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การลดภาวะความดันเลือดสูงและภาวะเครียดออกซิเดชันโดยสารสกัดจากเยื่อหุ้มเมล็ดฟักข้าวในหนูแรทที่ขาดไนตริกออกไซด์

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Alleviation of Hypertension and Oxidative Stress by *Momordica cochinchinensis* Aril Extract in Rats with Nitric Oxide Deficiency

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หลักการและวัตถุประสงค์: มีหลักฐานจากรายงานการศึกษามากมายแสดงให้เห็นว่าอาหารที่มีสารต้านออกซิแดนท์สามารถป้องกันโรคหัวใจร่วมหลอดเลือดได้ *Momordica cochinchinensis* ซึ่งรู้จักกันทั่วไปว่า แก๊กฟรุ๊ต หรือชื่อไทยว่า ฟักข้าว ถูกนำมาใช้เป็นอาหารและยาพื้นบ้าน เนื่องจากมีสารต้านออกซิแดนท์สูงและมีฤทธิ์ทางเภสัชวิทยาสาร N^{ω}-nitro-L-arginine methyl ester (แอลเนม) ซึ่งเป็นสารยับยั้งการสร้างไนตริกซ์ออกไซด์ที่สามารถเหนี่ยวนำให้เกิดภาวะความดันเลือดสูงและภาวะเครียดออกซิเดชัน จึงนิยมนำมาใช้เพื่อจำลองรูปแบบภาวะความดันเลือดสูงในการทดลอง การศึกษานี้มีวัตถุประสงค์เพื่อตรวจสอบผลของสารสกัดเยื่อหุ้มเมล็ดฟักข้าวต่อการป้องกันการเพิ่มความดันเลือดและรักษาสภาวะต้านออกซิเดชันในหนูแรทที่เหนี่ยวนำให้เกิดความดันเลือดสูงด้วยสารแอลเนม

วิธีการศึกษา: การเหนี่ยวนำให้เกิดความดันเลือดสูงโดยการให้หนูแรทเพศผู้ พันธุ์ Sprague-Dawley ได้รับสารแอลเนมขนาด 50 มก./กก./วัน ผสมในน้ำดื่มเป็นเวลา 3 สัปดาห์ในขณะเดียวกันหนูแรทจะได้รับน้ำกลั่นปราศจากอิออนหรือ สารสกัดเยื่อหุ้มเมล็ดฟักข้าว ขนาด 100 หรือ 500 มก./กก./วัน โดยการป้อนพร้อมๆกับได้รับสารแอลเนม ส่วนหนูแรทที่ได้รับน้ำประปา และป้อนด้วยน้ำกลั่นเป็นหนูทดลองกลุ่มควบคุมความดันเลือดปกติ

Background and Objectives: There is considerable evidence that dietary antioxidants can protect against cardiovascular disease. *Momordica cochinchinensis,* commonly known as gac fruit or fak-khao (in Thai), is used as food and traditional medicine. It possesses strong antioxidant and pharmacological activities. N^{ω}-nitro-L-arginine methyl ester (L-NAME) is a nitric oxide synthase (NOS) inhibitor that induces hypertension and enhances oxidative stress. L-NAME-induced hypertension is a well-established experimental model of hypertension. This study aimed to investigate the effect of *M. cochinchinensis* aril extract (MCE) on prevention of the progression of high blood pressure and preservation of antioxidant status in rats with L-NAME-induced hypertension.

Methods: Male Sprague-Dawley rats received L-NAME (50mg/kg/day) via drinking water for 3 weeks, together with intragastrically administration of deionized water (DI) or MCE at dose of 100 or 500 mg/kg/day. Rats received tap water and intragastrically administered with DI were served as normotensive controls.

Results: It is found that blood pressure, vascular

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ผลการศึกษา: พบว่าหนูทดลองความดันเลือดสูงแอลเนมมีค่าความดันเลือด การสร้างซุปเปอร์ออกไซด์ในเนื้อเยื่อหลอดเลือด และระดับมาลอนไดอัลดีไฮด์ที่สูงขึ้นอย่างมาก นอกจากนี้ยังพบว่าระดับของสารต้านออกซิแดนท์กลูตาไธโอนในเลือดยังลดลงอย่างมากหลังจากให้สารแอลเนม สารสกัดเยื่อหุ้มเมล็ดฟักข้าวสามารถลดผลเสียต่างๆที่เกิดขึ้นเหล่านี้ โดยลดค่าความดันเลือด ลดภาวะเครียดออกซิเดชันและเพิ่มสารต้านออกซิแดนท์กลูตาไธโอนได้อย่างมีนัยสำคัญ ($p<0.05$)

สรุป: ผลการทดลองจากการศึกษานี้บ่งชี้ถึงประโยชน์ของฟักข้าวในการป้องกันโรคความดันเลือดสูง

คำสำคัญ: สารต้านออกซิเดชัน, ความดันเลือดสูง, แอลเนม, ฟักข้าว, ไนตริกออกไซด์

superoxide production and plasma malondialdehyde level were markedly increased in L-NAME hypertensive rats. Moreover, the blood glutathione level was also decreased after L-NAME administration. MEC significantly alleviated these deleterious effects by reducing blood pressure, decreasing oxidative stress and enhancing antioxidant glutathione ($p<0.05$).

Conclusion: The findings of this study suggest the beneficial effect of fak-khao on preventing hypertension.

Keywords: Antioxidant, Hypertension, L-NAME, *Momordica cochinchinensis*, Nitric oxide

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Introduction

Oxidative stress is enhanced in hypertension and other forms of cardiovascular disease and participates in the mechanisms of vascular injury. It is induced by enhancing reactive oxygen species (ROS) and reactive nitrogen species (RNS)[1]. It is also well known that oxidative stress in the vascular system is resulted from a decrease in nitric oxide (NO) bioavailability, which leads to vascular injury and inflammation[2]. Oxidative stress and NO deficiency play an important role in the pathogenesis of cardiovascular disease, such as hypertension and atherosclerosis. Furthermore, an imbalance between antioxidant defense system and free radical production has been found in animal and human with hypertension[3]. Inhibition of NO production by various factors induces elevation of arterial blood pressure, increases in peripheral resistance, and reduces antioxidant status[4-7]. N^{ω}-nitro-L-arginine methyl ester (L-NAME), a NO synthase inhibitor, causes impairment of the endothelial-dependent relaxation and enhances oxidative stress which leads to hypertension[8]. Therefore, L-NAME-induced hypertension is a well-established model of hypertension.

There are several studies describing that administration of natural antioxidants such as vitamin C, vitamin E [9], beta-carotene, lycopene [10, 11], flavonoid and phenolic acid are useful for restoration of the antioxidant defense, preservation of the endothelial function, prevention and reduction risk of hypertension. Various medicinal plants have been investigated for prevention and treatment of hypertension. Some of them have been validated while others disproved.

M. cochinchinensis is botanically classified in the Cucurbitaceae family. It has been used as a food and traditional medicine in Asia such as Vietnam, Thailand, Laos, Myanmar, Philippine, Bangladesh, India and Kampuchea. It is named in Thai as “fak-khao”. Previous studies reported that *M. cochinchinensis* has high antioxidants such as carotenoids, lycopene, betacarotene, lutein, and flavonoid[12-14]. Since antioxidant is beneficial for cardiovascular health, especially protection against hypertension[1], therefore, antioxidant found in *M. cochinchinensis* may be effective in reducing blood pressure and oxidative stress in L-NAME-induced hypertensive rats.

Method

Chemicals

L-NAME, 5, 5 dithio-bis-2-nitrobenzoic acid (DTNB), ethylenediamine tetraacetic acid (EDTA), thiobarbituric acid (TBA), sodium dodecylsulfate (SDS), butylated hydroxyluene (BHT) were purchased from Sigma-Aldrich

Pte. Ltd. (Singapore). Trichloroacetic acid (TCA), 1-methyl-2 vinyl-pyridinumtrifate (M2VP), lucigenin, glutathione (GSH), glutathione reductase, metaphosphoric acid (MPA), nicotinamideadenine dinucleotide phosphate (NADPH), N-1-nepthylethylenediamine, and glucose-6-phosphate dehydrogenase were obtained from Roche Applied Sciences (Mannheim, Germany). All other chemicals used were of analytical grade quality.

Preparation of *M. cochinchinensis* extract

Fresh ripe fruits of *M. cochinchinensis* were collected from Ban Phai district, Khon Kaen, 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.

Animals and experimental protocol

Adult male Sprague-Dawley rats weighing 200- 230 g were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom, Thailand. All animals were housed in the HVAC (Heating, Ventilation and Air-Conditioning) system with a 12 hours dark/light cycle at the Northeast Laboratory Animal Center, Khon Kaen University, Thailand. To induce hypertension, rats were administered with L-NAME (50 mg/kg/day) in drinking water for 3 weeks, whereas the control rats 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=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 sacrificed. All animal procedures and experimental protocols were approved by the Institutional 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 catheter and connected to a pressure transducer for continuous monitoring of blood pressure (BP) and heart rate (HR) using an AcqKnowledge Data Acquisition Analysis software (BIOPAC Systems Inc., Goleta, CA, USA). Baseline values of BP and HR were monitored for 20 min. At the end of the experiments, rats were sacrificed by an overdose of an anesthetic drug. Blood samples were collected from abdominal aorta for assessment 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.[15] Assay of malondialdehyde (MDA), a lipid peroxidation marker, and blood glutathione (GSH) level were measured as previously described[8].

Data analysis

Results are expressed as mean ± SEM. The differences among various groups were compared by using one-way analysis of variance (ANOVA) followed by a Student Newman-Keuls 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 was progressively increased in the L-NAME-treated group throughout the periods of treatments. MCE at high dose (500 mg/kg) significantly reduced SBP compared to L-NAME controls ($p<0.05$, Figure 1). There were no changes in SBP in the normal control group (Figure 1). Changes in the arterial blood pressure of rats in all groups at the end of experiments are summarized in table 1. There were significant increases in SBP, diastolic blood pressure (DBP), and mean arterial pressure (MAP) in

L-NAME-treated rats when compared with normotensive controls (p<0.05, Table 1). Although the blood pressure did not return to normal values after MCE treatment, MCE dose-dependently reduced blood pressure of L-NAME hypertensive rats (p<0.05, Table 1). Meanwhile, there were no significant differences in HR among all experimental groups.

Effect of MCE on vascular $O_2^{\cdot-}$ production and MDA level

L-NAME significantly increased the level of $O_2^{\cdot-}$ production in carotid arteries compared to the untreated group (p<0.05, Figure 2). Increased oxidative stress was found in L-NAME hypertensive rats indicated by the increase of MDA levels in plasma, kidney and heart tissues compared to normal controls (p<0.05, Figure 3A, B and C). Significant reduction in $O_2^{\cdot-}$ production and MDA levels were found in L-NAME rats treated with MCE (p<0.05, Figure 2 and 3).

Effect of MCE on antioxidant status

The antioxidant defense system was depleted in the L-NAME treated rats, e.g. GSH and the ratio of GSH/ glutathione disulfide (GSSG) in blood were decreased compared to normal controls (p<0.05, Figure 4). MCE in a dose-dependent manner prevented the reduction of blood GSH and restored the redox status of L-NAME

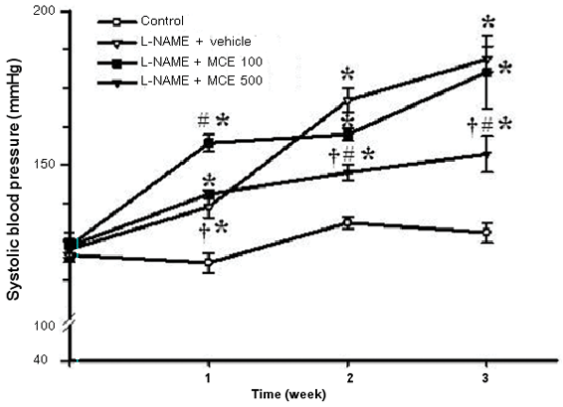

Figure 1 Effect of MCE on systolic blood pressure during L-NAME administration for 3 weeks. Data are expressed as mean ± SEM. (n=6/group),*p< 0.05 vs. control group, #p<0.05 vs. L-NAME group and †p<0.05 vs. L- NAME+MCE100 group.

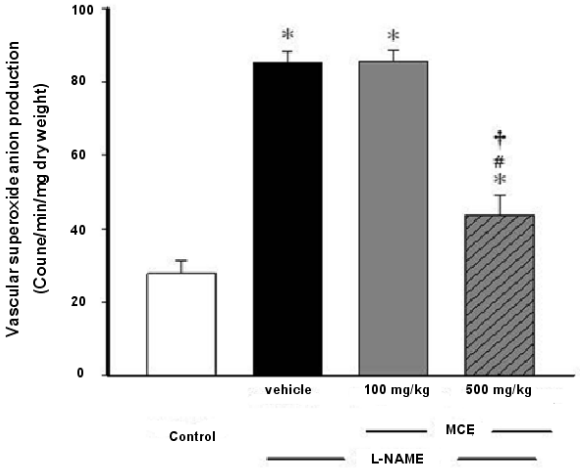

Figure 2 Effect of MCE on vascular superoxide production in carotid arteries of rats. Data are expressed as mean ± SEM. (n=6/group),*p< 0.05 vs. control group, #p<0.05 vs. L-NAME group and †p<0.05 vs. L- NAME+MCE100 group.

Table 1 Effect of MCE on arterial blood pressure of rats in all experimental groups.
Data are expressed as mean ± SEM. (n=6/group). SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure. *p<0.05 vs. control group, #p<0.05 vs. L-NAME group and † p<0.05 vs. L-NAME+MCE100 group.

Group	SBP (mmHg)	DBP (mmHg)	MAP (mmHg)
Normal	125.6 ± 2.1	82.8 ± 2.1	99.04 ± 1.4
L-NAME	194.4 ± 2.4*	134.2 ± 5.0*	156.7 ± 4.9*
L-NAME+MCE 100	174.3 ± 3.9*	119.6 ± 3.50*	140.6 ± 3.2*
L-NAME+MCE 500	155.9 ± 2.2*#†	96.0 ± 2.0*#†	123.8 ± 2.6*#†

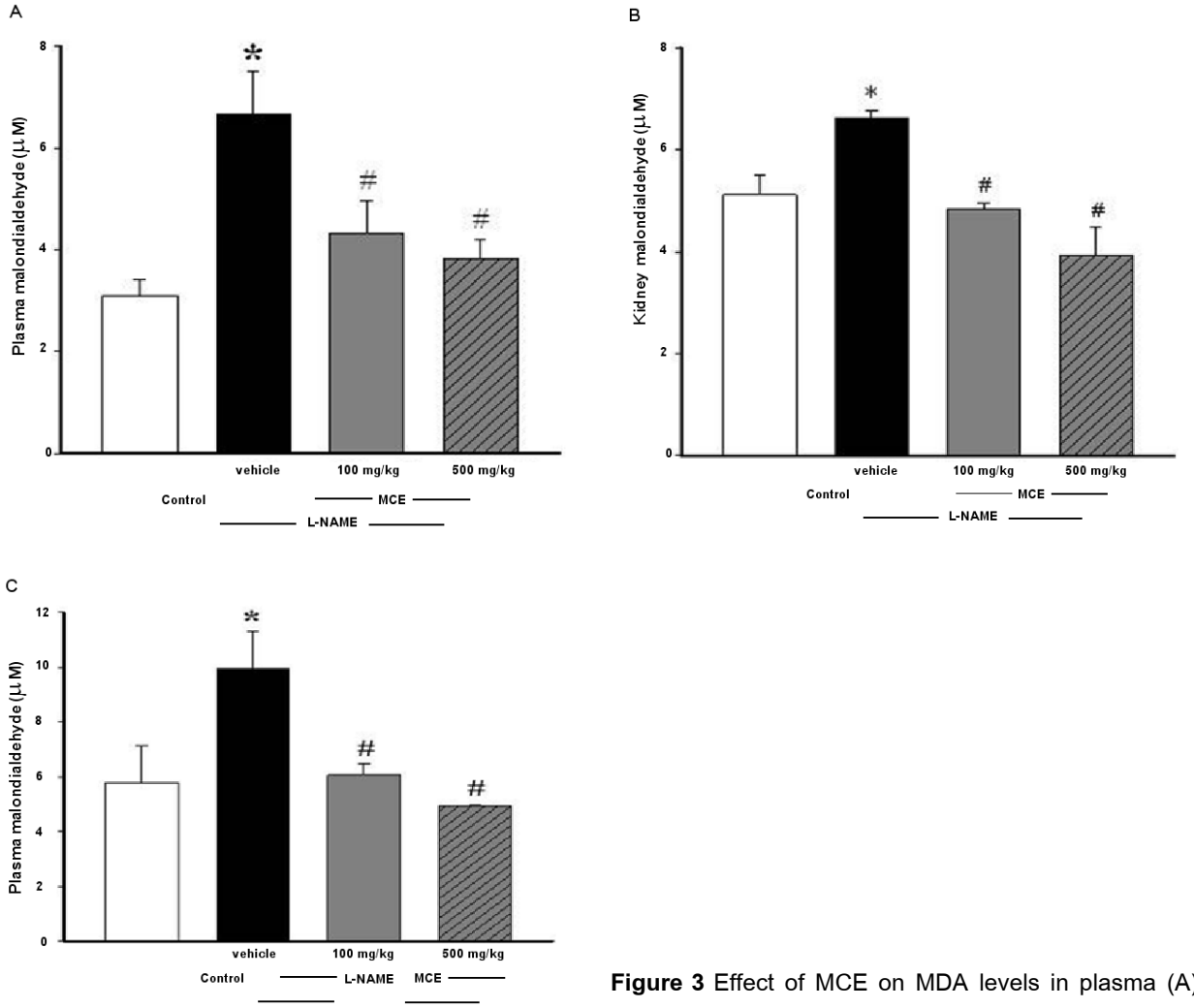

Figure 3 Effect of MCE on MDA levels in plasma (A) Kidney (B) and Heart (C) of rats. Data are expressed as mean ± SEM. (n=6/group),*p< 0.05 vs. control group, #p<0.05 vs. L-NAME group.

hypertensive rats (p<0.05, Figure 4). Interestingly, it was found that a reduction in oxidative stress and restored antioxidant status was associated with a reduction of high blood pressure in L-NAME rats treated with MCE, especially at high dose.

Discussion

In this study, we found that administration of L-NAME for 3 weeks induced hypertension and oxidative stress. Supplementation of MCE (100 and 500 mg/kg) prevented the progression of high blood pressure and reduced oxidative stress indicated by a decrease in blood pressure, $O_2^{\cdot-}$ production and the levels of MDA in plasma and tissues. Moreover, the antioxidant GSH and the redox status were also enhanced after MCE supplement. A reduction in antioxidant bioactivity in the biological system can enhance cellular oxidative stress and is implicated in cardiovascular oxidative damage that is associated with hypertension[1,2]. Previous study has demonstrated that chronic administration of L-NAME-induced high blood pressure is associated with increased oxidative stress in the vascular system[8]. Inhibition of NO production causes increase of $O_2^{\cdot-}$ production in vasculature[7] A decrease in NO bioavailability and

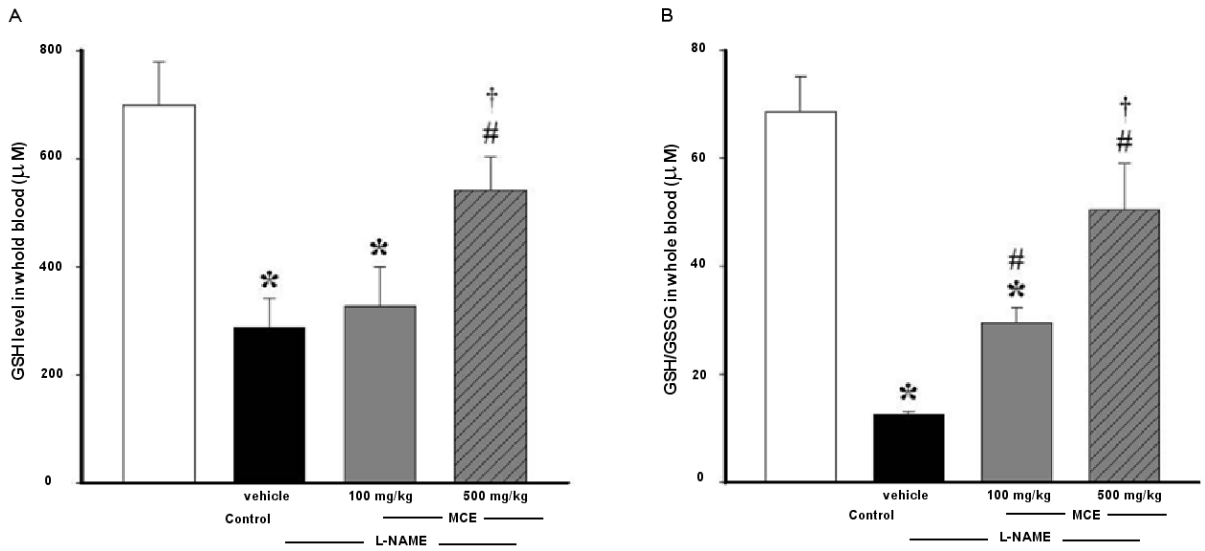

Figure 4 Effect of MCE on the blood glutathione levels of rats. Data are expressed as mean ± SEM. (n=6/group), *p< 0.05 vs. control group, #p<0.05 vs. L-NAME group.

increased vascular resistance are contributable to increased BP[16]. Supplementation with exogenous antioxidants could reduce oxidative stress and prevent development of hypertension[3]. Oxidative stress as evidenced by a marked increase in lipid peroxidation in L-NAME-treated rats led to a reduction in GSH and the redox status of GSH. Results of this study demonstrated that MCE markedly restored antioxidant GSH as well as reduced oxidative stress in the L-NAME hypertensive rats. Nevertheless, the GSH levels were still below those of the controls.

In conclusion, the present study has demonstrated that MCE prevents the progression of hypertension, reduced oxidative stress and restored antioxidant status in a rat model of L-NAME-induced hypertension. Additionally, the findings of this study support the idea of consuming fak-khao as a supplement to protect against hypertension resulted from NO deficiency. However, the mechanistic effect of MCE on blood pressure reduction warrants further investigation.

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