นิพนธ์ต้นฉบับ ▪ Original Article

ผลของสารสกัดน้ำดอกอัญชันต่อความดันเลือดและภาวะเครียดออกซิเดชั่นในหนูความดันเลือดสูงแบบหลอดเลือดไต

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Effect of Clitoria ternatea L. Aqueous Extract on Blood Pressure and Oxidative Stress in Renovascular Hypertensive Rats

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หลักการและวัตถุประสงค์: อัญชันเป็นสมุนไพรที่ใช้กันอย่างแพร่หลายในเอเชียตะวันออกเฉียงใต้ มีฤทธิ์ต้านการอักเสบและต้านอนุมูลอิสระ การศึกษาครั้งนี้เพื่อตรวจสอบว่าสารสกัดดอกอัญชันสามารถลดความดันเลือดสูง ลดอนุมูลอิสระและปรับปรุงการทำงานของหลอดเลือดในหนูความดันเลือดสูงแบบ 2K-1C หรือไม่

วิธีการศึกษา: หนูแรทเพศผู้ถูกชักนำให้เกิดความดันเลือดสูงโดยหนีบหลอดเลือดรีนอลข้างซ้าย หลังผ่าตัด 3 สัปดาห์ หนูความดันเลือดสูงถูกป้อนด้วยน้ำ และสารสกัดดอกอัญชัน 500 มก./กก. ในขณะที่หนูกลุ่มความดันเลือดปกติถูกป้อนด้วยน้ำเป็นเวลา 4 สัปดาห์ จากนั้นวัดความดันเลือด การทำงานของหลอดเลือด และภาวะเครียดออกซิเดชั่น

ผลการศึกษา: หนูที่ได้รับสารสกัดดอกอัญชัน 500 มก./กก. ความดันเลือดลดลงอย่างมีนัยสำคัญทางสถิติ ($p<0.01$) การคลายตัวตอบสนองของหลอดเลือดต่อสารอะซิติลโคลีนลดลงในหนู 2K-1C สารสกัดดอกอัญชันปรับปรุงการตอบสนองของหลอดเลือดต่อสารอะซิติลโคลีน ($p<0.01$) และลดการสร้างสารอนุมูลอิสระในหนู 2K-1C ($p<0.05$)

สรุป: สารสกัดดอกอัญชันมีฤทธิ์ลดความดันเลือด ซึ่งสัมพันธ์กับการลดการสูญเสียหน้าที่ของเซลล์ชั้นเอนโดทีเลียม ซึ่งผลต่อหลอดเลือดอาจเกี่ยวข้องกับการเพิ่มไนตริกออกไซด์ จากการลดการสร้างอนุมูลอิสระ

Background and objectives: *Clitoria ternatea* L. (CT) is known as Anchan in Thai. It has been extensively used for food coloring and a traditional herbal medicine in South East Asia. Several studies reported its biological activities including, anti-inflammatory and antioxidant. This study was to investigate whether CT extract could reduce blood pressure, oxidative stress and improve vascular function in two-kidneys, one-clip (2K-1C) hypertensive rats.

Methods: Male Sprague Dawley rats were induced hypertension by clipping the left renal artery. After three weeks of surgery, hypertensive rats were continuously treated with distilled water and CT extract 500 mg/kg while sham-operated group received distilled water for four weeks. After that rats were anesthetized to measure blood pressure, vascular function and oxidative stress biomarkers.

Results: The rats received CT extract 500 mg/kg significantly decreased systolic blood pressure, diastolic blood pressure and mean arterial pressure ($p<0.01$). Vasorelaxation responses to acetylcholine (ACh) were blunted in 2K-1C hypertensive rats while treated with CT extract 500 mg/kg significantly

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คำสำคัญ: อัญชัน, หนูแรทที่ถูกชักนำให้เกิดความดันเลือดสูงด้วยการหนีบหลอดเลือดรีนอลข้างซ้าย, ภาวะเครียดออกซิเดชั่น, การทำงานของหลอดเลือด

improved vasorelaxation response to ACh ($p<0.01$). In addition, CT extract significantly decreased vascular superoxide production in 2K-1C hypertensive rats ($p<0.05$).

Conclusion: These findings showed that CT extract had an antihypertensive effect, which associated with improving endothelial dysfunction. Its vascular effect is likely to be increased NO bioavailability as a result of reducing superoxide production.

Keywords: *Clitoria ternatea* L., 2K-1C induced hypertensionoxidative stress, oxidative stress, vascular function

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Introduction

Hypertension is the most common cardiovascular disorder and risk factor for mortality worldwide. It can predispose to various diseases such as stroke, heart failure, myocardial infarction and chronic kidney disease[1]. Renovascular hypertension is a major cause of secondary hypertension that associates with renal artery stenosis and contributes to the renin angiotensin system (RAS) activation. Angiotensin II (Ang II), the main effector of RAS system, is a potent vasoconstrictor. It also promotes vascular reactive oxygen species production via nicotinamide adenine dinucleotide phosphate [NAD(P)H] oxidase, which impairs nitric oxide (NO) bioavailability and results in endothelial dysfunction[2]. For this reason, using the experimental animal model is very useful to investigate the pathophysiology of hypertension and develop in new antihypertensive drugs. Two-kidney, one-clip (2K-1C), a RAS-dependent hypertensive animal model, has been developed to mimic secondary hypertension[3]. This animal model is characterized with high level of plasma Ang II, oxidative stress and vascular dysfunction[4].

Clitoria ternatea L. (CT) is known as butterfly pea or Anchan in Thai. It has been extensively used for food coloring and a traditional herbal medicine in South East Asia. CT is rich in flavonoid glycosides, anthocyanins[5], ternatin, acylated anthocyanins[6], taraxerol[7] and taraxerone[8]. Several studies reported its biological activities, including antimicrobial, antipyretic, anti-inflammatory, antiasthmatic, hepatoprotective analgesic, diuretic, local anesthetic, antidiabetic and antioxidant, however, no previous study has examined the effect of CT on cardiovascular disease especially hypertension. This study aimed to investigate whether CT could reduce blood pressure and oxidative stress in rat renovascular hypertension.

Methods

Animals

Male Sprague Dawley rats weighing 180-200 g were obtained from Nomura Siam International Co., Ltd., Bangkok, Thailand. The animals were housed under standard conditions (25±2 °C temperature and 12 h dark-light cycle) at Northeast Laboratory Animal Center. All experimental procedures were approved by Animal Ethics Committee of Khon Kaen University, Khon Kaen, Thailand (AEKKU-NELAC 72/2561).

Drugs and Chemicals

Dry CT was purchased from Vejpong Pharmacy, Co., Ltd. (Bangkok, Thailand). Acetylcholine chloride (ACh), sodium nitroprusside (SNP) and lucigenin were obtained from Fluka Chemika (Buchs, Switzerland). Phenylephrine (Phe) was purchased from Sigma-Aldrich Corp (St Louis, MO, USA).

Plant preparation

CT flowers were purchased from Vejpong Pharmacy Co., Ltd. (Bangkok, Thailand). The dried CT flowers 500 g were boiled in water at temperature 60 °C for 1 hour. The CT was remained with warm water and then filtered. The water extract of CT was freeze-dried by the lyophilizer (Labconbo, Becthai Bangkok Equipment & Chemical Co., Ltd). The samples were packed in containers and kept at - 20 °C (yield 27.78% w/w) until used.

Experimental designs

Rats were induced hypertension by clipping the left renal artery with a silver clip (0.2 mm) following the 2K-1C Goldblatt model while sham-operated rats were underwent the same procedure except for the placement of renal artery clip. After three weeks of the surgery, the rats were randomly divided into 3 groups (n=4-6/group); sham-operated group received distilled water, 2K-1C hypertensive groups received distilled water and 2K-1C hypertensive groups received CT extract 500 mg/kg for the last four weeks.

Direct measurement of blood pressure

At the end of the experimental period, animals were anesthetized with sodium thiopental 65 mg/kg. The femoral artery was identified and cannulated by polyethylene tube. Baseline values of systolic blood pressure, diastolic blood pressure, mean arterial pressure and heart rate were continuously monitored for 20 mins by a way of a pressure transducer and record using Acknowledge Data Acquisition software (Biopac Systems Inc., Santa Barbara, CA, USA).

Experimental protocols in isolated aortic rings

To assess vasoactive performance of the large arteries, the thoracic aorta was rapidly removed and cut into rings 2-3 mm long for tension measurement. They were mounted in 15 ml baths containing Krebs' solution at 37°C and gassed with 95% O2 and 5% CO2 gas mixture. Isometric contractions were recorded with a resting tension of 1 g using a transducer connected to a 4-channel bridge amplifier and a PowerLab A/D converter and a PC running Chart v5 (PowerLab System, ADInstruments, Australia). ACh (0.01-3 μM) and SNP (0.01-3 μM) induced endothelial mediated-relaxations were assessed by pre-contracting with phenylephrine (10 μM) and relaxation was expressed as % of the phenylephrine-induced contraction.

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

Carotid arteries were rapidly excised and used for analysis $O_2^{\bullet-}$ production which was determined by luciginin-enhanced chemiluminescence. The vessel segments (1-2 mm) were placed in Krebs-KCL buffer and allowed to equilibrate at 37°C for 30 min. Lucigenin was added to sample tube and placed in a luminometer (Turner Biosystems, Sunnyvale, CA, USA). The photo counts were integrated every 30 s for 5 min and averaged. The vessels were dried at 45°C for 24 hour to determine a dry weight. $O_2^{\bullet-}$ production in vascular tissue was expressed as relative light unit counts per minutes per milligram of a dry tissue.

Statistical analysis

The result of this study was expressed as mean ± S.E.M. Statistical analysis was used one-way ANOVA analysis of variance follow by Least Significant Difference (LSD) post-hoc tests for comparing between groups. A probability value < 0.05 was considered statistically significant.

Results

Effect of CT extract on SBP, DBP and MAP in 2K-1C hypertensive rats

There was no significant different in baseline SBP among the experimental groups. 2K-1C hypertensive group significantly increased SBP (196.07 ± 6.02 mmHg) when compared to the shamed group (114.67 ± 2.22 mmHg) ($p<0.01$). Administration of CT extract 500 mg/kg significantly decreased SBP (141.52 ± 9.63 mmHg) compared to hypertensive control group ($p<0.01$). Similarly, CT extract 500 mg/kg significantly decreased DBP and MAP (83.55 ± 8.42 and 107.08 ± 9.03 mmHg, respectively) compared to 2K-1C rats (126.95 ± 5.99 and 153.05 ± 5.50 mmHg, respectively) (Figure 1, $p<0.01$).

Effect of CT extract on vascular reactivity in aortic rings in 2K-1C hypertensive rats

Vasorelaxation responses to ACh were blunted in 2K-1C hypertensive rats.Treating with CT extract 500 mg/kg significantly improved vasorelaxation response to ACh (0.01-3 μM) when compared to 2K-1C hypertensive group (3 μM ACh, 69.01 ± 6.56 vs 32.85 ± 5.80 % of relaxation) (Figure 2A, $p<0.01$). In addition, vasorelaxation response to SNP was not significantly different among group, indicating normal vascular muscle function (Figure 2B).

Effect of CT extract on superoxide production in 2K-1C hypertensive rats

2K-1C hypertensive group significantly increased superoxide production (134.47 ± 5.72 count/mg dry wt/min) when compared to sham rats (58.88 ± 5.96 count/mg dry wt/min) ($p<0.01$). CT extract 500 mg/kg

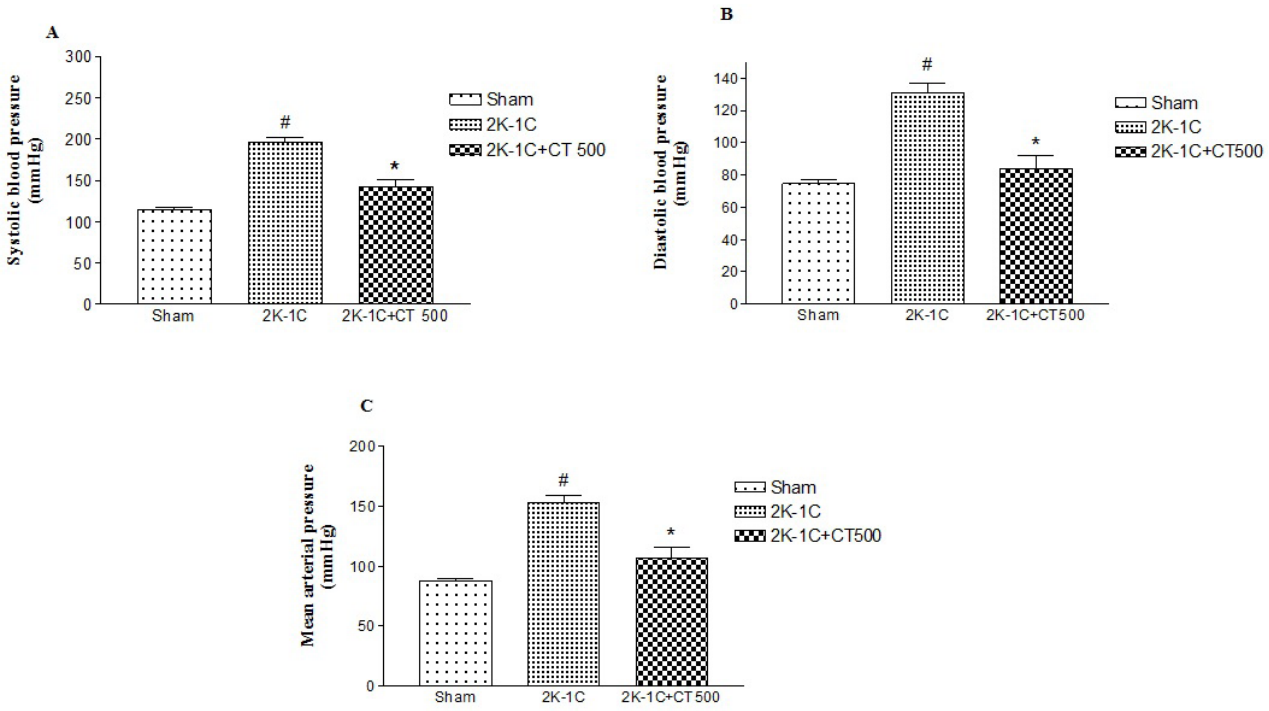

Figure 1 Effect of CT extract on SBP (A), DBP(B) and MAP(C) in 2K-1C hypertensive rats. Data were expressed as Mean ± S.E.M. (n=4-6/group). #p <0.01 vs sham group, *p<0.01 vs 2K-1C.

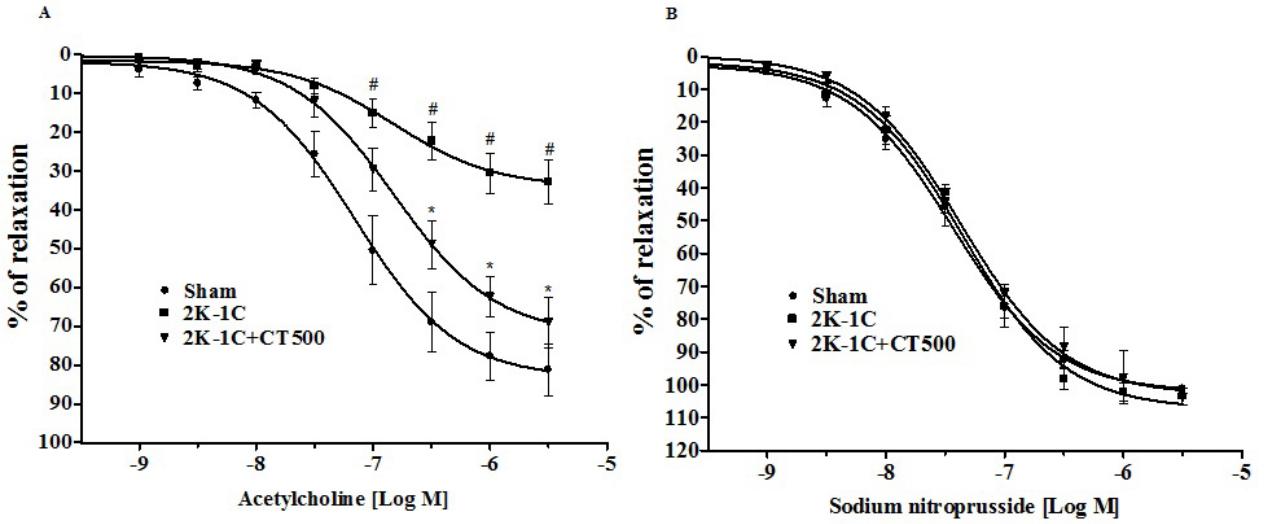

Figure 2 Effect of CT extract on vascular response to acetylcholine(A) sodium nitroprusside(B) in aortic rings in 2K-1C hypertensive rats. Data were expressed as Mean ± S.E.M. (n=4-6/group). #p <0.01 vs sham, *p<0.01 vs 2K-1C

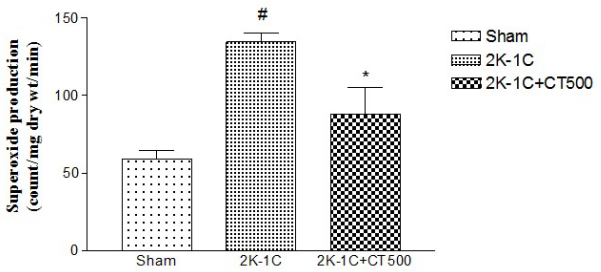

Figure 3 Effect of CT extract on superoxide production in 2K-1C hypertensive rats. Data were expressed as Mean ± S.E.M. (n=4-6/group). #p < 0.01 vs Sham, *p < 0.05 vs 2K-1C.

(87.84 ± 17.43 count/mg dry wt/min) significantly decreased superoxide production compared to hypertensive control (Figure 3, p<0.05).

Discussion

The findings of this study are that CT extract reduced blood pressure, improved endothelium-dependent vasorelaxation in aortic rings and decreased vascular superoxide production. After clipping the left renal artery, the rats developed hypertension through renin-angiotensin system as the previous studies[9]. It

is well established that hypertension in 2K-1C hypertensive rats is resulted from activation of RAS[10]. It is known that renal artery stenosis causes decreased renal perfusion pressure and activates renin production in juxtaglomerular cell, resulting in increase in circulating Ang II. Enhanced activity of RAS plays a major role in the pathogenesis of hypertension since Ang II activate AT1R to promote vascular reactive oxygen species production, sympathetic overactivity, vasoconstriction, and blood volume retention[10]. We found an impairment endothelium-dependent vasorelaxation in 2K-1C hypertensive rats. There is substantial evidence to indicate that Ang II mediated superoxide production via an AT1R-NADPH cascade[11]. Superoxide rapidly reacts with NO to produce peroxynitrite (ONOO-), a powerful oxidant[12] reaction causes a reduction of NO bioavailability, resulting in endothelial dysfunction[13].

CT significantly decreased blood pressure in 2K-1C hypertensive rats. The possible mechanism might relate with improvement of endothelium-dependent vasorelaxation observed in CT treated hypertensive rats. Little information of vasodilatory effect of CT extract was reported[14]. This study firstly demonstrated vasorelaxant effect of CT extract that could be involved its antioxidant properties. Nithianantham and coworkers found that CT extract had radical scavenging and antioxidant capacity[15], which can increase NO bioavailability and contribute to improve endothelial dysfunction.

Conclusion

In conclusion, our finding showed that CT extract had an antihypertensive effect, which associated with improving endothelial dysfunction. Its vascular effect is likely to be increased NO bioavailability as a result of reducing superoxide production.

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