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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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, Nω-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...
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\(^7\), 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
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. 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$_2^-$) production. Plasma NOx was determined by an enzymatic conversion method with the Griess reaction as previously described.

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$_2^-$ 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, † 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, † 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. Moreover, supplementation with vitamin E or tocopherols also reduced blood pressure in hypertensive and non-alcoholic fatty liver disease patients. The mechanism of blood pressure reduction is likely due to increase in NO production.
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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