นิพนธ์ต้นฉบับ ▪ Original Article

น้ำมันรำข้าวกล้องลดความดันเลือดโดยการเพิ่มระดับไนตริกออกไซด์และบรรเทาภาวะเครียดออกซิเดชันในหนูแรทความดันเลือดสูงจากการพร่องไนตริกออกไซด์

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Whole Grain Essential Oil Reduces Blood Pressure by Increasing Nitric Oxide Level and Alleviating Oxidative Stress in Nitric Oxide Deficient Hypertensive Rats

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หลักการและวัตถุประสงค์: ความดันเลือดสูงเป็นปัจจัยเสี่ยงสำคัญต่อการเกิดโรคหัวใจและหลอดเลือด (CVD) ภาวะเครียดออกซิเดชันมีความสัมพันธ์กับการเกิดภาวะความดันเลือดสูง การรับประทานอาหารที่มีสารต้านออกซิแดนท์พบว่าลดความดันเลือด เพิ่มการทำงานของเซลล์เอนโดทีเลียม และลดความเสี่ยงต่อการเกิด CVD น้ำมันรำข้าวกล้อง (WEO) มีคุณสมบัติต้านออกซิเดชันและมีรายงานว่าลดความเสี่ยงต่อการเกิด CVD การศึกษานี้มีวัตถุประสงค์เพื่อประเมินคุณสมบัติต้านความดันเลือดสูงของ WEO โดยศึกษาในรูปแบบหนูทดลองความดันเลือดสูงจากการพร่องไนตริกออกไซด์ (NO)

วิธีการศึกษา: หนูแรทเพศผู้ พันธุ์ Sprague-Dawley ได้รับสารแอลเนมซึ่งเป็นสารยับยั้งการทำงานของ NOS ใน ขนาด 50 มก/กก/วัน ผสมกับน้ำดื่ม เป็นเวลา 3 สัปดาห์ ทำการป้อน WEO (1 หรือ 2 มล/กก/วัน) ให้แก่หนูแรทในช่วงที่ได้รับสารแอลเนม

ผลการศึกษา: หนูแรทความดันเลือดสูงจากการได้รับสารแอลเนมมีค่าความดันเลือดสูงขึ้นมาก ความต้านทานการไหลเวียนเลือดไปยังอวัยวะท่อนล่างเพิ่มขึ้น และอัตราการไหลเวียนเลือดไปยังอวัยวะท่อนล่างลดลง ($p < 0.05$) นอกจากนี้ยังพบว่าเนื้อเยื่อหลอดเลือดของหนูแรทที่ได้รับแอลเนมมีการสร้างซุปเปอร์ออกไซด์มากขึ้น ภาวะเครียดออกซิเดชันเพิ่มขึ้นและระดับเมแทบอไลต์ของไนตริกออกไซด์ (NOx) ลดลง ($p < 0.05$) เมื่อให้ WEO พร้อมๆกับสารแอลเนม พบว่า WEO ให้ผลตามระดับความเข้มข้น ในการปรับพลศาสตร์การไหลเวียนเลือดให้

Background and Objective: Hypertension is the important risk factor for cardiovascular disease (CVD). Oxidative stress is associated with the development of hypertension. Consumption of dietary antioxidants appears to decrease blood pressure, improve endothelial function and reduce the risk of CVD. Whole grain essential oil (WEO) possesses strong antioxidant property and has been reported to reduce the CVD risk. This study aimed to evaluate the antihypertensive property of WEO using a rat model of nitric oxide (NO) deficiency hypertension.

Methods: Male Sprague-Dawley rats received a nitric oxide synthase (NOS) inhibitor, N^{ω}-nitro-L-arginine methyl ester (L-NAME) at dose of 50 mg/kg/day in drinking water for 3 weeks. WEO (1 or 2 ml/kg/day) were intragastrically administered during L-NAME administration.

Results: A markedly increased arterial blood pressure, elevated hindlimb vascular resistance and decreased hindlimb blood flow were found in L-NAME-induced hypertensive rats ($p < 0.05$). Enhanced vascular superoxide production, increased oxidative stress and decreased NO metabolites (NOx) levels were also found in L-NAME-treated rats. Concurrent treatment

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ดีขึ้น ลดผลเสียของแอลเนมต่อการเกิดภาวะเครียดออกซิเดชัน และการกดการสร้าง NO ได้อย่างมีนัยสำคัญทางสถิติ ($p < 0.05$)

สรุป: ข้อมูลจากการศึกษานี้บ่งชี้ว่า WEO เป็นสารต้านออกซิแดนท์ที่มีประสิทธิภาพที่ช่วยลดภาวะความดันเลือดสูง ผลต้านภาวะความดันเลือดสูงของ WEO มีแนวโน้มผ่านการเพิ่มฤทธิ์ต้านออกซิเดชัน และฟื้นฟูฤทธิ์ทางชีวภาพของไนตริกออกไซด์

คำสำคัญ: น้ำมันรำข้าวกล้อง ความดันเลือดสูง แอลเนม ไนตริกออกไซด์ ภาวะเครียดออกซิเดชัน

with WEO resulted in a dose-dependent improved hemodynamic status and significantly reversed the L-NAME-induced oxidative stress and suppression of NO production ($p < 0.05$).

Conclusions: The data revealed that WEO is an effective antioxidant that helps to reduce high blood pressure. The antihypertensive effects of WEO are likely to be mediated by increasing antioxidant activities and restoring NO bioavailability.

Keywords: Whole grain essential oil, Hypertension, L-NAME, Nitric oxide, Oxidative stress

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Introduction

Hypertension is a high prevalent pathological condition that is considered as one of the important cardiovascular risk factors. A large amount of evidence shows that oxidative stress plays a central role in the pathophysiology of hypertension[1]. Oxidative stress is defined as an imbalance between free radicals and antioxidants in the body, and leads to a decrease in NO bioavailability, which is associated with the impairment of endothelial function[2].

NO is a simple diatomic gas and free radical that is endogenously synthesized by a family of enzymes called NOS. NO plays an important role in modulation of physiological responses, especially in the cardiovascular system[3]. Inhibition of NO production disturbs vascular homeostasis, thereby decreases vasodilation, elevates blood pressure and increases oxidative stress. In various animal models of hypertension, inhibition of NO production by L-NAME, a NOS inhibitor, causes impairment of the endothelial-dependent relaxation and enhances oxidative stress[4]. Reduction in NO bioavailability and increased reactive oxygen species (ROS) generation are the major features of hypertension[5]. Therefore, L-NAME-induced hypertension is widely used as an experimental model of hypertension to mimic hypertension in human.

In the past decades, there is increasing evidence suggesting that consumption of a diet rich in phytochemicals and antioxidants could reduce the risk for CVD[6]. Dietary antioxidants restore the antioxidant defense system, preserve endothelial function and increase vasodilation, thus reducing the risk of hypertension[4, 6]. Several studies have been conducted on the beneficial effects of rice bran oil for prevention and treatment of chronic diseases[7]. WEO extracted from whole grain rice bran contains high levels of bioactive phytochemicals, including phytosterols, γ-oryzanol, tocopherols and tocotrienols. Previous study reported that rice bran oil possesses anti-oxidation, anti-hypercholesterolemia and anti-inflammation properties, thereby reducing the risk of CVD[8]. Since there is very limited information regarding the antihypertensive effect of rice bran oil, the present study was designed to evaluate whether WEO could prevent hypertension induced by L-NAME in rats.

Methods

Chemicals

WEO was obtained from Medifoods (Thailand) Co. Ltd. (Chaiyaphum Province, Thailand). L-NAME, thiobarbituric acid (TBA), β-nicotinamide adenine dinucleotide phosphate (NADPH), glucose-6-phosphate dehydrogenase (G-6-PD) and nitrate reductase were purchased from Sigma-Aldrich (MO, USA). Lucigenin was purchased from Fluka Chemika (Buch, Switzerland). Pentobarbital sodium was from Ceva Animals Health (Bangkok, Thailand). All other chemicals were analytical grade quality.

Animals

Male Sprague-Dawley rats weighing 200–220 g were obtained from the National Laboratory Animal Center, Nakhon Pathom Province, Thailand. The rats were housed in the Heating, Ventilation and Air-Conditioning (HVAC) System with 12 h dark/light cycle in the Northeast Laboratory Animal Center, Khon Kaen University, Thailand, and were fed with a standard chow diet (Chareon Pokapan Co. Ltd., Samutprakarn Province, Thailand).

The study protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Khon Kaen University (AEKKU19/2557). All surgical procedures were performed under standard anesthesia, and all efforts were made to minimize suffering. After one week of acclimatization, rats were randomly divided into two main groups; the normotensive group received tap water and the L-NAME hypertensive group received L-NAME (50 mg/kg/day) in drinking water for 3 weeks[4]. Concurrently, rats from each group (n=6-8/group) were orally administered with WEO 1 ml/kg (WEO1) or WEO 2 ml/kg (WEO2) or with deionized water (DI) as vehicle, once daily.

Measurement of hemodynamic status

After 3 weeks, rats were anaesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg). A tracheotomy was performed for spontaneous breathing, and left femoral artery was cannulated with polyethylene catheter connected to a pressure transducer for continuous monitoring of blood pressure (BP) using the Acqknowledge data acquisition analysis software (BIOPAC Systems Inc., California, USA). Baseline BP values were monitored in animals for 10 min. Hindlimb blood flow (HBF) was continuously measured by placing electromagnetic flow probe around the abdominal aorta connected to an electromagnetic flow meter (Carolina Medical Electronics, North Carolina, USA). Hindlimb vascular resistance (HVR) was calculated from the mean arterial pressure (MAP) divided by HBF. At the end of experiment, rats were sacrificed by overdose of the anesthetic drug. Blood samples were withdrawn from abdominal aorta for assays of oxidative stress markers and NO metabolites (NOx). The carotid arteries were rapidly excised from the animals and used for analysis of superoxide anion ($O_2^{\bullet -}$) production.

Assay of oxidative stress markers and NO metabolites

Vascular $O_2^{\bullet -}$ production was measured using lucigenin-enhanced chemiluminescence method as described previously[9]. In addition, lipid peroxidation, as measured by malondialdehyde (MDA) level, was estimated using thiobarbituric acid (TBA) as described previously[10]. Moreover, protein oxidation was assessed as the protein carbonyl and it was measured as described previously[10]. NOx or nitrite and nitrate, the end products of NO metabolism, were used as indicators of NO production. Plasma NOx was determined by an enzymatic conversion method with the Griess reaction as previously described[4].

Statistical analysis

Data are presented as means ± SEM. Statistical differences were evaluated by one-way analysis of variance (ANOVA) and followed by Student Newman–Keul's test to show specific group differences. All analysis was performed using SigmaStat software version 3.1. Statistical significance was determined at a level of $p < 0.05$.

Results

Effect of WEO on hemodynamic status

A marked increase in systolic, diastolic and mean arterial pressure were found in L-NAME treated rats ($p < 0.05$, Fig. 1). Meanwhile, there were no significant changes in heart rates among all experimental groups (Fig. 1). The elevation of arterial blood pressure was accompanied by decreasing HBF and increasing HVR ($p < 0.05$, Fig. 2). Rats receiving WEO together with L-NAME for 3 weeks showed a significant decrease in blood pressure and HVR whereas HBF increased ($p < 0.05$, Fig. 1 & 2). The improvement of hemodynamic status in L-NAME hypertensive rats treated with WEO was found in a dose-dependent manner. These data indicate that WEO supplementation reduced arterial blood pressure and total peripheral resistance, which could prevent the progression of high blood pressure in L-NAME-induced hypertensive rats.

Effect of WEO on oxidative stress and plasma NO metabolites

Increased oxidative stress was found in L-NAME-induced hypertensive rats as shown by increasing vascular $O_2^{\bullet -}$ production and elevating plasma MDA and protein carbonyl (Fig. 3). Treatment with WEO, particularly at the high dose significantly alleviated oxidative stress of L-NAME hypertensive rats ($p < 0.05$, Fig. 3). It appeared that alleviation of oxidative stress after WEO treatment was associated with a partial restoration of hemodynamics. A depletion of NO production in L-NAME-induced hypertensive rats was confirmed by a decrease in plasma NOx (Fig. 4). Interestingly, WEO significantly increased the levels of plasma NOx of L-NAME hypertensive rats ($p < 0.05$, Fig. 4).

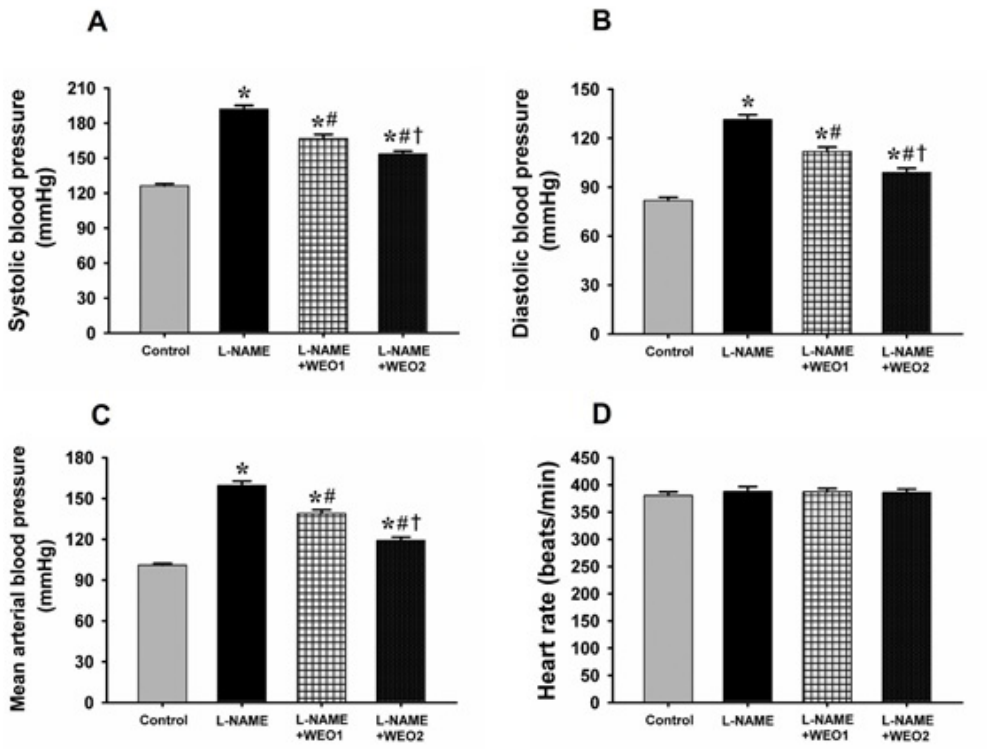

Figure 1 Effect of WEO on systolic blood pressure (A), diastolic blood pressure (B), mean arterial blood pressure (C) and heart rate (D) in all experimental groups. Data are expressed as mean ± SEM. (n = 6-8/group), $^{*}p < 0.05$ vs. control group, $^{\#}p < 0.05$ vs. L-NAME group, $^{\dagger}p < 0.05$ vs. L- NAME+WEO (1 ml/kg).

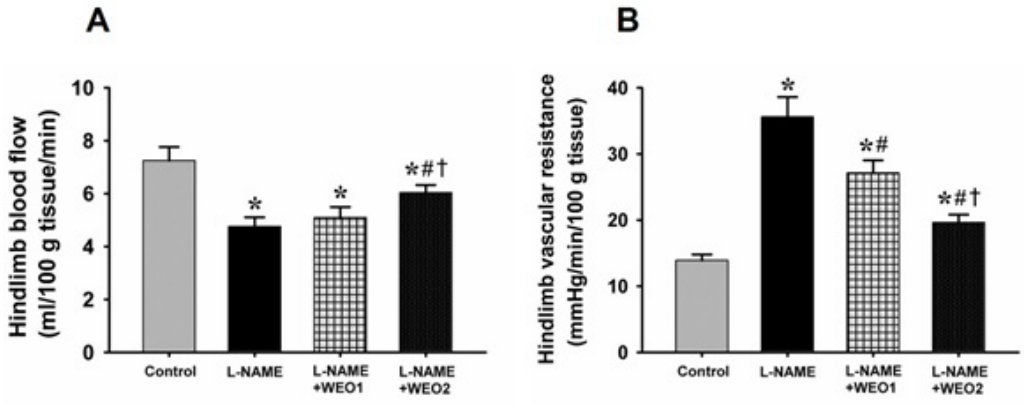

Figure 2 Effect of WEO on hindlimb blood flow (A) and hindlimb vascular resistance (B) in all experimental groups. Data are expressed as mean ± SEM. (n = 6-8/group), $^{*}p < 0.05$ vs. control group, $^{\#}p < 0.05$ vs. L-NAME group, $^{\dagger}p < 0.05$ vs. L- NAME+WEO (1 ml/kg).

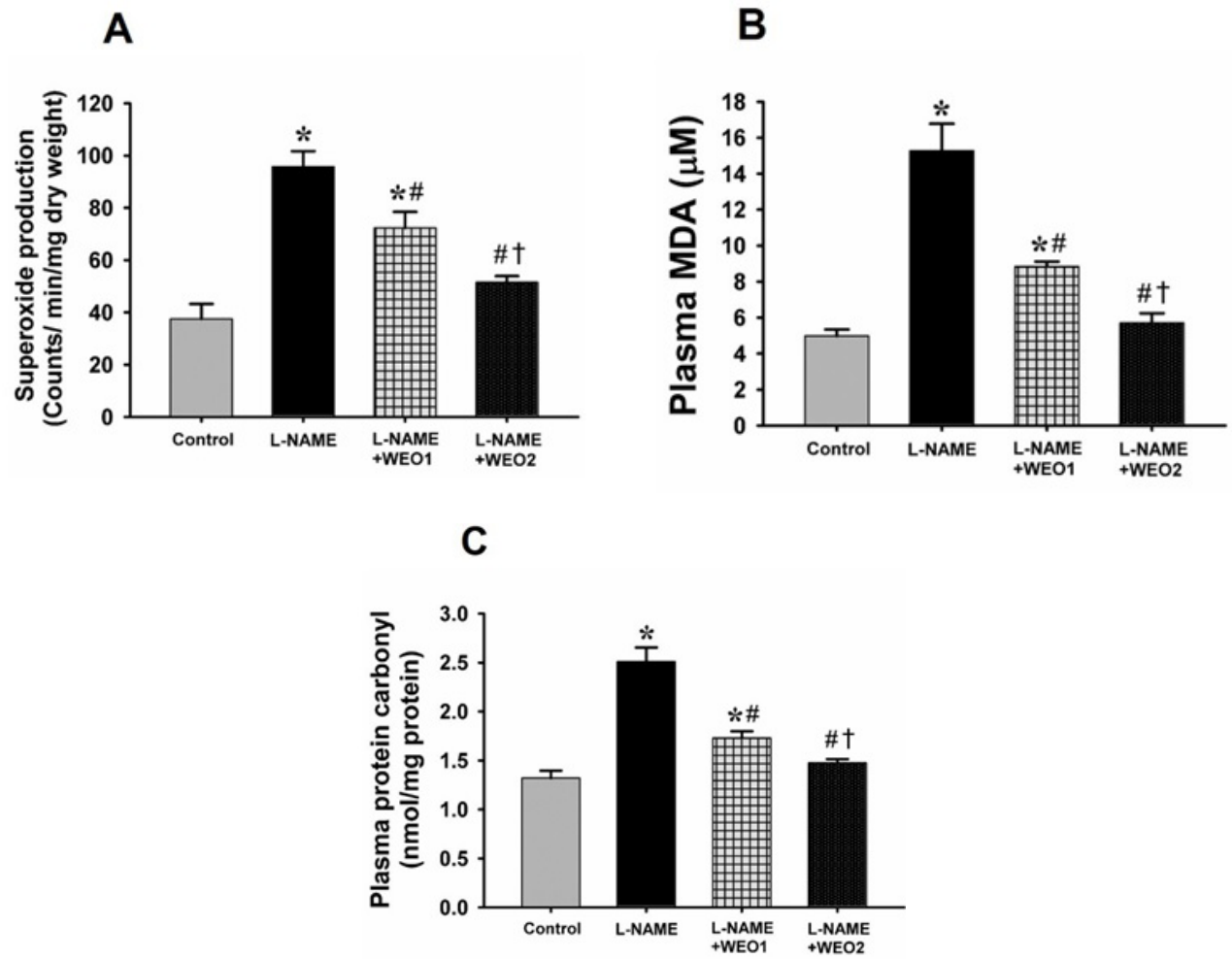

Figure 3 Effect of WEO on vascular superoxide production (A), plasma malondialdehyde (MDA) (B) and plasma protein carbonyl (C) in all experimental groups. Data are expressed as mean ± SEM. (n = 6-8/group), $^{*}p < 0.05$ vs. control group, $^{\#}p < 0.05$ vs. L-NAME group, $^{\dagger}p < 0.05$ vs. L- NAME+WEO (1 ml/kg).

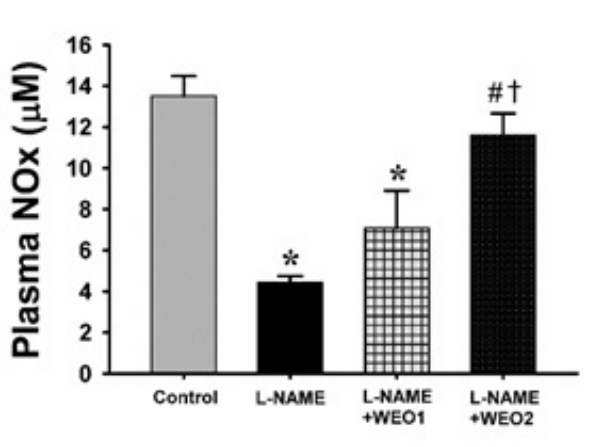

Figure 4 Effect of WEO on plasma nitric oxide metabolites (NOx) in all experimental groups. Data are expressed as mean ± SEM. (n = 6-8/group), $^{*}p < 0.05$ vs. control group, $^{\#}p < 0.05$ vs. L-NAME group, $^{\dagger}p < 0.05$ vs. L- NAME+WEO (1 ml/kg).

Discussion

This study confirms that inhibition of NO synthesis by L-NAME induced hypertension, increased peripheral vascular resistance, increased oxidative stress and decreased NO production. Concurrent treatment of WEO partially ameliorated all of these deleterious effects induced by L-NAME. The plausible mechanisms underlying these improvements might be contributable to increase in NO bioavailability and antioxidant activities of WEO. Previous studies demonstrated that phytosterol and γ -oryzanol which are the bioactive compounds found in WEO could reduce oxidative stress by suppressed ROS production and increased NO activity[11, 12]. Moreover, supplementation with vitamin E or tocopherols also reduced blood pressure in hypertensive and non-alcoholic fatty liver disease patients[13, 14]. The mechanism of blood pressure reduction is likely due to increase in NO production[15].

Conclusion

The present study demonstrated that WEO prevents the progression of hypertension, improves hemodynamics, alleviates oxidative stress and increases NO production in a rat model of L-NAME-induced hypertension. The overall findings support the idea of using whole grain essential oil as a food supplement to prevent hypertension and reduce oxidative stress.

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