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Asiatic Acid Reduces Blood Pressure and Improves Vascular Function in Nitric Oxide Deficient Hypertensive Rats

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Background and Objective: It has previously been reported that hypertension induced by the chronic blockade of NO (nitric oxide) production is characterized by increased blood pressure and impairment of endothelial-dependent vasorelaxation. Asiatic acid is widely found in dietary fruits and vegetables, and is a major component of various medicinal plants used in Asian countries. Thus, this study was to examine the effect of asiatic acid on the development of blood pressure in chronic nitric oxide-deficient rats.

Method: Male Sprague-Dawley rats were administered with N$^{\omega}$-nitro-L-arginine methyl ester (L-NAME) (40 mg/kg/day) in drinking water for five weeks. Asiatic acid (20 mg/kg/day) or vehicle was orally administered in thelast two weeks. Then hemodynamic parameters and vascular reactivity were measured.

Results: Daily administration of L-NAME for five weeks showed significant increases in blood pressure, heart rate, hindlimb vascular resistance, a decrease in hindlimb blood flow and impairment of vascular function ($p<0.05$). However, the supplementation of asiatic acid significantly alleviated the impairment of hemodynamic status and improved vascular function in L-NAME treated rats ($p<0.05$).

Conclusion: This study suggests that asiatic acid exhibit an anti-hypertensive effects in rats treated with L-NAME and its effect is likely to be linked with the restoration of endothelial function.

Key words: Asiatic acid, Hypertension, Vascular function

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Proceeding

Introduction

Chronic blockade of NO (nitric oxide) formation by the administration of L-NAME, a nitric oxide synthase inhibitor induces a dose-dependent increase in blood pressure and its physiological and pathological characteristics resemble essential hypertension [1,2]. Thus, this animal model of hypertension has been extensively used *in vivo* study [3,4]. Moreover, the impairment of vascular function with defective response to endothelial-dependent vasodilators also presented in L-NAME treated rats [5].

Asiatic acid, a triterpenoid compound, is one of the constituent triterpenes derived from the medicinal plant *Centella asiatica*. Several recent studies reported the pharmacological activities of asiatic acid such as antioxidant, anti-hyperglycemia, anticancer and anti-inflammatory properties [6-9]. Nevertheless, there is no investigation the effects of asiatic acid supplementation

on blood pressure and vascular function in L-NAME induced hypertensive rats. We hypothesized that asiatic acid treatment might prevent the increasing of blood pressure associated with restoration of endothelial-dependent vasorelaxation.

Objective

This present study is aimed to evaluate the effect of asiatic acid on hemodynamic parameters and vascular function in L-NAME induced hypertensive rats.

Methods

Chemicals

Asiatic acid (Figure 1) was obtained from Sigma-Aldrich (St. Louis, MO, USA) (purity >95%).

Animals and experimental protocols

Male Sprague-Dawley rats (220-240 g) were purchased from the National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom. Rats were maintained in an air-conditioned room (25 $\pm$ 2 C°) with a 12 h dark-light cycle at Northeast Laboratory Animal Center. All procedures are complied with the standards for the care and use of experimental animals and approved by Animal Ethics Committee of Khon Kaen University, Khon Kaen, Thailand (AEKKU 37/2555).

After one week of acclimatization, the animals were randomly divided into two main groups. Group 1, the normal control group; received tap water throughout an experimental period, Group 2, the L-NAME-treated group; received L-NAME (40 mg/kg) in their drinking water for five weeks to induce hypertension. The animals in all experimental groups were fed with a standard chow diet (Chareon Pokapan Co. Ltd., Thailand). After three weeks of L-NAME treatment, normal control rats were divided into two groups (n = 6/group); control rats plus vehicle (polyethylene glycol) and control rats plus asiatic acid (20 mg/kg), and hypertensive rats were divided in to two groups (n = 6/group); hypertensive rats plus vehicle (polyethylene glycol) and hypertensive rats plus asiatic acid (20 mg/kg) for two weeks.

Hemodynamic assessments

At the end of experiment, the animals were anesthetized by peritoneal injection of pentobarbital-sodium (60 mg/kg) and placed on heating pad. Subsequently, a tracheotomy was made to assist respiration. The femoral artery was identified, cleaned of connective tissue and calnulated with a polyethylene tube. Systolic blood pressure (SP), diastolic blood pressure (DP), mean arterial blood pressure (MAP) and heart rate (HR) were continuously monitored by a way of a pressure transducer and recorded using the acknowledge data acquisition with analysis software (Biopac System Inc., California, USA.). Hindlimb blood flow (HBF) was continuously measured by placing electromagnetic flow probes around the abdominal aorta connected to an electromagnetic flow meter (Carolina Medical Electronics, North Carolina, USA). Hindlimb vascular resistance (HVR) was calculated from the MAP and mean HBF.

Vascular reactivity assessments

Femoral vein was also canulated with a polyethylene tube. To test the endothelial and smooth muscle cell functions, a vascular responsiveness was carried out by intravenous infusion of acetylcholine (ACh), an endothelium dependent vasodilator, (3, 10, 30 nmol/kg), sodium nitroprusside (SNP), an endothelium independent vasodilator, (1, 3, 10 nmol/kg) and phenylephrine (Phe), an $_1$ adrenoceptor agonist, (0.01, 0.03, 0.1 ติmol/kg).

Statistical analysis

Data were expressed as mean $\pm$ S.E.M. The differences among treatment groups were analyzed by one-way analysis of variance (ANOVA) followed by post-hoc Duncan's multiple range tests. A *p*-value of less than 0.05 was considered a statistical significance.

Results

Rats with chronic L-NAME treatment showed a significant increase in SP, DP, MAP and HR (p<0.05) compareed to normal control rats (Table 1). In addition, the arterial blood flow was decreased in L-NAME treated rats (4.35 ± 0.1 ml/min/100 g tissue) comparing to normal control rats (7.63 ± 0.32 ml/min/100 g tissue) (p<0.05) (Figure 1A). This low arterial blood flow was observed in L-NAME rats consistent with high vascular resistance (39.50 ± 1.62 mmHg/ml/min/100 g tissue) (Figure 1B). Interestingly, a concomitants administration of asiatic acid for two weeks significantly reduced SP, DP, MAP and HR in hypertensive rats (p<0.05). Moreover, treatment with asiatic acid for two weeks also significantly increased HBF (5.93 ± 0.36 ml/min/100 g tissue) as well as decreased HVR (22.34 ± 1.19 mmHg/ml/min/100 g tissue) when compared to those of hypertensive rats (p<0.05).

Vasodilation responses to ACh (3, 10, 30 nmol/kg) was significantly blunted in hypertensive rats received vehicle when compared to control rats (29.36 ±1.9 % vs. 59.97 ± 1.2 %), (41.83 ±0.5 % vs. 56.57± 1.3 %) and (49.20 ± 2.5 % vs. 65.61 ± 1.2 %) dose respectively (p<0.05) (Figure 2A). Treatments of asiatic acid to L-NAME rats markedly restored the vasodilation response to Ach at all concentration (p<0.05). However, there was no significant difference in vascular responses to SNP in all groups of rats (Figure 2B). In addition, vasoconstriction responses to Phe was significantly impaired in hypertensive rats comparing to control rats (22.55± 3.8 % vs. 36.15 ± 2.1 %), (28.94 ± 5.2 % vs. 50.07 ± 3.1 %) and (37.65 ± 5.2 % vs. 66.06 ± 3.5 %) dose respectively (p<0.05) (Figure 2C). The restoration of vascular responses to Phe was oberved in L-NAME hypertensive rats treated with asiatic acid.

Conclusion

This study demonstrates that administration of L-NAME in drinking water for five weeks induces a progressive increase in blood pressure, heart rate, hindlimb vascular resistance and impairment of v ascular function. These impairment of hemodynamic

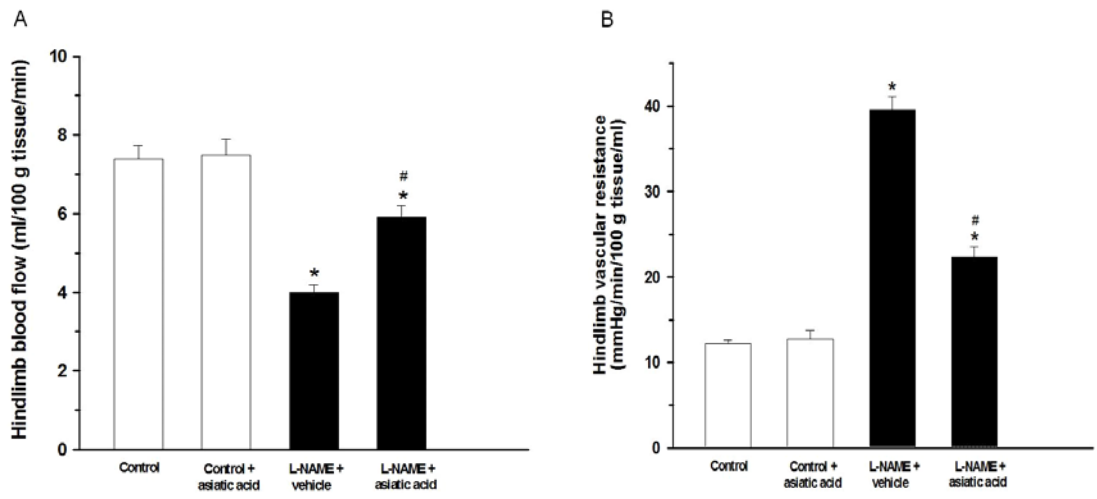

Figure 1 Effects of asiatic acid on HBF (A) and HVR (B) in all experimental groups. Data are presented as mean ± S.E.M. (n = 6/group). * p<0.05 vs. control, # p<0.05 vs. L-NAME + vehicle

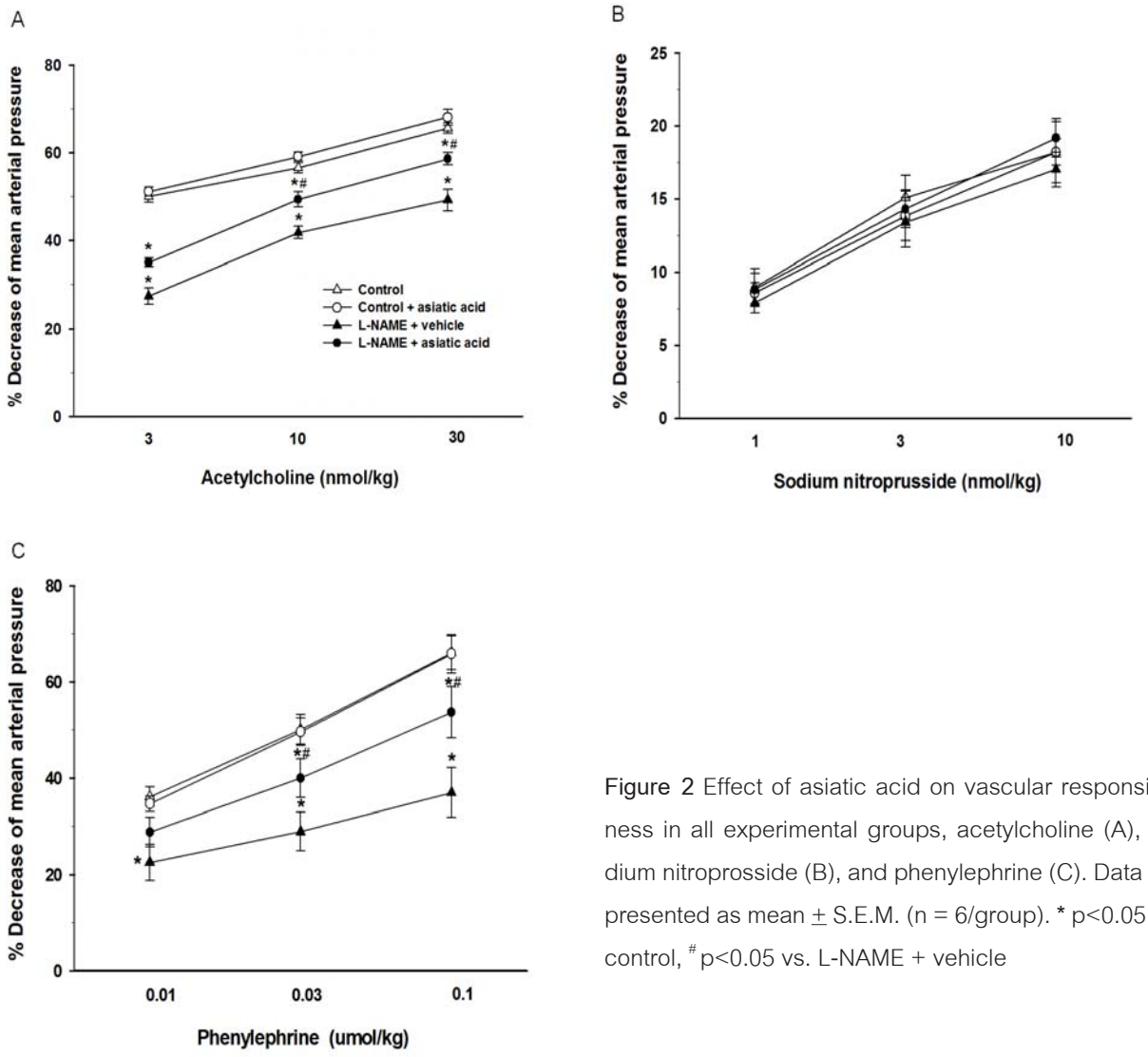

Figure 2 Effect of asiatic acid on vascular responsiveness in all experimental groups, acetylcholine (A), sodium nitroprosside (B), and phenylephrine (C). Data are presented as mean ± S.E.M. (n = 6/group). * p<0.05 vs. control, # p<0.05 vs. L-NAME + vehicle

Table 1 Effects of Asiatic acid on SP, DP, MAP and HR in all experimental groups (n = 6).

Parameters	Control	Control + asiatic acid	L-NAME + vehicle	L-NAME + asiatic acid
SP (mmHg)	120.3 ± 1.9	121.9 ±2.7	208.1 ± 6.5*	167.2 ± 3.2*#
DP (mmHg)	78.8 ± 2.2	81.7 ± 2.2	152.3 ± 7.5*	112.1± 3.2*#
MAP (mmHg)	92.6 ± 2.0	95.1 ± 2.3	170.9 ± 7.2*	130.4 ± 2.9*#
HR (beat/min)	346.88 ±7.1	339.80 ± 5.2	413.01 ± 9.9*	359.34 ± 4.9#

*p<0.05 vs control, #p<0.05 vs L-NAME + vehicle.

status evidently improved by treatment of asiatic acid. Additionally, the restoration of vascular function in L-NAME induced hypertensive rats was presented after asiatic acid supplementation. The results from this study could suggest that beneficial effect of asiatic acid which is present in several edible plants and it is feasible to alleviate hypertension and other vascular diseases.

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