นิพนธ์ต้นฉบับ • Original Article

ผลของสารสกัดเอทานอลจากดอกคำฝอยต่อการเปลี่ยนแปลงพลศาสตร์การไหลเวียนเลือดในหนูแรทที่มีภาวะความดันเลือดสูงที่ถูกเหนี่ยวนำโดยสารแอลเนม

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Effect of Ethanolic Extract of *Carthamus tinctorius* on Hemodynamic Changes in L-NAME Hypertensive Rats

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หลักการและวัตถุประสงค์: คาร์ทาร์มัสทินค์ทอเรียส (Carthamus tinctoris; CT) หรือดอกคำฝอยเป็นสมุนไพรที่ถูกใช้อย่างแพร่หลายในทางการแพทย์แผนโบราณ ได้รายงานว่าดอกคำฝอยมีความสามารถสูงในการเป็นสารต้านอนุมูลอิสระ การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาว่าสารสกัดเอทานอลจากดอกคำฝอยมีความสามารถทำให้การเปลี่ยนแปลงของพลศาสตร์การไหลเวียนเลือดในหนูแรทที่มีความดันเลือดสูงที่ถูกเหนี่ยวนำด้วยสารแอลเนม (L-NAME) ดีขึ้น
วิธีการศึกษา: หนูแรทเพศผู้ได้รับน้ำผสมสารแอลเนม (40 มก./กก./วัน) เป็นเวลา 5 สัปดาห์เพื่อเหนี่ยวนำให้เกิดภาวะความดันเลือดสูงและหนูแรทได้รับสารตัวทำละลายเป็นกลุ่มควบคุม หนูกลุ่มความดันเลือดสูงได้รับสารสกัดเอทานอลจากดอกคำฝอย (300 มก./กก./วัน) หรือเคอร์ซิติน (quercetin) (25 มก./กก./วัน) ใน 2 สัปดาห์สุดท้าย วัดความดันเลือดซิสโตลิกทางหางสัปดาห์ละครั้ง วันสุดท้ายของการทดลองวัดความดันเลือด อัตราการเต้นของหัวใจ อัตราการไหลของเลือดที่อวัยวะท่อนล่างและขาหลัง ความต้านทานการไหลของเลือดที่ไปเลี้ยงอวัยวะท่อนล่างและขาหลัง มาลอนไดอัลดีไฮด์ในพลาสมา (plasma malondialdehyde) ได้รับการตรวจวัด
ผลการศึกษา: หนูแรทที่ได้รับบสารแอลเนมเป็นเวลา 5 สัปดาห์ พบว่ามีความดันเลือดสูง ความต้านทานการไหลของเลือดที่อวัยวะท่อนล่างและขาหลังสูง อัตราการไหล

Background and Objective: *Carthamus tinctorius* (CT) or Safflower is extensively used as herb in traditional medicine. It has been reported to have a strong antioxidant capacity. This study aimed to investigate whether CT ethanolic extract could improve hemodynamic alterations in L-NAME induced hypertensive rats.
Methods: Male Sprague-Dawley rats received L-NAME (40 mg/kg/day) in drinking water for five weeks to induce hypertension or vehicle to serve as control. Hypertensive rats were treated with CT ethanolic extract (300 mg/kg/day) or quercetin (25 mg/kg/day) for the last 2 weeks. Systolic blood pressure (SP) was monitored using a tail cuff method once a week. At the end of experimental day, the blood pressure (BP), heart rate (HR), hindlimb blood flow (HBF) and hindlimb vascular resistance(HVR) were measured. Plasmamalondialdehyde (MDA) was also measured.
Results: Rats treated with L-NAME for five weeks had high blood pressure (BP), heart rate (HR), high hindlimb vascular resistance (HVR), low hindlimb blood flow (HBF) ($p<0.05$). MDA level were increased in L-NAME hypertensive rat ($p<0.05$). Treatment with either CT

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ของเลือดที่ไปเลี้ยงอวัยวะท่อนล่างและขาหลังต่ำ ($p<0.05$) ตัวบ่งชี้ภาวะเครียดออกซิเดชันสูงขึ้น ในหนูแรทที่มีภาวะความดันเลือดสูงที่ถูกเหนี่ยวนำโดยสารแอลเนม ($p<0.05$) การให้สารสกัดเอทานอลจากดอกคำฝอย หรือเคอร์ซิตินใน 2 สัปดาห์สุดท้ายสามารถลดความดันเลือด อัตราการเต้นของหัวใจ ความต้านทานการไหลของเลือดที่อวัยวะท่อนล่างและขาหลัง และมาลอนไดอัลดีไฮด์ในพลาสมา ($p<0.05$)

สรุป: ผลการศึกษานี้แสดงให้เห็นว่าสารสกัดเอทานอลจากดอกคำฝอยสามารถช่วยบรรเทาการเปลี่ยนแปลงพลศาสตร์การไหลเวียนเลือดในหนูแรทที่มีภาวะความดันเลือดสูงที่ถูกเหนี่ยวนำโดยสารแอลเนม ผลนี้อาจเกี่ยวข้องกับความสามารถในการเป็นสารต้านอนุมูลอิสระของดอกคำฝอย

คำสำคัญ: ความดันเลือดสูง แอลเนม ดอกคำฝอย คาร์ทาร์มัส ทินค์ทอเรียส

ethanolic extract or quercetin for the last two weeks significantly reduced BP, HR, HVR, and plasma MDA level.

Conclusions: These results suggest that the CT ethanolic extract can alleviate hemodynamic in L-NAME hypertensive rats. These effects are possibly related to its antioxidant capacity.

Keyword: Hypertension, L-NAME, Safflower, *Carthamus tinctorius*

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Introduction

Nitric oxide (NO) is a vasodilator that plays an important to control the diameter of blood vessels, vascular resistance and blood pressure. N -nitro-L-arginine methyl ester (L-NAME) is a nitric oxide synthase inhibitor. It inhibits NO production leading to increased vascular resistance and hypertension. L-NAME has been widely used to induce hypertension in animals to mimic essential hypertension [1]. It has been reported that chronic administration of L-NAME causes a sustained increase in blood pressure is associated with an increase in peripheral vascular resistance[2, 3] and cardiovascular remodeling [4]. Furthermore, there are several studies to show the association between oxidative stress status and L-NAME hypertensive rats[2, 5]. Pakdeechote and coworkers (2014) demonstrated that in L-NAME hypertensive rats, there was an increase in plasma MDA and vascular superoxide production. *Mentha Cordifolia* which its antioxidant activity reduced blood pressure by suppressing oxidative stress markers, which possibly increased NO bioavailability [6]

Safflower or scientific name, *Carthamus tinctorius* (CT) is extensively used as a herb in traditional medicine. Several current studies have been demonstrated the potential effects of CT extract such as antidiabetic [7], hepatoprotective [8], and anticoagulant effects [9]. Recent studies have reported antioxidant activities of the CT extract[8, 10, 11]. However, little information regarding the antihypertensive effects of the CT extract has been demonstrated. This study aimed to investigate the effect of CT extracts on the hemodynamic alterations and mechanisms responsible in the L-NAME hypertensive rats.

Materials and Methods

Plant extracts

The CT was purchased from Vejpongosot (V.P. Pharmacy, Bangkok, Thailand). The CT extract was prepared using ethanol. CT was soaked in 95% ethanol for 4 hours. The ethanol extract was filtered through nylon cloth and then dried using a spray dry machine. The yield (calculated on the dried powder extract) was 11.25 % of the dry CT.

Animal and experimental protocols

Male sprague-dawley rats weighing 230-260 g were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom, Thailand.

Rats were housed in stainless cages under the condition of a light/dark cycle of 12:12 h 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 randomly divided into four groups of six rats, each. Group 1: Control + vehicle, Group 2: L-NAME + vehicle, Group 3: L-NAME + CT ethanolic extract (300 mg/kg/day) and Group 4: L-NAME + quercetin (25mg/kg/day)

The control rats received tap water, whereas the L-NAME rats received 40 mg of L-NAME /kg/day dissolved in drinking water for 5 weeks. Rats in the control groups were given distilled water (DW) as a vehicle; rats in the L-NAME hypertensive groups were given CT ethanolic extract or quercetin for the last 2 weeks. Quercetin was used as a positive control in this study.

Indirect blood pressure measurement

In conscious rats, systolic blood pressure (SP) was measured once a week using a tail cuff plethysmography (IITC/Life Science Instrument model 229 and model 179 amplifiers, Woodland Hills, CA, USA) throughout experimental period.

Hemodynamic measurements

At the end of experimental day the animal were anesthetized with pentobarbital sodium (60 mg/kg, ip) and then the left femoral artery was cannulated by a polyethylene tube connecting 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 measured by using an electromagnetic flow meter (CarolinaMedical Electronics, Carolina, NC, USA) connected to an electromagnetic flow probe placed around the abdominal aorta. Hindlimb vascular resistance (HVR) was calculated from the MAP divided by the HBF expressed in 100 g tissue.

Plasma malondialdehyde (MDA) assay

Blood samples were collected from abdominal aorta. It was mixed with EDTA and placed on ice for plasma MDA measurement. The concentration of plasma MDA will be measured as thiobarbituric acid (TBA) reactive substances by a spectrophotometric method previously described [2].

Statistical analysis

Data are expressed as means ± SEM. The differences between experimental groups were analyzed by one-way ANOVA followed by post-hoc Student-Newman-Keuls multiple range tests. Statical significance was determined at a level of $p < 0.05$.

Results

Effects of CT ethanolic extract on hemodynamic parameters in L-NAME hypertensive rats

The alterations of SP in five weeks of experiment periods are showing Fig. 1. At the beginning of the experiments, baseline SP was similar in all experimental groups. Administration L-NAME for five weeks significantly increased SP (196.08 ± 2.28 mmHg) compared to the control rats (120.27 ± 1.90 mmHg,) ($p<0.05$). Treatment of the CT ethanolic extract or quercetin for the last two weeks in the hypertensive rats significantly lowered SP (162.00 ± 0.57 and 155.50 ± 4.41 mmHg) compared to that of the hypertensive rats without treatment ($p<0.05$) (Fig 1).

There were significant increaseds in SP, DP, MAP, and HR in L-NAME-treated rats (Table 1). The CT extract, ethanolic extract or quercetin also improved hemodynamic alterations in hypertensive rats (Table 1). A decrease HBF in the L-NAME-induced hypertension was observed (3.60 ± 0.25 ml/min/100 g tissue, respectively). The low HBF in hypertensive rats was improved following the CT extract ethanolic extract or quercetin treatment (4.96 ± 0.11 and 7.01 ± 0.21 ml/min/100 g tissue) ($p<0.05$) (Fig 2).

Moreover, the HVR in hypertensive rats was high (48.29 ± 1.97 mmHg/ml/min/100 g tissue) compared to that of the normotensive rats (13.13 ± 0.95 mmHg/ml/min/100 g tissue). It was obseved that the CT ethanolic

Table 1 Effect of CT ethanolic extract and quercetin 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 + quercetin 25 (mg/kg/day)
SP (mmHg)	122.61 ± 2.13	194.82 ± 2.96*	161.41 ± 3.67*#	166.36 ± 3.76*#
DP (mmHg)	80.605 ± 4.917	135.73 ± 2.81*	105.99 ± 2.27*#	113.81 ± 3.13*#
MAP (mmHg)	94.60 ± 3.26	155.42 ± 2.75*	124.47 ± 2.36*#	131.33 ± 3.33*#
HR (beat/min)	355.10 ± 10.30	422.19 ± 2.71*	365.63 ± 11.27#	363.05 ± 3.84#

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

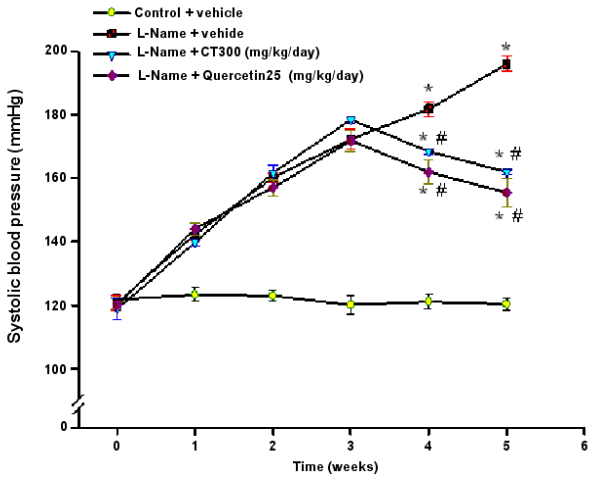

Figure 1 Effect of CT ethanolic extract and quercetin on Systolic blood pressure in L-NAME hypertensive rats. Data are expressed as means ± SEM. (n = 6/group) * p<0.05 vs. control group, # p<0.05 vs. L-NAME group

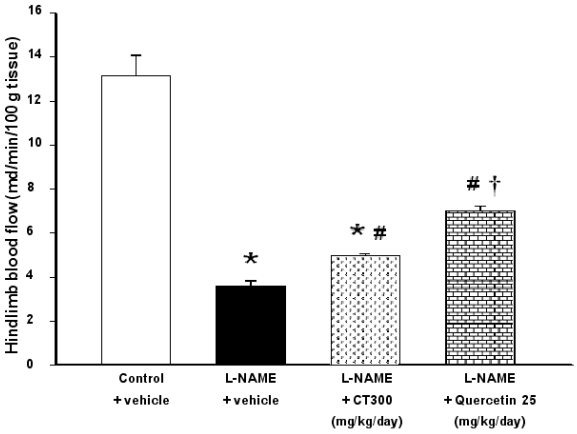

Figure 2 Effect of CT ethanolic extract and quercetin on hindlimb blood flow in L-NAME hypertensive rats. Data are expressed as means ± SEM. (n = 6/group) * p<0.05 vs. control group, # p<0.05 vs. L-NAME group,†p<0.05 vs. L-NAME+CT extract treated group

extract or quercetin also reduced the HVR in the hypertensive rats (24.81 ± 0.83 and 18.86 ± 0.93 mmHg/ml/min/100 g tissue) compared to those of the untreated hypertensive rats (p<0.05) (Fig 3).

Effect of CT ethanolic extract on plasma MDA

The level of plasma MDA was significantly increased in the L-NAME hypertensive rats compared to that of the control rats (10.69 ± 0.62 vs. 4.48 ± 0.67 μM) (p<0.05). Administration of the CT ethanolic extract or quercetin for the last 2 weeks in the L-NAME hypertensive rats significantly attenuated the plasma MDA level (6.37 ± 0.51 and 5.98 ± 0.41 μM) compared to the hypertensive rats without treatment (p<0.05) (Fig 4).

Discussion

In the present study, our findings indicate that L-NAME treatment for 5 weeks caused increases BP, HR, HVR but low HBF. These hemodynamic alterations were associated with the increased oxidative stress marker in this animal. CT ethanolic extract and quercetin significantly improved hemodynamic alterations and oxidative stress marker in the L-NAME-induced hypertensive rats.

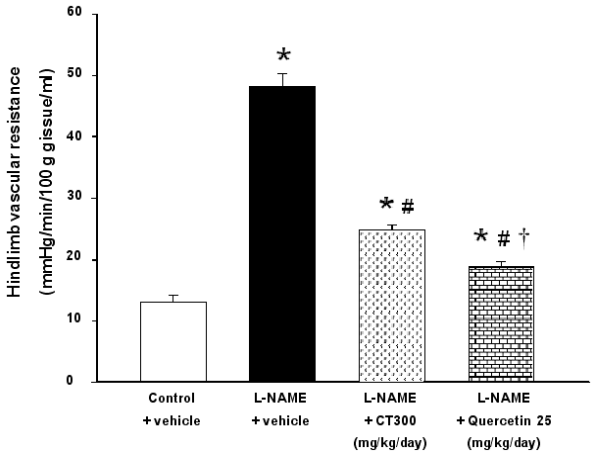

Figure 3 Effect of CT ethanolic extract and quercetin on hindlimb vascular resistance in L-NAME hypertensive rats. Data are expressed as means ± SEM. (n = 6/group) * p<0.05 vs. control group,# p<0.05 vs. L-NAME †group,p<0.05 vs. L-NAME+CT extract treated group

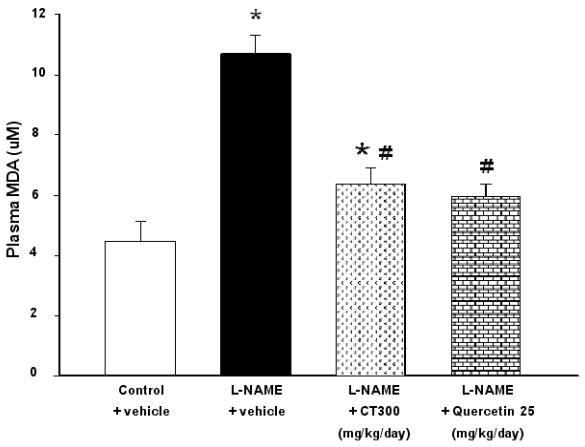

Figure 4 Effect of CT ethanolic extract and quercetin on plasma MDA level in L-NAME hypertensive rats. Data are expressed as means ± SEM. (n = 6/group) * p<0.05 vs. control group, # p<0.05 vs. L-NAME group

Blood pressure is influenced by cardiac output and total peripheral resistance. L-NAME causes a sustained increase in BP by blocking NO synthesis resulting in systemic vasoconstriction, and there by increased vascular resistance and hypertension[6]. We found that there was an increase in BP in the L-NAME hypertensive rats being consequent the high HVR and low HBF

The CT ethanolic extract significantly reduced BP as well as HVR in the hypertensive rats. The mechanism by which the CT ethanolic extract exhibited antihypertensive effects could be associated with decrease of plasma MDA level. This was associatied with the evidence that the CT extract contains phenolic compound with the antioxidant property[10]. Furthermore, quercetin, a flavonoid with antioxidant property, also improved blood pressure and other hemodynamic alterations in the L-NAME hypertensive rats. This was consistent with the studies that quercetin had antihypertensive and antioxidant effects in the hypertensive rats[6, 12].

Conclusion

The present study demonstrates that the CT ethanolic extract has an antihypertensive effect by reducing vascular resistance which is probably related to its antioxidant capacity.

Acknowledgements

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