

Effect of Aqueous Garlic (*Allium sativum* L.) Extract on Acetylcholine and Isosorbide-Induced Relaxation of Isolated Aorta in Rat

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ABSTRACT

The hypotensive effect of garlic has been well-documented in human subjects and animals. Since endothelial activity regulates vascular reactivity in physiological and pathophysiological conditions, the aim of the present study was to investigate the effect of garlic on endothelium-dependent and independent relaxation of rat aorta for elucidation of mechanism of the garlic anti-hypertensive effect. Four and eight weeks after treatment with garlic extract, aortic rings were studied for relaxation response to acetylcholine and isosorbide dinitrate. The obtained results showed that endothelium-dependent relaxation response of aortic rings to acetylcholine, from rats treated with garlic for 4 and 8 weeks, increases about 5-24% and 3-27%, respectively compared to controls. But, endothelium-independent relaxation response to isosorbide showed no difference in all groups. Moreover, the relaxant effect of garlic extract was time-dependent. The obtained findings strongly suggest that garlic exerts its relaxant effect through endothelium by production and/or releasing of endothelium-derived relaxing factor. *Iran. Biomed. J.* 7 (1): 23-27, 2003

Keywords: Aqueous garlic extract, Aortic rings, Acetylcholine, Isosorbide dinitrate

INTRODUCTION

Epidemiologic studies have suggested an inverse relationship between nutritional garlic intake and the occurrence of cardiovascular disorders that could be attributed to protective effect of garlic against these diseases [1]. Evidence from numerous studies has pointed at that garlic can bring about plasma lipid normalization [2], enhance fibrinolytic activity [3], inhibit platelet aggregation and thromboxane formation [4]. Several reports have suggested that garlic has protective actions against stroke, coronary thrombosis and atherosclerosis [5]. Fresh garlic extracts have been found to lower blood pressure in spontaneously hypertensive rats [6, 7] and in anesthetized dogs [8]. The hypotensive activity of garlic has been repeatedly confirmed in human subjects [1] and animals [6]. On the other hand, it is evident that endothelial cells can regulate basal vascular tone and vascular reactivity in physiological and pathological conditions by responding to mechanical forces and neurohumoral mediators via

the release of a variety of relaxing and contracting factors [9].

Acetylcholine and several other vasodilators require an intact endothelium to elicit their relaxant effect [10-12]. These agents induce the formation and the release of endothelium-derived relaxing factor (EDRF) that identified as nitric oxide [13]. Conversely, it is postulated that the organic nitrate vasodilators including isosorbide dinitrate are prodrug and independent of endothelium such that it transforms to the active inorganic metabolite, nitric oxide, prior to the onset of vasodilation [14]. Since the mechanism of the hypotensive effect of garlic has not been fully elucidated, therefore, the purpose of the present study was to investigate the possible role of endothelium in the anti-hypertensive effect of aqueous extract of garlic administered intraperitoneally to rats.

MATERIALS AND METHODS

Preparation of garlic extract. Fresh garlic was

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purchased from a local grocery store, identified by botanists in the herbarium of Shaheed Beheshti University of Medical Sciences (Tehran, Iran), and specimen voucher No. 270 was assigned. The garlic extract was prepared according to the method reported by Alnaqeeb *et al.* [15]. Briefly, the 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 $2000 \times g$ for 10 min and the clear supernatant was made up to 100 ml with normal saline. The concentration of the garlic preparation was considered to be 500 mg/ml. The prepared garlic extract was stored at -20°C until use.

Drugs. The following pharmacological agents were used: phenylephrine hydrochloride, Isosorbide dinitrate, acetylcholine hydrochloride. All drugs were purchased from Sigma (Sigma, St. Louis, Mo, USA).

Animals. Male Sprague-Dawley rats (animal house of Iran University of Medical Sciences) weighing 220-250 g were used in this study. All groups of rats were maintained under standard housing conditions for a period of 4 to 8 weeks with free access to food and water. Body weight of all the rats was determined before and 4 to 8 weeks after intraperitoneal injection of saline or aqueous garlic extract. Two groups of normal rats were selected for obtaining the concerned normal values. In this study, totally four groups of the rats were used (each group $n = 8$):

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 garlic extract (100 mg/kg body weight/day) for 4 weeks. Group D: Extract-treated rats received garlic extract (100 mg/kg body weight/day) for 8 weeks.

Preparation of aortic rings. Four and eight weeks after injection, the animals were weighed and anesthetized with diethyl ether followed by decapitation. Thoracic aortas were excised and trimmed free of adhering fat and connective tissues, then the aortic tissues was placed in a Petri dish filled with Krebs solution with the following composition (in mM): NaCl, 118.5; KCl, 4.74; CaCl_2 , 2.5; MgSO_4 , 1.18; KH_2PO_4 , 1.18; NaHCO_3 , 24.9 and glucose 10.0. The dissected aorta was cut transversely into rings of 3-4 mm in length. One ring of each pair was left intact and in the other

ring, endothelium was mechanically removed. The rings with or without endothelium were mounted in an organ bath of 50 ml capacity filled with Krebs solution which was kept at 37°C and continuously bubbled with a 95% O_2 and 5% CO_2 gas mixture. Preparation was allowed to equilibrate for 60 min under a resting tension of 2 g. 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 (10^{-5} M/L)-induced relaxation in precontracted rings by phenylephrine (10^{-6} M/L) [16]. To evaluate isosorbide dinitrate and acetylcholine-induced vasodilation, rings with or without endothelium were precontracted to their EC_{80} value with phenylephrine to obtain a stable plateau. Then, the cumulative dose-response curve to isosorbide dinitrate and acetylcholine was obtained [17]. EC_{80} values were calculated from the cumulative doses of phenylephrine that produced 80% of its maximal response for each aorta preparation with or without endothelium. Consecutive dose-response curves were taken at minimum 30-min intervals, during which the Krebs solution was changed at least three times. The responses of the aortic rings were recorded on a physiological recorder (Physiograph, MK-IV-P, Narco-Biosystems) using isometric transducer (F-60 myograph, Narco-Biosystem).

Data analysis. The relaxation response to acetylcholine and isosorbide was expressed as a percentage of the precontraction induced by phenylephrine. Statistical significance was indicated by $P < 0.05$, which was obtained from paired and unpaired student's *t*-test.

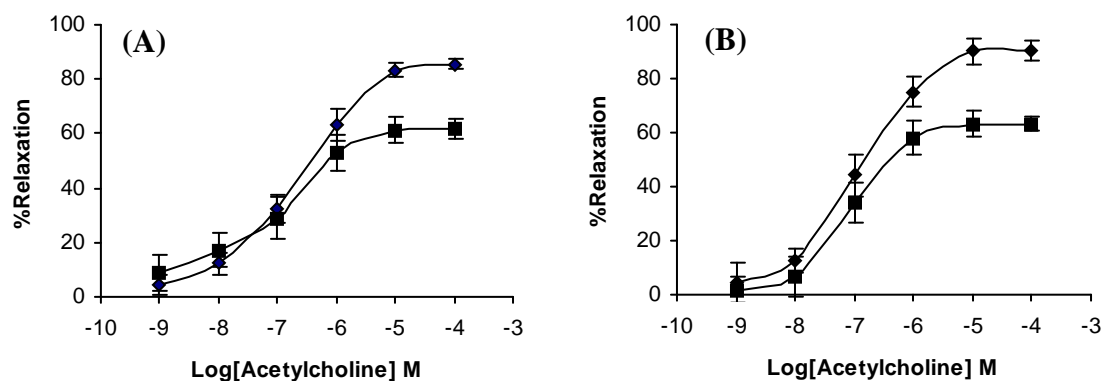
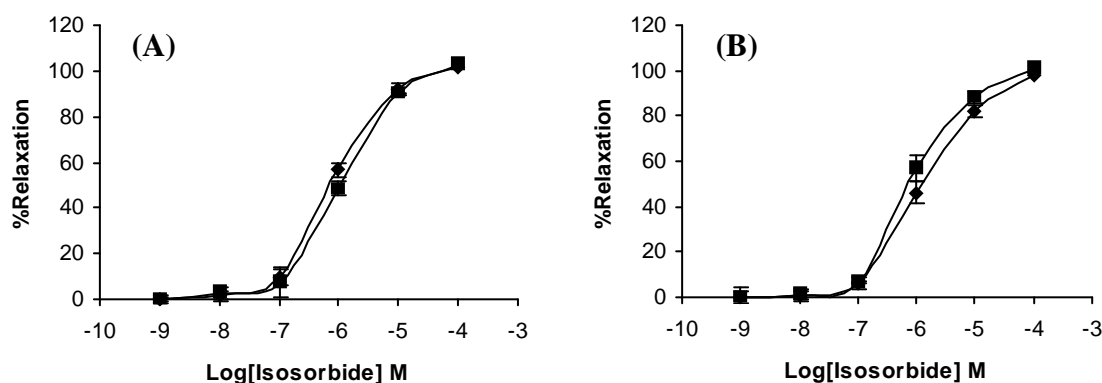
RESULTS

Body weight. The data of the recorded weight of rats before and after experimental periods showed a reduction in weight in groups C and D (extract treated) in comparison with groups A and B as control ($P < 0.05$ Table 1).

Vascular reactivity endothelium-dependent relaxation response. Cumulative dose-response curve to acetylcholine (10^{-9} - 10^{-4} M) from aortic rings with endothelium has been shown in Figure 1. Endothelium-dependent relaxation response of aortic rings from rats treated for 4 and 8 weeks significantly increased at doses greater than 10^{-6} M of acetylcholine.

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

Time	Body Weight			
	Control		Extract-Treated	
	Week 4	Week 8	Week 4	Week 8
Before treatment	235.1 ± 1.1	230.7 ± 2.4	239.1 ± 3.2	241.3 ± 2.9
Week 1	238.1 ± 3.2	237.7 ± 3.8	233.7 ± 2.8	234.3 ± 2.6
Week 4	261.5 ± 4.1	243.6 ± 5.9	212.7 ± 5.9*	205.2 ± 6.7*
Week 8	-	241.0 ± 6.2	-	187.7 ± 6.7*

* $P < 0.05$ (Compared to Control)**Fig. 1.** Cumulative concentration-response curves for acetylcholine. The endothelium-dependent relaxation response after garlic extract treatment for 4 (A) and 8 (B) weeks. ■ Control; ♦ Extract-treated; * $P < 0.05$ compared to controls**Fig. 2.** Cumulative concentration-response curves for isosorbide dinitrate. The endothelium-independent relaxation response after garlic extract treatment for 4 (A) and 8 (B) weeks. ■ Control; ♦ Extract-treated.**Endothelium-independent relaxation response.**

Cumulative dose-response curve to isosorbide dinitrate (10^{-9} - 10^{-4} M) from aortic rings without endothelium has been shown in Figure 2. Endothelium-independent relaxation response of aortic rings showed no considerable difference in all groups. On the other hand, there was no significant

difference in endothelium-independent relaxation response of aortic rings to isosorbide in rats treated with garlic extract for 4 and 8 weeks (Fig. 2). But, the endothelium-dependent relaxation response of aortic rings to acetylcholine in rats treated for 8 weeks was 6-11% more than that of rats treated for 4 weeks (Fig. 1).

DISCUSSION

The study provided evidence that both acetylcholine and isosorbide dinitrate were found to produce relaxation of aortic rings in a dose-dependent manner. It is well-documented that relaxant effect of acetylcholine on vascular smooth muscles is exerted through the release of EDRF [18] and muscle-derived relaxing factor (MDRF) [19]. On the other hand, it has been reported that both in normal and hypertensives subjects, isosorbide dinitrate caused an increase in aortic diameter together with an increase in arterial distensibility [20]. In the present study, the *in vivo* effect of aqueous garlic extract on endothelium-dependent and -independent relaxant activity of acetylcholine and isosorbide was investigated on the isolated rat aorta in the presence and absence of endothelium. There are several explanations for interpretation of the obtained results.

It has been previously shown that the garlic extract has an endothelium-dependent relaxant effect on the vascular smooth muscle [16]. Hypotensive activity of garlic has been reported in a variety of experimental studies [1, 6]. It has been shown that garlic extract produces a significant fall in systolic, diastolic and mean blood pressure of dogs which is not due to depression of vasomotor center and heart and atropine is ineffective on this anti-hypertensive activity of garlic in dogs [21, 22]. As the results showed, the relaxant effect of acetylcholine on rat aorta was accentuated after administration of garlic extract for a period of 4 and 8 weeks. The augmentation effect of garlic extract on relaxing action of acetylcholine could be explained as follows: i) Since garlic extract had no effect on relaxant action of isosorbide in rat aorta after removing endothelium, it seems plausible that garlic potentiated the relaxant action of acetylcholine through interference with production and/or release of EDRF or adaptive alterations in muscarinic receptors on the endothelial cells, ii) since it has been demonstrated that relaxation in rat aorta is mediated through endothelium-derived hyperpolarizing factor (EDHF), therefore, it is possible that garlic enhances relaxing action of acetylcholine through the release of this factor [23], iii) it has also been reported that a vasoactive factor i.e. the auricular vasorelaxing factor (AVF) produces vasodilatation [24], thus garlic may elicit its relaxant effect via AVF. Moreover, the garlic extract has been known to contain essential oils and such oils may have the capacity to penetrate cell membranes [25]. Thus, the required time for

penetration may explain the optimal and prolonged duration that garlic extract needs to produce its maximal effect on the vascular system. Further experiments are needed to unravel the relaxant action of garlic extract and its mechanism on rat aorta.

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