Effect of *Momordica cochinchinensis* Extract on Hemodynamics and Oxidative Stress in L-NAME-induced Hypertensive Rats

ผลของสารสกัดฟักข้าว (*Momordica cochinchinensis*) ต่อพลศาสตร์การไหลเวียนเลือดและภาวะเครียดออกซิเดชันในหนูแรทที่ทำให้เกิดภาวะความดันเลือดสูงด้วยสารแอลเนม

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ABSTRACT

Consumption of diets rich in antioxidants is beneficial in preventing cardio vascular diseases. *Momordica cochinchinensis* (named in Thai "Fak khao") is usually known as food and traditional medicinal plant which possesses strong antioxidants. This study aimed to investigate the effect of *M. cochinchinensis* extract (MCE) on hemodynamic changes and oxidative stress in rats with N^{ω}-nitro-L-arginine methyl ester (L-NAME)-induced hypertension. Male Sprague-Dawley rats received L-NAME at 50 mg/kg body weight/day via drinking water for 3 weeks. MCE (100 or 500 mg/kg/day) was intragastrically administered to rats simultaneously with L-NAME. A marked increase in blood pressure (BP), hindlimb vascular resistance (HVR) and oxidative stress was found in L-NAME treated rats. MCE significantly alleviated these deleterious effects by reducing BP, HVR and oxidative stress (P<0.05). Overall findings suggest that MCE is beneficial for cardiovascular health.

บทคัดย่อ

การรับประทานอาหารที่มีสารต้านอนุมูลอิสระสูงเป็นประโยชน์ต่อการป้องกันโรคหัวใจร่วมหลอดเลือด *Momordica cochinchinensis* (ชื่อไทย "ฟักข้าว") รู้จักกันในรูปของอาหารและสมุนไพรพื้นบ้านที่มีสารต้านอนุมูลอิสระในปริมาณสูง การศึกษานี้มีวัตถุประสงค์เพื่อตรวจสอบผลของสารสกัดฟักข้าว (MCE) ต่อการเปลี่ยนแปลงพลศาสตร์การไหลเวียนเลือด และภาวะเครียดออกซิเดชันในหนูแรทที่เหนี่ยวนำให้เกิดความดันเลือดสูงด้วยสารแอลเนม หนูแรทเพศผู้ พันธุ์ Sprague- Dawley ได้รับสารแอลเนม ขนาด 50 มก./กก.น้ำหนักตัว/วัน โดยผสมในน้ำดื่ม เป็นเวลา 3 สัปดาห์ หนูทดลองได้รับ MCE 100 หรือ 500 มก./กก./วัน ด้วยการป้อนพร้อมๆกับได้รับสารแอลเนม ผลการทดลองพบว่าหนูทดลองที่ได้รับแอลเนม มีค่าความดันเลือด ความต้านทานการไหลเวียนเลือดที่ไปเลี้ยงอวัยวะท่อนล่างและขาหลัง และภาวะเครียดออกซิเดชันเพิ่มขึ้นอย่างมาก MCE สามารถลดผลเสียต่างๆที่เกิดขึ้นเหล่านี้ได้อย่างมีนัยสำคัญ (P<0.05) ข้อมูลทั้งหมดนี้ชี้แนะว่าฟักข้าวมีประโยชน์ต่อสุขภาพหัวใจและหลอดเลือด

Key Words: Gac fruit, Hypertension, Oxidative stress

คำสำคัญ: ฟักข้าว ความดันเลือดสูง ภาวะเครียดออกซิเดชัน

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Introduction

Hypertension is a major risk factor for cardiovascular disease. It is a silent, invisible killer that rarely causes symptoms. In Thailand, hypertension is one of the major risk factors of cardiovascular diseases (CVD), and it is attributable to 600,000 losses, which is approximately 6.6% of Thai population each year (Bundhamcharoen et al., 2011).

Oxidative stress together with nitric oxide (NO) deficiency plays an important role in CVD, including hypertension, diabetes, atherosclerosis and chronic kidney disease. Inhibition of NO production by using N^{ω}-nitro-L-arginine methyl ester (L-NAME); a NO synthase inhibitor, causes impairment of the endothelial-dependent relaxation, elevates blood pressure and increases oxidative stress (Nakmareong et al., 2011; Priviero et al., 2007). In recent years, there is strong evidence suggesting that consumption of diets rich in antioxidants is useful for restoration of the antioxidant defense system, prevention of hypertension and preservation of the cardiovascular function (Kizhakekuttu and Widlansky, 2010; Kukongviriyapan *et al.*, 2007).

M. cochinchinensis (or Fak Kao) is classified under Cucurbitaceae family. It is a traditional medicinal plant grown in many countries of Asia such as Vietnam, Thailand, Laos, and China. A previous study reported that *M. cochinchinensis* has high antioxidants, including carotenoids, lycopene, beta-carotene, lutein, and flavonoid (Kubola and Siriamornpun, 2011). As fruits and vegetables are the main source of natural antioxidants (Paran *et al.*, 2009), they may prevent and reduce the risk of CVD (Utsugi et al., 2008). Several lines of evidence suggested that the antioxidant property of carotenoids can preserve vascular health by increasing NO bioavailability (Wolak and Paran, 2013). Moreover, lycopene also reduces blood pressure and improves endothelial function (Xaplanteris et al., 2012). As increased oxidative stress has been observed in rats with L-NAME-induced hypertension, antioxidants found in *M. cochinchinensis* may be effective in reducing of oxidative stress in L-NAME-induced hypertensive rats.

Objectives

The present study aimed to investigate the antihypertensive and antioxidant effects of *M. cochinchinensis* extract in L-NAME-induced hypertensive rats.

Methodology

Fresh ripe fruits of *M. cochinchinensis* were collected from Amphur Ban Phai, Khon Kaen Province, Thailand. The seed membranes or aril were separated and extracted with 95% ethanol. Ethanol was removed by using the rotary vacuum evaporator. The crude aril ethanolic extract of *M. cochinchinensis* (MCE) was lyophilized and kept in a tight, light-protected container and stored at -20°C until use.

Adult male Sprague-Dawley rats weighing 200- 230 g were obtained from National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom, Thailand. All animals were housed in the HVAC (Heating, Ventilation and Air-Conditioning) system with 12 hours dark/light cycle at the Northeast Laboratory Animal Center, Khon Kaen University Thailand. To induce hypertension, rats were administered L-NAME (LN) 50 mg/kg/day by drinking water for 3 weeks, whereas rats in the control group received standard chow diet and tap

water. MCE (100 or 500 mg/kg/day) was intragastrically administered to animals simultaneously with L-NAME. Rats were divided into 4 groups (n=4-6/group): normal control treated with deionized water (DI) as vehicle, L-NAME treated with DI, MCE 100 and 500 mg/ kg/day, respectively. Body weight and blood pressure of rats were measured before and during the periods of treatments until sacrifice. All animal procedures and experimental protocols were approved by the intuitional Animal Ethics Committee of Khon Kaen University.

Blood pressure measurement and biochemical assay

Systolic blood pressure was measured weekly in conscious rats using a tail cuff plethysmography (BP analyzer, model 179, IITC, Woodland hills, CA, USA). After 3 weeks of treatments, rats were anesthetized with pentobarbital sodium (60 mg/kg i.p.). A tracheotomy was performed for spontaneous breathing. The femoral artery was catheterized with polyethylene catheters and connected to a pressure transducer for continuous monitoring of blood pressure (BP) and heart rate (HR) using Acqknowledge data acquisition analysis software (BIOPAC Systems Inc., Goleta, CA, USA). Baseline values of BP and HR were monitored for 20 min. Subsequently, hindlimb blood flow (HBF) was continuously measured by opening the abdominal cavity and placing an electromagnetic flow probe around the abdominal aorta connected to an electromagnetic flowmeter (Carolina Medical Electronics Inc., East Bend, NC, USA). Hindlimb weight was obtained by cutting rat's hindlimb and weighing. Hindlimb vascular resistance (HVR) was calculated from mean arterial pressure (MAP) and HBF and expressed per 100 g tissue. At the end of the experiments, rats were sacrificed by an overdose of an anesthetic drug. Blood samples were collected from abdominal aorta for the assaying of oxidative stress markers. The carotid arteries were rapidly excised from the animal and used for analysis of vascular superoxide ($O_2^{\cdot-}$) production using the Lucigenin-enhanced chemiluminescence method as previously described (Nakmareong et al., 2011). Plasma malondialdehyde (MDA), a lipid peroxidation marker, and plasma protein carbonyl, oxidizing protein damage were measured as previously described (Nakmareong et al., 2012).

Data analysis

Results were expressed as mean ± S.E.M. The differences among various groups were compared by using one-way analysis of variance (ANOVA) followed by a Post hoc Tukey test. A value of $P < 0.05$ was considered statistically significant.

Results

Effect of MCE on blood pressure in conscious rats

At the beginning of the experiments, there were no significant differences in baseline values of systolic blood pressure (SBP) in all experimental groups (Figure 1). SBP progressively increase in L-NAME-treated group throughout the three weeks of treatments. A daily MCE supplementation (500 mg/kg) showed a significant decrease of SBP in L-NAME-treated rats ($P<0.05$). There was no change in SBP in normal control group (Figure 1). MCE at high dose significantly reduced SBP when compared with L-NAME controls (Figure 1).

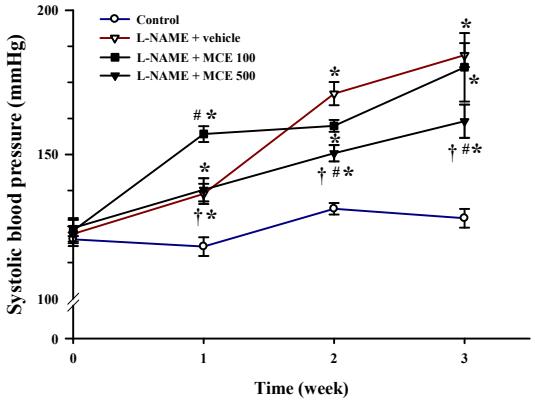

Figure 1 Effect of MCE on systolic blood pressure during L-NAME administration for 3 weeks. Data are expressed as mean ± S.E.M. (n=4-6/group),*P< 0.05 vs. control group, #P<0.05 vs. L-NAME group and †P<0.05 vs. L- NAME+MCE100 group.

Table 1 Effect of MCE on blood pressures of rats in all experimental groups at the end of experimental day (weeks 3rd).

Group	SBP (mmHg)	DBP (mmHg)	MAP (mmHg)
Normal	128.55±2.43	82.88±2.15	104.82±1.90
LN	186.26±2.43*	123.32±6.24*	150.78±4.88*
LN+MCE100	179.21±5.80*	122.05±5.72*	141.10±4.05*
LN+MCE500	163.42±4.11*#†	98.99±2.47*#†	121.86±2.09*#†

Data are expressed as mean ± S.E.M. (n=4-6/group), *P<0.05 vs. control group, #P<0.05 vs. L-NAME group and †P<0.05 vs. L-NAME+MCE100 group.

Effect of MCE on hemodynamic status

After 3 weeks of treatment, there was a significant increase in SBP, DBP, and MAP in rats-treated with L-NAME (P<0.05, Table 1). MCE at 500 mg/kg significantly reduced blood pressure, increased HBF and decreased HVR of L-NAME hypertensive rats as compared to L-NAME-treated controls (P<0.05; Table 1 and Figure 2 and 3). Meanwhile, there were no significant differences in HR among all experimental groups.

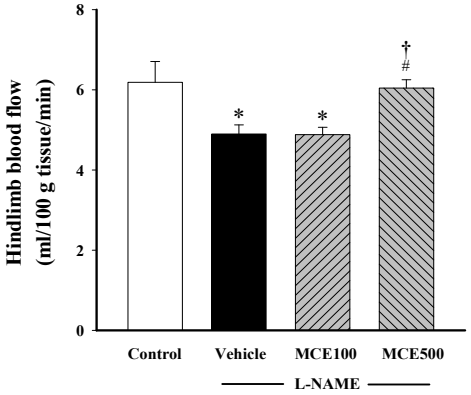

Figure 2 Effect of MCE on hindlimb blood flow in All experimental groups at the end of experimental day. Data are expressed as mean ± S.E.M. (n=4-6/group),*P<0.05 vs. control group, #P<0.05 vs. L-NAME group and †P<0.05 vs. L-NAME+MCE100 group.

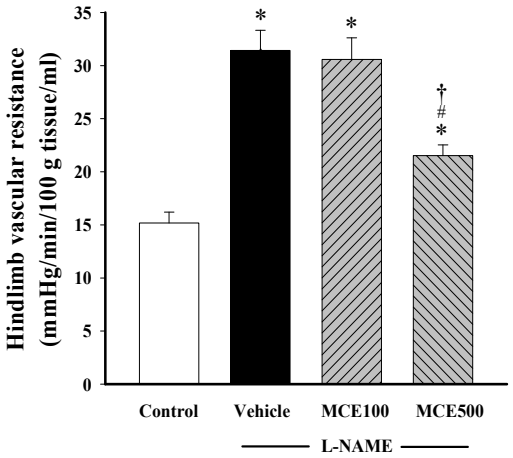

Figure 3 Effect of MCE on hindlimb vascular resistance in all experimental groups at the end of experimental day. Data are expressed as mean ± S.E.M. (n=4-6/group),*P< 0.05 vs. control group, #P<0.05 vs. L-NAME group and †P<0.05 vs. L-NAME+MCE100 group.

Effect of MCE on vascular $O_2^{\cdot-}$ production and oxidative stress markers

The level of $O_2^{\cdot-}$ production in carotid arteries was significantly increased in L-NAME hypertensive rats when compared with normal control (P<0.05, Figure 4). Increased oxidative stress was found in L-NAME hypertensive rats. This was indicated by a marked increase of plasma MDA and plasma protein carbonyl when compared with normal controls (P<0.05, Figure 5A and B). Vascular $O_2^{\cdot-}$ production was significantly decreased in L-NAME treated with MCE 500 mg/kg (P<0.05, Figure 4). Moreover, a significant reduction in oxidative stress as indicated by reducing plasma MDA and protein carbonyl was found in L-NAME rats treated with MCE at dose of 100 or 500 mg/kg (P<0.05, Figure 5A and B). Interestingly, it is found that a reduction in oxidative stress is associated with an improvement of hemodynamics in L-NAME rats treated with MCE, especially at high dose.

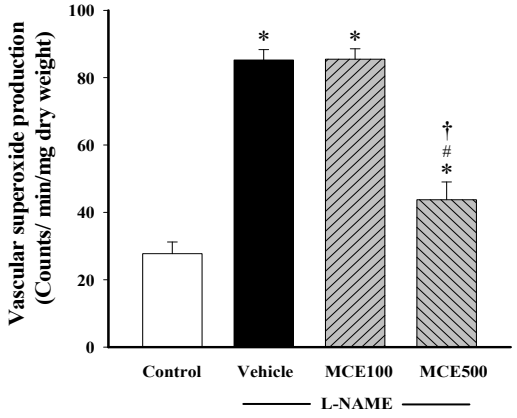

Figure 4 Effect of MCE on vascular superoxide production in all experimental groups at the end of experimental day. Data are expressed as mean ± S.E.M. (n=4-6/group),*P< 0.05 vs. control group, #P<0.05 vs. L-NAME group and †P<0.05 vs. L-NAME+MCE100 group.

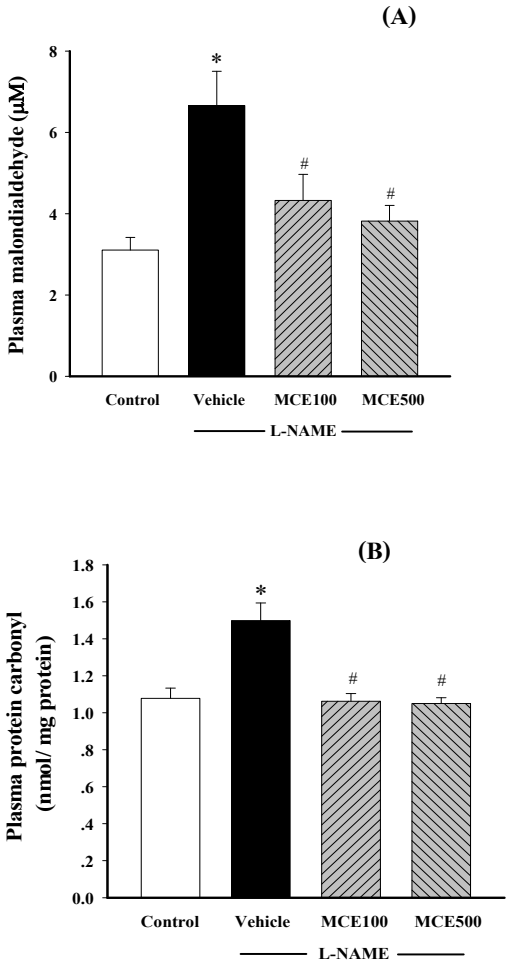

Figure 5 (A) Effect of MCE on plasma MDA (B) and plasma protein carbonyl in all experimental groups at the end of experimental day. Data are expressed as mean ± S.E.M.(n=4-6/group), *P< 0.05 vs. control group, #P< 0.05 vs. L-NAME group.

Discussion and Conclusions

The present study has confirmed that chronic administration of L-NAME induces increases of BP, total peripheral resistance and oxidative stress.

Supplementation of MCE (500 mg/kg) prevented the progression of high blood pressure and reduced oxidative stress as indicated by improving hemodynamic status, decreasing $O_2^{\cdot-}$ production and

alleviating plasma MDA and protein carbonyl. Previous studies have demonstrated that L-NAME-induced increased BP is associated with increased oxidative stress in vasculature. It is suggested that disruption of NO production causes increase of $O_2^{\cdot-}$ production in vascular system (Nakmareong et al., 2011). Overproduction of vascular $O_2^{\cdot-}$ rapidly reacts with NO to produce peroxynitrite, which consequently reduces NO bioavailability and increases vascular resistance that contributes to increase BP (Briones and Touyz, 2010).

Results of this study showed that MCE reduced oxidative stress load in vascular system during the development of hypertension in L-NAME treated rats, indicating that these effects may be attributable to the antioxidant properties of MCE. It has been demonstrated that *M. cochinchinensis* consists of high natural antioxidants, such as carotenoid, lycopene, beta-carotene, phenolic acid, and flavonoids (Kubola and Siriamornpun, 2011). Therefore, the antioxidant property of MCE might explain how MCE attenuates the deleterious effects in this animal model.

In conclusion, the present study has demonstrated that MCE prevents the progression of hypertension and hemodynamic changes in a rat model of L-NAME-induced hypertension. The beneficial effect of MCE on blood pressure reduction warrants further investigation.

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