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Original Article

# Hypotensive effect of *Centella asiatica L.* extract in acute Angiotensin II-induced hypertension

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## Abstract

*Centella asiatica L. (C.asiatica)* is a plant with a hypotensive effect. Since this effect has several mechanism(s), therefore in the current study we investigated the effects of hydroalcoholic extract of *C.asiatica* on cardiovascular parameters in acute hypertensive rats. Animals were divided into four groups; 1) control, 2) Angiotensin II (AngII) that received (50 ng/kg) intravenously (i.v.), 3 and 4) two groups of *C.asiatica* extract (CA100, and CA200 mg/kg). In treated groups, 30 min after injection of the extract, AngII was injected. Cardiovascular parameters were recorded by the power lab system after the cannulation of the femoral artery. The maximum changes (Δ) of systolic blood pressure (SBP), mean arterial pressure (MAP), and heart rate (HR) were calculated and used for statistical analysis. In the AngII group, ΔSBP and ΔMAP significantly increased compared to the control group. The HR also decreased compared to the control group but it was not significant. In CA100+AngII group ΔSBP and ΔMAP significantly decreased compared to the AngII. The use of these doses of CA with AngII compared to AngII alone had a significant effect on the HR. In CA200+AngII group ΔSBP and ΔMAP significantly decreased compared to the AngII. The change of HR in this dose was not significant to the AngII. In a recent study, we concluded that *C.asiatica* decreased the cardiovascular responses in hypertensive rats received AngII. Also between two doses of *C.asiatica* did not observe any significant difference. Therefore, one of the most important hypotensive mechanism(s) of the considered plant can be the effect on Renin-angiotensin system.

*Keywords*: *Angiotensin II, Blood pressure, Centella asiatica L.*, Cardiovascular, Hypertension, Rat.

## 1. Introduction

Hypertension that sometimes was called a silent killer is an important and serious risk factor for cardiovascular diseases such as myocardial infarction, arteriosclerosis, heart failure, and kidney failure [1]. The prevalence of hypertension is increasing worldwide and it is anticipated that number of adults that

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contract to hypertension will increase by about 1.5 billion in 2025 [2]. The etiology of hypertension is unknown exactly, however different mechanisms have been proposed to causes this disorder. For example proliferation of smooth muscle cells that lead to an increase in total peripheral resistance, the function of endothelial cells that by releasing of local factors can regulate vascular tone, mostly and the well-known mechanism is Renin-angiotensin system (RAS) [3]. The main product of RAS is Angiotensin II (AngII) that which has an acute and chronic regulatory role in the cardiovascular system. AngII is strong vasoconstriction and also it induces the proliferation of smooth muscle cells that are involved in the pathogenesis of cardiovascular diseases [4]. Today use of herbal medicines by the people worldwide due to lesser side effects, availability, safety, being cheaper is increasing [5]. With the regarded role of AngII in hypertension, investigating these plants can be useful in the management of hypertension. The *Centella asiatica L.* (*C.asiatica*) is a small herbaceous plant that it is belongs to the Apiaceae family (Figure 1). This plant is native to South East Asia, parts of China, India and other regions. The main bioactive compounds of this plant are triterpene acids, glycosides, asiatic acids, alkaloids, flavonoids, volatile and fatty oil and others. According to the pharmacological studies, this plant has different biological activities such as gastric ulcer healing, memory enhancing, antitumor, neuroprotective, cardioprotective, hepatoprotective, and several effects on different organs [6].

In the previous studies, we investigated the hypotensive effects of several plants in AngII-induced hypertensive rats. These plants were including *Nigella sativa* [7], *Ziziphus jujuba* [8], and *Ribes khorasanicum* [9].

Intharachatorn *et al.* showed that *C.asiatica* extract decreased elevated blood pressure in hypertensive rats induced by $N^G$-nitro-L-arginine methyl ester (LNAME). Also, the antihypertensive effects of this plant in hypertensive rats induced with phenylephrine were demonstrated [10]. Although the hypotensive effects of *C.asiatica* have been reported but exact mechanisms of *C.asiatica* need to be studied more. Thus, in the current study, we investigated the effects of hydroalcoholic extract of *C.asiatica* on cardiovascular parameters in acute hypertensive rats.

## 2. Materials and Methods

### *2.1. Extract Preparation*

*C.asiatica* was collected in the spring season from Bandar Anzali lagoon, Gilan, Iran. This plant was identified by a botanist at Medicinal Research Center, Gilan University of Medical Sciences, Gilan, Iran (Voucher No: 13335). *C.asiatica* was grounded and 100 gr of its powder was added to 1800 ml of ethanol 70%. This solution was mixed up for 72 h at

37℃. A filter paper was used to separate the soluble waste. The solvent was evaporated by using an oven at 40°C. Finally, the desired concentrations were obtained by adding saline [8].

### *2.2. Animals and Surgery*

Male Wistar rats were obtained from Mashhad Laboratory Animals Reproduction and Conservation Center. Twenty four rats were kept under standard conditions. The animals were anesthetized with urethane (1.5 g/kg, i.p). After confirming the anesthesia to record cardiovascular parameters the femoral artery and for drug injection, the femoral vein was cannulated. The femoral artery was cannulated with a blue angiocath filled with heparinized saline and connected to a pressure transducer and blood pressure (BP) and heart rate (HR) were continuously recorded by a power lab system (ID instrument, Australia) [11]. The femoral vein was also cannulated in the same way as the femoral artery.

### *2.3. Drug and Animal Groups*

Urethane and AngII were bought from Sigma, USA. All drugs were dissolved in saline. Twenty four male rats with weight range (250-300 gr) were divided randomly into 5 experimental groups (n = 6 in each group) including:

1. Control: saline was injected (i.v, 0.5cc)
2. AngII: AngII was perfused slowly (50 ng/kg, i.v.) [12]

3 and 4: *C.asiatica* +AngII: doses of 100, 200 mg/kg of *C.asiatica* extract (CA100 and CA200) were injected intraperitoneally[13] and AngII was injected after 30 min.

In all groups, systolic blood pressure (SBP), mean arterial pressure (MAP) and heart rate (HR) were measured during the experiment.

### *2.4. Data Analysis*

The changes (Δ) of the cardiovascular parameters (MAP, SBP, and HR) were calculated and indicated as mean±standard error of means (SEM). Statistical analysis was performed by Instat software. $P<0.05$ was significant.

## 3. **Results and Discussion**

In the control group, saline after stabilizing of cardiovascular parameters was injected intravenously and then responses of cardiovascular for 15 min were recorded. Injection of saline had no significant changes on the cardiovascular parameters (ΔHR; 5.4 ± 3.4 beats/min), (ΔSBP; 0.8 ± 2.3 mmHg) and (ΔMAP; 0.6 ± 1.7 mmHg).

In the AngII group, AngII (50 ng/kg) was perfused slowly and cardiovascular responses were measured. According to Fig. 2A, B and C, in the AngII group ΔSBP and ΔMAP significantly increased compared to the control group (ΔSBP; AngII: 53.4 ± 4.9 *vs* control: 0.8 ± 2.3 and ΔMAP; AngII: 43.3 ±3.7 *vs* control: 0.6 ± 1.7 ($P<0.001$)). The HR also decreased compared to the control group but it was not significant (ΔHR:-17.8 ± -8.6 *vs* control: 5.4 ± 3.4 beats/min).

Changes in CA100+AngII group was demonstrated that ΔSBP and ΔMAP significantly decreased compared to the AngII (ΔSBP; CA100+AngII: 12.6 ± 1 *vs* AngII: 53.4 ± 4.9 (P<0.001) and ΔMAP; CA100+AngII: 18.9 ± 7.7 *vs* AngII: 43.3 ± 3.7 (P<0.05)). The use of this doses of CA with AngII compared to AngII alone, had a significant effect on the HR (ΔHR; CA100+AngII: 22.5 ± 9.2 *vs* AngII: -17.8 ± 8.6 (P<0.01)).

Results in CA200+AngII group were indicated that ΔSBP and ΔMAP significantly decreased compared to the AngII (ΔSBP; CA200+AngII: 17.2 ± 4.3 *vs* AngII: 53.4 ± 4.9 (P<0.001) and ΔMAP; CA200+AngII: 13.5 ± 4 *vs* AngII: 43.3 ± 3.7 (P<0.01)). The change of HR in this dose was not significant than the AngII (ΔHR; CA200+AngII: 2.2 ± 4.3 *vs* AngII: -17.8 ± 8.6).

In Fig. 3 a cardiovascular record sample was observed.

Usually, antihypertensive agents can work at several levels in the RAS. These levels include regulation of renin activity as angiotensinogen converting enzyme to angiotensin I, regulation of angiotensin-converting enzyme (ACE) activates AngI to AngII, regulation of AngII level, and ultimately the effect of AngII on their receptors, including AT1 and AT2 [14].

Compounds of the *C.asiatica* include flavonoids (quercetin, kaempferol), various glycosides, terpenoids, acetic acid, phytosterols, and tannins. The main constituents of this plant are madecassoside and asiaticoside [15]. Asiaticoside and madecassoside are the major triterpenoid type of saponin derived from *C.asiatica* and widely used in antioxidant and anti-inflammatory applications. Asiaticoside and madecassoside from *C.asiatica* have an ACE inhibitory effect [16].

Different types of triterpenes including saponins and aglycons have properties such as renin enzyme inhibitory properties and block of AT1 receptor in addition to the ET1 receptor which ultimately causes hypotension [14]. Triterpenoids also result in the reduction of heart rate as well as blood pressure [17].

*C.asiatica* flavonoids also have an inhibitory effect on the ACE result in the reduction of AngII level cause to hypotension. Some studies have reported that quercetin and anthocyanins, active ingredients from most herbal plants, inhibit the RAS pathway by inhibiting ACE activity and reducing ACE mRNA generation in human embryonic kidney-293 cells. Proanthocyanidins inhibited the binding of AngII to the AT1 receptors [18]. Some flavonoids such as can reduce the heart rate [19].

Angiotensin II also causes oxidative stress through increased ROS. Therefore, the antioxidant activity of the above-mentioned substances can also help reduce the damage caused by oxidative stress [20].

Intharachatorn *et al*. have indicated that *C.asiatica* has a hypotensive effect in hypertension induced by L-NAME in rats [21]. Therefore, we can conclude that nitric oxide also has an important role in addition to RAS in hypotension induced by *C.asiatica* extract

administration. Also between two doses of *C.asiatica* did not observe any significant difference. Probably, because these doses have been selected closely, the *C.asiatica* hypotensive effect is near together. Therefore, we suggest that higher doses of *C.asiatica* in addition ECG be investigated for evaluation of cardiovascular responses in animals.

## 4. Conclusion

In the recent study, we concluded that *C.asiatica* decreased the cardiovascular responses in hypertensive rats received AngII. Also between two doses of *C.asiatica* did not observe any significant difference. Therefore, one of the most important hypotensive mechanism(s) of considering plant can be the effect on RAS.

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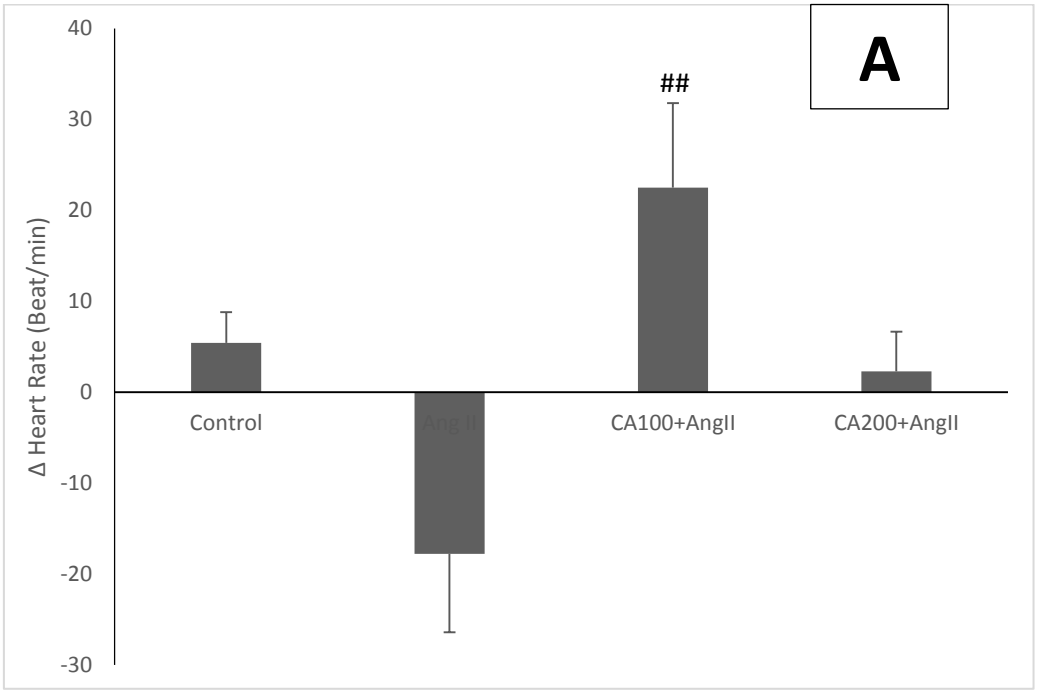
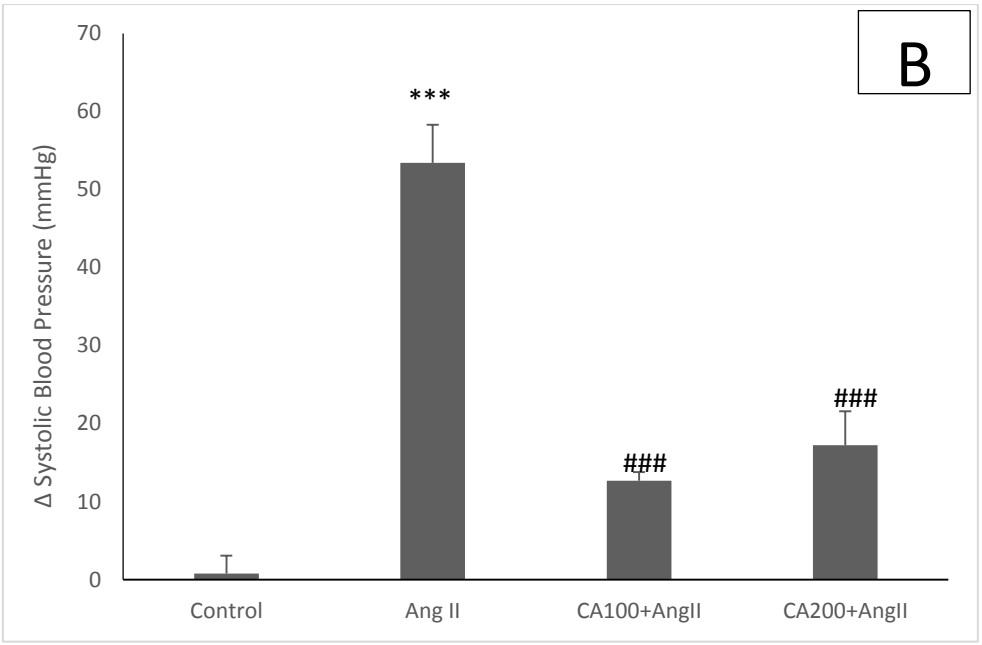
# Figures

**Figure 1**. Natural image for *Centella asiatica L.*





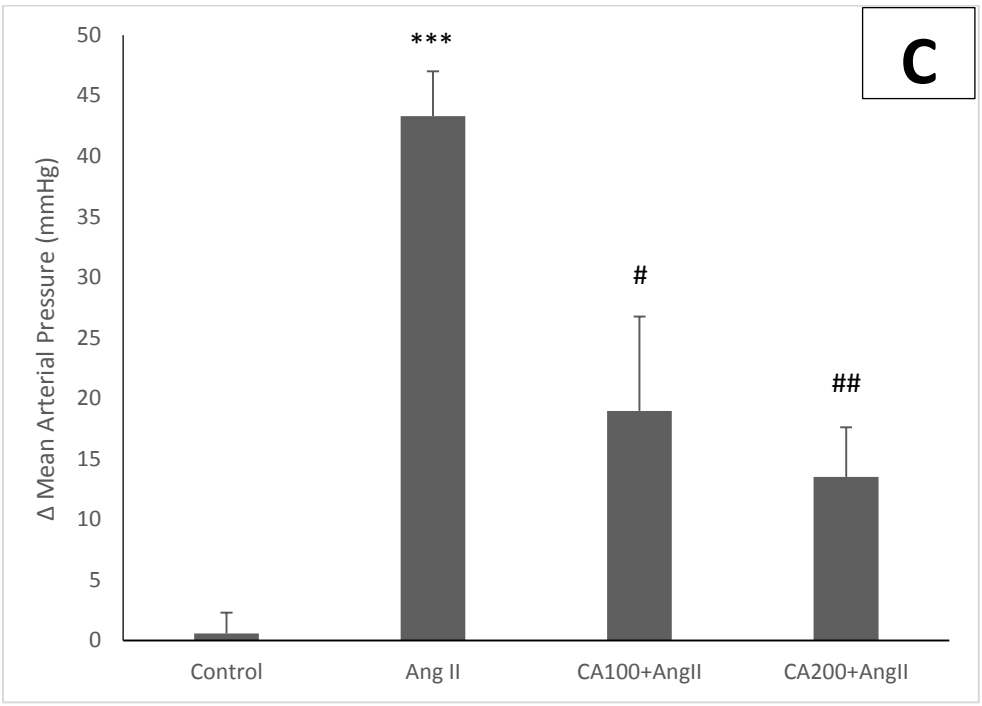




**Figure 2**. Effects of two doses of hydroalcoholic extract of *Centella asiatica L.* (CA 100 and 200 mg/kg) with AngII on the ΔHR(a), ΔSBP(b), ΔMAP(c) in anesthetized rats.***P<0.001 comparison between AngII and control groups # P<0.05, ## P<0.01, ### P<0.001 comparisons between AngII and CA 100 and 200 with AngII The data were expressed as mean ± SEM. One‑way ANOVA used for statistical analysis.

SBP: Systolic blood pressure, MAP: mean arterial pressure, HR: heart rate

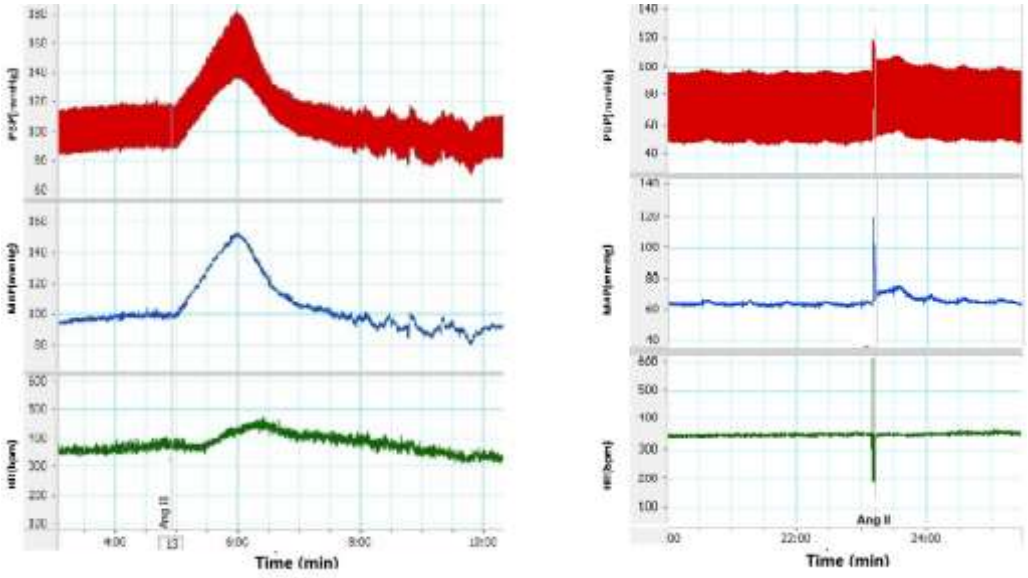



**Figure 3**. The cardiovascular record samples from AngII (left) and treated rats with of hydroalcoholic extract of *Centella asiatica L*. (CA200mg/kg) (right).

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