นิพนธ์ต้นฉบับ ▪ Original Article

สารสกัดผักเม็กบรรเทาการเกิดพังผืดในไต ในหนูแรทความดันเลือดสูงที่ถูกเหนี่ยวนำด้วยสารแอลเนม

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Syzygium gratum Extract Attenuates Renal Fibrosis in L-NAME Induced-Hypertensive Rats

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หลักการและวัตถุประสงค์: สารสกัดผักเม็กมีฤทธิ์ต้านอนุมูลอิสระ ต้านมะเร็ง และป้องกันหลอดเลือด การศึกษานี้มีวัตถุประสงค์เพื่อตรวจสอบผลของสารสกัดผักเม็กต่อความดันเลือด และการเปลี่ยนแปลงโครงสร้างของไต ในหนูแรทความดันเลือดสูงที่ถูกเหนี่ยวนำด้วยสารแอลเนม

วิธีการศึกษา: หนูแรทเพศผู้สายพันธุ์ Sprague-Dawley ได้รับสารแอลเนม (40 มก./กก./วัน) ในน้ำดื่มเป็นเวลา 5 สัปดาห์เพื่อเหนี่ยวนำให้เกิดภาวะความดันเลือดสูง หนูทดลองที่มีภาวะความดันเลือดสูงจะได้รับการป้อนด้วยสารสกัดผักเม็ก (300 มก./กก./วัน) หรือสารหลอกใน 2 สัปดาห์สุดท้ายของการทดลอง เมื่อสิ้นสุดการทดลองทำการประเมินความดันเลือดซิสโตลิกและการเกิดพังผืดในไต

ผลการศึกษา: หนูแรทที่ได้รับแอลเนมมีความดันเลือดซิสโตลิกสูง (187.8 ± 5.1 และ 124.4 ± 2.8 มม.ปรอท) ร่วมกับการเพิ่มขึ้นของพังผืดใน (ร้อยละ 3.8 ± 0.1 และ 2.1 ± 0.2) tubulointerstitial และ corpuscular (ร้อยละ 11.3 ± 1.1 และ 4.8 ± 0.4) ($p<0.05$) สารสกัดผักเม็กลดความดันเลือดซิสโตลิก (154.7 ± 2.7 มม.ปรอท) และบรรเทาการเกิดพังผืดใน tubulointerstitial และ corpuscular (ร้อยละ 2.4 ± 0.1 และ 5.5 ± 0.4 ตามลำดับ) ในหนูแรทความดันเลือดสูงอย่างมีนัยสำคัญทางสถิติ ($p<0.05$)

สรุป: จากผลการศึกษาแสดงให้เห็นว่าสารสกัดผักเม็กลดความดันเลือด และบรรเทาการเกิดพังผืดในไต ในหนูแรทความดัน

Background and Objectives: Syzygium gratum (SG) extract has antioxidant, anticancer and vascular protective activities. This study aimed to investigate the effect of SG extract on blood pressure and renal remodeling in N^{ω}-Nitro-L-arginine methyl ester hydrochloride (L-NAME)-induced hypertensive rats.

Methods: Male Sprague-Dawley rats were administered with L-NAME (40 mg/kg/day) in drinking water for 5 weeks in order to induce hypertension. Hypertensive rats were treated with SG extract (300 mg/kg/day) or vehicle for the last 2 weeks. Systolic blood pressure and renal fibrosis were evaluated.

Results: Rats treated with L-NAME had high systolic blood pressure (187.8 ± 5.1 mmHg vs. 124.4 ± 2.8 mmHg) and increases in renal tubulointerstitial (3.8 ± 0.1 % vs. 2.1 ± 0.2 %) and corpuscular fibrosis (11.3 ± 1.1 % vs. 4.8 ± 0.4 %) compared with control rats ($p<0.05$). Treatment of hypertensive rats with SG extract significantly reduced systolic blood pressure (154.7 ± 2.7 mmHg), and alleviated renal tubulointerstitial and corpuscular fibrosis (2.4 ± 0.1 % and 5.5 ± 0.4 % respectively) ($p<0.05$).

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เลือดสูงที่ถูกเหนี่ยวนำด้วยสารแอลเนม

คำสำคัญ: ผักเม็ก, สารต้านอนุมูลอิสระ, ภาวะความดันเลือดสูง, พังผืดในไต

Conclusion: Our results indicate that SG extract decreased blood pressure and alleviated renal fibrosis in L-NAME-induced hypertensive rats.

Keywords: *Syzygium gratum*, antioxidant, hypertension, renal fibrosis

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Introduction

Hypertension is a chronic medical alteration with a high prevalence in adults worldwide, and the common cause of renal disease[1]. A sustained elevation of blood pressure can cause renal structural remodeling and resulting progression of chronic renal disease[2]. N^{ω}-Nitro-L-arginine methyl ester hydrochloride (L-NAME), a nitric oxide synthase inhibitor, is frequently used to induce hypertension in experimental animals[3]. Previous studies have demonstrated that chronic inhibition of nitric oxide synthesis by the administration of L-NAME in rats has been reported to produce high blood pressure, together with renal morphological alterations including, glomerular damage, glomerulosclerosis and tubulointerstitial fibrosis[4-6].

Syzygium gratum (SG), a member of family Myrtaceae, is a widely consumed local dietary and herbal plant in the Southeast Asian countries. The several bioactive compounds that present in Syzygium species, such as gallic acid, proanthocyanidins, and conjugated flavonoids, which suggest they are rich sources of antioxidant compounds[7]. There are substantial data to show the pharmacological effects of the extract from SG including antioxidant, anticancer and vascular protection activities[8,9]. Additionally, previous study has shown that SG extract reduced blood pressure and improved vascular endothelial function in nitric oxide-deficient hypertensive rats[10].

Although a wide range of potentially therapeutic effects of SG extract have been reported, the effects of SG extract on renal remodeling after chronic administration of L-NAME in rats remain unknown. Therefore, the aim of this present study was to investigate whether SG extract can reduce blood pressure and alleviate renal fibrosis in L-NAME-induced hypertensive rats.

Materials and Methods

Chemicals

L-NAME and paraformaldehyde were obtained from Sigma-Aldrich (St Louis, MO, USA). Picrosirius red stain kit was obtained from Polysciences, Inc. (Warrington, PA, USA). All chemicals used were of analytical grade quality.

Preparations of SG extract

The SG extract was supplied by Dr. Senggunprai, faculty of medicine, Khon Kaen University, Khon Kaen, Thailand. Fresh leaves of SG were collected from local agricultural fields in Khon Kean Province, Thailand. To prepare the aqueous extract of SG, the leaves of plant were weighed, chopped and boiled in deionized water for 30 min, then filtered. The filtrates were combined, and then freeze-dried yielding residues of 3.34% per wet weight[8]. Dried SG extract was then packed in tight containers and kept at -20°C until used.

Animals and experimental protocols

Male Sprague–Dawley rats (180–200 g) were purchased from Nomura Siam International Co., Ltd., Bangkok, Thailand. They were housed at 25 ± 2 °C with a 12 h dark–light cycle at Northeast Laboratory Animal Center, Khon Kaen university, Khon Kaen, Thailand. All procedures complied with the standards for the care and use of experimental animals and were approved by the Animal Ethics Committee of Khon Kaen university (AEKKU-NELAC 37/2559).

After a week of acclimatization, rats were randomly divided into three groups of 6 rats each: Group I. control + vehicle (distilled water, 0.15 mL/100 g), Group II. L-NAME + vehicle (distilled water, 0.15 mL/100 g), and Group III. L-NAME + SG extract (300 mg/kg/day). Over the 5-week study course, control rats received distilled water, while hypertensive rats received L-NAME (40 mg/kg/day) in their drinking

water in order to induce hypertension. SG extract or vehicle was intragastrically administered daily for the last 2 weeks of the experiment. The concentrations of SG extract used in the present study were influenced by the previous study[10].

Indirect measurement of blood pressure in conscious rats

Systolic blood pressure was measured at the start of the study, week 3rd and week 5th using non-invasive tail-cuff plethysmography (IITC/Life Science Instrument model 229 and model 179 amplifiers; Woodland Hills, CA, USA). For 5 days prior to experiments, conscious rats were familiarized with the procedures for tail-cuff blood pressure measurement, including heat and restraint. Rats were warmed in a hot box at 38°C for 10 min, and then placed in a restraining apparatus which was also kept at 38°C. The tail of each rat was placed inside the tail cuff, and the cuff was automatically inflated and released. For each rat, blood pressure was recorded as the mean value from the three measurements with 15 min intervals.

Kidney weights and tissue sampling isolation

At the end of study, rats were killed by over dosage of the anesthetic drug. Kidneys were collected and right kidney weight (RKW) and left kidney weight (LKW) were measured, and RKW to body weight (BW) ratio (RKW/BW) and LKW to BW ratio (LKW/BW) were calculated. Samples of the kidney were fixed in 4% paraformaldehyde and used for histological analysis.

Histological analysis

The kidney tissues were fixed for 24 h in 4% paraformaldehyde processed routinely in paraffin, and cut to a thickness of 5 μm. Tubulointerstitial and corpuscular fibrosis were assessed using picrosirius red stained. Images were obtained under light microscopy (DS-2Mv, Nikon, Tokyo, Japan). Morphometric evaluations were analyzed with the Image-pro plus software (Media Cybernetics, MD, USA) and data were expressed as a percentage area fraction of the stained areas to total medial areas.

Statistical analysis

Data are expressed as mean ± standard error of mean (SEM). The differences among treatment groups were analyzed by one-way analysis of variance (ANOVA) with a post-hoc Student Newman–Keul's test. A p-value of less than 0.05 was considered statistically significant.

Results

Effects of SG extract on systolic blood pressure in conscious rats

At the beginning of the study, average baseline values of systolic blood pressure among all groups of rats were not significantly different (Figure 1). Administration of L-NAME for 5 weeks significantly increased systolic blood pressure compared with control group (187.8 ± 5.1 mmHg vs. 124.4 ± 2.8 mmHg) ($p<0.05$; Figure 1). Treatment with SG extract significantly reduced systolic blood pressure in L-NAME hypertensive rats compared with untreated group (154.7 ± 2.7 mmHg) ($p<0.05$).

Effect of SG extract on body weight and kidney weight

Body weight and kidney weight from the different experimental groups are shown in Table 1. At the conclusion of the 5 week experiment, rat body weights did not differ among groups of animal. Similarly, there also were no significant differences in RKW/BW and LKW/BW between groups.

Effect of SG extract on renal fibrosis

Renal tubulointerstitial and corpuscular fibrosis were assessed from the picrosirius red-stained kidney sections. Chronic L-NAME treatment resulted in a significant increase in tubulointerstitial fibrosis

Figure legends

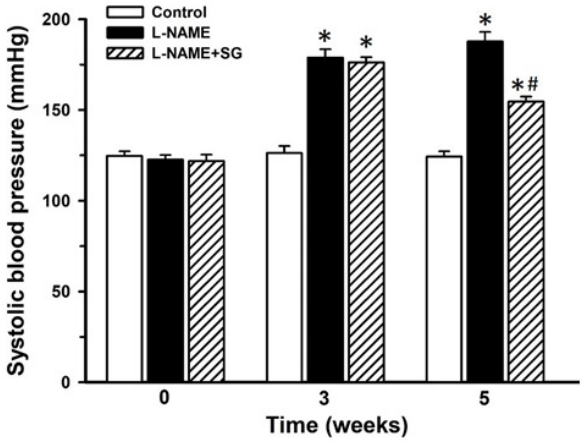

Figure 1 Effect of SG extract on systolic blood pressure in L-NAME-induced hypertensive rats. Results are expressed as mean ± SEM. *p<0.05 vs. control group, #p<0.05 vs. L-NAME group (n = 6).

compared with control group (3.8 ± 0.1 % vs. 2.1 ± 0.2 %) ($p<0.05$; Figure 2). Moreover, an increase in corpuscular fibrosis was presented in L-NAME hypertensive group compared with control group (11.3 ± 1.1 % vs. 4.8 ± 0.4 %) ($p<0.05$; Figure 3). However, treatment with SG extract showed a significant reduction in tubulointerstitial and corpuscular fibrosis compared to untreated rats (2.4 ± 0.1 % and 5.5 ± 0.4

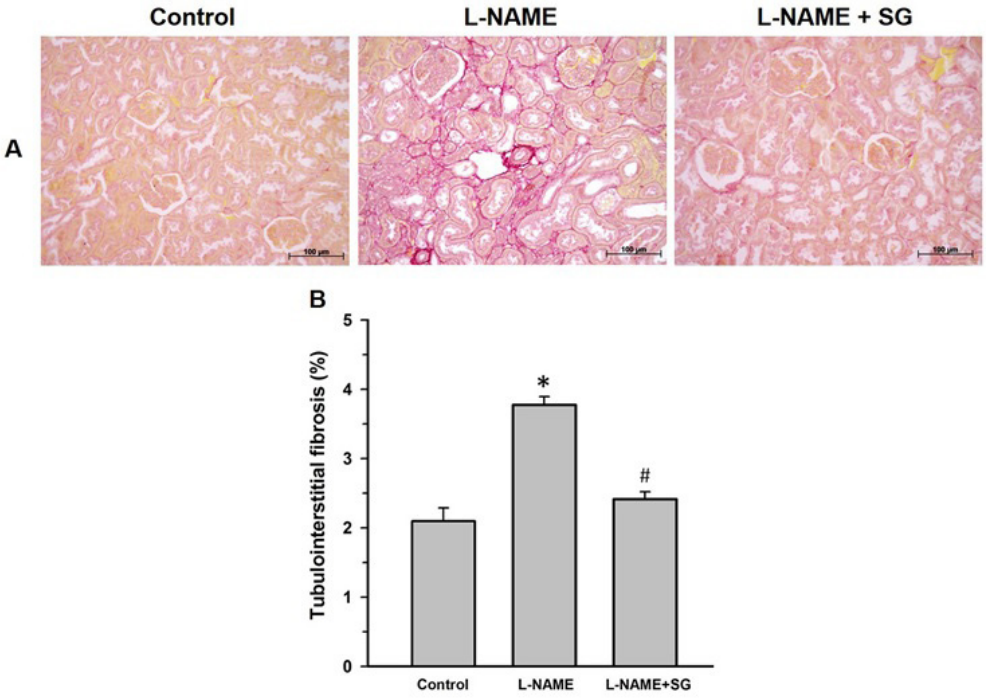

Figure 2 Effect of SG extract on renal tubulointerstitial fibrosis in L-NAME-induced hypertensive rats. Representative images of tubulointerstitial fibrosis under the light microscopeusing a 20x objective lens (A) and values of percentage area of tubulointerstitial fibrosis (B). Results are expressed as mean ± SEM. *$p<0.05$ vs. control group, #$p<0.05$ vs. L-NAME group (n = 6).

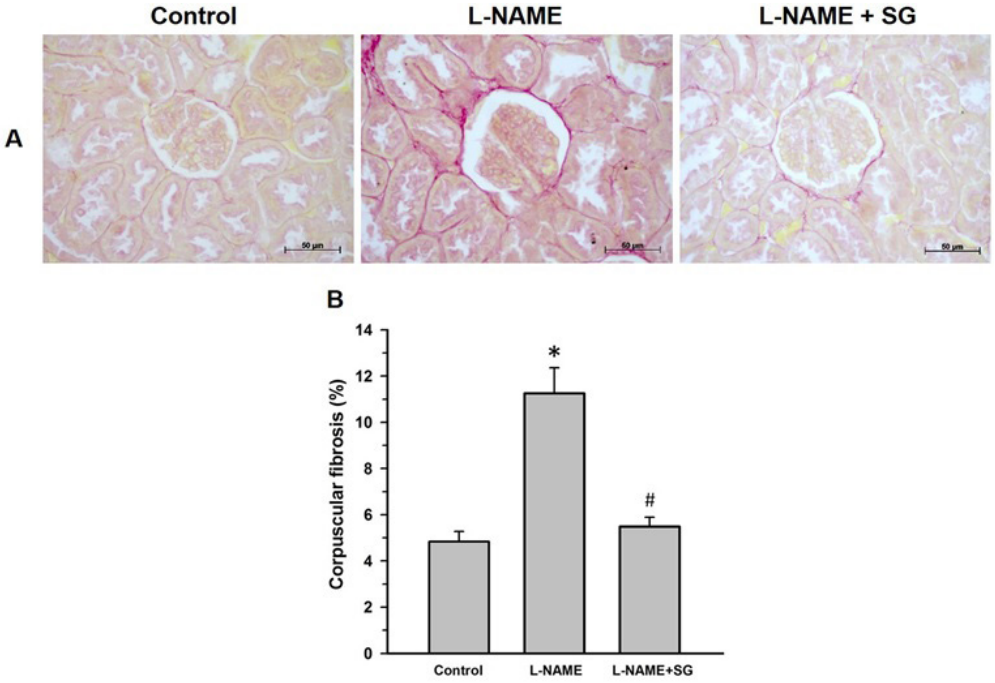

Figure 3 Effect of SG extract on renal corpuscular fibrosis in L-NAME-induced hypertensive rats. Representative images of corpuscular fibrosis under the light microscopeusing a 40x objective lens (A) and values of percentage area of corpuscular fibrosis (B). Results are expressed as mean ± SEM. *$p<0.05$ vs. control group, #$p<0.05$ vs. L-NAME group (n = 6).

Table 1 Body weight and kidney weight in all experimental groups.

Parameters	Control	L-NAME	L-NAME + SG
Body weight (g)	425.3 ± 16.5	422.0 ± 33.1	429.3 ± 24.1
LKW/BW (mg/g)	3.56 ± 0.10	3.82 ± 0.21	3.67 ± 0.04
RKW/BW (mg/g)	3.75 ± 0.12	4.12 ± 0.21	3.59 ± 0.09

Data are shown as mean ± SME.

BW: body weight; LKW: left kidney weight; RKW: right kidney weight.

% respectively) ($p<0.05$).

Discussion

This present study demonstrates the therapeutic effect of SG extract on blood pressure and renal fibrosis in L-NAME-induced hypertensive rats. We have shown that chronic administration of L-NAME produced hypertension, together with progression of renal remodeling. Treatment of L-NAME-induced hypertensive rats with SG extract partially lowered systolic blood pressure, which reduced fibrosis in renal tubulointerstitial and corpuscular area.

It is well known that chronic administration of a nitric oxide synthase inhibitor is widely used as an animal model to induce a sustained arterial hypertension[3,11]. Similarly, our result showed that administration of L-NAME in rats for 5 weeks increase systolic blood pressure. However, oral supplementation with SG extract decreased systolic blood pressure in L-NAME-induced hypertensive rats. This result support previous findings that SG extract reduced blood pressure and restored vascular endothelium-dependent vasorelaxation in nitric oxide-deficient hypertensive rats10. This indicates that one possible antihypertensive mechanism of SG extract might involve with the improvement vascular endothelial function.

Our results confirm and extend previous studies that L-NAME induces hypertension and concomitant renal remodeling, including renal tubulointerstitial and corpuscular fibrosis[4,6]. It is known that the alterations of renal epithelial cells structural occurs when the kidney are continuously exposed to fluid shear stress[12,13]. Our results showed an increase in systolic blood pressure after L-NAME treatment, representing high intraglomerular pressure and fluid shear stress that can provoke renal remodeling. Administration of SG extract decreased systolic blood pressure in L-NAME hypertensive rats and alleviated renal fibrosis. These results suggest that supplementation with SG extract might result in a lower fluid shear stress and alleviate renal structural alterations. Oxidative stress is a factor that plays an important role in the progression of hypertensive rats and is associated with the development of renal fibrosis[14]. Experimental studies have demonstrated that oxidative stress is closely implicated in pathological processes of hypertensive kidney disease including renal tissue inflammation, renal cell proliferation, as well as glomerular and tubular fibrosis[15,16]. Additionally, treatment with antioxidant has been shown to suppress renal oxidative stress and improve renal structural alterations in rats with hypertension[17,18]. In our experimental animals, we found that treatment of L-NAME-induced hypertensive rats with SG extract attenuated renal tubulointerstitial and corpuscular fibrosis. A previous study has shown that many antioxidant phytochemicals, especially phenolic compounds, have been found in extracts of Syzygium species[7]. SG extract also exhibits potent antioxidant properties with directly quantify the free radical scavenging activity and suppressed oxidative stress markers in phenylhydrazine (PHZ)-induced vascular dysfunction rats[8]. Moreover, a clinical study demonstrated that the total antioxidant capacity in the plasma of thalassemia patients significantly increased after SG extract supplementation[19]. Therefore, antioxidative activity of SG extract might be responsible for the alleviation of renal fibrosis in nitric oxide deficiency hypertensive rats.

Conclusion

In conclusion, the present study demonstrates that SG extract is able to reduce blood pressure, and alleviate renal tubulointerstitial and corpuscular fibrosis in L-NAME-induced hypertensive rats. These findings support the further development of the SG extract as a complementary medicine in hypertensive kidney disease management.

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