

Asiatic Acid Decreases Blood Pressure and Oxidative Stress Markers

in L-NAME-induced Hypertensive Rats

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ความดันเลือดสูงที่ถูกเหนี่ยวนำด้วยสารแอลเนม

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ABSTRACT

This study aimed to investigate the effect of asiatic acid on blood pressure and oxidative stress status in N-nitro-L-arginine-methylester (L-NAME) induced hypertensive rats. Daily administration of L-NAME (40 mg/kg) in male Sprague-Dawley rats for 5 weeks showed significant increases in systolic blood pressure (SP), vascular superoxide production, plasma and heart tissue malondialdehyde (MDA) ($p<0.05$). However, the supplementation of asiatic acid (20 mg/kg) or L-arginine (100 mg/kg) for 2 weeks significantly reduced the elevation of SP, and oxidative stress markers ($p<0.05$) in L-NAME treated rats. This study suggests that asiatic acid had an anti-hypertensive effects in rats treated with L-NAME and its effect is likely to be linked with an alleviation of oxidative stress status.

บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของสารเอเชียติก แอซิด ต่อความดันเลือดและภาวะเครียดออกซิเดชัน ในหนูขาวความดันเลือดสูงที่ถูกเหนี่ยวนำด้วยสารแอลเนม ผลการทดลองพบว่าหนูทดลองที่ได้รับสารแอลเนม (40 มก./กก.) เป็นเวลา 5 สัปดาห์ มีการเพิ่มขึ้นของความดันเลือด การผลิต superoxide ในหลอดเลือด ระดับของ malondialdehyde (MDA) ในพลาสมาและเนื้อเยื่อหัวใจ ($p<0.05$) อย่างไรก็ตามการให้เอเชียติก แอซิด (20 มก./กก.) หรือ L-arginine (100 มก./กก.) เป็นเวลา 2 สัปดาห์ สามารถลดระดับความดันเลือด และสารบ่งชี้ภาวะเครียดออกซิเดชัน ($p<0.05$) ในหนูทดลองความดันเลือดสูง การศึกษาครั้งนี้แสดงให้เห็นว่า เอเชียติก แอซิด มีฤทธิ์ต้านความดันเลือดสูงในหนูทดลองที่ได้รับแอลเนม โดยผลดังกล่าวอาจจะมีความสัมพันธ์กับการลดลงของภาวะเครียดออกซิเดชัน

Key Words: Hypertension, Oxidative stress, Asiatic acid

คำสำคัญ: ภาวะความดันเลือดสูง ภาวะเครียดออกซิเดชัน เอเชียติก แอซิด

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Introduction

N-nitro-L-arginine-methylester (L-NAME) is a nonspecific inhibitor of all three nitric oxide synthase (NOS) including neuronal NO synthase (nNOS), inducible NO synthase (iNOS), and endothelial NO synthase (eNOS). Administration of L-NAME can induce high blood pressure in an animal model (Arnal *et al.*, 1993; Bernatova *et al.*, 1999). This was related to the decrease in NO production which augments vasoconstriction (Attia *et al.*, 2001). Moreover, an increase in protein, lipid peroxidation and decrease antioxidant enzymes also have been reported in L-NAME hypertensive rats (Cardoso *et al.*, 2012; Nakmareong *et al.*, 2011).

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, hepatoprotective, anticancer and antiinflammatory properties (Liu *et al.*, 2006; Ma *et al.*, 2009; Shyun Huang-Shyh *et al.*, 2011; Soo Lee *et al.*, 2003). Asiatic acid has also reported to lower blood glucose level in type 1 diabetic rats (Liu *et al.*, 2010). However, there is no investigation the effects of asiatic acid supplementation on L-NAME induced hypertension and oxidative stress in rats. Therefore, the aimed of the present study was to evaluate whether asiatic acid could reduce blood pressure and oxidative stress status in L-NAME induced hypertensive rats.

Materials and methods

Chemicals

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

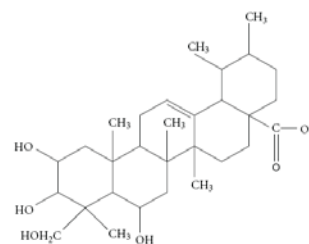


Figure 1 Chemical structure of asiatic acid.

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 ($25 \pm 2^\circ\text{C}$) 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.

After one week of acclimatization, the animals were randomly divided into 4 groups ($n = 7/\text{group}$). Group 1, the normal control group, received tap water throughout an experimental period, whereas in the L-NAME-treated group (Group 2-4) rats received L-NAME (40 mg/kg) in their drinking water for 5 weeks to induce hypertension. The animals in all experimental groups were fed with a standard chow diet (Chareon Pokapan Co. Ltd., Thailand). After 3 weeks of L-NAME treatment, normal rats were orally administered with polyethylene glycol and hypertensive rats, group 2, 3 and 4, were orally given polyethylene glycol, asiatic acid (20 mg/kg), L-arginine (100 mg/kg) respectively for 2 weeks. L-arginine is an α -amino acid and it is well known as a precursor of NO synthesis. Therefore, rats received L-arginine was a positive control group.

Indirect blood pressure measurement

Animals were determined systolic blood pressure (SP) using a tail-cuff plethysmography (IITC model 179 blood pressure analyzer) once a week to assess blood pressure changes during 5 weeks of experiment. At the end of treatment, rats were anesthetized with peritoneal injection of pentobarbital-sodium (60 mg/kg) and blood samples were collected for plasma MDA measurement. Carotid arteries were rapidly excised and used for analysis of $O_2^{\bullet-}$ production.

Assay of $O_2^{\bullet-}$ production

Vascular $O_2^{\bullet-}$ production was measured using lucigeninenhanced chemiluminescence method as described previously (Luangaram *et al.*, 2007). In brief, the carotid arteries were quickly dissected and cleaned of adherent fat and connective tissue on ice. The vessel segments (0.5-1 cm) were placed in Krebs-KCl buffer and allowed to equilibrate at 37 °C for 30 min. Lucigenine was added in sample tube and placed in luminometer (Turner Biosystems, 23 CA, USA). The photon counts were integrated every 30 s for 5 min and averaged. The vessels were dried at 45 °C for 24 h, for determination of dry weight. $O_2^{\bullet-}$ production in vascular tissue was expressed as relative light unit counts per minute per milligram of dry tissue weight.

Assay of malondialdehyde (MDA)

The level of MDA was assayed following a previous described method of Luangaram and coworkers (2007). In brief; 150 µl of plasma was reacted with 10 % TCA, 125 µl of 5 mM EDTA, 125 µl of 8 % SDS and 10 µl of 0.5 µl/ml of BHT. The mixture was left for 10 min, then 0.6 % TBA was added in an equal volume and the mixture was heated for 30 min in a boiling water bath. After cooling to room temperature, the mixture was centrifuged 10,000

g for 5 minutes at 25 °C. The absorbance of the supernatant was measured at the wavelength of 532 nm by spectrophotometry. The amount of MDA in tissue was calculated using a standard curve of 1,1,3,3-tetra-ethoxypropane (0.3–10 µmol/l). The MDA concentration in the tissues was normalized against the protein concentration. Protein was determined by the Bradford dye binding method.

Statistical analysis

Results were expressed as mean ± SEM. 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

Effect of asiatic acid and L-arginine on blood pressure

Blood pressure of rats was measured by the indirect tail-cuff method. As shown in Fig. 2, baseline values of SP at the beginning of the experiment were similar among all experimental groups. L-NAME administration caused a progressive increase in SP (194.58 ± 9.5 mmHg) after the 3 weeks of its administration comparing to those of normal control rats (117.60 ± 2.2 mmHg) ($p < 0.001$). Treatments of asiatic acid (20 mg/kg) for 2 weeks decreased SP (162.00 ± 2.8 mmHg) in hypertensive rats when compared to those of hypertensive rats without treatment (211.79 ± 6.6 mmHg) ($p < 0.05$). In addition, a reduction of SP (143.90 ± 4.3 mmHg) ($p < 0.05$) in hypertensive rats treated with L-arginine (100 mg/kg) for 2 weeks was also observed (Fig. 2).

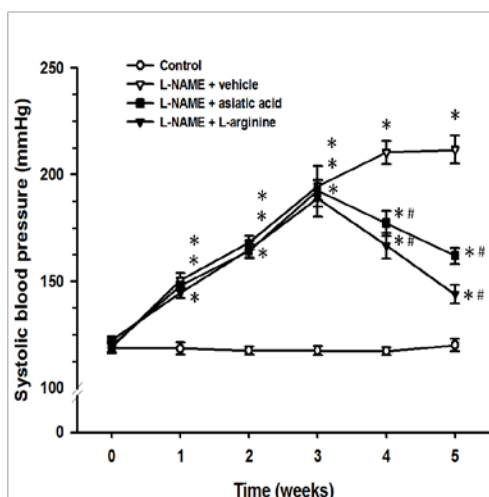


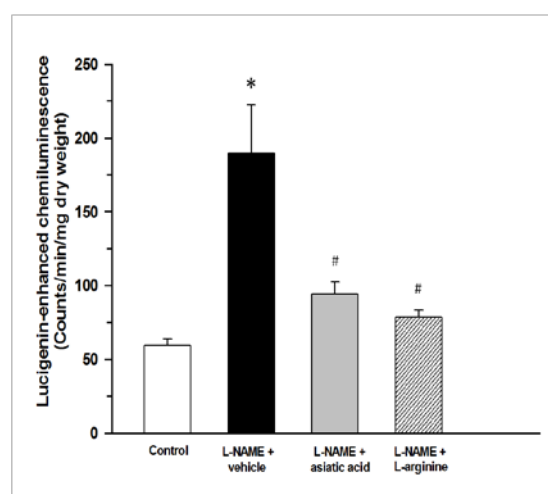
Figure 2 Effect of asiatic acid and L-arginine on SP during LNAME administration for 5 weeks. Results are expressed as mean \pm SEM. * $p < 0.05$ vs. normal control group, # $p < 0.05$ vs. L-NAME + vehicle (n = 5-7/group).

Effect of asiatic acid and L-arginine on oxidative stress status

Increased oxidative stress was found in rats receiving L-NAME. This was reflected by increase of vascular $O_2^{\cdot-}$ production (189.51 ± 32.90 counts/min/mg dry weight) in L-NAME induced hypertensive rats when compare to those of normal control rats (59.33 ± 4.51 counts/min/mg dry weight) ($p < 0.05$). Interestingly, administration of asiatic acid (20 mg/kg) for 2 weeks significantly reduced vascular $O_2^{\cdot-}$ production (94.37 ± 8.09 counts/min/mg dry weight) ($p < 0.05$) in L-NAME induced hypertensive rats. Moreover, daily treatment with L-arginine (100 mg/kg) for 2 weeks also significantly decreased vascular $O_2^{\cdot-}$ production (78.71 ± 4.83 counts/min/mg

dry weight) ($p < 0.05$) when compared to those of hypertensive rats (Fig. 3).

Figure 3 Effect of asiatic acid and L-arginine on vascular superoxide production in all experimental groups. Results are expressed as mean \pm SEM. * $p < 0.05$ vs. normal control group, # $p < 0.05$ vs. L-NAME + vehicle (n = 5-7/group).



Similarly, plasma and tissue MDA levels were higher in L-NAME induced hypertensive rats (plasma; 11.21 ± 1.10 μ M, heart; 4.06 ± 0.29 μ M/mg protein) comparing to those of normal rats (plasma; 2.75 ± 0.28 μ M, heart; 1.03 ± 0.10 μ M/mg protein) ($p < 0.05$). However, increasing of plasma and heart tissue MDA levels in L-NAME induced hypertensive rats was attenuated by asiatic acid supplementation (plasma; 4.57 ± 1.19 μ M, heart; 1.71 ± 0.10 μ M/mg protein) ($p < 0.05$). As well, treatment of L-arginine for 2 weeks suppressed plasma and tissue MDA (plasma; 4.46 ± 1.21 μ M, heart; 1.32 ± 0.12 μ M/mg protein) ($p < 0.05$) in L-NAME treated rats (Fig. 4A and 4B).

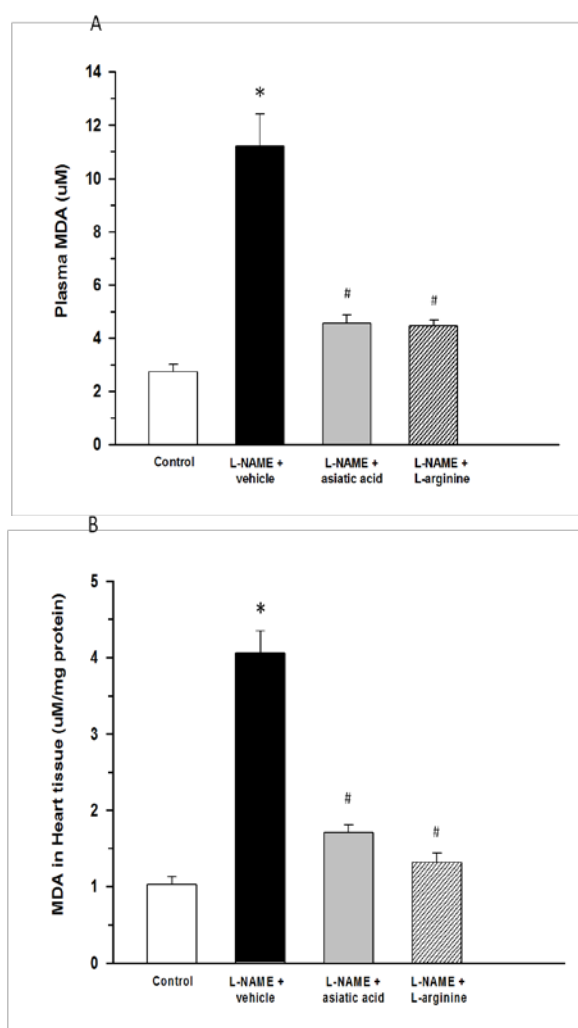


Figure 4 Effect of asiatic acid and L-arginine on plasma MDA (A) and heart tissue (B) in all experimental groups. Results are expressed as mean \pm SEM. * $p < 0.05$ vs. normal control group, # $p < 0.05$ vs. L-NAME + vehicle (n = 5-7/group).

Discussion

This present study found that administration of L-NAME in rats for 5 weeks led to increases in blood pressure and oxidative stress as an increase in

vascular $O_2^{\bullet-}$ production, plasma and heart tissue MDA levels. However supplementation of either asiatic acid or L-arginine evidently reduced blood pressure, which was associated with an improvement of oxidative stress status by reducing vascular $O_2^{\bullet-}$ production and lipid peroxidation.

Chronic inhibition of NO synthesis with L-NAME showed an increase in blood pressure. It is known that L-NAME induces a sustained hypertension which is primarily due to the loss of both basal and stimulated NO production (Baylis *et al.*, 1992; Manning *et al.*, 1993). The decreasing of NO production contributes to imbalance of vessel homeostasis by stimulating vascular smooth muscle contraction and growth, platelet aggregation, and leukocyte adhesion to the vascular endothelium cells which finally lead to increase total peripheral resistance and blood pressure (Rongen *et al.*, 1994). Moreover, the increasing in oxidative stress status by increases lipid peroxidative products such as thiobarbituric acid reactive substances, and lipid hydroperoxides also enhance L-NAME induced hypertensive rats (Kumar *et al.*, 2012; Nakmareong *et al.*, 2011). Our finding is consistent with above that in L-NAME induce hypertensive rats had the excessive of vascular $O_2^{\bullet-}$ production and lipid peroxidation by increasing in plasma and heart tissue MDA.

In the present study, asiatic acid supplementation reduced blood pressure and oxidative stress biomarkers in hypertensive rats induced by chronic inhibition of NO synthesis with L-NAME. These results indicated that antihypertensive effect of asiatic acid may involve its antioxidant capacity. There is evidence support that asiatic acid supplementation increased the activities of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) in

the liver tissue and attenuated tissue MDA concentration in λ -carrageenan induced edema in mice (Shyun Huang-Shyh *et al.*, 2011). It has been suggested that the excessive of reactive oxygen species interacting with NO and reducing its bioavailability (De Gennaro Colonna *et al.*, 2005). Bioavailability of NO can be preserved by inhibition of oxidative stress (Kumar S *et al.*, 2010). Therefore, antioxidant properties of asiatic acid may decrease vascular tone by enhancing bioavailability of NO and contributing to decrease blood pressure.

This study we use L-arginine as a positive control and the result showed that L-arginine had the antihypertensive effect as well as antioxidant capacity in L-NAME treated rats. L-arginine is the substrate for NO synthesis and it has been demonstrated that chronic L-arginine administration attenuated high blood pressure in rats with chronic inhibition of NO synthesis (Luciano Ramos *et al.*, 2006).

Conclusions

The main finding of this study is that chronic administration of L-NAME induced elevation of blood pressure and oxidative stress in rats. Supplementation of asiatic acid exhibited antihypertensive effect in L-NAME induced hypertensive rats. This might be related to the alleviation of oxidative stress.

Acknowledgements

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