Carthamus tinctorius Linn. Reduces Blood Pressure and Oxidative Stress Markers in Nitric Oxide-Deficient Hypertensive Rats

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Background and Objective: *Carthamus tinctorius* L. (CT) has been reported to have antioxidant, anti-inflammatory and antidiabetic effects. N^{ω}-Nitro-L-arginine methyl ester (L-NAME)-induced hypertension in rats was reported to increased reactive oxygen species. This study aimed to investigate whether CT extract could reduce blood pressure and oxidative stress markers in L-NAME hypertensive rats.

Methods: Male Sprague-Dawley rats, weighing 230-260 g were divided into three groups. 1) Normal control group, 2) L-NAME control group and 3) L-NAME+CT extract group (n=6,each). Normotensive rats received drinking water for five weeks and vehicle for the last two weeks. Hypertensive rats received L-NAME (40 mg/kg/day) and vehicle or CT extract (500 mg/kg/day) for the last two weeks. Systolic blood pressure (SP) was monitored using tail cuff method once a week. At the end of study blood pressure, heart rate (HR), hindlimb blood flow (HBF) and hindlimb vascular resistance (HVR) were measured. Superoxide production in carotid arteries and plasma malondialdehyde (MDA) were detected.

Results: Daily administration of L-NAME for five weeks showed significant increases in BP, HVR and decreased HBF. Furthermore, increases in vascular superoxide production and plasma MDA were observed in hypertensive rats ($p<0.05$). CT extract significantly reduced BP, HVR, and significantly increased HBF in hypertensive rats ($p<0.05$). Additionally, oxidative stress markers were reduced in hypertensive rats treated with CT extract.

Conclusions: The present investigation suggests that CT extract exhibited an antihypertensive effect. This might be related with its antioxidant capacity.

Key Words: Hypertension, Oxidative stress, Carthamus tinctorius Linn., Safflower

Proceeding

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Introduction

It is well documented that L-NAME-induced high blood pressure was associated with tachycardia and increased in vascular resistance[1]. Furthermore, increased free radicals leading to vascular dysfunction and hypertension have been observed in L-NAME treated rats[2]. Nitric oxide is a vasodilator and modulates vascular tone. Chronic administration of nitric oxide inhibitors provides a model of hypertension in animals[3].

Carthamus tinctorius L. is commonly known as safflower. It is a widely used as traditional Chinese medicine. More than 200 compounds have been isolated from CT [4] . Several current studies have been demonstrated its beneficial effects such as anti-inflammatory, anti-coagulant, hepatoprotective and antidiabetic effects[5]. Since the anti-hypertensive and antioxidant activity of CT extract have not been elucidated yet, the purpose of the present study is to investigate the effects of CT extract on cardiovascular parameters in L-NAME induced hypertensive rats.

Materials and methods

Plant extract

Carthamus tinctorius L. (CT) extract was prepared using ethanol. In brief, CT was soaked in 95% ethanol for four 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 fresh CT.

Animal and experimental protocols

Male Sprague-Dawley rats weighing 230-260 g were purchased from the the National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom, Thailand. Rats were housed in stainless cages with controlled temperature (25 ± 2 °C), under a 12-h light-dark cycle at Northeast Laboratory Animal Center, Khon Kaen University, Thailand. 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 (AEKKU 5/2557). The rats were randomly assigned to three group with six rats in each group.1) Normal control group: received tap water all over experimental period. 2) L-NAME control group, rats received L-NAME (40 mg/kg/day) in drinking water for five weeks to induce hypertension. 3) Treated group, rats received L-NAME (40 mg/kg/day) in drinking water and were orally administered with CT extract (500 mg/kg/day) for the last two weeks. All rats were fed with a standard rat chow diet.

Indirect blood pressure measurement

In conscious rats, Systolic blood pressure (SP) was measured using tail-cuff plethysmography (IITC model 179 blood pressure analyser) method once a week for 5 weeks to monitor blood pressure.

Hemodynamic measurements

At the end of study, rats were anesthetized with pentobarbital sodium (60 mg/kg, ip.), and then placed on heating pads. The temperature was constantly kept at 37°C throughout the study period. The left femoral artery was identified, cleaned connective tissue out off and cannulated by a polyethylene tube of which connected to a pressure transducer for measuring SP, DP, MAP, and heart rate (HR) and recorded using the Acknowledge Data Acquisition with analysis software (Biopac Systems Inc., Santa Barbara, CA, USA). Hindlimb blood flow (HBF) was continuously measured by placing electromagnetic flow probes on the abdominal aorta connected to an electromagnetic flow meter (Carolina Medical Electronics, Carolina, NC, USA). Hindlimb vascular resistance (HVR) was caculated as MAP and mean HBF. Blood samples were collected, and placed on ice for plasma MDA measurement. Carotid arteries were excised for superoxide production measurement.

Assay of superoxide ($O_2^{\bullet-}$) production

Carotid arteries were rapidly excised for the analysis of $O_2^{\bullet-}$ production which determined by lucigininenhanced chemiluminescence as described previously [6]. The carotid arteries were quickly dissected, The adherent fat and connective tissue were cleaned on ice. The vessel segments (3–5mm) were placed in Krebs-KCl buffer and allowed to equilibrate at 37°C for 30min. Lucigenin was added to the sample tube and placed in a luminometer (Turner Biosystems, Sunnyvale, CA, USA). The photon counts were integrated every 30 s for 5 min. The vessels were dried at 45°C for 24 h to determine a dry weight. $O2^{\bullet-}$ production in vascular tissue was expressed as relative light unit counts per minute per milligram of dry tissue weight.

Assay of plasma malondialdehyde (MDA)

Blood samples were collected, mixed with EDTA and placed on ice for plasma MDA measurement. The concentration of plasma MDA was measured as TBA reactive substances by a spectrophotometric method as previously described [7]. In brief, 150-μL plasma samples were reacted with 10% TCA, 5 mmol/L EDTA, 8% SDS, and 0.5 - μg/mL BHT. The mixture was incubated for 10 min at room temperature, then 0.6% TBA was added, and the mixture was boiled in a water bath for 30 min. After cooling to room temperature, the mixture was centrifuged at 10,000 g for 5 min. The absorbance of the supernatant was measured at 532 nm. A standard curve was generated at different concentrations from 0.3 to 10 -μL mol/L using 1,1,3,3-tetraethoxypropane.

Statistical analysis

Data were expressed as means ± SEM. Comparisons between groups were performed using

one-way ANOVA followed by post-hoc Student-Newman-Keuls multiple range tests. Differences between groups were considered a significant difference at $p < 0.05$ levels.

Results

Effects of CT extract on cardiovascular parameters

Figure 1 shows SP of all groups in five weeks of experiment periods. SP value of all experimental groups was not different at baseline. L-NAME administration continuously increased SP from week 0 to week three (175.51 ± 6.10 mmHg), comparing to those of control group (120.63 ± 2.83 mmHg) ($p<0.05$). Daily treatment with CT extract (500 mg/kg/day) for two weeks showed a reduction SP in hypertensive rats (157.66 ± 2.82 mmHg) comparing to those of untreated rats (207.41± 4.24 mmHg) ($p<0.05$). After five weeks of treatment SP, DP, MAP and HVR were decreased in hypertensive rats received CT extract, this was consistent with the increases of HBF. There was no significant difference of HR among groups (Table 1).

Effect of CT extract on oxidative stress status

It was found that vascular $O2^{\cdot-}$productions (110.58 ± 6.52 counts/min/ mg dry weight) in hypertensive rats were significantly increased comparing to those of normal control rats (44.55 ±15.66 counts/min/mg dry weight) ($p<0.05$) while the hypertensive rats receiving CT extract showed a significant reduction of vascular $O2^{\cdot-}$ productions (67.51 ± 3.63 counts/min/mg dry weight) ($p<0.05$) (Fig 2). Similarly, plasma MDA levels in hypertensive rats receiving CT extract were lower (7.60 ± 0.53 μM) than those of in untreated hypertensive rats (12.65 ± 1.02 μM) ($p<0.05$) (Fig 3).

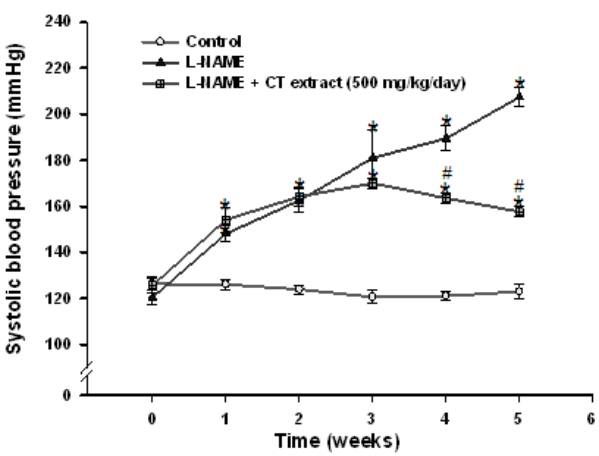

Figure 1 Effects of CT extract on SP in L-NAME induced hypertension. Data were expressed as means ± SEM. (n = 6/group) * $p<0.05$ vs. control, # $p<0.05$ vs. L-NAME

Discussion

The main finding of this study is that CT extract reduced blood pressure which is consistent with an improvement of HBF and HVR in L-NAME hypertensive rats. This study provided evidence that CT extract decreased vascular resistance and then reduced blood pressure. Since CT extract had an antioxidant effect, this was supported by decreasing vascular superoxide production and plasma MDA in hypertensive treated rats. Increased free radical, superoxide, can develop hypertension as superoxide react with NO to produce peroxynitrite and decrease NO bioavailability[8]. Therefore, antioxidant property of CT extract might be one of the possible mechanisms for reducing blood pressure in L-NAME-induced hypertensive rats.

Table 1 Effects of CT extract on SP, DP, MAP, HR, HBF and HVR in all experimental groups (n = 6, each).

Parameters	Control	L-NAME	L-NAME + CT extract
SP (mmHg)	122.86 ± 2.13	204.53 ± 7.16*	167.86 ± 2.26*#
DP (mmHg)	73.11 ± 3.53	145.78 ± 7.16*	115.99 ± 2.58*#
MAP (mmHg)	89.69 ± 2.36	165.36 ± 7.07*	133.28 ± 2.43*#
HR (beat/min)	334.17 ± 6.33	350.95 ± 4.66	342.90 ± 12.87
HBF (ml/min/100 g tissue)	6.80 ± 0.58	3.64 ± 0.31*	4.86 ± 0.22*#
HVR(mmHg/ml/min/100g tissue)	13.66 1.18	46.97 4.40*	27.66 1.12*#

*$p<0.05$ vs control, #$p<0.05$ vs L-NAME

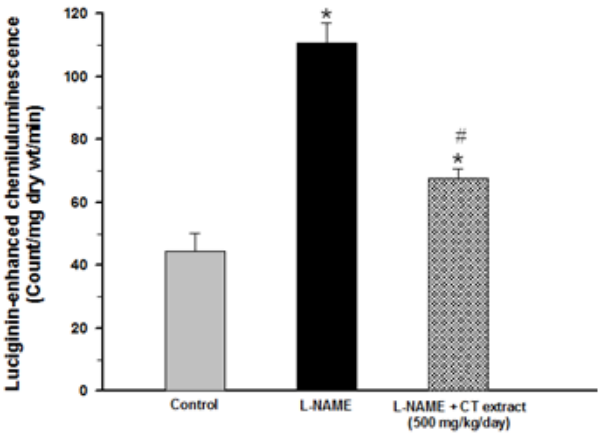

Figure 2 Effects of CT extract on vascular $O_2^{\cdot-}$ productions in all experimental groups. Data were expressed as means ± SEM. (n = 4-6/group). * p<0.05 vs. control, # p<0.05 vs. L-NAME

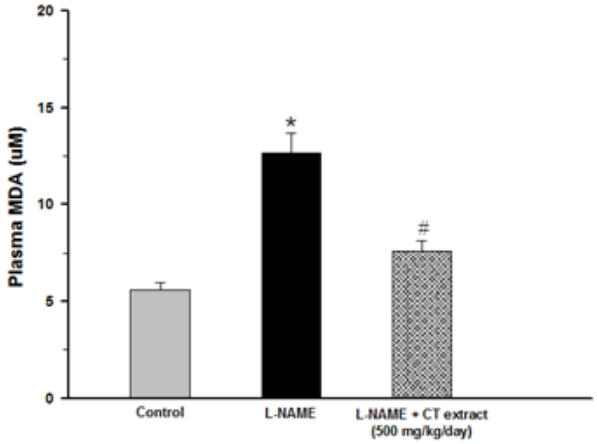

Figure 3 Effects of CT extract on plasma MDA in all experimental groups. Data were expressed as means ± SEM. (n = 4-6/group). *p<0.05 vs. control, # p<0.05 vs. L-NAME

Conclusions

Supplementation of CT extract showed improving hemodynamics status in L-NAME induce hypertensive rats. This effect might be related with its antioxidant capacity. It may be concluded that CT extract exhibited anti-hypertensive effects and antioxidant activity.

Acknowledgements

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