

The Effects of Hydroalcoholic Extract of *Teucrium polium L.* on Hypertension Induced by Angiotensin II in Rats

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ABSTRACT

Background: Antispasmodic and vasorelaxant effects of *Teucrium polium L.* (TP) were mentioned in former studies, so we attempted to evaluate the eventual preventive effect of TP in an acute experimental model of hypertension induced by angiotensin II (Ang II).

Methods: Forty-eight male Wistar rats were divided randomly into six groups ($n = 8$); control Group (C), which received only saline, group Ang II; which received Ang II (300 ng/min, IV), group losartan (Los); which received Los (10 mg/kg, IV) before Ang II injection, three groups of TP 100, TP 200, and TP 400; which received different doses of TP extract (100, 200 and 400 mg/kg, IP, respectively) before Ang II application. After cannulation of the femoral artery, mean arterial blood pressure (MAP) and heart rate (HR) was continuously measured and recorded during the experiments. Comparisons were performed using *t*-test with SPSS software, version 16 (SPSS, Chicago, IL).

Results: MAP and HR in Ang group were significantly higher than the control group ($P < 0.001$), MAP in group Los significantly was lower than Ang group ($P < 0.001$) and pretreatment with three doses of TP extract also inhibited increasing of MAP after Ang II injection ($P < 0.001$). Los also inhibited the increase of HR due to Ang II ($P < 0.001$), but none of three doses of TP extract had a protective effect on tachycardia induced by Ang II.

Conclusions: It seems TP extract could be effective in preventing of high blood pressure induced by Ang II pathway activation but could not have remarkable efficacy for improving the created tachycardia.

Keywords: Angiotensin II, hypertension, rat, *Teucrium polium*

INTRODUCTION

Hypertension is a highly prevalent cardiovascular risk factor worldwide. High blood pressure can induce coronary artery disease, congestive heart failure, stroke, impaired vision, and kidney disease; untreated hypertension affects all organ systems and can shorten people's life expectancy.^[1]

Common clinical strategies to achieve a lowering of blood pressure include the use of beta adrenergic antagonists, calcium channel blockers, diuretics, angiotensin II (Ang II) receptor antagonists and Ang converting enzyme (ACE) inhibitors.^[2]

Activation of the renin-Ang system (RAS) both systemically and locally seems to be of importance for cardiovascular function. The octapeptide Ang II definitively plays a central role. In a reversal, for example, of left ventricular hypertrophy, so far the most important independent risk factor for an adverse outcome, blocking of the RAS with ACE inhibition has been shown to be particularly effective. A more complete blockade of the Ang II Type 1 receptor would offer more effective attenuation of the unfavorable effect of Ang II.^[3]

In recent years, there has been a growing interest and demand in using medicinal plants for treating and preventing various diseases including cardiovascular diseases. Traditional medicines of plants origin have received much attention due to several factors such as easy availability, affordable cost, safety, and efficacy as well as cultural acceptability. *Teucrium polium L.* (TP) is a flowering plant belonging to the family Labiate and is found abundantly in South Western Asia, Europe, and North Africa. *Teucrium* species have been used as medicinal herbs for over 2000 years as diuretic, diaphoretic, tonic, antipyretic, antispasmodic, antidiabetic, and many of them are used in folk medicine.^[4] Antiinflammatory,^[5] antinociceptive,^[6] and anorexic^[7] effects are other reported activities of TP.

There is increasing evidence of cardiovascular effects of TP such as positive inotropic and chronotropic,^[8] decreasing of blood pressure,^[9-11] and lowering blood lipid.^[12] Nevertheless, the exact effect of TP extract on the vascular system has not been clarified. Therefore, in the present study, we aimed to investigate the effects of the hydroalcoholic extract of TP on Ang II-induced hypertension and related heart rate (HR) variability.

METHODS

Plant material and preparation of the extract

Stems and leaves of TP were collected in October 2012 from Khorasan Province, Ferdows, Iran, and identified by Ferdowsi University Herbarium (Voucher No. 152-2016-4) and then

dried at room temperature. Aerial parts (300 g) of the plant were soaked in ethanol (50%) for 48 h and paper filter was used to filter the solute after mixing. The solution was then dried using a 40°C oven for 72 h. The dried extract was dissolved in the distilled water to make desired doses.

Experimental animals

Male Wistar rats, 200-250 g were housed in colony rooms with 12/12 h light/dark cycle at 21°C ± 2°C and had free access to food and water. The experimental protocol was approved by Ethical Committee at Mashhad University of Medical Sciences (Process Number 900559).

Drugs and reagents

The following reagents were used: Ang II and urethane (Sigma, USA), losartan (Los) (a gift from Daru pakhsh, I.R. Iran).

Experimental groups

Forty-eight male Wistar rats were divided into six groups as following order ($n = 8$ in each group)

- Control group; received saline (intravenous [I.V])
- Ang group; received Ang II (300 ng, I.V)
- Los group; received Los (10 mg/kg, I.V 0.5 ml) 30 min before injection of Ang II
- TP 100 group; received 100 mg/kg of TP extract (i.p.) 30 min before injection of Ang II
- TP 200 group; received 200 mg/kg of TP extract (i.p.) 30 min before injection of Ang II
- TP 400 group; received 400 mg/kg of TP extract (i.p.) 30 min before injection of Ang II.

Experimental procedure

Rats were anesthetized with urethane (1.4 g/kg, i.p. with 0.7 g/kg as a supplementary dose). Temperature was kept at 37.5°C with a heating lamp. A polyethylene catheter-50 filled by heparinized saline was inserted in the femoral artery. The catheter connected to a pressure transducer then mean arterial pressure (MAP), and HR, were continuously recorded by a power lab system (ID instrument, Australia). Another similar catheter was inserted in the jugular vein for injection of Ang II. Volume of injection in I.V and i.p. methods was 0.5 ml.

Data analysis

The data of the blood pressure and HR were expressed as mean ± standard error of the mean time

course alterations of HR and arterial pressure was plotted. The maximum change was compared with the control or Ang group (independent *t*-test) values. Repeated measures ANOVA were used to compare the time course changes between groups. $P < 0.05$ were considered to be statistically significant.

RESULTS

Effects of injection of saline on blood pressure and heart rate

Baseline MAP and HR before injection of saline were recorded then saline injected into the jugular vein. Injection of saline had no significant effects on MAP (before: 95 ± 1.6 mmHg and after: 99 ± 2.17 mmHg) or HR (before: 264 ± 7.32 beats/min and after: 262 ± 7.1 beats/min).

Cardiovascular responses to injections of angiotensin II

Injection of 300 ng Ang II significantly increased MAP and HR. The time course changes of MAP and HR after injection of Ang are shown in Figure 1. Maximal change in MAP was statistically significant compared with the control group (Δ : 32.37 ± 3.37 , $P < 0.001$). Moreover, significant HR changes were shown after injection of Ang (Δ : 72.79 ± 5.1 , $P < 0.001$) [Figure 2].

Effect of systemic intravenous pretreatment with losartan on the cardiovascular effects of angiotensin II

Losartan pretreatment (10 mg/kg, I.V) inhibited cardiovascular effects induced by Ang II so the cardiovascular responses to Ang II after pretreatment with Los did not differ significantly with those observed before injection of Ang II (Δ MAP: -9.05 ± 5.63 mmHg and Δ HR: 15.76 ± 5.7 beats/min). In 4th min after injection of Ang II, maximum significant changes of MAP and HR were observed in Los group compared with simultaneous changes in other two groups, independent *t*-test, $P < 0.001$. Meanwhile, MAP changes in Los group were significantly lower than those of control and Ang groups (repeated measures ANOVA, $P < 0.001$ for both) [Figure 1a]. As well as HR changes in Los group were lower compared to Ang group (repeated measures ANOVA, $P < 0.001$) [Figure 1b].

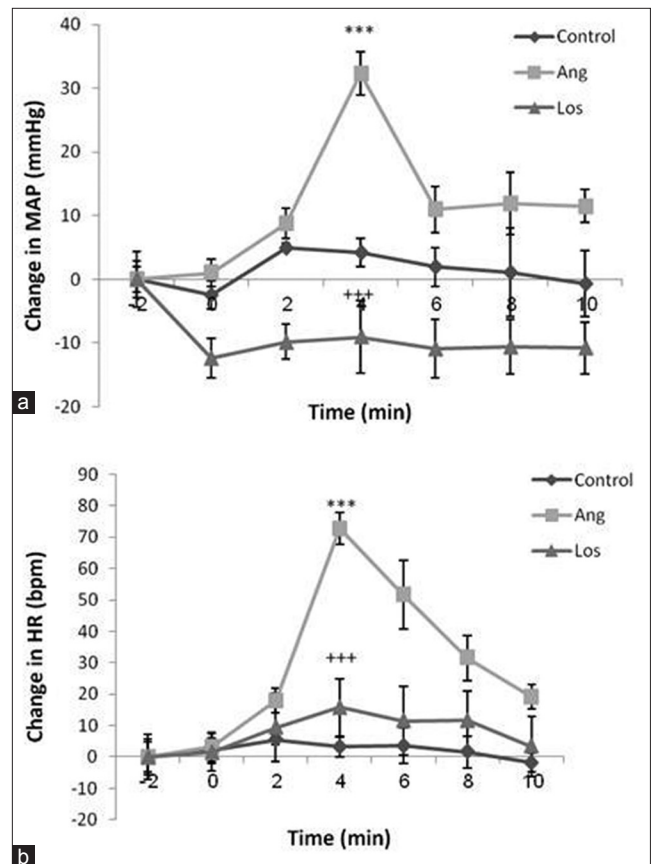


Figure 1: Time courses of changes in mean arterial pressure (MAP) and heart rate (HR) in response to injection of angiotensin II (Ang II) (300 ng) in groups of control, Ang II and losartan. For each group, $n = 8$. (a) MAP, $***P < 0.001$ compared to control and $+++P < 0.001$ compared to Ang group. (b) HR, $***P < 0.001$ compared to control and $+++P < 0.001$ compared to Ang group

Effect of systemic i.p. pretreatment with different doses of *Teucrium polium* on the cardiovascular effects of angiotensin II

Pretreatment of normotensive rats with different doses of TP (100, 200, and 400 mg/kg, i.p.) reduced significantly MAP compared to baseline (paired *t*-test, $P < 0.001$ for all three doses, data not shown) but had no effect on HR.

The cardiovascular responses to Ang II injection antagonized by TP so that in these groups the maximum changes of MAP were significantly lower than those of induced in Ang group (Δ MAP: 14.73 ± 3.94 , 13.44 ± 4.93 , 14.19 ± 1.9 compared to 32.37 ± 3.37 mmHg, respectively, *t*-test, $P < 0.001$ for all three groups). Moreover, MAP changes in all three groups of TP extract were significantly lower than Ang group (repeated

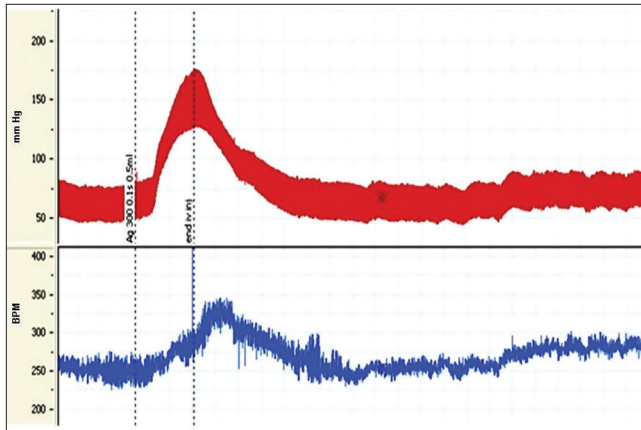


Figure 2: Typical recording of blood pressure and heart rate in response to angiotensin II injection. The vertical lines indicate the injection time

measures ANOVA, $P < 0.001$) [Figure 3a] and HR changes in two groups of TP 100 and TP 200 showed a significant reduction compared to Ang group (repeated measures ANOVA, $P < 0.001$ and $P < 0.01$, respectively) [Figure 3b].

However, no significant HR changes were observed between these pretreated groups and Ang group in the maximum level of effect [Δ HR: 55.6 ± 3.8 , 53.62 ± 5.25 , 60.12 ± 8.87 compared to 72.79 ± 5.10 beats/min, respectively; Figure 3].

DISCUSSION

In the present study, the injection of normal saline with the same volume of TP extract had not significant effects on blood pressure and HR of the control group. All three doses of TP extract (100, 200, and 400 mg/kg) significantly reduced MAP in normotensive condition as well as inhibited of increase in blood pressure after injection of Ang II which indicated a relaxation effect on vascular smooth muscles.

Angiotensin II regulates blood pressure and plasma volume via aldosterone-regulated sodium excretion, sympathetic nervous activity, and thirst responses.^[13]

As previous studies have been shown, Ang II-mediated hypertension could be induced through different signaling events; stimulation of phospholipase C and phosphatidylinositol hydrolysis, increased intracellular free calcium concentration, activation of protein kinase C, increased intracellular free concentrations of Na^+ ,

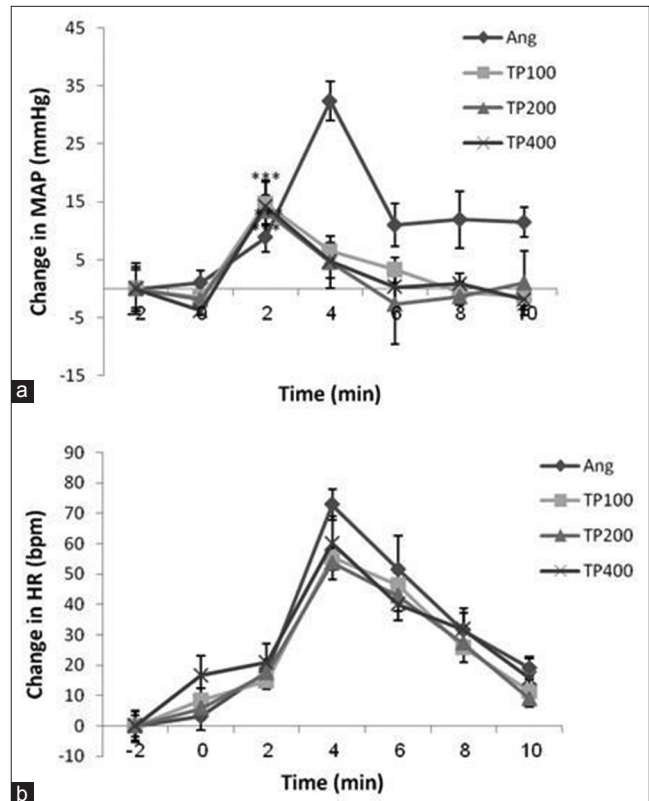


Figure 3: Time courses of changes in mean arterial pressure (MAP) and heart rate (HR) in response to injection of angiotensin II (Ang II) (300 ng) in groups of Ang II and three doses of *Teucrium polium* L. (TP) extract (TP 100, TP 200 and TP 400). For each group, $n = 8$. (a) MAP, $***P < 0.001$ compared to Ang group. (b) HR

and decreased intracellular free concentrations of Mg^{2+} , activation of tyrosine kinases, implication of mitogen-activated protein kinase pathways or phospholipase D activation,^[13,14] so in our study the hypotensive effect of TP extract could be attributed to affect on each of these pathways. Besides, former reports have also demonstrated that TP extract had relaxation effects on ileum^[15] and vascular smooth muscles.^[11] Moreover, TP contains important ingredients such as salvigenin, cirsiolol, pinen- α and β , sabinene, myrcene, germacrene D, limonene, β -caryophyllene, spathulenol which can influence on vascular smooth muscle tone. For instance, in many studies, the antispasmodic effect of some components such as α - and β -pinen,^[16,17] cirsiolol,^[18,19] spathulenol,^[20] limonene,^[21] and salvigenin^[22] from other members of Lamiaceae family have been demonstrated. Altogether, it seems that the TP ingredients can induce the hypotensive effects in rats.

On the other hand, abundant evidence now suggests that a key mechanism by which Ang II influences blood pressure is via its ability to produce reactive oxygen species (ROS).^[23,24] Most investigations of Ang II hypertension and oxidant stress have focused on the vasculature as a key player, and in particular the notion that increased levels of O₂ lead to diminished bioactivity of nitric oxide and thus vasoconstriction.^[25]

An important role for ROS-mediated vascular smooth muscle hypertrophy and remodeling in Ang II-dependent hypertension has also received considerable attention so considering to known antioxidant properties of TP,^[26-29] it can impress on hypertension through this mechanism.

Our data showed that in spite of the effect of TP on lowering of blood pressure it could not improve tachycardia induced by Ang, but even if TP could reduce HR, it seems activation of baroreflex, which may be had a role in blood pressure reduction conceal impact of TP on HR, in addition, since the baroreflex response is impaired in anesthesia^[30] therefore, no changes of HR may at least in part, be due to the blunted of baroreflex activity.

CONCLUSIONS

Taking together upon to our findings TP can prevent hypertension induced by activation of RAS through antagonizing the effects of Ang II and according to its various ingredients as well as different implicated pathways involve in this type of hypertension, the mechanisms of TP action are different.

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