

ผลต่อการลดความดันเลือดและการต้านออกซิเดชันของสารสกัดเอทานอลจากดอกคำฝอยในหนูเรทความดันเลือดสูงที่เกี่ยวข้องกับหลอดเลือดไต

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Antihypertensive and Antioxidant Effects of Safflower

Ethanollic Extract in Renovascular Hypertensive Rats

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หลักการและวัตถุประสงค์ : การศึกษาก่อนหน้านี้แสดงให้เห็นว่าสารสกัดจากดอกคำฝอย มีผลต่อการลดความดันเลือดในหนูเรทที่ชักนำให้เกิดความดันเลือดสูงจากการยับยั้งสารไนตริกออกไซด์แบบเรื้อรัง จุดมุ่งหมายของการศึกษาค้นคว้าคือการตรวจสอบว่าสารสกัดจากดอกคำฝอยสามารถลดความดันเลือดในหนูเรทที่มีภาวะความดันเลือดสูงแบบสองไตหนึ่งคลิปลและกลไกที่เกี่ยวข้อง

วิธีการศึกษา : การศึกษานี้ใช้หนูเรทเพศผู้สายพันธุ์ Sprague-Dawley ถูกเหนี่ยวนำให้เกิดความดันเลือดสูงแบบ 2K-1C ซึ่งหนูความดันเลือดสูงนี้ได้รับสารสกัดจากดอกคำฝอย (500 มิลลิกรัม/กิโลกรัมต่อวัน) หรือ captopril (5 มิลลิกรัม/กิโลกรัมต่อวัน) เป็นเวลา 4 สัปดาห์ ในขณะที่กลุ่มผ่าตัดหลอดเลือด (กลุ่มควบคุม) และ 2K-1C ได้รับเพียงแค่น้ำกลั่น ความดันเลือดซิสโตลิก (SBP) และอัตราการเต้นของหัวใจ (HR) ของหนูทดลองจะถูกวัดสัปดาห์ละครั้งโดยใช้วิธีการวัดที่หาง ในวันสุดท้ายของการทดลอง ผู้วิจัยทำการวัดการผลิตซูเปอร์ออกไซด์ ในหลอดเลือด และความเข้มข้นของมาลอนไดอัลดีไฮด์ ในพลาสมา

ผลการศึกษา : ผลการทดลองพบว่าหนูเรทที่มีภาวะความดันเลือดสูงแบบ 2K-1C มีความดันเลือดสูง (SBP = 222.83 ± 11.85 มิลลิเมตรปรอท) เมื่อเปรียบเทียบกับกลุ่มควบคุม (SBP = 134.80 ± 2.16 มิลลิเมตรปรอท) ไม่มีความแตกต่างของอัตราการเต้นของหัวใจในหนูกลุ่มควบคุมและความดันเลือดสูง มีการเพิ่มขึ้นของการผลิตซูเปอร์ออกไซด์ในหลอดเลือด

Background and Objectives : Previous studies demonstrated that safflower extract had antihypertensive effect in chronic NO synthase inhibition induced hypertensive rats. The aim of the present study was to investigate whether safflower extract could reduce blood pressure and oxidative stress in 2K-1C hypertensive rats and mechanism involved.

Methods : Male Sprague-Dawley rats were induced hypertension using two-kidney one-clip (2K-1C) model. Hypertensive rats were fed with safflower extract (500 mg/kg BW per day) or captopril (5 mg/kg BW per day) for four weeks while sham-operated control group and 2K-1C untreated group received distilled water. Systolic blood pressure (SBP) and heart rate (HR) were measured once a week using tail cuff method. At the end of experiment, vascular superoxide ($O_2^{\cdot -}$) production and plasma malondialdehyde (MDA) concentration were evaluated.

Results : 2K-1C hypertensive rats had high blood pressure (SBP = 222.83 ± 11.85 mmHg) comparing to those of control (SBP = 134.80 ± 2.16 mmHg). There was no significant difference of HR between control and 2K-1C hypertensive rats. Increases in vascular superoxide production and plasma MDA were observed in 2K-1C hypertensive rats. Interestingly, the safflower extract

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และมาลอนไดอัลดีไฮด์ในพลาสมาพบในหนูแรทที่มีภาวะความดันเลือดสูงแบบ 2K-1C สารสกัดจากดอกคำฝอยสามารถลดความดันเลือด ลดการผลิตซูเปอร์ออกไซด์ในหลอดเลือด และลดความเข้มข้นของมาลอนไดอัลดีไฮด์ในพลาสมาได้อย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) นอกจากนี้สารแคปโตพริลลดความดันเลือดและตัวบ่งชี้ภาวะเครียดออกซิเดชันในหนู 2K-1C ความดันเลือดสูง

สรุป : ผลการทดลองแสดงให้เห็นว่าสารสกัดจากดอกคำฝอยมีฤทธิ์ลดความดันเลือดในหนูความดันเลือดสูงที่เกี่ยวข้องกับหลอดเลือดไตโดยมีความสัมพันธ์กับการลดลงของภาวะเครียดออกซิเดชัน

คำสำคัญ : ดอกคำฝอย, หนูแรทที่มีภาวะความดันเลือดสูงแบบ 2K-1C, ภาวะเครียดออกซิเดชัน

significantly reduced blood pressure, vascular superoxide production and plasma MDA concentration ($p < 0.05$). Furthermore, captopril also reduced SBP and oxidative stress markers in 2K-1C hypertensive rats.

Conclusion : These findings suggested that the safflower extract exhibited an antihypertensive effect in renovascular hypertensive rats. This was associated with reducing oxidative stress.

Key words : Safflower, 2K-1C hypertensive rats, Oxidative stress

ศรีนครินทร์เวชสาร 2559; 31 (1): 75-81. ♦ Srinagarind Med J 2016; 31 (1): 75-81.

Introduction

Hypertension is the most common risk factor for myocardial infarction, stroke, heart failure and chronic kidney disease. Nowadays, the researchers focus on pathophysiology of hypertension and cardiovascular disease which cause mortality and mobility in the worldwide. Although many therapeutic agents can be used for management of hypertension. However, the antihypertensive drugs may result in side-effects. On the other hand, there are many studies regarding the use of herb supplementation in reducing high blood pressure and cardiovascular disease¹⁻³.

Safflower has been grown for centuries from China to the Mediterranean region⁴. It is a widely used in Traditional Chinese Medicines (TCM). Many studies showed variety potentials of safflower such as anti-oxidation, anti-inflammation, neuroprotection and hepatoprotection. Previous study reported that safflower extract had antihypertensive effect in L-NAME induced hypertensive rats⁵. However, there is no evidence showing its antihypertensive effect in two kidney-one clip (2K-1C) hypertensive rats. 2K-1C hypertensive rats

mimicsecondary hypertension since this animal model, one renal artery is constricted to chronically reduce renal perfusion while another kidney was not clipped. Chronic reduction of renal blood flow activates renin-angiotensin system⁶.

The present study is aimed to investigate the effects of safflower ethanolic extract on blood pressure and oxidative stress in renovascular hypertensive rats. Moreover, captopril, an angiotensin converting enzyme (ACE) inhibitor, is used for antihypertensive drugs or a positive control.

Methods

Preparation of safflower extract

Dry safflower was purchased from Vejpong Pharmacy, Co., Ltd. (Bangkok, Thailand). The safflower extract was prepared using ethanol. In brief, safflower was soaked in 95% ethanol for four hours. The ethanolic extract was filtered through nylon cloth and then dried using spray dry machine. The yield was 11.25% of the dried safflower.

Animals

Male Sprague-Dawley rats (150-180 g) were purchased from the National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom. Rats were maintained in an air-conditioned room ($25 \pm 2^\circ\text{C}$) with a 12 h dark-light cycle at Northeast Laboratory Animal Center. All procedures are 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 (AEKKU-NELAC 12/2558).

Induction of 2K-1C hypertensive rats

Rats were anesthetized and then a silver clip (0.2 mm i.d.) was placed on to the left renal artery. Sham-operated group was performed the same surgical procedure except the clip was not applied on to the left renal artery. A sustain hypertension was observed after 4 weeks of the operation. Thereafter, the rats were randomized in to sham-operated, 2K-1C and 2K-1C treated groups.

Experimental designs

At 4 weeks after the surgery, the 2K-1C rats were divided into 4 groups of 5-6 rats: Group 1-sham operated control rats received distilled water (0.5 ml/100 mg; p.o.); Group 2-2K-1C rats received distilled water (0.5 ml/100 mg; p.o.); Group 3-2K-1C rats received safflower extract (500 mg/kg/day; p.o.); Group 4: 2K-1C rats received captopril (5 mg/kg/day; p.o.). Safflower extract (500 mg/kg/day) or captopril (5 mg/kg/day) or distilled water was intragastrically administered daily for 4 weeks.

Indirect measurement of blood pressure and heart rate in conscious rats

Indirect blood pressure was measured once a week for 8 weeks. Systolic blood pressure (SBP) and heart rate (HR) were measured in conscious rats by tail-cuff plethysmography (IITC model 179 blood pressure analyser) method.

Measurement of oxidative stress markers

At the end of experiment, the animals were killed by sodium pentobarbital overdose followed by exsanguinations. Carotid arteries were rapidly excised use for analysis $\text{O}_2^{\cdot -}$ production which determined by lucigenin-enhanced chemiluminescence as described previously⁷ with some modification⁸. 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 described⁹.

Statistical analysis

Results were reported as means \pm SEM. Comparisons between groups were performed using one-way ANOVA followed by Least Significant Difference (LSD) post hoc tests. A probability value of less than 0.05 was considered statistically significant.

Results

Effects of safflower extract on blood pressure and heart rate (HR) in 2K-1C hypertensive rats

Changes in SBP during the experimental period were shown in Figure 1. At the beginning of the experiments, baseline SBP was similar in all experimental groups. After induction of hypertension for 1 weeks, the SBP was significantly high in the 2K-1C hypertensive compared to that of the sham-operated control rats. Blood pressure was gradually increased over 4 weeks of the experiment. Treatment with safflower extract for 4 weeks significantly decreased blood pressure in 2K-1C group (191.5 ± 12.25 mmHg) ($p < 0.05$) comparing to those of untreated group (222.83 ± 11.85 mmHg). Captopril markedly reduced SBP (167.87 ± 6.27 mmHg) ($p < 0.05$) in hypertensive rats comparing to that of hypertensive rats. There was no significant difference of HR among groups (Fig 2)

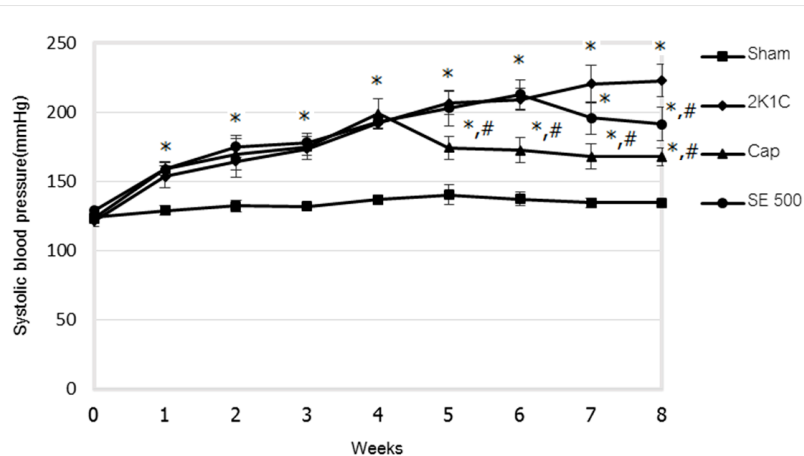


Figure 1 Effect of safflower extract (SE) on blood pressure in 2K-1C hypertensive rats. Data were presented as mean \pm S.E.M. (n = 6/group). * p<0.05 vs. Sham, # p<0.05 vs. 2K-1C.

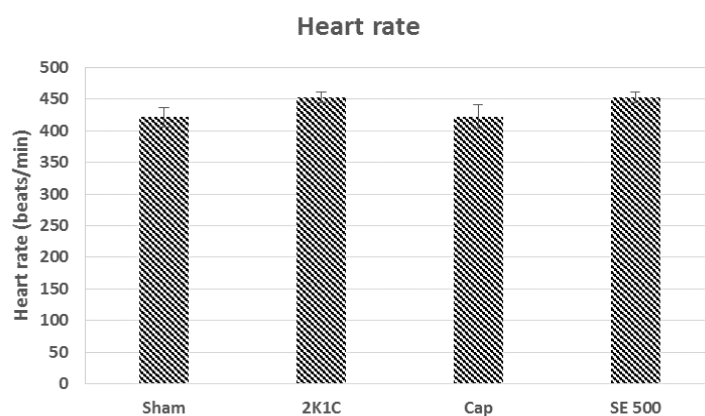


Figure 2 Effect of safflower extract (SE) on heart rate in 2K-1C hypertensive rats after treatment for 4 weeks. Data were presented as mean \pm S.E.M. (n = 6/group).

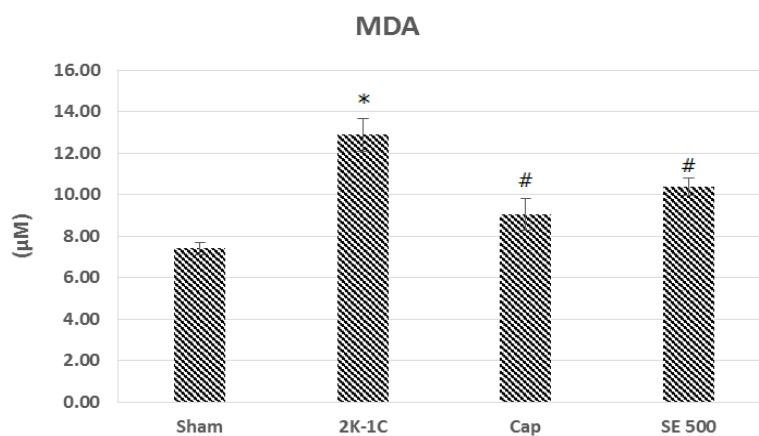


Figure 3 Effect of safflower extract (SE) on the level of plasma MDA in 2K-1C hypertensive rats. Data were presented as mean \pm S.E.M. (n = 5-8/group). * p<0.05 vs. Sham, # p<0.05 vs. 2K-1C.

Effects of safflower extract on plasma MDA

There was an increase in plasma MDA level in the 2K-1C hypertensive rats ($12.89 \pm 0.79 \mu\text{M}$) compared to that the sham-operated control rats ($7.40 \pm 0.30 \mu\text{M}$) ($p < 0.05$). This increased plasma MDA level was significantly attenuated by safflower extract ($10.37 \pm 0.42 \mu\text{M}$) and captopril ($9.03 \pm 0.76 \mu\text{M}$) ($P < 0.05$) (Fig 3).

Effects of safflower extract on superoxide production

High level of superoxide production was shown in 2K-1C hypertensive rats. Treatment of safflower

extract for 4 weeks significantly reduced vascular superoxide production (103.83 ± 23.83 count/mg dry wt/min) ($p < 0.05$) comparing to those of 2K-1C hypertensive rats without treatment (170.97 ± 20.61 count/mg dry wt/min). Furthermore, captopril also exhibited anti-oxidative effect by reducing vascular superoxide production in 2K-1C hypertensive rats (94.00 ± 15.24 count/mg dry wt/min) (Fig 4).

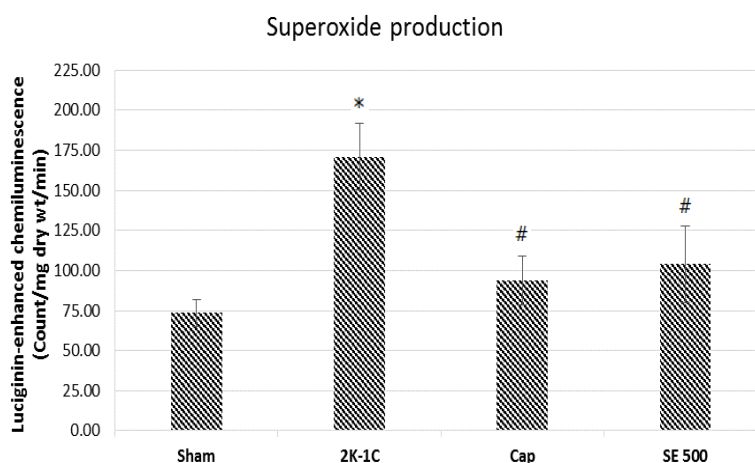


Figure 4 Effect of safflower extract (SE) on the superoxide production in 2K-1C hypertensive rats. Data were presented as mean \pm S.E.M. ($n = 6/\text{group}$). * $p < 0.05$ vs. Sham, # $p < 0.05$ vs. 2K-1C.

Discussion

The findings of this study is that safflower extract reduced blood pressure, plasma MDA, and vascular superoxide production in 2K-1C hypertensive rats. Captopril, an antihypertensive drug, also decreased SBP and oxidative stress markers in renovascular hypertensive rats. It was known that the 2K-1C model was characterized by an increase in blood pressure via activation of renin-angiotensin-system (RAS) activity¹⁰. Ang II was primary active product of the RAS and a potent vasoconstrictor¹¹ that caused high vascular resistance and high blood pressure. The present study demonstrated that the safflower extract reduced blood pressure in 2K-1C

hypertensive rats. There was evidence to show that safflower might inhibit renin-angiotensin system since antihypertensive effect of safflower yellow in spontaneous hypertensive rats mediated by reducing plasma renin activity and angiotensin II level¹². Furthermore, recent study reported the association of oxidative stress and hypertension-induced RAS activation¹³. Wei and coworkers indicated that Ang II could increase reactive oxygen species (ROS) production by activating NADPH oxidases¹⁴. Therefore, an increase in RAS activity resulted to increasing of blood pressure and oxidative stress in 2K-1C hypertensive rats. The result of the present study

showed an antioxidant activity of safflower extract by reducing of plasma MDA level and vascular superoxide production. These results suggested safflower extract improved hypertension in 2K-1C hypertensive rats by reducing of oxidative stress. Captopril reduced SBP and oxidative stress markers in 2K-1C hypertensive rats that was observed in the present study. This can be mediated by its ACE inhibitors activity. Furthermore, the antioxidant activity of captopril was also reported with its thiol group¹⁵.

Conclusion

In conclusion, the findings of the present study indicates that ethanolic extract of safflower exhibited an antihypertensive effects in 2K-1C renovascular hypertensive rats. The mechanism is likely to involve its antioxidant activity.

Acknowledgments

This study was supported by Invitation Research Fund (IN 59143), Faculty of Medicine, Khon Kaen University, Thailand.

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