นิพนธ์ต้นฉบับ ▪ Original Article

สารสกัดติ้วขาวบรรเทาหัวใจห้องล่างซ้ายโตในหนูแรทที่มีภาวะพร่องไนตริกออกไซด์

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Cratoxylum formosum Extract Alleviates Left Ventricular Hypertrophy in Nitric Oxide-Deficient Rats

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หลักการและวัตถุประสงค์: ติ้วขาวเป็นพืชพื้นเมืองที่พบว่ามีผลต้านอนุมูลอิสระและต้านการอักเสบ การศึกษานี้มีวัตถุประสงค์เพื่อตรวจสอบผลของสารสกัดติ้วขาวต่อภาวะหัวใจห้องล่างซ้ายโตและภาวะเครียดออกซิเดชั่นในหนูแรทที่มีภาวะพร่องไนตริกออกไซด์

วิธีการศึกษา: หนูแรทเพศผู้สายพันธุ์ Sprague-Dawley ถูกแบ่งเป็น 3 กลุ่ม ได้แก่ กลุ่มควบคุมได้รับน้ำดื่มปกติ กลุ่มแอลเนมได้รับสารแอลเนม 40 มก./กก./วัน เป็นเวลา 5 สัปดาห์ และกลุ่มแอลเนมที่ได้รับสารสกัดติ้วขาว 500 มก./กก./วัน ใน 2 สัปดาห์สุดท้าย เมื่อสิ้นสุดการทดลอง ทำการวัดน้ำหนักหัวใจ ความหนาและพื้นที่หน้าตัดของหัวใจห้องล่างซ้าย การสร้าง superoxide ในหลอดเลือด และ malondialdehyde ในพลาสมา

ผลการศึกษา: หนูแรทที่ได้รับแอลเนมมีหัวใจห้องล่างซ้ายโตและมีการเพิ่มขึ้นของตัวบ่งชี้ของภาวะเครียดออกซิเดชั่น ($p<0.05$) การรักษาด้วยสารสกัดติ้วขาวบรรเทาภาวะหัวใจห้องล่างซ้ายโตอย่างมีนัยสำคัญทางสถิติ ซึ่งสัมพันธ์กับการลดภาวะเครียดออกซิเดชั่นในหนูแรทที่ได้รับแอลเนม

สรุป: สารสกัดติ้วขาวมีผลบรรเทาภาวะหัวใจห้องล่างซ้ายโตในหนูแรทที่ได้รับแอลเนม ซึ่งอาจเกี่ยวข้องกับคุณสมบัติในการต้านอนุมูลอิสระ

คำสำคัญ: ติ้วขาว; หัวใจห้องล่างซ้ายโต; หนูแรทที่มีภาวะพร่องไนตริกออกไซด์; ภาวะเครียดออกซิเดชั่น

Background and Objectives: *Cratoxylum formosum* (CF), Tiew Khao, is a local plant that exerts antioxidant and anti-inflammatory effects. This study aimed to investigate the effects of CF extract on left ventricular (LV) hypertrophy and oxidative stress in nitric oxide-deficient rats.

Methods: Male Sprague-Dawley rats were divided into 3 groups including control group received normal drinking water, N^{ω}-nitro L-arginine methyl ester hydrochloride (L-NAME) group received L-NAME (40 mg/kg/day) for five weeks and L-NAME group treated with CF extract (500 mg/kg/day) for the last two weeks. At the end of experiment, heart weight, LV wall thickness and cross-sectional area, vascular superoxide production and plasma malondialdehyde were measured.

Results: Rats received L-NAME showed LV hypertrophy and increases in oxidative stress markers ($p<0.05$). Treatment with CF extract significantly alleviated LV hypertrophy associated with reducing oxidative stress markers in L-NAME treated rats.

Conclusion: CF extract alleviated LV hypertrophy in L-NAME rats which might be related with its antioxidant properties.

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Keywords: *Cratoxylum formosum*; LV hypertrophy; nitric oxide-deficient rats; oxidative stress

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Introduction

Nitric oxide is derived from many cell types and plays an important role on cardiovascular morphology and function[1]. There is evidence to show that nitric oxide (NO)-deficiency cause cardiac hypertrophy in rats [2]. N^{ω}-nitro L-arginine methyl ester hydrochloride (L-NAME) is a nitric oxide synthase inhibitor that cause NO deficiency in rats[3]. It has been reported that chronic treatment with L-NAME caused hypertension associated with left ventricular (LV) hypertrophy[4, 5]. LV hypertrophy is consistently associated with increased cardiovascular morbidity and mortality worldwide. In addition, oxidative stress play role in pathogenesis of LV hypertrophy with excessive production of reactive oxygen species leading to maladaptation of cardiomyocytes[6]. Recently, it has been confirmed that an increased oxidative stress markers contributed to cardiac alterations was found in L-NAME treated rats [7].

Cratoxylum Formosum (CF), Tiew Khao, is a local plant that widely consumed in Thailand[8]. Senggunprai and coworkers (2016) demonstrated that aqueous extract of CF are comprise of several phenolic compounds for example, syringic acid, protocatechuic acid, vanillic acid, and caffeic acid[9]. A wide range of potentially effects of CF extract have been reported such as antioxidant[10] and anti-inflammation[8]. However, the effect of CF extract on LV hypertrophy has not been investigated. Therefore, the proposed of this study was to find out the effect of CF extract on LV hypertrophy in NO-deficient rats.

Methods

Preparation of the CF extract

To prepare the CF aqueous extract, fresh leaves were weighed, chopped and boiled in distilled water for 30 minutes, then filtrated. The filtrates were dried into powder by using a freeze dryer (Labconco, USA). The extraction process yielded residues of 11.5% per dry weight of CF [9]. The crude extract was kept in a light-protected container and stored at -20 °C until used. The extract was dissolved in distilled water before used.

Animals

Male Sprague-Dawley rats weighing 220-250 g were purchased from Nomura Siam International Co, Ltd., Bangkok, Thailand. Rats were housed in the HVAC (Heating, Ventilation and Air-Conditioning) System (25±2 °C) with a 12 h dark-light cycle at Northeast Laboratory Animal Center. All procedures were 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 (ACUC-KKU-29/60).

Experimental protocol

Rats were divided into three groups (n=8/group) as following.

1. Control group received drinking water and intragastrically administrated distilled water 1.5 ml/kg/day as vehicle.

2. L-NAME group received L-NAME (40 mg/kg/day) in their drinking water for five weeks and intragastrically administrated distilled water 1.5 ml/kg as vehicle.

3. L-NAME-treated group received L-NAME (40 mg/kg/day) in their drinking water for five weeks and intragastrically administrated CF extract 500 mg/kg/day for the last two weeks.

Morphometric analysis of heart

Body weight (BW), heart weight (HW) and left ventricular weight (LVW) were measured and calculated as HW/BW and LVW/BW ratio. Left ventricle was bisected coronally at the midventricular position, middle between base and apex. Then, the tissues were fixed 24 h in 4% paraformaldehyde and embedded in paraffin block. The sections were consecutively cut at 5 µm of thickness and stained with H&E (Bio-Optica Milano SpA., Milano, Italy) to investigate the general appearance of the heart ventricle. The whole heart section and a known length of calibrator were captured by using stereoscope. After that ImageJ morphometric software (National Institutes of Health, Bethesda, MD, USA) was used for evaluation of LV wall thickness and cross sectional area (CSA).

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

The carotid artery was rapidly removed, clean off connective tissues, cut into 1 cm in length and incubated with 1 mL oxygenated Krebs-KCl solution at pH 7.4, 37 °C for 30 minutes. Then, lucigenin 100 mM was added in sample tube and measured in a luminometer (Turner Biosystems, CA, USA). Luminometer count was integrated every 30 second for 5 minutes and averaged. Data were expressed as relative light unit count per minute per dried weight of vascular tissues.

Assay of plasma malondialdehyde (MDA)

Blood was collected into EDTA tube and placed on ice for plasma MDA measurement. Briefly, 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 minutes and then 0.6 % TBA was added in an equal volume and the mixture was heated for 30 minutes 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 spectrophotometer. A standard curve was generated using appropriate concentrations of standard TEP (0.3-10 mmol/1).

Statistical analysis

Data are expressed as mean ± S.E.M. and analyzed by one-way analysis of variance (ANOVA) followed by Tukey HSD post hoc test. A p-value of less than 0.05 was considered statistically significant.

Results

Effects of CF extract on cardiac mass indices

BW was not different among groups. HW/BW and LVW/BW ratios significantly increased in L-NAME hypertensive rats comparing to the control rats ($p<0.05$). Treatment with CF extract significantly reduced HW/BW and LVW/BW ratios compared to untreated rats ($p<0.05$; Table1).

Table 1 Effect of CF extract on cardiac mass indices

Parameters	Control	L-NAME	L+CF500
BW (g)	443.44±10.33	441.13± 0.92	439.00± 3.13
HW/BW (mg/g)	2.62 ±0.06	3.01±0.07 *	2.57± .04#
LVW/BW (mg/g)	1.79 ± 0.05	2.17± 0.07 *	1.89± .04#

HW: heart weight; BW: body weight; LVW: left ventricular weight. Data are expressed as means ± S.E.M. (n = 6/group). *p<0.05 vs. control, #p<0.05 vs. L-NAME.

Effects of CF extract on ventricular morphology

It was found that administration with L-NAME for five weeks caused significant increases in LV wall thicknesses (3.09 ± 0.05 vs. 2.45 ± 0.04 mm) and CSA (63.28 ± 1.04 vs. 51.49 ± 1.43 mm^2) comparing to the control group ($p<0.05$). CF extract significantly reduced these alterations of LV (LV wall thickness 2.50 ± 0.04 mm and LV CSA 55.98 ± 1.12 mm^2) compared with untreated group ($p<0.05$, Figure 1B and C).

Effect of CF extract on vascular $O_2^{\bullet-}$ production

A significant increase in vascular $O_2^{\bullet-}$ production was found in L-NAME hypertensive group compared to control group (150.1 ± 10.1 vs. 62.5 ± 5.5 count/mg dry wt/min, $p<0.01$). The rise of $O_2^{\bullet-}$ production was significantly reduced (79.7 ± 11.4 count/mg dry wt/min, $p<0.05$) by treatment with CF extract (Figure 2).

Effect of CF extract on plasma MDA

Long-term L-NAME administration produced high plasma MDA level compared with control group (18.4 ± 1.7 vs. 8.9 ± 1.6 µM, $p<0.05$). CF extract treatment significantly decreased plasma MDA level in L-NAME hypertensive rats (12.5 ± 0.5 µM, $p<0.05$) (Figure 3).

Discussion

The results of this study demonstrated that long-term administration of L-NAME caused LV hypertrophy and oxidative stress in rats. Cardiac alterations including increases in cardiac mass indices and LV hypertrophy were alleviated by treatment with CF extract. Moreover, CF extract reduced vascular $O_2^{\bullet-}$ production and plasma MDA level in L-NAME treated rats.

It is well documented that chronic administration of L-NAME produced LV hypertrophy. We found that rats received L-NAME have an increased in HW/BW, LVW/BW ratio, LV wall thickness and LV CSA. Recently, Wunpathe and coworkers (2020) demonstrated that given L-NAME for five weeks caused LV dysfunction and hypertrophy in rats [11]. An increase in LV wall stress in response to chronic hemodynamic overload is the major determinant of

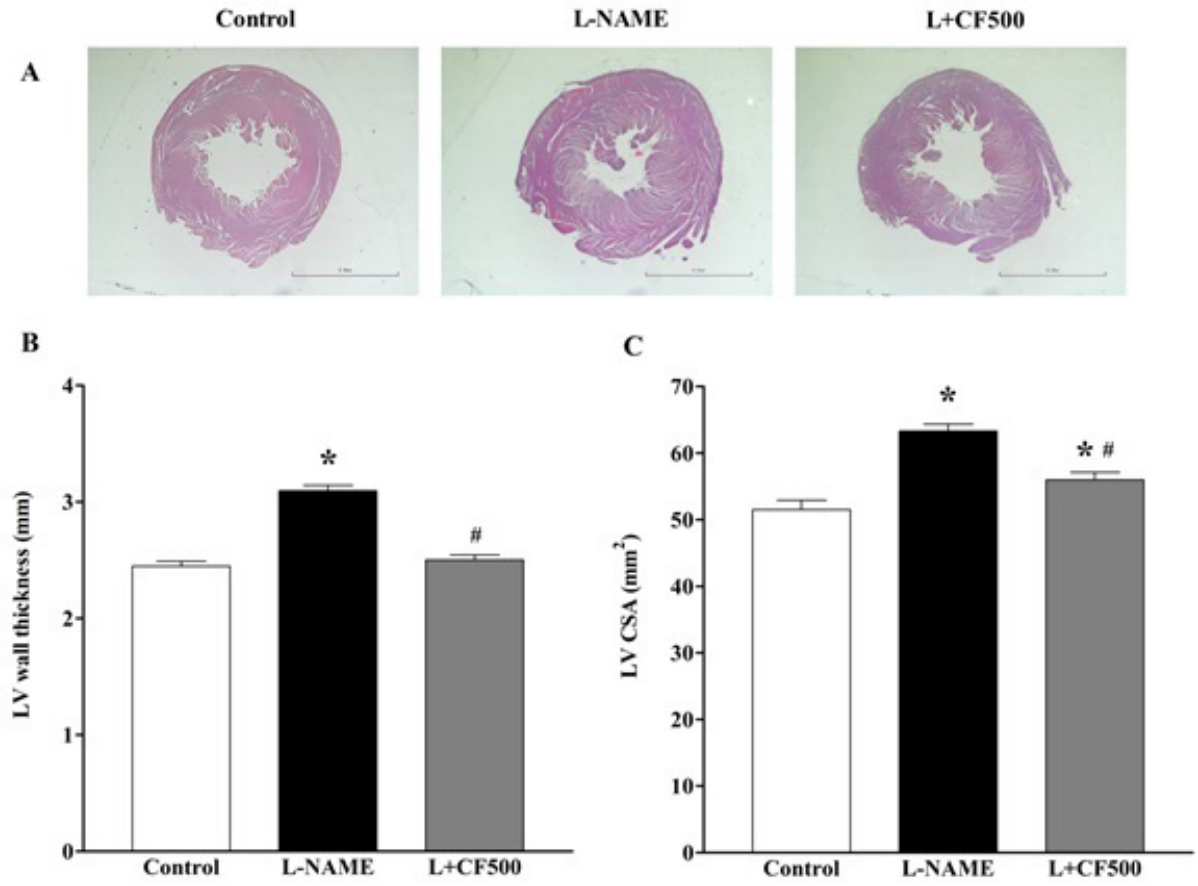

Figure 1 Effect of CF extract on LV morphology in rats. Representative figures of LV sections stained with hematoxylin and eosin under stereomicroscopy using a 1x objective lens, scale bars = 5.0 mm (A), LV wall thickness (B) and LV CSA (C). Results are shown as means ± S.E.M. (n = 6/group), *p<0.05 vs. control, #p<0.05 vs. L-NAME.

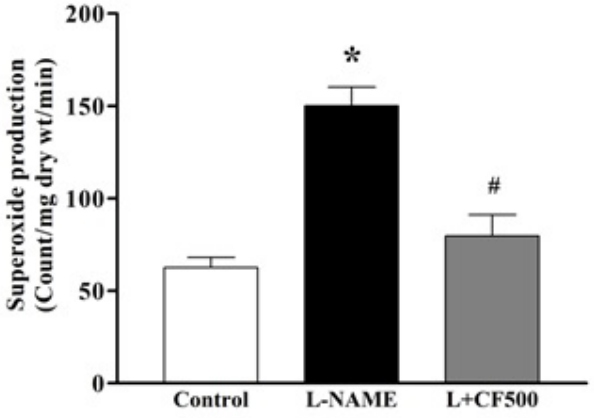

Figure 2 Effects of CF extract on vascular $O_2^{\bullet-}$ production. Data are expressed as mean ± S.E.M. (n=6 /group), *p<0.05 vs. control, #p<0.05 vs. L-NAME.

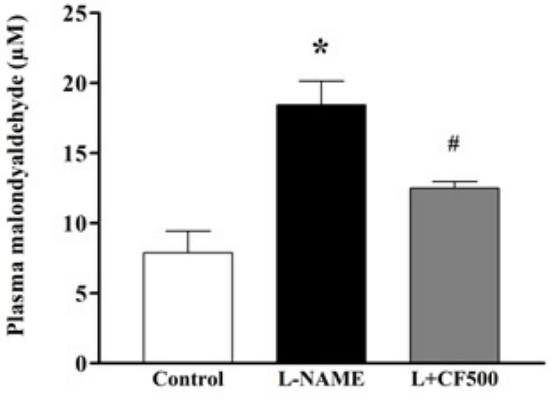

Figure 3 Effects of CF extract on plasma MDA. Data are expressed as mean ± S.E.M. (n=6 /group), *p<0.05 vs. control, #p<0.05 vs. L-NAME.

LV hypertrophy in hypertension. However, a reduction in NO is non-hemodynamic factors that have been also modulated the hypertrophic response[12, 13]. In present study, treatment with CF extract at dose 500 mg/kg decreased cardiac alterations in L-NAME treated rats. This effect might be related to a reduction of oxidative stress markers.

Oxidative stress is characterized in rats treated with L-NAME since there is an increase in oxidative stress markers and decreased antioxidant enzymes in this animal model[14]. In this study, an increases in vascular $O_2^{\bullet-}$ production and plasma MDA level were observed. It has been suggested that oxidative stress activate a variety of intracellular signaling cascades in

cardiomyocytes leading to cardiac hypertrophy[6]. We found that oxidative stress induced by L-NAME was attenuated with CF extract supplementation. Several studies confirmed the components of CF extract which exert free radical scavenging activity[10, 15]. The results of this study were consistent with several studies that medicinal plant with antioxidant effect was able to improved cardiovascular remodeling and oxidative stress status in nitric oxide-deficient animal model[16, 17]. The possible mechanism might be involved with strong antioxidant properties of CF extract[10].

Conclusion

The results of this study indicated that CF extract improved LV hypertrophy associated with antioxidant effect in nitric oxide-deficient rats induced by L-NAME.

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