cause or contribute to some of the cardiovascular disease such as hypertension (1, 2, 3), in which adrenergic responsiveness and adrenoceptors undergo some modifications (4). There is some evidence concerning the role of alpha-adrenoceptors as a mediator in blood pressure maintenance, and occurrence of contractile response in aorta (5). Recent advances in the use of pre-hypertensive rats, spontaneously hypertensive rats with one kidney and renal hypertension, suggest that vascular alpha-adrenoceptor involvement in the contraction of a variety of vessels and its role in the control of blood pressure (6). Treatment strategies of hypertension continue to be carried out by drugs combined with alteration in nutritional habits. Yet, hypertension management did not improve, and recent research has focused on the possibility of preventing the disease.

Today, herbs have been as an integral part of society, valued for both their culinary and medicinal properties. Among them, alliums such as garlic have been studied extensively for their health benefits. Garlic has demonstrated multiple beneficial effects on the cardiovascular system through reduction of blood pressure (7-14), inhibiting platelet aggregation and thromboxane synthesis (15-18), enhancing fibrinolytic activity (13), and lowering serum cholesterol and triglyceride levels (19-22). Interestingly, in one cross-section observation study of older patients, garlic intake was found to reduce age-related increase in aortic stiffness (23). In recent years, research has focused on anti-hypertensive effect of garlic and its use in preventing atherosclerosis (24). While, the hypotensive effect of garlic has been repeatedly confirmed in human (7) and animals (8), however, there has been no report concerning garlic extract effect on blood vessels reactivity to alpha<sub>1</sub>-adrenoceptor agonists. Therefore, the present study was carried out to investigate the effect of subchronic administration of aqueous garlic extract on alpha<sub>1</sub>-adrenoceptor agonist-induced contraction. Aortic rings from rats pretreated with aqueous garlic

extract were used as a model in this study.

## Materials and Methods

### \* Preparation of aqueous garlic extract:

Fresh garlic was purchased from a local grocery store and garlic extract was prepared according to the method described previously by alnaqeeb et al. (25). Briefly, fresh garlic cloves were peeled on crushed ice, and 50 g of garlic was homogenized in 75 ml of cold, sterile 0.9% saline in the presence of some crushed ice. The filtered homogenized mixture was then centrifuged at 2000×g for 10 min and the clear supernatant was made up to 100 ml with normal saline. The concentration of garlic extract was 500 mg/ml and it was stored at -20°C until use.

### \* Animals

Male albino rats of the Sprague-Dawley strain (Razi institute, Tehran) weighing 220-250 g were housed in an air-conditioned colony room on a light/dark cycle at 21±3°C and supplied with a standard pellet diet and tap water ad libitum. Procedures involving care were conducted in accordance with the NIH guidelines. Their body weight was initially determined and also 4 to 8 weeks later, after receiving intraperitoneal injection of saline or garlic extract. Two groups of normal rats were also selected for obtaining the concerned normal values. In the experiment, totally 4 groups (eight rats each) were used:

Group A: Control rats received saline (0.5 ml/ kg body weight/ day) for 4 weeks

Group B: Control rats received saline (0.5 ml/ kg body weight / day) for 8 weeks

Group C: Extract-treated rats received 0.5 ml of garlic extract (100 mg/kg body weight/day) for 4 weeks

Group D: Extract-treated rats received 0.5 ml of garlic extract (100 mg/ kg body weight / day) for 8 weeks

### \* Preparation of aortic rings

Four and eight weeks after the experiment, the animals were weighed and anesthetized with diethyl ether and sacrificed by decapitation. Thoracic aortas

were excised and trimmed free of adhering fat and connective tissues. Then, they were placed in a petri dish filled with Krebs solution of the following composition (in mmol/L): NaCl, 118.5; KCl, 4.74; CaCl<sub>2</sub>, 2.5; MgSO<sub>4</sub>, 1.18; KH<sub>2</sub>PO<sub>4</sub>, 1.18; NaHCO<sub>3</sub>, 24.9 and glucose 10.0. The dissected aorta was cut transversely into rings of 3-4 mm in width. One ring of each pair was left intact and in the other ring, the endothelium was mechanically removed. The rings, with or without endothelium, were mounted in an organ bath of 50 ml capacity filled with Krebs solution that was kept at 37°C and continuously bubbled with a 95% O<sub>2</sub> and 5% CO<sub>2</sub> gas mixture. Preparations were allowed to equilibrate for 60 min under a resting tension of 2g. During the equilibration period, the solution of tissue bath was replaced every 30 min. Successful removal of the endothelium was confirmed by loss of acetylcholine-induced relaxation (10<sup>-5</sup> mol/L) in precontracted rings by phenylephrine (10<sup>-6</sup> mol/L). Following the equilibrium period, dose-response curves were obtained with phenylephrine and acetylcholine. Phenylephrine was added in a cumulative manner (10<sup>-9</sup> -10<sup>-4</sup> mol/L) until maximal response was achieved. After addition of each dose plateau response was obtained before addition of a subsequent dose. To evaluate acetylcholine-induced vasodilation, the rings with the endothelium were precontracted to their EC<sub>80</sub> value with phenylephrine to obtain a stable plateau and then the cumulative dose-response curve to acetylcholine was obtained. EC<sub>80</sub> values were calculated from the cumulative doses of phenylephrine that produced 80% of its maximal response for each aorta preparation. Consecutive dose-response curves for phenylephrine and acetylcholine were taken at minimum 30-min intervals, during which the Krebs solution was changed at least three times. Aortic rings contractions and relaxations were recorded on a physiological recorder (Physiograph, MK-IV-P, Narco-Biosystems) using isometric transducers (F-60 myograph, Narco-Biosystem).

※ **Drugs**

Phenylephrine hydrochloride and acetylcholine hydrochloride were purchased from Sigma (Sigma, St

Louis, Mo, USA). All other chemicals were from Merck (Germany).

※ **Data analysis**

Contractile responses of the aortic rings to phenylephrine with or without endothelium were expressed as grams of tension per milligram of tissue, and relaxation responses for acetylcholine were expressed as a percentage decrease of the maximum contractile response induced by phenylephrine. The sensitivity to the agonist was evaluated as EC<sub>50</sub>, which is the negative logarithm of the drug concentration required to produce 50% of the maximum response. For phenylephrine and acetylcholine, maximum responses (E<sub>max</sub>) are presented as g/mg tissue and percentage decrease of the maximum contractile response induced by phenylephrine respectively. All values were given as means±S.E.M. Statistical significance was indicated by a P value less than 0.05, which was obtained using paired and unpaired Student's t-test.

**Results**

※ **Body weight**

The data of recorded weight of rats before and after experimental periods showed that there was a reduction in weight in groups C and D in comparison with groups A and B (P<0.05; Table 1).

Table 1. Body weight of the control and the aqueous garlic extract-treated rats.

	Body weight (g)		
	Week +1	Week +4	Week +8
Control			
4 weeks	238.1±3.2	261.5±4.1	
8weeks	237.7±3.8	243.6±5.9	241±6.2
Extracted			
treated			
4 weeks	233.7±2.8	212.7±5.9*	
8 weeks	234.3±2.6	205.2±6.7*	187.7±6.7*

\* P<0.05 is significantly different from the controls

※ **Vascular contractile response**

Cumulative dose-response curves to phenylephrine (10<sup>-9</sup> -10<sup>-4</sup> M) from aortic rings with or without endothelium is shown in Fig 1 and 2, respectively. Treatment with aqueous garlic extract for a period of

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4-week caused a 32-39% reduction in the maximum contractile response to phenylephrine in aortic rings with endothelium compared to the controls. In addition, 8-week treatment with garlic extract caused a significant decrease in contractile response compared to the controls ( $P < 0.05$ ) (Fig 1). Denuded aortic rings from rats treated with garlic for 4 and 8 weeks showed an 11-16% and 22-24% decrease in contractile response respectively, compared to controls (Fig 2).

**\* Endothelium-dependent relaxation response**

Cumulative dose-response curve to acetylcholine ( $10^{-9}$  -  $10^{-4}$  M) from the aortic rings with endothelium is shown in Fig 3. Endothelium-dependent relaxation response of the aortic rings from rats treated with garlic for 4 and 8 weeks increased by 3-24% and 5-27% respectively, compared to the controls. In addition,  $E_{max}$  and  $EC_{50}$  for phenylephrine and acetylcholine in the related groups have been shown in Table 2.

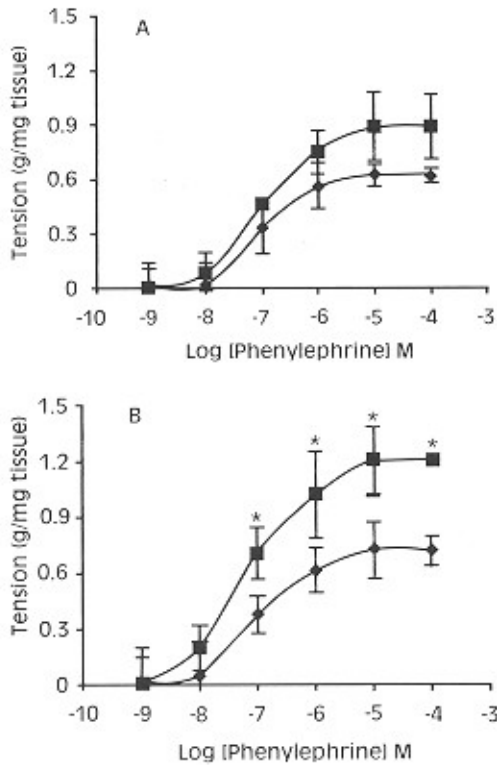


Fig 1: (A) Cumulative concentration-response curve for phenylephrine in the aorta with endothelium from the rats treated with garlic for 4 weeks (♦) compared to the controls (■). (B) Cumulative concentration-response curve for phenylephrine in aorta with endothelium from the rats treated with garlic for 8 weeks (♦) compared to the controls (■).  $P < 0.05$  is significantly different from the controls.

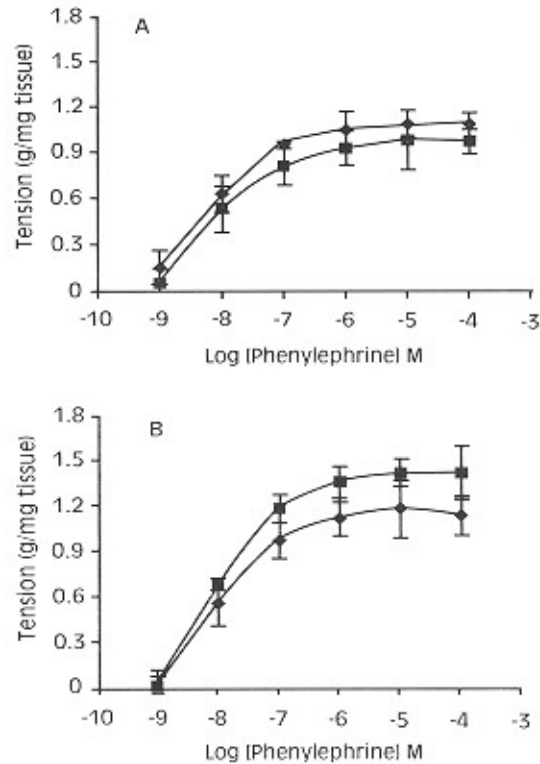


Fig 2: (A) Cumulative concentration-response curve for phenylephrine in the aorta without endothelium from the rats treated with garlic for 4 weeks (♦) compared to the controls (■). (B) Cumulative concentration-response curve for phenylephrine in aorta without endothelium from the rats treated with garlic for 8 weeks (♦) compared to the controls (■).

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Table 2: Maximum responses ( $E_{max}$ ) and  $EC_{50}$  of phenylephrine and acetylcholine in the aortic rings from the control and the aqueous extract-treated rats.

Group	$EC_{50}$		Acetylcholine	$E_{max}$		Acetylcholine
	Phenylephrine			Phenylephrine		
	With endo	Without endo		With endo	Without endo	
After 4 weeks	6.95 ± 0.17	8.6 ± 0.12	7.12 ± 0.17	0.89 ± 0.18	1.09 ± 0.12	61.79 ± 3.68
A						
C	6.54 ± 0.15	8.4 ± 0.18	6.65 ± 0.11	0.63 ± 0.04	0.98 ± 0.08	85.65 ± 1.72*
After 8 weeks	7.63 ± 0.19	7.87 ± 0.08	7.70 ± 0.22	1.21 ± 0.01	1.44 ± 0.87	63.21 ± 2.68
B						
D	7.18 ± 0.13	8.2 ± 0.14	7.15 ± 0.25	0.730.1	1.16 ± 0.13*	90.17 ± 3.7*

Data are expressed as means ± SEM. For phenylephrine and acetylcholine, maximum responses ( $E_{max}$ ) are presented as g/mg tissue and percentage decrease of the maximum contractile response induced by phenylephrine respectively. \*  $P < 0.05$  is significantly different from the control.

With endo: Aortic rings with endothelium. Without endo: Aortic rings without endothelium.

**\* Time dependent effect of garlic extract on vascular contractile and relaxation response:**

There was a significant difference in vascular contractile response of aortic rings with or without endothelium from the rats treated for 4 weeks in comparison with that of those treated for 8 weeks ( $P < 0.05$ ) (Fig 3).

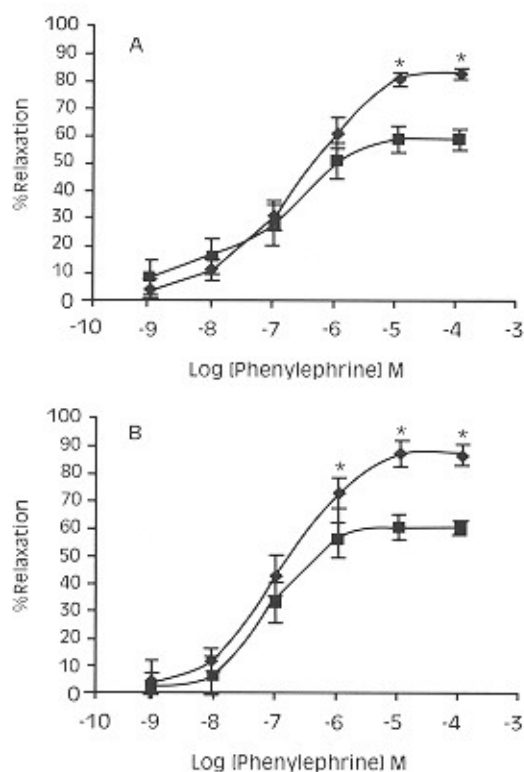


Fig 3. (A) Cumulative concentration-response curve for acetylcholine in the aortic rings with endothelium from the rats treated with garlic for 4 weeks (◆) compared to the controls (■). (B) Cumulative concentration-response curve for acetylcholine in the aortic rings with endothelium from the rats treated with garlic for 8 weeks (◆) compared to the controls (■).  $P < 0.05$  is significantly different from the controls.

The results showed that both 4 and 8-week treatment with aqueous garlic extract caused a decrease in contractile response of aortic rings to phenylephrine, but the attenuating effect of garlic extract on vascular contractile response in group D was about 45% more than that of group C (Fig 4). On the other hand, the endothelium-dependent relaxation response of aortic rings from the rats treated for 8 weeks was 6-11% more than that of those treated for 4 weeks (Fig 5).

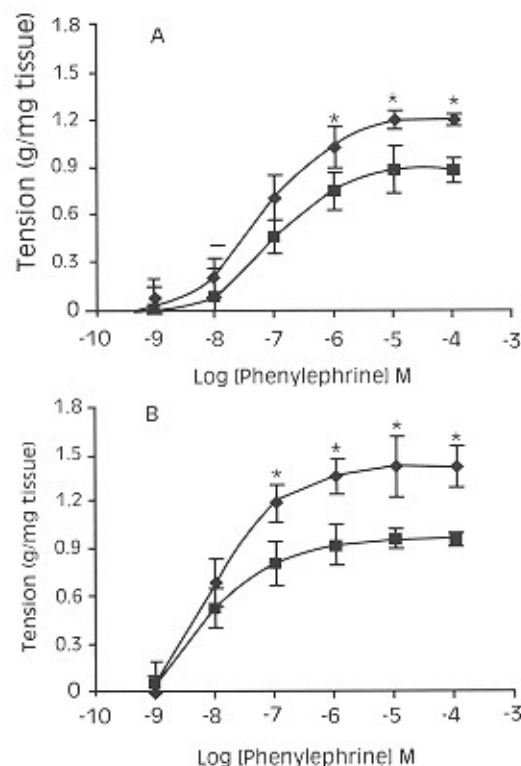


Fig 4. (A) Cumulative concentration-response curve for phenylephrine in the aorta with endothelium from the rats treated with garlic for 4 weeks (◆) and the rats treated with garlic for 8 weeks (■). (B) Cumulative concentration-response curve for phenylephrine in aorta without endothelium from rats treated with garlic for 4 weeks (◆) and the rats treated with garlic for 8 weeks (■).  $P < 0.05$  is significantly different from the controls.

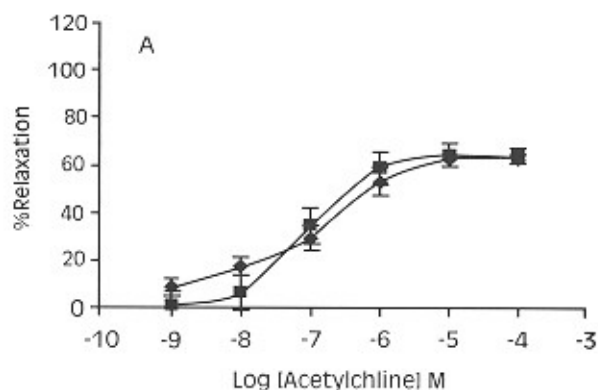


Fig 5: Cumulative concentration-response curves for acetylcholine in the aortic rings from the rats treated with garlic for 4 (◆) and 8 weeks (■).

**Discussion**

The goal of the present study was to investigate the effect of subchronic administration of aqueous garlic extract on  $\alpha_1$ -adrenoceptor agonist-induced contraction of isolated aorta in an in vivo model. The

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results showed that the administration of aqueous extract of garlic significantly inhibited the phenylephrine-induced contraction and potentiated the endothelium-dependent relaxation of rat aortic rings in Krebs solution. Furthermore, the effect of garlic extract on the vascular reactivity of the aortic rings in rats treated for 8 weeks was greater than that of those treated for 4 weeks. These findings strongly suggested that *in vivo* garlic extract treatment for a period of two months could strongly attenuate contractile reactivity and could accentuate the relaxation response of the aortic rings. The beneficial effect of long-term and/or subchronic garlic extract treatment on contraction and endothelium-dependent relaxation responses may be specific for rats aortas. Several mechanisms could explain the beneficial effects of garlic extract on the functional reactivity of the vascular system. It was shown that *in vitro* garlic extract treatment can improve impairment in endothelium-dependent relaxation evoked by Ach (26), and reduces increased lipid peroxidation induced by oxygen-free radicals (27). Therefore, a possible mechanism by which garlic extract administration can improve vascular reactivity may depend on inhibiting oxidative stress. Consistent with this idea, it has been shown that *in vivo* treatment with garlic extract reduces end-products of lipid peroxidation (28). However, whether the lipid peroxidation-lowering effect of garlic extract results from its direct superoxide scavenging properties or indirectly by increasing NO synthesis, is controversial since NO has also antioxidant activity *per se* (29). In addition, the ameliorating effect of garlic extract on vascular responsiveness may be closely related to its anti-hypertensive activity (30). In this respect, aqueous extracts of fresh garlic inhibits efficiently adenosine deaminase (ADA) activity of cultivated endothelial cells. The inhibition of endothelial ADA seems to contribute to the hypotensive activity and vessel protective effects

of garlic (31). Garlic powder extract may also inhibit modified low-density lipoprotein uptake and degradation of lipoprotein-derived cholesteryl esters, thus considerably reducing the intracellular accumulation of cholesteryl esters. This suggests the mechanism responsible for the prevention of lipid accumulation in aortic cells (32). It is also believed that garlic juice may exert a direct relaxant effect on smooth muscles (33). In addition, it is likely that garlic potentiates the endothelium-relaxation response of the aortic rings via an additive effect on NO release. Moreover, since garlic could exert a part of its relaxant effect in the absence of endothelium, therefore, it is possible that anti-hypertensive action of garlic is mediated through a direct relaxant effect on smooth muscle or by interfering with the production and/or release of vasodilators or vasoconstrictors derived from smooth muscle. Finally, the extract has been known to contain some essential oils (34) and such oils may have some beneficial effects through penetrating into cell membrane like that of endothelial cells.

In conclusion, the obtained findings clearly showed that aqueous garlic extract could attenuate agonist-induced contraction of rat aorta and produce a direct or an indirect vasorelaxant effect. Since it can reduce risk factors of some cardiovascular disorders including hypertension, therefore, it is highly recommended that garlic extract may be administered as a complementary therapeutic regimen for patients with cardiovascular abnormalities. Further studies are to be undertaken to investigate the possible beneficial effect of garlic extract administration and the related mechanisms for its efficacy.

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