นิพนธ์ต้นฉบับ • Original Article

ผลของสารสกัดผักเม็กต่อความดันเลือดและการขยายตัวของหลอดเลือดขึ้นกับเซลล์ชั้นเอนโดทีเลียมในหนูแรทที่มีภาวะพร่องไนตริกออกไซด์

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Effect of *Syzygium gratum* Extract on Blood Pressure and Endothelium-Dependent Vasorelaxation in Nitric Oxide-Deficient Rats

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หลักการและวัตถุประสงค์: ผักเม็กมีฤทธิ์ต้านอนุมูลอิสระและป้องกันหลอดเลือดในสัตว์ทดลอง การศึกษานี้ต้องการทดสอบว่าสารสกัดผักเม็กสามารถบรรเทาความดันเลือดสูงและการทำงานของหลอดเลือดที่ผิดปกติจากการชักนำของแอลเนมในหนูแรท

วิธีการศึกษา: หนูแรทเพศผู้ น้ำหนัก 180-200 กรัม ได้รับสารแอลเนม (40 มก./กก./วัน) ในน้ำดื่มนาน 5 สัปดาห์ และได้รับสารสกัดผักเม็ก (300 มก./กก./วัน) ใน 2 สัปดาห์สุดท้าย เมื่อสิ้นสุดการทดลองความดันเลือดแดงเฉลี่ยและอัตราการเต้นของหัวใจถูกวัด การตอบสนองของหลอดเลือดต่อสารอะซิติลโคลีน (1 นาโนโมลาร์-0.01 ไมโครโมลาร์และโซเดียมไนโตรปรัสไซด์ 1 นาโนโมลาร์-0.01 ไมโครโมลาร์) ถูกวัดในหลอดเลือดมีเซนเทอริก

ผลการศึกษา: หนูแรทที่ได้รับแอลเนมมีความดันเลือดสูง (153.85 ± 7.7 มิลลิเมตรปรอท เทียบกับ 91.4 ± 2.72 มิลลิเมตรปรอท) และอัตราการเต้นของหัวใจสูง (415.0 ± 1.0 ครั้ง/นาที เทียบกับ 355.8 ± 15.3 ครั้ง/นาที) เทียบกับหนูปกติ ($p<0.05$) การคลายตัวของหลอดเลือดตอบสนองต่อสารอะซิติลโคลีนลดลงในหนูที่ได้รับสารแอลเนม ($p<0.05$) ขณะที่การตอบสนองต่อสารโซเดียมไนโตรปรัสไซด์ไม่แตกต่างระหว่างกลุ่ม สารสกัดผักเม็กลดความดันเลือด

Background and Objectives: *Syzygium gratum* (Pakmek) show antioxidant and vascular protective effects in experimental animal models. This study was to investigate whether *Syzygium gratum* extract could alleviate high blood pressure and vascular dysfunction in N^{ω}-Nitro-l-arginine methyl ester (L-NAME)-induced hypertension in rats.

Methods: Male Sprague-Dawley rats weighing 180-200 g were treated with L-NAME (40 mg/kg/day) in drinking water for five weeks and orally treated with *Syzygium gratum* (300 mg/kg BW per day) for the last two weeks. Mean arterial blood pressure (MAP) and heart rate (HR) were measured at the end of experiment. Vascular responses to acetylcholine (ACh, 1 nM-0.01 μM) and sodium nitroprusside (SNP, 1 nM-0.01 μM) were performed in isolated mesenteric vascular beds.

Results: Rats treated with L-NAME had high blood pressure (MAP; 153.85 ± 7.7 mmHg vs.91.4 ± 2.72 mmHg) and HR (415.0 ± 1.0 beats/min vs. 355.8 ± 15.3 beats/min) comparing to control rats ($p<0.05$). Vasorelaxation to ACh was blunted in L-NAME treated rats ($p<0.05$) while the response to SNP was not different among groups. Furthermore, *Syzygium gratum* extract significantly reduced

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(121.75 ± 8.1 มิลลิเมตรปรอท) และอัตราการเต้นของหัวใจ (380.3 ± 16.5 ครั้ง/นาที) เทียบกับหนูที่ไม่ได้รับสารสกัด หนูแอลเนมที่ได้รับสารสกัดผักเม็กมีการตอบสนองของหลอดเลือดต่อสารอะซิติลโคลีนที่ดีขึ้น ($p<0.05$)

สรุป: สารสกัดผักเม็กสามารถลดความดันเลือดและอัตราการเต้นของหัวใจที่สูงได้ ซึ่งผลนี้อาจเกี่ยวข้องกับการเพิ่มขยายตัวของหลอดเลือดซึ่งขึ้นกับเซลล์เอนโดทีเลียมในหนูแรทที่ถูกชักนำให้เกิดความดันเลือดสูงด้วยสารแอลเนม

คำสำคัญ: ผักเม็ก, หนูแรทที่ถูกชักนำให้เกิดความดันเลือดสูงด้วยสารแอลเนม, การทำงานของหลอดเลือดที่ผิดปกติ

blood pressure in L-NAME hypertension (121.75 ± 8.1 mmHg) and HR (380.3 ± 16.5 beats/min) comparing to those of untreated rats. L-NAME rats received *Syzygium gratum* extract had an improvement of vascular response to ACh ($p<0.05$).

Conclusion: These findings suggested that *Syzygium gratum* extract reduced blood pressure and HR in hypertension induced by L-NAME. This antihypertensive effect could involve the improvement of endothelium-dependent vasorelaxation in L-NAME hypertension.

Keywords: *Syzygium gratum*, L-NAME-induced hypertensive rats, Vascular dysfunction

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Introduction

Vascular endothelial cells play an important role in regulation of vascular tone as it can synthesize and release vasodilators, nitric oxide and prostacyclin, and vasoconstrictors, endothelin and thromboxane A2[1, 2]. NO synthesized from L-arginine by endothelial nitric oxide synthase (eNOS) mediates vasorelaxation to influence vascular tone and therefore blood pressure[3]. Acetylcholine produces vasorelaxation through inducing NO production from vascular endothelium[4]. Depletion of NO mainly causes endothelium dysfunction and high blood pressure[5, 6]. L-NAME is a nitric oxide synthase (NOS) inhibitor that reduces NO production in vascular endothelial cells[7]. Thus, rats that receive L-NAME are developed hypertension because of systemic vasoconstriction and vascular dysfunction[3, 8, 9]

Syzygium gratum is a dietary and herbal plant mainly found in Southeast Asian countries. The shoot and young leave are eatable and helpful for several disorders including arthralgia, dyspepsia, indigestion, peptic ulcer, diarrhea, bacterial infection, asthma, and cardiovascular diseases[10,11]. There are substantial data to show the beneficial effects of *Syzygium gratum* since Senggunpria and coworkers (2010) found that *Syzygium gratum* exhibits potent direct antioxidant properties and can induce cytoprotective enzyme in mice[12]. Moreover *Syzygium gratum* had strong antioxidant property and improved vascular function in phenylhydralazine-induced circulatory shock[13]. There is no evidence regarding to antihypertensive effect of *Syzygium gratum* in nitric oxide-deficient hypertensive rats.

This study aimed to investigates the effect of *Syzygium gratum* extract on blood pressure and endothelium-dependent vasorelaxation in L-NAME-induced hypertensive rats.

Materials and Methods

Animals

Male Sprague-Dawley rats (180-200 g) were obtained from Nomura Siam International Co., Ltd., Bangkok, Thailand. Rats were maintained in an air-conditioned room (25 ± 2 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 37/2559).

Drugs and chemical

Acetylcholine chloride (ACh) and sodium nitroprusside (SNP) were obtained from Fluka Chemika (Buchs, Switzerland). L-NAME and Methoxamine were obtained from Sigma-Aldrich Corp (St Louis, MO, USA).

Preparations of *Syzygium gratum* extract

Fresh leaves of *Syzygium gratum* were collected from local agricultural fields in and around Khon Kean Province, Thailand. It was identified as *Syzygium gratum* by the same method as described in our previous study[13].

To prepare the aqueous extract of *Syzygium gratum*, the leaves of plant was weighed, chopped and boiled in purified water for 30 min, and then was dried into a powder using a spray-drying technique which yielded a residue of 16.7% by dry weight of plant[12]. The dry extract that was used in the present study is a gift from Dr. Senggunprai.

Experimental designs

After one week of acclimatization, rats were randomly divided into 3 groups with 5 animals in each group, Group I; Control (water 0.15 ml/100 mg), Group II; L-NAME (L-NAME + water 0.15 ml/100 mg) and Group III; L+SG 300 (L-NAME + *Syzygium gratum* extract 300 mg/kg/day; p.o.). Control rats received distilled water for five weeks while L-NAME rats received L-NAME (40 mg/kg/day) in their drinking water for five weeks in order to induce hypertension. *Syzygium gratum* extract and vehicle were daily intragastrically administered during the last two weeks of study.

Hemodynamic measurements

At the end of the experimental period, rats were anesthetized with pentobarbital sodium (60 mg/kg, ip.). The left femoral artery was identified, cleaned connective tissue out off and cannulated by a polyethylene tube. Baseline values mean arterial pressure (MAP), and heart rate (HR) was continuously monitored for 20 min by a way of a pressure transducer and recorded using Acknowledge Data Acquisition software (Biopac Systems Inc., Santa Barbara, CA, USA).

Experimental protocols in isolated mesenteric vascular beds

Mesenteric vascular beds were carefully isolated and then placed on a stainless steel grid (7x5 cm) in a humid chamber. The preparations were perfused with physiological Krebs' solution at a constant flow rate of 5 ml/min, using a peristaltic pump. The solution was maintained at 37 °C and continually gassed with a mixture of 95% O_2 and 5% CO_2 gas. The preparations were allowed to equilibrate for 30 min before the next trial. Thereafter, methoxamine (5-7 μM) was added into Kreb's solution to raise tone (70-90 mmHg above baseline). To determine vasoactive performance of resistance small arteries, different doses of vasoactive agents, ACh (Endothelium-dependent vasodilator, 1 nM-0.01 μM) or SNP (NO donor, 1 nM-0.01 μM), were injected through neoprene rubber tubing proximal to the tissue. The relaxation response was expressed as decrease in perfusion pressure (mmHg).

Statistical analysis

Data are expressed as mean ± S.E.M. The differences among treatment groups were analyzed by one-way analysis of variance (ANOVA). A p-value of less than 0.05 was considered statistically significant.

Results

Effect of *Syzygium gratum* extract on blood pressure in L-NAME hypertensive rats

Chronic administration of L-NAME for five weeks caused significant increase in MAP (153.85 ± 7.7 mmHg), comparing to control group (91.4 ± 2.72 mmHg) ($p<0.05$). Treatment with *Syzygium gratum* extract (300 mg/kg/day) significantly reduced MAP (121.75 ± 8.1 mmHg, $p<0.05$) comparing to L-NAME treated group (Figure 1).

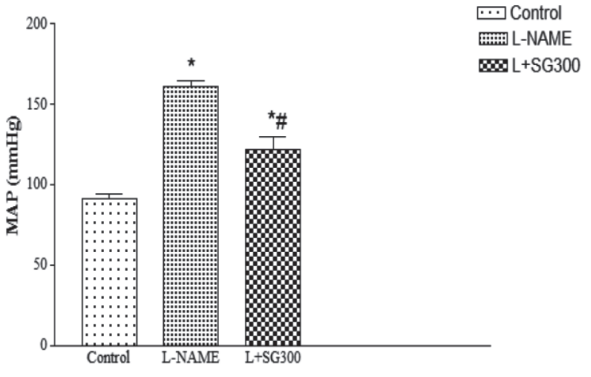

Figure 1 Effect of *Syzygium gratum* (SG) extract on Mean arterial blood pressure in L-NAME hypertensive rats. Data are expressed as mean ± S.E.M. (*n*= 5 in each group).*$p<0.05$ vs. control,# $p<0.05$ vs. L-NAME.

Effect of *Syzygium gratum* extract on HR in L-NAME hypertensive rats

Chronic administration of L-NAME for five weeks caused a significant increase in heart rate (415.0 ±

1.0beats/min), comparing to control group (355.8±15.3 beat/min) ($p<0.05$). Treatment with *Syzygium gratum* extract (300 mg/kg/day) significantly decreased heart rate (380.3 ± 16.5 beats/min, $p<0.05$) in L-NAME hypertensive rats comparing to L-NAME untreated group (Figure 2).

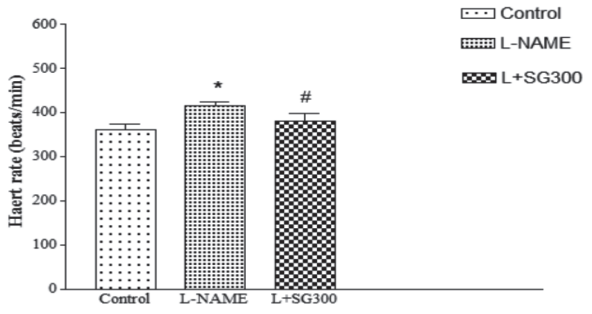

Figure 2 Effect of *Syzygium gratum* (SG) extract on HR in L-NAME hypertensive rats. Data are expressed as mean ± S.E.M. (n = 5 in each group). *$p<0.05$ vs. control, #$p<0.05$ vs. L-NAME.

Effect of *Syzygium gratum* extract on vascular reactivity in isolated mesenteric vascular beds

Vasorelaxation response to ACh (0.1 μM-0.1 mM) in the mesenteric vascular bed was significantly blunted in L-NAME treated group compared to those of control group (0.1 μM ACh, 4.1 ± 1.05 *vs.* 43.9 ± 4.5 mmHg) ($p<0.05$). Treatment with *Syzygium gratum* extract at dose 300 mg/kg/day significantly improved the response to ACh in L-NAME hypertensive rats compared to those of untreated group (0.1 μM ACh, 25.4 ± 5.9 mmHg) ($p<0.05$; Figure 3A). In addition, there was no significant difference in the vasorelaxation responses to SNP among groups, indicating normal vascular smooth muscle cell function in all groups of rats (Figure 3B).

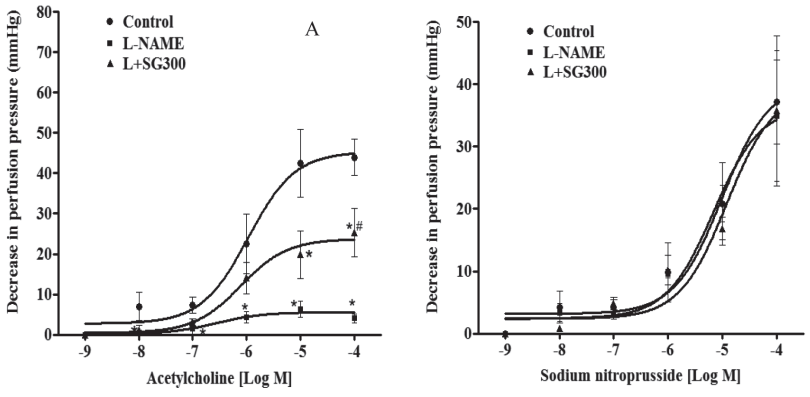

Figure 3 Effect of *Syzygium gratum* (SG) extract on vascular responses to acetylcholine (A) and sodium nitroprusside (B) in isolated mesenteric vascular beds. Data are presented as mean ± S.E.M. (n = 4-5/group). *$p<0.05$ vs. control, #$p<0.05$ vs. L-NAME.

Discussion

The present findings show that rats received L-NAME had high blood pressure and HR as well as an impairment of endothelium-dependent vasorelaxation. L-NAME rats treated with *Syzygium gratum* extract for two weeks had reduction of MAP and HR. This was accompanied with and improvement of endothelium-dependent vasorelaxation in L-NAME rats treated with *Syzygium gratum* extract.

It is well established that chronic administration of L-NAME in rats is widely used as an animal model to induce a sustained hypertension with increased total peripheral resistance, which is associated with inhibition of NO synthesis[3]. Furthermore, sympathetic over-activation was demonstrated as a result of activation of central sympathetic nervous outflow[14].Our results confirmed the study above that chronic administration of L-NAME in rats induced a high arterial blood pressure which was

associated with increased HR. In addition, high blood pressure found in L-NAME-treated rats was associated with endothelium dysfunction, as shown by the decreased responses to ACh while the response to the NO donor SNP is not changed. This is consistent with the studies that hypertension in rats received L-NAME are associated with endothelial dysfunction[9, 15].

Treatment with *Syzygium gratum* extract alleviated hypertension and HR induced by L-NAME. This was consistent with improvement of vascular function in L-NAME rats-treated with *Syzygium gratum* extract. There is study to show that *Syzygium gratum* extract had strong radical scavenging activities[12] that might increase NO bioavailability. Subsequently, Kukongviriyapan and coworkers (2007) reported that *Syzygium gratum* extract improved vascular function that was involved reducing oxidative stress markers, plasma malonaldehyde and vascular superoxide production in phenylhydrazine-induced hemolysis and hemodynamic disturbances in rats[13].

Conclusion

In summary, *Syzygium gratum* extract exhibits antihypertensive effect in L-NAME hypertensive rats. This effect might involve with its ability to improve vascular dysfunction in L-NAME-induced hypertensive rats.

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