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Effect of Subchronic Administration of Aqueous *Artemisia annua* Extract on α_1-Adrenoceptor Agonist-Induced Contraction of Isolated Aorta in Rat

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ABSTRACT

***Artemisia* species, growing in almost all the northern hemisphere, is used in folk medicine of some countries as a remedy for hypertension. Since some cardiovascular disorders including hypertension are accompanied with altered responsiveness of vascular alpha-adrenergic receptors, the effect of aqueous *Artemisia annua* extract (100 and 200 mg kg^{-1}) on α_1-adrenoceptor agonist-induced contraction of rat aorta was investigsted. After 4 weeks, the contraction and endothelium-dependent and -independent relaxation response of extract-treated rats were recorded. The results showed that the contractile response of aortic rings with endothelium to phenylephrine in extract-treated rats decreases (P<0.01-0.0001) and their endothelium-dependent relaxation response to acetylcholine increases (P<0.01-0.001) significantly in comparison with controls. Endothelium-denuded aortic rings from extract-treated rats also showed a significant decrease (P<0.05) in contractile response, but there was no considerable difference in their endothelium-independent relaxation response to isosorbide dinitrate. These data suggest that the aqueous extract of *Artemisia annua* could attenuate contractile response and enhance the endothelium-dependent relaxation response of aortic rings from normal rats.** *Iran. Biomed. J. 9 (2): 57-62, 2005*

Keywords: Aqueous *Artemisia annua* extract, Aortic rings, α_1-adrenoceptor agonist, Phenylephrine, Rat

INTRODUCTION

One approach to the discovery of new drugs is the study of the bioactive constituents of higher plants. The investigation of plants used as remedies in traditional folk medicine can be an interesting tool to identify several biologically active molecules from the 250,000 higher plant species [1]. The genus *Artemisia* is a rich source of bioactive constituents with anti-inflammatory [2, 3], anti-tumor [4, 5], anti-ulcerogenic [6], diuretic [7]. *A. abrotanum* was found to possess spasmolytic activity on the carbacholine-induced contraction of guinea-pig trachea [8]. In addition, ultrasonic studies show that *Artemisia* decoction intravenous infusion has remarkable effects on the contractility of gallbladder [9]. An alkaloid of *Artemisia* which was metabolized to small molecules in digestive tract and then could pass through the blood-brain barrier, appeared to be an acetylcholinesterase inhibitor with a blocker of neurotoxicity induced by beta amyloid in human brain causing Alzheimer's disease [10-11]. The standardized extract of *A. asiatica* (DA-9601) has an inhibitory effects on cyclooxygenase-2 expression, inducible nitric oxide synthease expression in mouse skin [12]. The antioxidant activities of various fractions of *Artemisia* species were investigated, especially, the n-butanol extract that causes significant increase in the rat liver cytosolic superoxide dismutase (SOD) and catalase [13-14]. Some studies have shown that aqueous and chloroform extracts from leaves of *A. vulgaris* have anti-hypertensive actions but have no significant effects on cardiovascular hemodynamics under basal conditions [15].

Although the extract of *Artemisia* has numerous biological effects, there is no report concerning its effect on reactivity of blood vessels on α_1-

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adrenoceptor agonists. Therefore, the present study was carried out to investigate the effect of subchronic administration of aqueous extract of *A. annua* on α_1-adrenoceptor agonist- induced contraction of isolated aorta in an *in vivo* model.

MATERIALS AND METHODS

Preparation of aqueous Artemisia extract. The aerial parts of *Artemisia annua* (Dermaneh*)* were collected in Babolsar, a northen city of Iran, during the late spring season. The plant was taxonomically identified by botanists in the Department of Biology (Tehran University, Iran). The powder of air-dried herb (100 g) was added to one liter of boiled distilled water and kept the temperature at 60°C for 15 min and then filtered three times through filter paper. The resulted liquid was dried at 37°C, until a concentrated residue (62% w/w) was obtained. This stock extract was maintained at -20°C until being used and the diluted concentration of the extract were made up with normal saline [1].

Animal. Male Albino Wistar rats, weighing 220-250 g, were obtained from the Pasteur Institute of Iran (Tehran), and 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. All groups of rats were maintained under standard housing conditions for a period of 4 weeks with free access to food and water. Body weight and serum glucose of all rats were determined before and 4 weeks after intraperitoneal injection of saline or *Artemisia* extract (100 and 200 mg kg^{-1}) [16, 17]. One group of normal rats was also selected for obtaining the concerned normal values. In the experiment, totally four groups of eight rats were used:

Group A) Control rats for obtaining the normal values.

Group B) Vehicle-treated rats received saline (0.5 ml/daily) for four weeks.

Group C) Extract-treated rats received *Artemisia* extract (100 mg kg^{-1}/0.5 ml saline daily) for four weeks.

Group D) Extract-treated rats received 0.5 ml of *Artemisia* extract (200 mg kg^{-1}/0.5 ml saline daily) for four weeks.

Preparation of aortic rings. The applied procedure has been described before [18]. Briefly, four weeks after the experiment, the animals were anesthetized with diethyl ether and thoracic aortas were excised and trimmed free of adhering fat and connective tissues. Then, the aortic tissues were placed in a Petri dish filled with Krebs solution of the following composition (in mmol/L): 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. 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_2 and 5% CO_2 gas mixture. Following the equilibrium period, dose-response curves were obtained with phenylephrine and acetylcholine. Phenylephrine was added in a cumulative manner (10^{-9} -10^{-4} mol/L) until a maximal response was achieved. After the addition of each dose, a plateau response was obtained before addition of a subsequent dose. To evaluate acetylcholine-induced vasodilation, rings with endothelium were preconstricted to their EC_{80} value with phenylephrine to obtain a stable plateau and then the cumulative dose-response curve to acetylcholine was obtained. EC_{80} 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. The contraction and relaxation response of the aortic rings were digitized on-line on the computer using isometric transducers (F-60 myograph, Narco-Biosystem) and signals were stored for offline analysis.

Drugs. Phenylephrine hydrochloride and acetylcholine hydrochloride were purchased from Sigma (St. Louis, Mo, USA). All other chemicals were from Merck!(Germany).

Data analysis. The contractile responses of 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. All values were given as means ± S.E.M. Statistical significance was indicated by a $P<0.05$, which was obtained from student's paired and unpaired *t*-test.

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

	Body weight (g)		Serum glucose (mg/dl)	
	Week 1	Week 4	Week 1	Week 4
VC	200.66 ± 3.49	214.33 ± 3.88	100.34 ± 1.99	98.40 ± 2.44
AT100	205.50 ± 3.20	250.16 ± 3.08*	110.22 ± 4.43	69.07 ± 2.15*
AT200	219.83 ± 7.30	259.33 ± 6.68¶	104.12 ± 7.81	80.15 ± 10.09¶

Data are expressed as means ± SEM. (VC, AT100 and AT200 stand for vehicle-untreated control, 100 mg kg^{-1} *Artemisia*-treated and 200 mg kg^{-1} *Artemisia*-treated rats, respectively). Body weight: *, $P<0.0001$(AT100 comparing to VC); ¶, $P<0.001$ (AT200 comparing to VC). Serum glucose: *, $P<0.01$(AT100 comparing to VC); ¶, $P<0.005$ (AT200 comparing to VC).

RESULTS

Body weight and serum glucose. The data of recorded weight of rats before and after experimental periods showed that there exists an increasing in weight in groups C and D in comparison with groups A and B ($P<0.001$-$P<0.0005$; Table 1). On the other hand, the serum glucose of extract-treated rats were decreased significantly comparing to normal rats ($P<0.01$-$P<0.005$; Table 1).

Vascular contractile response. Cumulative dose-response curves to phenyephrine (10^{-9} -10^{-4} M) from aortic rings with or without endothelium have been shown in Figure 1. Treatment with aqueous *Artemisia* extract (100 and 200 mg kg^{-1}) for a period of four weeks caused a significant reduction in the maximum contractile response to phenylephrine in aortic rings with endothelium when compared to vehicle-treated rats ($P<0.01$-$P<0.0001$) (Fig.1A). Denuded aortic rings from rats treated with aqueous *Artemisia* extract (100 and 200 mg kg^{-1}) for four weeks showed a considerable decrease in contractile response to phenyephrine at concentrations of 10^{-7} to 10^{-4} when compared to controls ($P<0.05$) (Fig. 1B).

Endothelium-dependent and -independent relaxation response. Cumulative dose-response curve to acetylcholine and isosorbide dinitrate (10^{-9} -10^{-4} M) from aortic rings with and without endothelium has been shown in Figure 2. Endothelium-dependent relaxation response of aortic rings from rats treated with aqueous *Artemisia* extract (100 and 200 mg kg^{-1}) for a period of four weeks caused a significant increase in the maximum contractile response to acetylcholine when compared to vehicle-treated rats ($P<0.01$-$P<0.001$) (Fig. 1A). On the other hand, there was no significant difference in the endothelium-independent relaxation response of aortic rings to isosorbide dinitrate between all of groups.

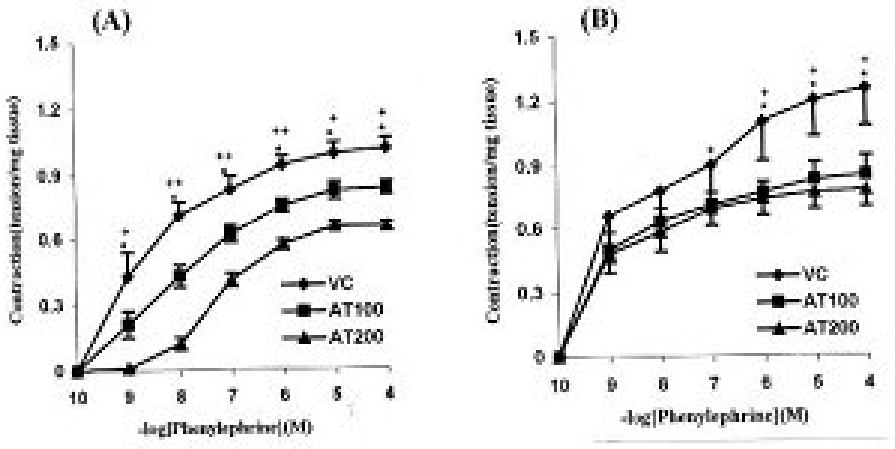


Fig. 1. Cumulative concentration-response curve for phenylephrine in aorta with endothelium **(A)** and without endothelium **(B)** from rats treated with *Artemisia* for four weeks when compared with controls. (VC, AT100 and AT200 stand for vehicle-untreated control, 100 mg kg^{-1} *Artemisia*-treated and 200 mg kg^{-1} *Artemisia*-treated rats, respectively). A, *, $P<0.01$ (AT100 comparing to VC); $^{+}$, $P<0.005$, $^{++}$, $P<0.0001$ (AT200 comparing to VC); B, *, $P<0.05$ (AT100 comparing to VC); $^{+}$, $P<0.05$ (AT200 comparing to VC).

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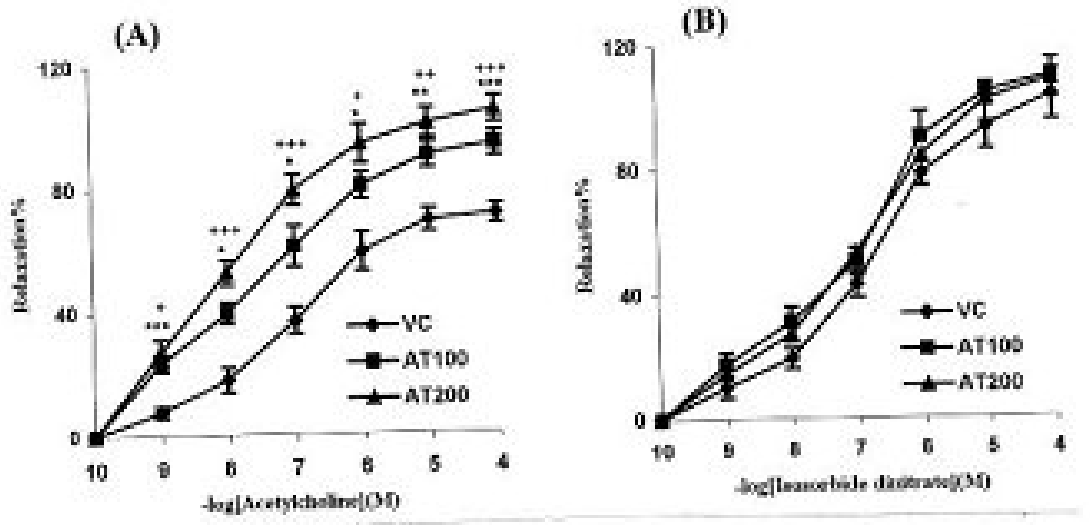


Fig. 2. Cumulative concentration-response curve for acetylcholine **(A)** and isosorbide dinitrate **(B)** in rats treated with *Artemisia* for four weeks when compared with controls. A, *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$ (AT100 comparing to VC); $^{+}$, $P<0.01$; $^{++}$, $P<0.005$; $^{+++}$, $P<0.0001$ (AT200 comparing to VC).

Dose-dependent effect of Artemisia extract on vascular contractile and relaxation response. There was a significant difference ($P<0.05$) in vascular contractile response of aortic rings with endothelium from rats treated with 100 mg kg^{-1} in comparison with that of rats treated with 200 mg kg^{-1}. In the other words, the attenuation effect of the dose 200 mg kg^{-1} of the *Artemisia* on phenylephrine -induced contraction of aortic rings was more than that of 100 mg kg^{-1}. On the other hand, the endothelium-dependent relaxation response of aortic rings from rats treated with 200 mg kg^{-1} was considerably more than that of rats treated with 100 mg kg^{-1} for four weeks.

DISCUSSION

It is demonstrated that feeding diabetic rats and rabbits with 100-390 mg kg^{-1} of the aqueous extract of the aerial parts of some species of *Artemisia* for 2-4 weeks could cause a significant reduction in blood level. This action prevents elevation of glycosylated haemoglobin level and possesses a hypoliposis effect, in addition to the protective effect against body weight loss of diabetic animals [16, 17].

In the present study, we determined that the subchronic administration of 100 and 200 mg kg^{-1} aqueous *Artemisia annua* extract significantly inhibited the phenylephrine-induced contraction and potentiated the endothelium-dependent relaxation of rat aortic rings in Krebs solution. Furthermore, the effect of *Artemisia* extract on the vascular reactivity of aortic rings in rats treated with 200 mg kg^{-1} was greater than that of rats treated with 100 mg kg^{-1}. The beneficial effect of long-term and/or subchronic *Artemisia* extract treatment on contraction and endothelium-dependent relaxation responses may be specific for aortas of rats.

Several mechanisms could explain the effects of *Artemisia* extract on the functional reactivity of vascular system. In the *in vitro* functional studies, it has been shown that extract has a marked vasorelaxing effect, which is competitively inhibited by atropin, typical of the interaction between this alkaloid and the muscarinic receptor. Since the cyproheptadine does not reduce the effects of the extracts, excluding the involvement of histamine and of the 5-HT receptors. Since L-NAME, an L-arginine analogue and inhibitor of the synthesis of NO by endothelial cells, fully abolished the vascular relaxation, it seems that extract of *Artemisia* act through production and/or release NO [1].

Other studies show that the extract of *Artemisia* has a stimulatory effect on the proliferation of cultured endothelial cells [19]. It seems that the extract enhanced the cell growth promotion by basic fibroblast growth factor [20]. In addition, the ameliorating effect of *Artemisia* extract on the vascular responsiveness may be closely related to the presence of sesquiterpene ketones, moxartenone, moxartenolide, coumarins and flavonols. Moxartenolide was found to inhibit the contractions induced by a high concentration of K^{+} by

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norepinephrine and by serotonine in isolated aortic strips of rat [21]. The pharmacological evaluation of the responses evoked by an aqueous dried extract of *Artemisia* verlotorum on the blood pressure of anaesthetized rats and on *in vitro* rat isolated aortae showed a marked, but transient, hypotensive activity. This effect was mediated by a strong vasodilator action, closely linked to the release of endothelial nitric oxide and to the nitric oxide-guanosine 3'-5'-cyclic monophosphate (cGMP) pathway, caused by a muscarinic receptor agonism [22, 23]. Moreover, four flavonols with spasmolytic activity have been isolated from a methanol extract of *Artemisia* [8]. It has been demonstrated that the maximum contractions induced by acetylcholine or oxytocin were also inhibited by 7-O-methyl-eriodictyol, a flavanone isolated from *Artemisia* [24]. The spasmolytic flavanone 7-O-methyl-eriodictyol is claimed to interact with Ca^{2+} metabolism [8].

The essential oil of *Artemisia annua* aerial parts has shown an antioxidant activity equivalent to 18% of the reference compound (alpha-tocopherol) [25]. Finally, since in this study *Artemisia* extract exerted a part of its relaxant effect in the absence of endothelium, therefore, it is possible that vaso-relaxant action of *Artemisia annua* 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.

In conclusion, the obtained findings clearly showed that aqueous *Artemisia annua* extract could attenuate the α_1-adrenoceptor agonist-induced contraction of rat aorta and produce a direct or an indirect vasorelaxant effect. Since it can reduce the risk factors of some cardiovascular disorders including hypertension, therefore, it is highly recommended that *Artemisia annua* extract may be administered as a complementary therapeutic regimen for patients with cardiovascular abnor-malities. Further studies are to be undertaken to investigate the possible beneficial effect of administration of *Artemisia annua* extract and its constituents and the related mechanisms for their efficacy.

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