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Effects of Tubtim Chum Phae Rice Bran Hydrolysates on Blood Pressure and Oxidative Stress in L-NAME-induced Hypertensive Rats

ผลของไฮโดรไลเสทรำข้าวทับทิมชุมแพต่อความดันเลือดและภาวะเครียดออกซิเดชันในหนูแรทที่ถูกเหนี่ยวนำให้เกิดความดันเลือดสูงด้วยสารแอลเนม

Somphot Chumjit (สมโภชน์ ชุมจิตร)[1]* Dr.Weeropon Sangartit (ดร.วีระพล แสงอาทิตย์)**
Dr.Upa Kukongviriyapan (ดร.ยุพา คู่คงวิริยพันธุ์)*** Dr.Poungrat Pakdeechote (ดร.พวงรัตน์ ภักดีโชติ)***
Dr.Veerapol Kukongviriyapan (ดร.วีรพล คู่คงวิริยพันธุ์)****
Dr. Supawan Thawornchinsombat (ดร.ศุภวรรณ ถาวรชินสมบัติ)******

ABSTRACT

The present study aimed to investigate the effects of Tubtim Chum Phae rice bran hydrolysates (TCRH) against hypertension, oxidative stress and endothelial dysfunction in nitric oxide (NO)-deficient hypertensive rats. Hypertension was induced in male Sprague-Dawley rats by administrating N^{ω}-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO synthase at dose of 50 mg/kg b.w./day in drinking water for 3 weeks. Animals were randomly divided into 5 groups: normal control+deionized water (DI), normal control+TCRH 500 mg/kg, L-NAME+DI, L-NAME+TCRH 250 mg/kg, and L-NAME+TCRH 500 mg/kg, respectively. Results showed that TCRH in a dose-dependent manner significantly reduced blood pressure, decreased vascular resistance, alleviated oxidative stress, and improved vasorelaxation to acetylcholine in L-NAME-induced hypertensive rats (P<0.05). These data suggest the TCRH might be used for the prevention and /or treatment of hypertension.

บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อสำรวจผลของไฮโดรไลเสทรำข้าวทับทิมชุมแพ (TCRH) ต่อการต้านภาวะความดันเลือดสูง ภาวะเครียดออกซิเดชัน และภาวะเซลล์เอนโดทีเลียมทำงานผิดปกติในหนูแรทความดันเลือดสูง จากการขาดไนตริกออกไซด์ (NO) ภาวะความดันเลือดสูงได้เหนี่ยวนำในหนูแรท เพศผู้ สายพันธุ์ Sprague- Dawley ถูกเหนี่ยวนำให้เกิดภาวะความดันเลือดสูงโดยการให้สารแอลเนม (L-NAME) ซึ่งยับยั้งการสร้าง NO ขนาด 50 มก./กก. น้ำหนักตัว/วัน ผสมในน้ำดื่ม เป็นเวลา 3 สัปดาห์หนูทดลองถูกสุ่มและแบ่งออกเป็น 5 กลุ่ม ได้แก่ หนูทดลองปกติ+ได้รับน้ำปราศจากไอออน (DI) หนูทดลองปกติ +TCRH 500 มก./กก. หนูทดลองแอลเนม +DI หนูทดลองแอลเนม+ TCRH 250 250 มก./กก. และหนูทดลองแอลเนม +TCRH 500 มก./กก. ผลการทดลองพบว่า TCRH ตามขนาดความ

[1] *Correspondent author: Somphot_chum@hotmail.com*

* *Student, Master of Science Program in Medical Physiology, Department of Physiology, Faculty of Medicine, Khon Kaen University*

** *Lecturer, Department of Physiology, Faculty of Medicine, Cardiovascular Research Group, Khon Kaen University*

*** *Associate Professor, Department of Physiology, Faculty of Medicine, Cardiovascular Research Group, Khon Kaen University*

**** *Professor, Department of Pharmacology, Faculty of Medicine, Khon Kaen University*

****** *Assistant Professor, Faculty of Technology, Cardiovascular Research Group, Khon Kaen University*

เข้มข้นสามารถลดความดันเลือดลดความต้านทานหลอดเลือด ลดภาวะเครียดออกซิเดชัน และเพิ่มการคลายตัวของหลอดเลือดต่อยา acetylcholine ในหนูทดลองความดันเลือดสูงแอลเนมอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) ผลการศึกษานี้บ่งชี้ว่า TCRH อาจนำไปใช้เพื่อการป้องกันและ/หรือรักษาภาวะความดันเลือดสูง

Keywords: L-NAME hypertension, Antioxidant, Tubtim Chum Phae rice bran hydrolysates
คำสำคัญ: ความดันเลือดสูงแอลเนม ภาวะเครียดออกซิเดชัน ไฮโดรไลเสทรำข้าวทับทิมชุมแพ

Introduction

Rice is the main staple food of the Asian populations. Rice bran is a by-product of rice milling industry and constitutes around 10% of the total weight of rough rice. It is a rich source of proteins, vitamins, minerals, essential fatty acids, dietary fiber and bioactive phytochemicals [1-3]. A study in an *in vitro* model found that peptides-derived from Thai rice bran possess strong angiotension-converting enzyme (ACE) inhibitory and free radical scavenging effects [4]. Moreover, previous studies in many experimental animal models also demonstrated that rice bran hydrolysates-derived from Hom Mali white rice ameliorated cardiovascular risk factors by reducing oxidative stress, inflammation, dyslipidemia, insulin resistance and hypertension [5-9]. However, studies on the biological activities of rice bran-derived from Thai colored rice are still limited.

Tubtim Chum Phae rice is a new Thai rice strain, RD69. This rice is produced from hybridization between Hom Mali rice or Jasmin rice and Sung Yod Patthalung rice. The name of "Tubtim Chum Phae" refers to the color of the rice, which is red as ruby. Tubtim Chum Phae rice can be grown all year round and in all regions of Thailand. Previous studies reported that rice with dark red or purple color contains high amount of anthocynanins, a photosynthetic pigment found deep purple or reddish fruits and vegetables [10]. Meanwhile, rice with red color consists high amount of polyphenols, phenolics and flavonoids [11-12].

It is well established that nitric oxide (NO) plays a crucial role in regulating a wide spectrum of functions in the cardiovascular system [13-14]. The formation of NO can be interrupted by giving L-arginine analoque such as N^{ω}-nitro-L-arginine methyl ester (L-NAME), which is the most common NO synthase inhibitor used to induce hypertension, endothelial dysfunction and oxidative stress [15-17]. Interestingly, there are several evidences supported that oxidative stress plays an important role in pathogenesis of hypertension [18-19]. Therefore, amelioration of oxidative stress might be the possible therapeutic strategy that could prevent or treat hypertension.

Objective of the study

The present study aimed to evaluate the effect of Tubtim Chum Phae rice bran hydrolysates (TCRH) on blood pressure, oxidative stress and endothelial dysfunction in L-NAME-induced hypertensive rats.

Methodology

Tubtim Chum Phae rice (RD69) is approved by the Rice Research and Development Committee of Thailand in 2016. Tubtim Chum Phae rice used in this study is grown and harvested from Chum Phae District, Khon

Kaen, Thailand. TCRH were prepared at Department of Food Technology, Faculty of Technology, Khon Kaen University, Thailand. Briefly, the defatted rice bran of Tubtim Chum Phae rice was prepared by using the cold pressing method. The maillard reaction products were prepared from defatted rice bran by using two-step process; hydrothermolysis under mild subcritical alkaline water and enzymatic hydrolysis by protease G6 (Genencor International Inc., CA, USA). Thereafter, the enzyme was inactivated by heating in the microwave. After centrifugation, the protein hydrolysates were lyophilized using a freeze dryer. The rice bran hydrolysate powder was stored in air-tight containers and kept at -20°C for further studies. The yield of crude TCRH was 35 % by weight from the defatted rice bran. TCRH consist bioactive compounds, peptides, phenolic compounds and reducing sugars.

Male Sprague-Dawley, weighing 180- 220 g, were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom, Thailand. Animals were kept in the Northeast Laboratory Animal Center, Khon Kaen University, Thailand, under standard conditions (light/dark cycle; 12 h, humidity; 30-60%). The experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee of Khon Kaen University (AEKKU 13/2558).

Hypertension was induced in rats by administering L-NAME at dose of 50 mg/kg/day in drinking water for 3 weeks [16]. TCRH (250 or 500 mg/kg/day) was intragastrically administered to animals simultaneously with or without L-NAME. Rats were divided into 5 groups (n = 8): normotensive rats-treated with DI as vehicle, normotensive rats-treated with TCRH (500 mg/kg/day), hypertensive rats-treated with DI, TCRH 250 mg/kg/day and TCRH 500 mg/kg/day, respectively.

Hemodynamics and vascular responsiveness measurement

Systolic blood pressure was measured indirectly in conscious restrained animals once a week by using rat tail-cuff plethysmography (Blood pressure analyzer, model 179; IITC, Woodland Hills, CA, USA). At the end of experiment, rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg). The tracheotomy was performed to facilitate respiration. The left femoral artery was cannulated with polyethylene tubing connected to a pressure transducer for continuously monitoring of blood pressure including systolic (SBP), diastolic (DBP) and mean arterial blood pressure (MAP) (Biopac Systems Inc., CA, USA). The left femoral vein was cannulated for infusions of aceylcholine (ACh, 10 nmol/kg), an endothelium-dependent vasodilator and sodium nitroprusside (SNP, 3 nmol/kg), an endothelium-independent vasodilator). Changes in blood pressure were expressed as percentages of control values obtained immediately before the administration of the drug (baseline). Thereafter, the abdominal aorta was approached by minimal opening of intraperitoneal cavity for measurement of hindlimb blood flow (HBF) using an electromagnetic flowmeter (Carolina Medical Electronics, NC, USA). Hindlimb vascular resistance (HVR) was calculated from mean arterial pressure and mean HBF as following equation; HVR = MAP/HBF (mmHg/min/100 g tissue/mL or Peripheral Resistance Unit: PRU).

Biochemical assay

After hemodynamics and vascular responsiveness measurements, rats were euthanized with an overdose of pentobarbital sodium and plasma of the study rats were collected for the biochemical assay.

Assay of superoxide production

Vascular superoxide (O_2•-) production in the carotid artery was determined by using luciginin-enhanced chemiluminescence as previously described (Kukongviriyapan et al., 2014). The carotid arteries were rapidly excised and incubated in oxygenated Krebs-Ringer bicarbonate solution at 37°C for 30 min. The chemiluminescence signals were measured by adding lucigenin and using luminometer (Turner Biosystems, CA, USA). The photon counts were integrated every 15 s for 5 min. The data were expressed as relative light unit counts/mg dry wt/min.

Assay of malondialdehyde

The level of malondialdehyde (MDA) in plasma was assayed following a previous method [20]. The absorbance of the supernatant was measured at 532 nm by spectrophotometer (Ultrospec 6300 *pro*. Inc., Biochrom Ltd. UK). A standard curve was generated by using 1, 1, 3, 3-tetraethoxy propane (0.3-10 μM).

Assay of nitric oxide metabolites

The concentrations of nitric oxide metabolites (NOx) were measured following previously described methods [21-22]. In brief, plasma samples were deprotenizated by ultrafiltration using centrifugal concentrators (NANOSEP™, Pal Filtration, USA). In brief, the nitrate in plasma was converted to nitrite by nitrate reductase for 30 min at 30°C. The reaction was finished by the addition of Griess reagent (4% Sulfonamide in 0.3% Naphthalenediamin dihydrochloride. The final colored substance obtained was determined on an enzyme-linked immunosorbent assay (ELISA) plate reader with filter wavelength of 540 nm (Tecan GmbH., Grodig, Austria). A standard curve is generated with a set of serial dilution of $NaNO_2$.

Data analysis

Results were expressed as mean ± S.E.M., and indicated the number of animals. The differences among experimental groups were analyzed using one-way analysis of variance (ANOVA) followed by a post-hoc Turkey test (SigmaStat statistical analysis software version 3.5). A value of $P < 0.05$ is considered statistically significant.

Results

TCRH attenuated blood pressure of L-NAME-induced hypertensive rats.

At the beginning of experiments, the baseline values of systolic blood pressure (SBP) were similar in all experimental groups (Fig. 1). The SBP of L-NAME-induced hypertensive rats were progressively increased

throughout the study period of 3 weeks. A significant reduction of SBP was found in L-NAME-induced hypertensive rats-treated with TCRH in dose-dependent manner when compare with L-NAME group (P<0.05, Fig. 1). There was no change in SBP of normotensive rats-treated with vehicle or TCRH (Fig. 1). The MAP and HVR of L-NAME-induced rats-treated with TCRH were significantly lower than those of L-NAME controls (Fig. 2; P<0.05). Results indicate the antihypertensive effect of TCRH. Sinc e there were no differences in MAP, HBF and HVR of normal control groups either treated or untreated with TCRH, suggesting that TCRH had no hypotensive effect in normotensive rats (Fig. 2).

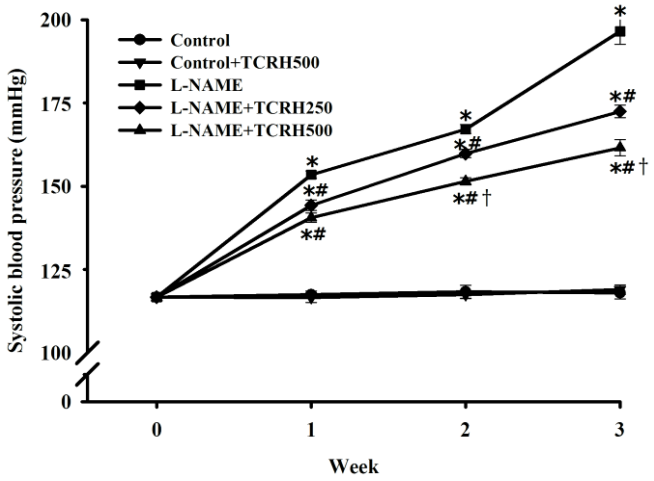

Figure 1 Systolic blood pressure measured by tail-cuff method throughout the study period in all experimental groups. Data are shown as mean ± S.E.M. (n= 8/group). * P<0.05 compared with normal control group, # P<0.05 compared with L-NAME group, † P <0.05 compared with L-NAME+TCRH 250 group.

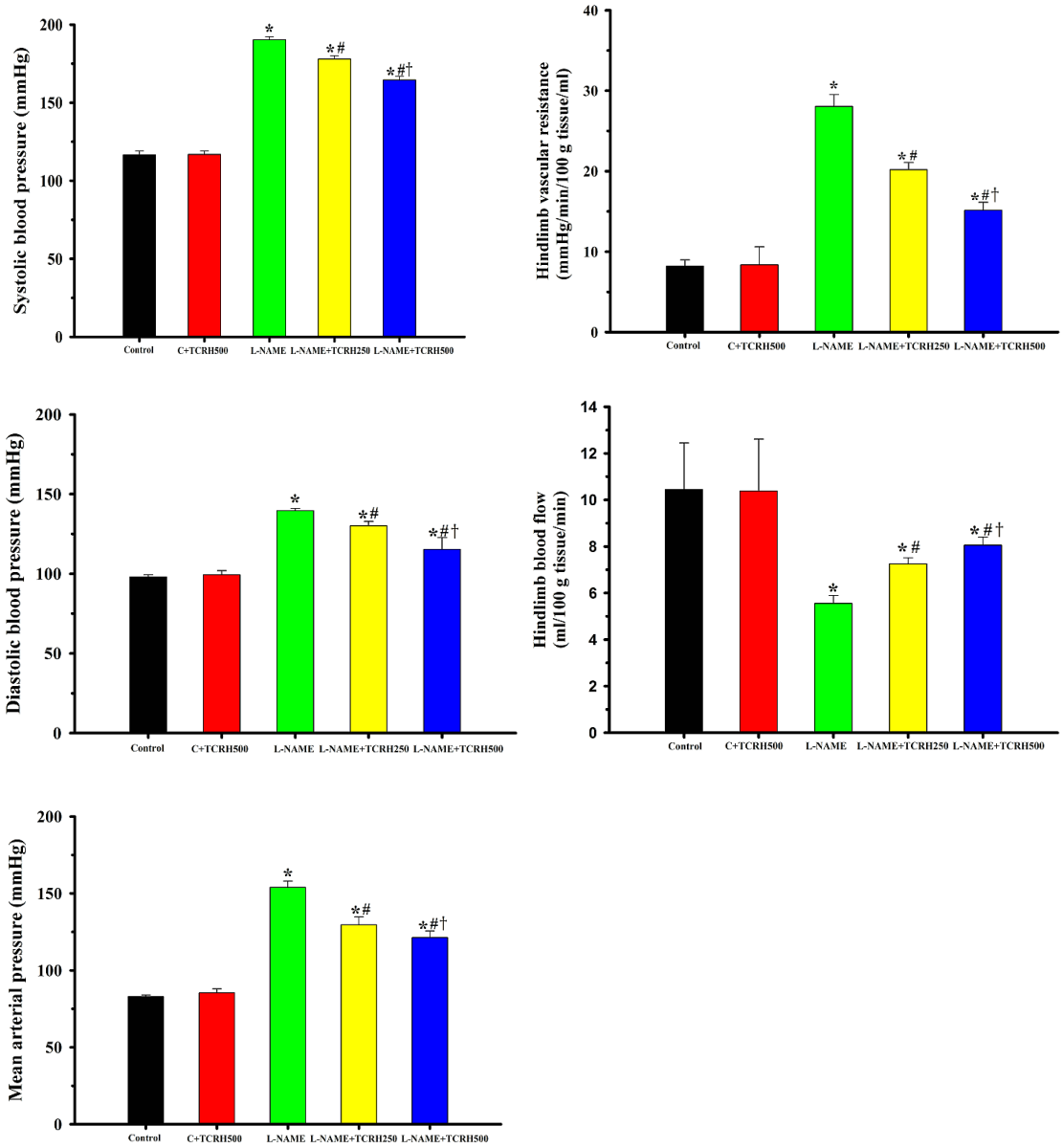

Figure 2 Effect of TCRH on hemodynamic status. Data are shown as mean ± S.E.M. (n= 8/group). * P<0.05 compared with normal control group, # P<0.05 compared with L-NAME group, † P <0.05 compared with L-NAME+TCRH 250 group.

TCRH improved endothelium-dependent relaxation by ACh

A significant blunted response to ACh was found in L-NAME-induced hypertensive rats when compared with normal controls (Figure 3; *P*<0.05), indicating the impairment of the endothelium-dependent relaxation after L-NAME treatment. Increased vasorelaxation to ACh was found in L-NAME rats-treated with TCRH at doses of 250 and 500 mg/kg when compared with L-NAME group. (Fig. 3; *P*<0.05). There were no differences in endothelium-independent relaxation induced by SNP in all study groups (data not shown).

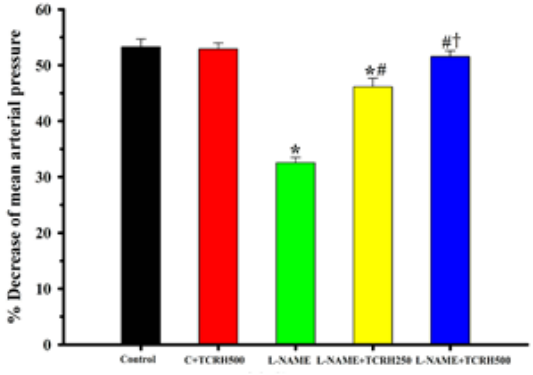

Figure 3 Effect of TCRH on endothelium-dependent relaxation induced by acetylcholine in all experimental groups. Data are shown as mean ± S.E.M. (n= 8/group). * P<0.05 compared with normal control group, #P<0.05 compared with L-NAME group, † P <0.05 compared with L-NAME+TCRH 250 group.

TCRH alleviated oxidative stress in L-NAME-induced hypertensive rats

The levels of O_2•- production in carotid arteries and plasma MDA were significantly increased, whereas the concentration of plasma NOx was dramatically decreased in L-NAME-induced hypertensive rats when compared with normal controls (Fig. 4; *P*<0.05), suggesting the occurrence of oxidative stress in L-NAME-induced hypertensive rats. TCRH in a dose-dependent manner alleviated oxidative stress by reducing O_2•- production, decreasing plasma MDA and increasing plasma NOx levels when compare with L-NAME group (Fig. 4; *P*<0.05). Interestingly, it is found that a reduction in oxidative stress is associated with a decrease in arterial blood pressure of L-NAME rats-treated with TCRH.

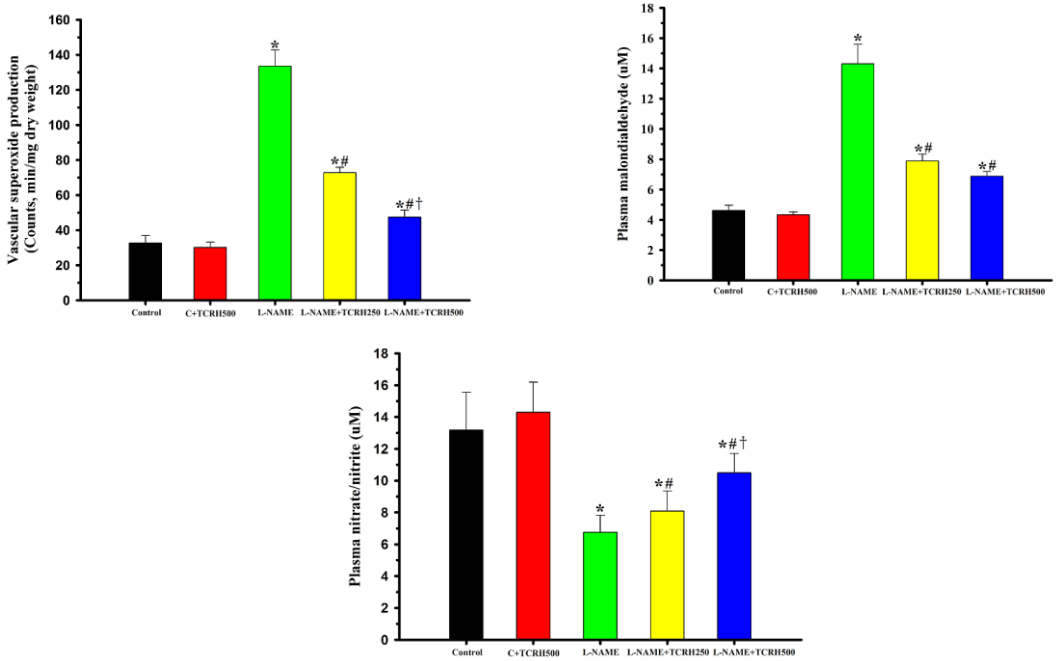

Figure 4 Effects of TCRH on oxidant and antioxidant status. Data are shown as mean ± S.E.M. (n= 8/group).* P<0.05 compared with normal control group, # P<0.05 compared with L-NAME group, † P <0.05 compared with L-NAME+TCRH 250 group.

Discussion

In the present study, we found the disruption of NO generation initiated by L-NAME. Rats received L-NAME showed a significant increase in blood pressure and oxidative stress, and impairment of endothelium-dependent relaxation. Administration of TCRH at doses of 250 and 500 mg/kg attenuated high blood pressure and reduced oxidative stress in L-NAME-induced hypertensive rats. In addition, L-NAME rats-treated with TCRH in a dose-dependent manner improved endothelial function by enhancing vasorelaxation to ACh.

Previous studies have been reported that L-NAME–induced high blood pressure is correlated with the overproduction of vascular O_2•-. Several lines of evidences support the concept that increased vascular O_2•- production is associated with increased expression of NADPH oxidase, a major source of O_2•- generation in hypertensive rats [23-24]. It has been demonstrated that excessive production of O_2•- can rapidly scavenge NO, resulting in formation of peroxynitrite ($ONOO^-$), which thereby reduced NO bioavailability and causing oxidative damage and endothelial dysfunction [25]. As TCRH reduced O_2•- production and improved endothelium-dependent relaxation to ACh, these results suggest that TCRH are able to restore NO bioavailability. Furthermore, the relaxant response to SNP, which is an NO donor, was comparable among experimental groups. The data also confirm impairment of endothelial-dependent relaxation in L-NAME-induced hypertensive rats.

It is well known that endothelium plays an important role in modulating the vascular tone and blood pressure. Therefore, increased NO bioactivity and improved endothelial-dependent relaxation during treatment with TCRH could in part to ameliorate of hypertension in rats with NO syntheses inhibition. Moreover, previous studies demonstrated that many peptides-derived from food protein possess ACE-inhibiting and free radical scavenging activities, such as whey protein [26], peptide derived from rice grain [27], rice bran [4] and rice bran protein hydrolysates [5, 8-9]. Several studies reported that rice with red color contains high bioactive compounds polyphenols, phenolic compounds and flavonoids [11-12]. We found that TCRH consist of bioactive compounds, peptides, phenolic compounds and reducing sugars. Therefore, the ACE-inhibiting activity and antioxidant properties might explain the antihypertensive effect of TCRH in this rat model of hypertension.

Conclusion

Overall results suggest that the antihypertensive effect of TCRH might be contributable to an improvement of endothelial function and a decrease in oxidative stress. The mechanisms involved with these beneficial effects of TCRH might be attributable to the ability to scavenge ROS, increase in antioxidant activity and NO bioavailability, together with a decrease in plasma ACE activity. Overall findings of this study support the idea of using TCRH as a food supplement in daily diet to prevent and control hypertension. However, the mechanistic effects of TCRH on blood pressure attenuation are required for further investigation.

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