Effect of Parboiled Germinated Brown Rice on Heart of L-NAME-induced Hypertensive Rats

ผลของข้าวกล้องงอกนึ่งต่อหัวใจของหนูทดลองที่ชักนำให้เกิดภาวะความดันโลหิตสูงด้วยสาร L-NAME

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ABSTRACT

The objective of the present study is to investigate the health benefits of parboiled germinated brown rice (PGBR), Khao Dawk Mali 105 variety on antihypertensive effects in N-nitro-L-arginine methyl ester (L-NAME) hypertensive rat model. Male Sprague-Dawley rats were administrated with L-NAME (20 mg/kg/day) for 8 weeks and were fed by control diet, white, brown, and PGBR rice. PGBR and BR groups had a lower systolic blood pressure (SBP) when compared to control diet treated hypertensive rats group. The nitrite/nitrate (NOx) concentration in plasma and endothelial NO synthase (eNOS) gene expression in heart tissue of the L-NAME-treated group were significantly decreased as compared with the controls. PGBR group showed the higher level of plasma NOx and eNOS gene expression level but not statistically significant when compared with L-NAME-treated group. These results suggest that PGBR may prevent the increase of SBP in the L-NAME-induced hypertension rats that the mechanism may be related to nitric oxide bioavailability.

บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลเชิงสุขภาพของข้าวกล้องงอกนึ่ง สายพันธุ์ขาวดอกมะลิ 105 ต่อฤทธิ์การต้านความดันโลหิตสูงในโมเดลหนูความดันโลหิตสูงจากสาร N-nitro-L-arginine methyl ester (L-NAME) หนูทดลองสายพันธุ์ Sprague-Dawley เพศผู้ถูกเหนี่ยวนำให้เกิดภาวะความดันโลหิตสูงด้วยสาร L-NAME (20 มก./กก./วัน) ผสมในน้ำดื่มเป็นเวลา 8 สัปดาห์ ร่วมกับการให้อาหารปกติ และอาหารที่ถูกเตรียมโดยการแทนที่แป้งข้าวโพดในอาหารสูตรปกติด้วยแป้งข้าวขาว ข้าวกล้อง และข้าวกล้องงอกนึ่ง ผลการศึกษาพบว่าหนูทดลองที่เหนี่ยวนำให้เกิดภาวะความดันโลหิตสูงกลุ่มที่ได้รับข้าวกล้องงอกนึ่ง และข้าวกล้องมีระดับความดันโลหิตต่ำกว่าหนูทดลองกลุ่มที่ได้รับอาหารสูตรปกติ ปริมาณของไนไตรท์/ไนเตรต (NOx) ในพลาสมาและยีน endothelial NO synthase (eNOS) ในเนื้อเยื่อหัวใจของกลุ่มที่ถูกเหนี่ยวนำด้วย L-NAME เพียงอย่างเดียวลดลงอย่างมีนัยสำคัญเมื่อเปรียบเทียบกับหนูทดลองกลุ่มปกติ หนูทดลองในกลุ่มที่ได้รับข้าวกล้องงอกนึ่งแสดงให้เห็นปริมาณของพลาสมา NOx และยีน eNOS ที่สูงกว่าหนูกลุ่มที่ถูกเหนี่ยวนำด้วย L-NAME เพียงอย่างเดียว ผลการศึกษาเหล่านี้แสดงให้เห็นว่าข้าวกล้องงอกนึ่งอาจจะสามารถป้องกันการเพิ่มของความดันโลหิตสูงในหนูทดลองที่ถูกเหนี่ยวนำให้เกิดภาวะความดันโลหิตสูงได้ โดยกลไกการออกฤทธิ์อาจเกี่ยวข้องกับ NOx bioavailability

Keywords: Parboiled germinated brown rice, Hypertensive rats, Nitric oxide
คำสำคัญ: ข้าวกล้องงอกนึ่ง หนูทดลองความดันโลหิตสูง ไนตริกออกไซด์

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Introduction

Hypertensive heart disease is a group of abnormalities that includes systolic and diastolic dysfunction, left ventricular hypertrophy (LVH) or cardiac fibrosis in response to elevated blood pressure, and their clinical symptoms (Drazner, 2011). Endothelium-derived nitric oxide (NO), synthesized by endothelial NO synthase (eNOS), has multifunctional physiological effects such as regulates vascular tone, inhibition of platelet aggregation and leukocyte adhesion, and modulation of vascular smooth muscle cell proliferation (Moncada and Higgs 1991; Radomski et al., 1987). The chronic administration of N-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthase (NOS), for long-term blockade of NO synthesis obtains a model of specific vascular dysfunction responsible for increasing systemic arterial hypertension and structural alterations of the heart and the arterial walls (Ribeiro et al., 1992; De Gennaro Colonna et al.; 2002a). This L-NAME-induced hypertension shows impairment of endothelium-dependent vasorelaxation that is associated with low levels of plasma NO and decreased eNOS protein expression and activity (Fu et al., 2011). Upregulation of eNOS mRNA expression attenuates cardiac and aortic wall remodeling in L-NAME-induced hypertension was reported (Kumar et al., 2014).

Bioavailability of NO can be maintained by inhibition of oxidative stress and antioxidant properties of the substance which eliminating free radicals can improve regulation of vascular tone and increasing bioavailability of NO (Saravanakumar et al., 2015; Silambarasan et al., 2014). On the other hand, several studies shown that oxidative stress contributes to the production or maintenance of hypertension by inactivation of NO and hypertension produced by NO synthesis inhibition is involved with increased oxidative stress (Raja et al., 2010).

Parboiled germinated brown rice (PGBR) was produced from Khao Dawk Mali 105 (KDML 105) rice variety by steaming germinated paddy rice, which has high levels of nutritive values, bioactive components, and antioxidant activities. The evidence suggests that several bioactive compounds such as ferulic acid, γ-amino butyric acid, γ-oryzanol and γ-tocotrienol have huge antioxidant potential (Wunjuntuk et al., 2015; Tuntipopipat et al., 2015). Previous studies by the Institute of Nutrition, Mahidol University in vitro studies indicate that PGBR against the enzyme that associated with hypertension and in vivo studies PGBR may prevent CCl_4-induced liver oxidative stress and injury by antioxidant capacities (Wunjuntuk et al., 2015). This study, therefore, it is possible to discover the health benefit of PGBR on blood pressure by enhancing nitric oxide bioavailability in L-NAME-induced hypertensive rats.

Objectives of the study

This study aimed to evaluate PGBR could attenuate blood pressure by enhancing nitric oxide bioavailability in hypertensive rats induced by L-NAME compare with white rice (WR), brown rice (BR), and drug treatment.

Methodology

Sample preparation

PGBR, BR and WR were produced from the same kind of Khao dok mali 105 (*Oryza. sativa L. cv.,*) rice variety that was planted and harvested in Kalasin province, Thailand in 2012. Then, cooking method was prepared by

according to the first subproject's method (Srichamnong et al., 2015) and was analyzed nutrients and energy. PGBR, BR, and WR diets were prepared by replacing corn starch in the basal formula diet (AIN 76A) with cooked PGBR, BR, and WR powders, respectively (Table 1). The components of each rice diet were designed based on the macronutrient compositions in the rice that displayed in Table 2 (Wunjuntuk et al., 2015).

Table 1 Compositions of basal formula diet (AIN-76A)

Diet ingredients	Percent
Sucrose	50
Casein-vitamin free	20
Corn starch	15
Powdered cellulose	5
Corn oil	5
AIN-76 mineral mix	3
AIN-76 vitamin mix	1
DL-methionine	0.3
Choline bitartrate	0.2

Table 2 Compositions of cooked white rice, brown rice, and parboiled germinated brown rice powders

Major composition/100 g	WR	BR	PGBR
Energy (Kcal)	382.6	389.5	389.2
Protein (g)	6.6	7.1	6.9
Fat (g)	0.4	2.4	2.6
Carbohydrate (g)	88.2	84.9	84.4
γ-amino butyric acid (GABA) (mg)	1.2	2.6	11.9
γ-oryzanol (mg)	ND	11.4	13.3
Total phenolic acid (mg)	8.4	37.1	69.6
Total vitamin E (mg)	ND	1.17	1.19
Dietary fiber (g)	0.8	3.0	3.5

ND; not detected.

Experimental design

The experimental protocols were approved for the use of animal by the Siriraj Animal Care and Use Committee (SiACUC) from Faculty of Medicine Siriraj Hospital, Mahidol University (SI-ACAP 008/2557, 2014)

and compliance with International Guidelines for Animal Research Protection such as International Guiding Principles for Biochemical Research Involving Animals.

Male Sprague-Dawley rats (5 week-old) with a body weight ranging 150-170 g were housed with controlled temperature on a 12/12 h light/dark cycles. After a 1 week acclimation period, treatment was initiated. Animals were randomly divided into 6 groups of 4 rats each. The experimental details are as follows:

Group I: control group, rats were fed with basal diet throughout the experiment.

Group II: L-NAME group, rats were received basal diet + 1%cholesterol and 20 mg/KgBW/day of L-NAME in the drinking water.

Group III: L-NAME + drug group, rats were received basal diet + 1%cholesterol, 20 mg/KgBW/day of L-NAME and Losartan in the drinking water.

Group IV: L-NAME + white rice (WR) group, rats were received basal diet that total starch in the recipe was all replaced with WR + 1%cholesterol, and 20 mg/KgBW/day of L-NAME in the drinking water.

Group V: L-NAME + brown rice (BR) group, rats were received basal diet that total starch in the recipe was all replaced with BR + 1%cholesterol, and 20 mg/KgBW/day of L-NAME in the drinking water.

Group VI: L-NAME + parboiled germinated brown rice (PGBR) group, rats were received basal diet that total starch in the recipe was all replaced with PGBR + 1%cholesterol, and 20 mg/KgBW/day of L-NAME in the drinking water.

The previous pilot study, systolic blood pressure (SBP) were recorded by two rats per group and the results found rising of SBP in L-NAME group when compare with control group, but SBP value in L-NAME + PGBR group is lower than L-NAME group. Thus, from this data indicate that PGBR tend to reduce BR in hypertensive rat.

At the end of the experimental period (8 weeks), the animals were anesthetized by inhalation of CO_2. The blood samples were collected from posterior vena cava and then centrifuged to obtain plasma which was stored at -20ºC. Heart was removed intact and weighed in each rat. Heart tissues were frozen for gene expression and stored at -80ºC until use.

General parameters measurement

Systolic blood pressure (SBP) was measured non-invasively using the tail-cuff plethysmography (CODA TM noninvacive blood pressure, 2 controller (Biolasco co. Ltd, USA). Body weight and heart weight were measured by digital scale.

Measurement of plasma nitrate/nitrite concentration

Nitrite and nitrate (NOx) levels in plasma samples were measured with colorimetric assay kit (Cayman Chemical, Michigan, USA) and the absorbance values at 540 nm were read in a plate reader**.**

Quantitative real-time PCR analysis

Total RNA was extracted from the heart by using high pure RNA tissue kit (Roche, Penzberg, Germany) according to the manufacturer's instructions. First-strand cDNA was prepared from total RNA by using transcriptor first strand cDNA synthesis kit (Roche, Penzberg, Germany) then, RNA was changed into cDNA. Each specific gene

product was amplified by real-time PCR using LightCycler® 480 SYBR Green I Master (Roche, Penzberg, Germany) according to the manufacturer's protocol. The highly specific measurement of cDNA was carried out for eNOS using the LightCycler system (Bio Rad Inc., USA). Each sample was analyzed in duplicate. The specific mRNA sequences were amplified using the following primer pairs; eNOS (forward) 5' -GGGCCAGGGTGATGAGCTCTG– 3' and (reverse) 5' –CCCTCCTGGCTTCCAGTGTCC- 3' (Zambrano et al., 2013). The RNA concentration in each sample was determined from the threshold cycle (Ct) values and calculated with the sequence detection software supplied by the manufacturer. The quantitative fold changes in gene expression were determined relative to β-actin mRNA levels in each corresponding group and calculated using the $2^{-\Delta\Delta_{CT}}$ method (Livak and Schmittgen, 2001).

Statistical analysis

Data are presented as means ± standard error of the mean (SEM). Statistical comparisons between groups done using analysis of variance (ANOVA) followed by Tukey test. All statistical analyses are performing applying SPSS version 16.0 package for windows. P value < 0.05 was considered statistically significant.

Results

Effect of PGBR on general parameters

At the end of the experimental period, heart to body weight ratio of the control group had significantly different from the L-NAME-treated group (0.317 ± 0.02 vs. 0.261 ± 0.01; P = 0.04). The L-NAME rats significantly increased the SBP compared with the control group (168.30 ± 0.44 vs. 120.54 ± 0.53; P = 0.00). Treatment with Losartan, BR, and PGBR significantly reduced SBP in hypertensive rats compared to hypertensive rats without treatment (120.89 ± 2.66, 153.03 ± 0.97, and 150.05 ± 1.51 respectively vs. 168.30 ± 0.44 mmHg; P = 0.00) (Table 3).

Table 3 General parameters measurement

Group	Heart/Body weight (g %)	SBP (mmHg)
Control	0.317 ± 0.02*	120.54 ± 0.53*
L-NAME	0.261 ± 0.01	168.30 ± 0.44
L-NAME + Losartan	0.315 ± 0.02	120.89 ± 2.66*
L-NAME + WR	0.292 ± 0.03	167.20±0.71
L-NAME + BR	0.288 ± 0.02	153.03±0.97*
L-NAME + PGBR	0.305 ± 0.03	150.05±1.51*

Results are expressed as mean ± SEM (n = 4 rats). *Significant difference (P<0.01) compared to L-NAME group.

Effect of PGBR on plasma NOx concentration

Plasma NOx concentration was significantly decreased in L-NAME-treated rats (15.89 ± 2.54 µmol/L) compared to the control group (37.86 ± 2.40 µmol/L; P = 0.00). The highest restore of NOx when compare to L-NAME-treated rat were drug and PGBR treated, respectively (Figure 1). However no statistical significant was observed.

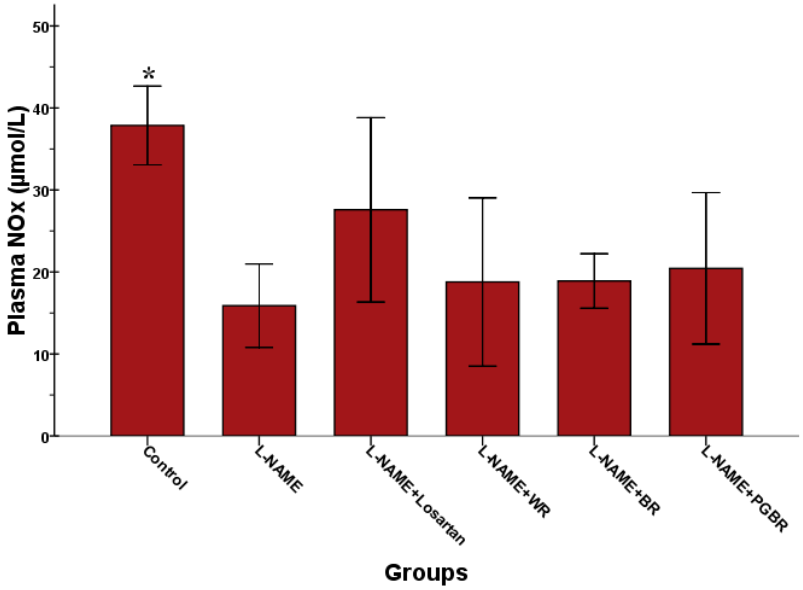

Figure 1 Effect of different diets on plasma NOx concentration in L-NAME-induced hypertensive rats. Values are means ± SEM (n = 4 rats). *Significant at P < 0.01 compared to L-NAME group.

Effect of PGBR on eNOS gene expression in heart tissues

In L-NAME-induced hypertensive rats group showed a significantly decreased of eNOS gene expression in heart tissue when compared to control group (P = 0.04). Treatment with Losartan significantly increased eNOS gene expression in heart tissue compared with L-NAME-treated group (P = 0.03). PGBR showed the higher level of eNOS gene expression when compared to L-NAME-treated group (Figure 2) but did not different in statistical significant.

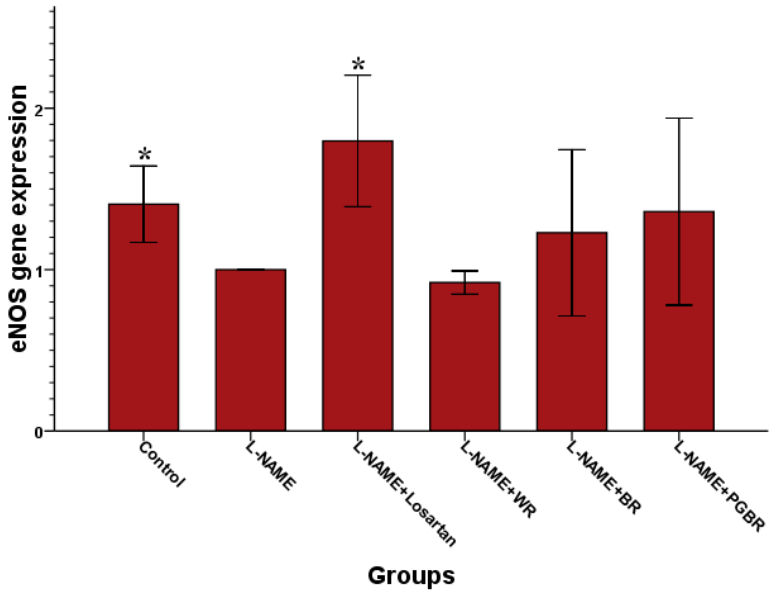

Figure 2 Effect of different diets on eNOS gene expression in heart tissue of hypertensive rats. Values are means ± SEM (n = 4 rats). *Significant at P < 0.05 compared to L-NAME group.

Discussion and Conclusions

The present study demonstrates the health benefits of PGBR on blood pressure, plasma NOx level and eNOS gene expression in L-NAME-induced hypertensive rats. Supplementation of PGBR reduced blood pressure, the plasma NOx concentration and the gene expression of eNOS may be increased that were associated with an improvement of NO bioavailability.

Nitric oxide synthesized in endothelial cells where L-arginine is converted to NO by eNOS is a major regulator of the vascular resistance. Hypertension, one of the most important risk factor for cardiovascular diseases or hypertension heart diseases, is characterized by the production of the higher amount of reactive oxygen species (ROS) interacting with NO and reducing its bioavailability (De Gennaro Colonna et al., 2005b). Besides, it is well established that acute inhibition of NO biosynthesis by in vivo administration of L-NAME, an L-arginine analog, leads to hypertension and vasoconstriction (Silambarasan et al., 2014). In this study, L-NAME rats showed significantly increased SBP, decreased plasma NOx concentration and downregulation of eNOS gene expression. Our findings confirm previous studies that chronic inhibition of NO synthesis with L-NAME induces sustained hypertension which is primarily due to the loss of both basal and stimulated NO production (Baylis et al. 1992; Manning et al. 1993). Thus, downregulation of eNOS may also participate at lower NO availability.

The present study results show PGBR is able to attenuate the increasing in blood pressure together with its likely increased plasma NOx concentration and might cause upregulation eNOS gene expression in heart of rats. Therefore, the reduction of blood pressure in this experiment might be related to enhancing of NO bioavailability (Bunbupha et al., 2014).

The antihypertensive effect of PGBR might be the effect of high content of bioactive ingredients in rice such as γ-amino butyric acid, ferulic acid, p-coumaric acid, γ-oryzanol and γ-tocotrienol (Wunjuntuk et al., 2015; Tuntipopipat et al., 2015). It was found that ferulic acid reduced SBP and increased antioxidant enzyme activity in heart by reduced interstitial fibrosis in hypertensive rats (Alam et al., 2013). Hence, PGBR decreased blood pressure, which may be mediated by reducing oxidative stress and retaining the bioavailability of NO in the L-NAME-induced hypertension rat model.

In conclusion, the administration of L-NAME with PGBR resulted that a reduction of blood pressure which may relate to an upregulation trend of eNOS gene expression in heart rat. The antihypertension effects of PGBR are likely to be mediated by enhanced generation of vascular NOx and improving NO bioavailability and mechanisms of oxidative stress should also be considered. The consumption of PGBR might be useful to reduce NCDs prevalence in the population. Limitations of the current study include sample size that too small thus the results in this study showed a high of SEM. And diastolic blood pressure and mean arterial pressure were not recorded.

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