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

Gulladawan Jan-on¹, Upa Kukongviriyapan², Poungrat Pakdeechote¹, Veerapol Kukongviriyapan³, Boontium Kongsaktrakoon⁴, Orachom Boonla¹

¹Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002
²Department of Physiology, Faculty of Pharmacy, Mahidol University, Bangkok 10400
³Faculty of Allied Health Sciences, Burapha University, Chonburi 20131

**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 was found that blood pressure, vascular
Alleviation of Hypertension and Oxidative Stress

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\(^4\)\(^-\)\(^7\). NO\(^\circ\)-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.

\textit{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, Philippines, Bangladesh, India and Kampuchea. It is named in Thai as "fak-khao". Previous studies reported that \textit{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 \textit{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.
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$_2^-$) production using the Lucigenin-enhanced chemiluminescence method as previously described.

Assay of malondialdehyde (MDA), a lipid peroxidation marker, and blood glutathione (GSH) level were measured as previously described.

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\(_2^–\) production and MDA level

L-NAME significantly increased the level of O\(_2^–\) 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\(_2^–\) 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](image1.png) **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](image2.png) **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>
<thead>
<tr>
<th>Table 1</th>
<th>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&lt;0.05 vs. control group, #p&lt;0.05 vs. L-NAME group and †p&lt;0.05 vs. L-NAME+MCE100 group.</th>
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<tbody>
<tr>
<td>Group</td>
<td>SBP (mmHg)</td>
</tr>
<tr>
<td>Normal</td>
<td>125.6 ± 2.1</td>
</tr>
<tr>
<td>L-NAME</td>
<td>194.4 ± 2.4*</td>
</tr>
<tr>
<td>L-NAME+MCE 100</td>
<td>174.3 ± 3.9*</td>
</tr>
<tr>
<td>L-NAME+MCE 500</td>
<td>155.9 ± 2.2 †</td>
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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$_2^-$ 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$_2^-$ production in vasculature$^7$. A decrease in NO bioavailability and
Increased vascular resistance are contributable to increased BP\textsuperscript{16}. Supplementation with exogenous antioxidants could reduce oxidative stress and prevent development of hypertension\textsuperscript{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.

Acknowledgements

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References


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.