Effect of *Carthamus tinctorius* Ethanolic Extract on Cardiovascular Parameters and Oxidative Stress Markers in Nitric Oxide-deficient Hypertensive Rats

ผลของสารสกัดเอทานอลจากดอกคำฝอยต่อตัวแปรในหัวใจร่วมหลอดเลือดและตัวบ่งชี้ภาวะเครียดออกซิเดชันในหนูแรทที่มีภาวะความดันเลือดสูงเนื่องจากภาวะพร่องไนตริกออกไซด์

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ABSTRACT

Carthamus tinctorius (CT) is commonly known as Safflower and widely used as traditional medicine. This study aimed to investigate whether CT extract could improve cardiovascular parameters and oxidative stress markers in L-NAME induced hypertensive rats. Male Sprague-Dawley rats receiving L-NAME (40 mg/kg/day) for 5 weeks had high blood pressure, high hindlimb vascular resistance (HVR), low hindlimb blood flow (HBF) and cardiac hypertrophy. Plasma malondialdehyde (MDA) level and vascular superoxide production were increased in L-NAME hypertension ($p<0.05$). Treatment with either CT extract (300 mg/kg/day) or captopril (30 mg/kg/day) for the last 2 weeks significantly improved blood pressure, HVR, HBF, cardiac hypertrophy and oxidative stress markers ($p<0.05$). This present investigation suggests that CT extract had an antihypertensive effect that was associated with its antioxidant capacity.

บทคัดย่อ

คาร์ทาร์มัส ทินค์ทอเรียส (CT) เป็นที่รู้จักกันในชื่อแซฟฟลาวเวอร์หรือดอกคำฝอย นิยมนำมาใช้ในการแพทย์แผนโบราณ การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาว่าสารสกัดจากดอกคำฝอยสามารถปรับปรุงตัวแปรในหัวใจร่วมหลอดเลือดและตัวบ่งชี้ภาวะเครียดออกซิเดชันในหนูแรทความดันเลือดสูงที่ถูกเหนี่ยวนำด้วยสารแอลเนมได้หรือไม่ โดยหนูแรทที่ได้รับสารแอลเนม (40 มก./กก./วัน) นาน 5 สัปดาห์ พบว่ามีความดันเลือดสูงความต้านทานการไหลของเลือดที่อวัยวะท่อนล่างและขาหลัง (HVR) สูง อัตราการไหลของเลือดที่ไปเลี้ยงอวัยวะท่อนล่างและขาหลัง (HBF) ต่ำ มีภาวะหัวใจโตและพบการเพิ่มขึ้นของตัวบ่งชี้ภาวะเครียดออกซิเดชั่น ($p<0.05$) การให้สารสกัดจากดอกคำฝอย (300 มก./กก./วัน) หรือยาแคปโตพริล (30 มก./กก./วัน) ในช่วง 2 สัปดาห์สุดท้าย สามารถลดระดับความดันเลือดและ HVR เพิ่ม HBF ลดภาวะหัวใจโตและตัวบ่งชี้ภาวะเครียดออกซิเดชันได้ ($p<0.05$) การศึกษานี้ชี้ให้เห็นว่าสารสกัดจากดอกคำฝอยมีฤทธิ์ต้านความดันเลือด ซึ่งเกี่ยวข้องกับฤทธิ์ต้านอนุมูลอิสระ

Key Words: Hypertension, Oxidative stress, Safflower

คำสำคัญ: ภาวะความดันเลือดสูง ภาวะเครียดออกซิเดชัน ดอกคำฝอย

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Introduction

Nitric oxide (NO) is an important vasodilator to control the caliber of blood vessels as well as vascular resistance. L-NAME is a nitric oxide synthase inhibitor that reduced NO production, leading to increased vascular tone and hypertension. It is usually used for an animal model of hypertension. There is evidence to support that L-NAME-induced hypertensive rats is normally characterized by high blood pressure and cardiovascular remodeling (Paulis *et al.*, 2008). Furthermore, there are several studies to show the association between oxidative stress status and L-NAME hypertensive rats (Bunbupha *et al.*, 2014; Nakmareong *et al.*, 2011).

Carthamus tinctorius L. is commonly known as safflower. It is widely used as traditional food or herbal medicine. Several current studies have demonstrated its beneficial effects such as anti-inflammatory (Jun *et al.*, 2011), antidiabetic (Wang *et al.*, 2011) and antioxidant effects (Kruawan and Kangsadalampai, 2006). However, the effects of CT extract on cardiovascular parameters and oxidative stress markers in nitric oxide-deficient hypertensive rats have not been previously reported.

Objectives

The present study is to investigate the effects of CT extract on cardiovascular parameters and oxidative stress markers in L-NAME induced hypertensive rats.

Methodology

Plant extract

Dry flower of CT was extracted using ethanol. In brief, CT was soaked in 95% ethanol for 4 hours. The ethanol extract was filtered through nylon cloth and then dried using spray dry machine. The yield (calculated on the dried powder extract) was 11.25 % of the dry CT.

Animals

Male sprague-dawley rats were purchased from the National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom, Thailand. They were housed in stainless cages under a 12:12 h light/dark cycle at 25 ± 2 °C at Northeast Laboratory Animal Center, Khon Kaen University, Thailand. The experiment was carried out according to the guidelines of Animal Ethics Committee of Khon Kaen University (AEKKU 5/2557).

The rats were divided randomly into 4 groups of 5-7 rats each.

Group I: Control + vehicle

Group II: L-NAME + vehicle

Group III: L-NAME + CT (300 mg/kg/day)

Group IV: L-NAME + captopril (CAP) (30 mg/kg/day)

Control rats received tap water, whereas L-NAME rats received 40 mg of L-NAME /kg/day dissolved in the drinking water throughout the experimental period (5 weeks) to induce hypertension. Rats were orally treated with CT or captopril or distilled water (vehicle) for the last 2 weeks. Captopril is an angiotensin-converting enzyme (ACE) inhibitor that is used for positive control of this study.

Indirect blood pressure measurement

In conscious rats, systolic blood pressure (SP) were determined by tail-cuff method (IITC model 179 blood pressure analyser) once a week for 5 weeks to monitor blood pressure.

Hemodynamic measurements

After 5 weeks of treatment, the rats were anesthetized with pentobarbital sodium (60 mg/kg, ip The left femoral artery was identified, cleaned off connective tissues, and cannulated by a polyethylene tube connected to a pressure transducer for measuring SP, diastolic blood pressure (DP), mean arterial pressure (MAP), and heart rate (HR) and recorded by the Acknowledge Data Acquisition with analysis software (Biopac Systems Inc., Santa Barbara, CA, USA). Hindlimb blood flow (HBF) was continuously measured by an electromagnetic flow meter (Carolina Medical Electronics, Carolina, NC, USA) connected to an electromagnetic flow probe placed around the abdominal aorta. Hindlimb vascular resistance (HVR) was calculated from MAP divided by HBF in 100 g tissue.

At the end of experimental period after measuring hemodynamic status, the rats were sacrificed and the body weight (BW), heart weight (HW), left ventricle weight (LVW) were determined. The LVW/BW and RVW/BW ratios were calculated

Measurement of oxidative stress markers

Carotid arteries were rapidly excised to use for analysis of $O_2^{\bullet-}$ production by luciginin-enhanced chemiluminescence as described previously (Lu *et al.*, 1996) with some modifications (Kukongviriyapan *et al.*, 2007). Blood sample were mixed with EDTA and placed on ice for plasma MDA measurement. The concentration of plasma MDA will be measured as TBA reactive substances by a spectrophotometric method as previously described (Nakmareong *et al.*, 2011).

Statistical analysis

Data are presented as mean ± SEM. Comparisons between groups are performed using one-way ANOVA followed by post-hoc Student-Newman-Keuls multiple range tests. All analysis was performed using SigmaStat software. P value <0.05 were considered significant.

Results

Effect of CT extract on cardiovascular parameters

L-NAME treatment for 5 weeks resulted in a significant increase in SP (207.41±4.24 mmHg) comparing to the control rats (122.99±3.33mmHg). Daily treatment of CT extract (300 mg/kg/day) or captopril (30 mg/kg/day) for the last 2 weeks showed a significant reduction of SP in hypertensive rats (166.3±2.35 and 117.8±4.26 mmHg, respectively) (Figure 1). SP, DP, MAP, HVR and HR were significantly increased following 5 weeks of L-NAME treatment compared to the normal control group ($P < 0.05$), while a decrease of HBF was observed in L-NAME treated rats. Treatment with either CT extract (300 mg/kg/day) or captopril (30 mg/kg/day) for two consecutive weeks significantly reduced SP, DP, MAP, HVR and HR in L-NAME hypertensive rats. This was consistent with the increases of HBF in L-NAME hypertensive rats receiving CT extract or captopril (Table1).

Table 1 Effect of CT extract and captopril on SP, DP, MAP and HR, in all experimental groups at weeks 5

Parameters	Control+vehicle	L-NAME+vehicle	L-NAME+CT 300 (mg/kg/day)	L-NAME+CAP 30 (mg/kg/day)
SP (mmHg)	121.36 ±4.24	195.49±0.93*†	159.98±5.28*#	138.98±5.74*#†
DP (mmHg)	75.06±7.55	135.20±1.52*†	105.80±3.71*#	82.20±2.32#†
MAP (mmHg)	90.49±5.38	155.30±1.17*†	123.86±3.97*#	101.13±3.22#†
HR (beat/min)	358.28±11.59	420.92±2.94*†	346.13±5.14#	357.80±19.15*#
HBF(ml/min/100 g tissue)	6.05±0.38	3.38±0.16*†	4.23±0.13*#	4.94±0.19*#†
HVR(mmHg/ml/min/100g tissue)	4.73±0.60	13.11±0.95*†	6.27±0.63#	4.89±0.44#

Data are expressed as means ± S.E.M. (n= 6 /group), *p< 0.05 vs. control groups, #p<0.05 vs. L-NAME group, †p<0.05 vs. L-NAME+CT treated group

Table 2 Effect of CT extract and captopril on general biological parameters of heart in all experimental groups at weeks 5

Parameters	Control+vehicle	L-NAME+vehicle	L-NAME+CT 300 (mg/kg/day)	L-NAME+CAP 30 (mg/kg/day)
BW (g)	436.5±4.15	438.5±11.21	438.0±9.57	425.8±4.10
HW/BW (mg/g)	3.18±0.04	3.59±0.11*†	3.31±0.10#	3.13±0.05#
LVW/BW (mg/g)	2.16±0.03	2.56±0.07*†	2.31±0.07#	2.14±0.03#

Data are expressed as means ± S.E.M. (n=5- 7 /group), *p< 0.05 vs. control groups, #p<0.05 vs. L-NAME group, †p<0.05 vs. L-NAME+CT treated group

Effect of CT extract and captopril on cardiac hypertrophy

The body weight was not significantly different in all groups. L-NAME induced significant increases in HW/BW and LV/BW ratios comparing to control rats. Treatment of either CT extract (300 mg/kg/day) or captopril (30 mg/kg/day) in the last 2 weeks showed significantly decreased HV/BW and LV/BW ratio in hypertensive rats, comparing to untreated rats (Table 2).

There was a significant increase vascular $O2^{\bullet-}$productions (115.76 ± 3.47 counts/min/ mg dry weight), comparing to control rats (42.93 ± 3.73 counts/min/mg dry weight) in L-NAME hypertensive rats. CT extract (300 mg/kg/day) or captopril (30 mg/kg/day) reduced vascular $O_2^{\bullet-}$productions in hypertensive rats (86.74 ± 11.02 and 87.37 ± 13.41 counts/min/mg dry weight, respectively) comparing to untreated rats (Fig 2).

Similarly, high level of plasma MDA in hypertensive rats was significantly decreased after CT extract (300 mg/kg/day) or captopril (30 mg/kg/day) treatment (6.27±0.64 and 4.89±0.44 µM, respectively) comparing to untreated hypertensive rats (13.11±0.95 µM) ($p<0.05$) (Fig 3).

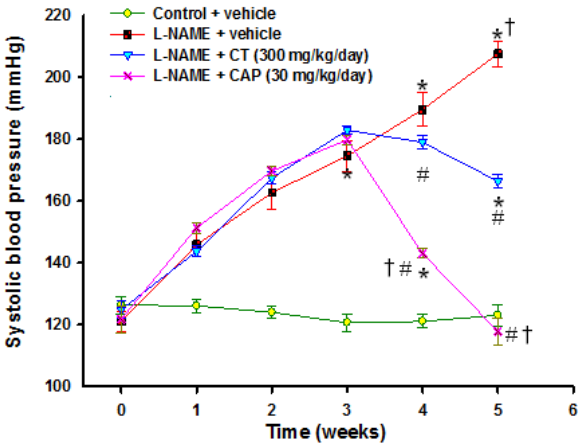

Figure 1 Effect of CT extract on SP in L-NAME induced hypertension. Data were expressed as means ± SEM. (n = 5-7/group) * $p<0.05$ vs. control group, # $p<0.05$ vs. L-NAME group, †$p<0.05$ vs. L-NAME+CT

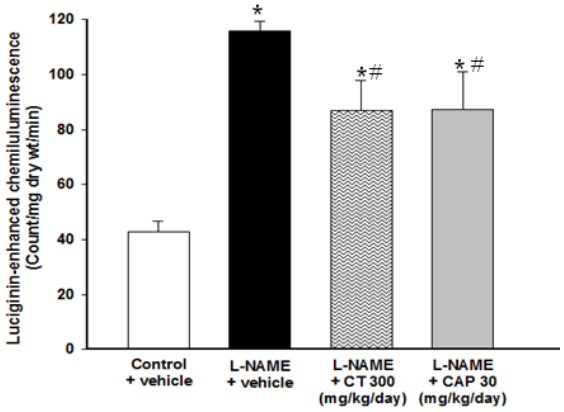

Figure 2 Effect of CT extract on vascular $O2^{\bullet-}$ productions in L-NAME induced hypertensive rats. Data were expressed as means ± SEM. (n = 5-7/group) * $p<0.05$ vs. control group, # $p<0.05$ vs. L-NAME group, †$p<0.05$ vs. L-NAME+CT treated group

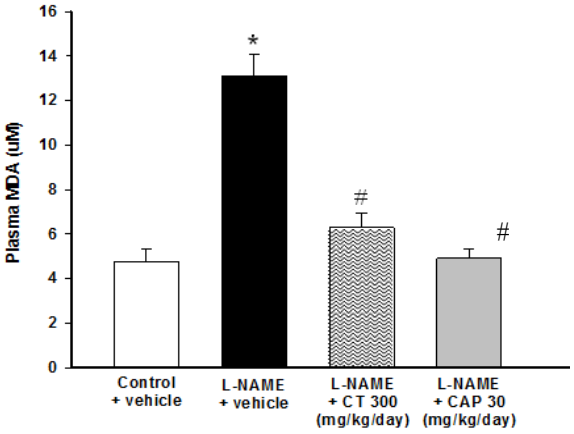

Figure 3 Effect of CT extract on plasma MDA in L-NAME induced hypertensive rats. Data were expressed as means ± SEM. (n = 5-7/group) * $p<0.05$ vs. control group, # $p<0.05$ vs. L-NAME group, †$p<0.05$ vs. L-NAME+CT treated group

Discussion and Conclusions

The main findings of this study are that L-NAME caused hypertension with cardiac hypertrophy and high levels of oxidative stress markers. CT extract and captopril improved cardiovascular parameters and oxidative stress status in L-NAME-induced hypertension.

It is well established that blood pressure is determined by cardiac output and total peripheral resistance. Under the conditions of this study, there was an elevation of blood pressure with an increase in HVR in L-NAME hypertensive rats. L-NAME blocks NO synthesis, produces systemic vasoconstriction, an increase in vascular resistance and high blood pressure (Pakdeechote *et al.*, 2014).

CT extract reduced blood pressure in L-NAME hypertensive rats, which is consistent with an improvement of HBF and HVR. This antihypertensive effect of CT extract may involve its antioxidant capacity. This was supported by this findings that CT extract supplementation decreased vascular superoxide production and plasma MDA in

hypertensive treated rats. Free radical can develop hypertension, especially superoxide ($O2^{\bullet-}$). Superoxide rapidly react with NO to produce peroxynitrite ($ONOO^-$) to decrease NO bioavailability (Bunbupha *et al.*, 2014), which caused endothelial dysfunction and tissue damage leading to hypertension.

Cardiac hypertrophy is observed in cardiac remodeling when heart suffered from high workload in L-NAME hypertensive rats. This present study revealed that heart weight and LVW in the L-NAME-treated rats were increased. These results indicated that the left ventricle of these rats have been remodelled by hypertrophy as cardiac adaptation to maintain the normal cardiac output. CT extract reduced cardiac hypertrophy in L-NAME hypertensive rats. The mechanism involve in CT extract inhibited left ventricle remodeling is unknown. However, there is evidence that antioxidant substance can attenuate cardiovascular remodeling (Silambarasan *et al.*, 2014).

Captopril, an angiotensin-converting enzyme inhibitor, it restored alterations of blood pressure, HR, HBF, HVR as well as cardiac hypertrophy in the L -NAME hypertensive rats. This was consistent with previous study that ACE inhibitors normalized blood pressure, vascular and left ventricular hypertrophy in L-NAME hypertension (Bernatova *et al.*, 2000; Khattab *et al.*, 2005). In addition, captopril can reduce vascular $O_2^{\bullet-}$ production and plasma MDA. Captopril reduces angiotensin II level, resulting in decreased free radical production and oxidative damage (Chengzhi *et al.*, 2012; Khattab *et al.*, 2005)

In conclusion, the present study demonstrated that CT extract is able to reduce vascular resistance, blood pressure, cardiac hypertrophy and oxidative stress markers in L-NAME hypertensive rats. Further studies are needed to explore the precise mechanism of this hypertensive effect.

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References

Bernatova I, Pechanova O, Pelouch V, Simko F. Regression of chronic L -NAME-treatment-induced left ventricular hypertrophy: effect of captopril. J Mol Cell Cardiol 2000; (2)32: 177-85.

Bunbupha S, Pakdeechote P, Kukongviriyapan U, Prachaney P, Kukongviriyapan V. Asiatic acid reduces blood pressure by enhancing nitric oxide bioavailability with modulation of eNOS and p47phox expression in L-NAME-induced hypertensive rats. Phytother Res 2014; (10)28: 1506-12.

Chengzhi L, Li W, Aubdool-Essackjee, Sherpa N, Di L. Angiotensin-II Induced Reactive Oxygen Species: Implications in Neurogenic Hypertension. J Hypertens 2012; (1)106: 2167-1095.

Jun MS, Ha YM, Kim HS, Jang HJ, Kim YM, Lee YS, et al. Anti-inflammatory action of methanol extract of Carthamus tinctorius involves in heme oxygenase-1 induction. J Ethnopharmacol 2011; (2)133: 524-30.

Khattab MM, Mostafa A, Al-Shabanah O. Effects of captopril on cardiac and renal damage, and metabolic alterations in the nitric oxide-deficient hypertensive rat. Kidney Blood Press Res 2005; (4)28: 243-50.

Kruawan K, Kangsadalampai K. Antioxidant activity, Phenolic compound contents and antimutagenic activity of some water extract of herbs. Thai J Pharm Sci 2006; 30: 28-35.

Kukongviriyapan U, Luangaram S, Leekhaosoong K, Kukongviriyapan V, Preeprame S. Antioxidant and vascular protective activities of Cratoxylum formosum, Syzygium gratum and Limnophila aromatica. Biol Pharm Bull 2007; (4)30: 661-6.

Lu FJ, Lin JT, Wang HP, Huang WC. A simple, Sensitive, non-stimulated photon counting system for detection of superoxide anion in whole blood. Experientia 1996; (2)52: 141-4.

Nakmareong S, Kukongviriyapan U, Pakdeechote P, Donpunha W, Kukongviriyapan V, Kongyingyoes B, et al. Antioxidant and vascular protective effects of curcumin and tetrahydrocurcumin in rats with L-NAME-induced hypertension. Naunyn Schmiedebergs Arch Pharmacol 2011; (5)383: 519-29.

Pakdeechote P, Prachaney P, Berkban W, Kukongviriyapan U, Kukongviriyapan V, Khrisanapant W, et al. Vascular and antioxidant effects of an aqueous Mentha cordifolia extract in experimental N(G)-nitro-L-arginine methyl ester-induced hypertension. Z Naturforsch C 2014; (1-2)69: 35-45.

Paulis L, Matuskova J, Adamcova M, Pelouch V, Simko J, Krajcirovicova K, et al. Regression of left ventricular hypertrophy and aortic remodelling in NO-deficient hypertensive rats: effect of L-arginine and spironolactone. Acta Physiol (Oxf) 2008; (1)194: 45-55.

Silambarasan T, Manivannan J, Krishna Priya M, Suganya N, Chatterjee S, Raja B. Sinapic Acid prevents hypertension and cardiovascular remodeling in pharmacological model of nitric oxide inhibited rats. PLoS One 2014; (12)9: e115682.

Wang CC, Choy CS, Liu YH, Cheah KP, Li JS, Wang JT, et al. Protective effect of dried safflower petal aqueous extract and its main constituent, carthamus yellow, against lipopolysaccharide-induced inflammation in RAW264.7 macrophages. J Sci Food Agric 2011; (2)91: 218-25.