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

ผลของไฮโดรไลเสทรำข้าวทับทิมชุมแพต่อความดันเลือดและภาวะเครียดออกซิเดชันในหนูแรทที่ความดันเลือดสูงด้วยสารแอลเนม

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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 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 และภาวะเซลล์เอนโดทีเลียมทำงานผิดปกติในหนูแรทความดันเลือดสูงจากการขาดไนตริกออกไซด์ (NO) ภาวะความดันเลือดสูงได้เหนี่ยวนำในหนูแรท เพศผู้ สายพันธุ์ Sprague- Dawley โดยการให้สารแอลเนม (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 มก./กก. และหนูทดลองแอลเนม+TCRH 500 มก./กก. ผลการทดลองพบว่า TCRH ตามขนาดความเข้มข้นสามารถลดความดันเลือด ลดความต้านทานหลอดเลือด 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

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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. (Sereewatthanawut et al., 2008; Min et al., 2011; Zhang et al., 2010). 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 (Kokaew and Thawornchinsombu 2011). 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 (Tuangpolkrung, 2012; Boonloh et al., 2015; Justo et al., 2013; Boonla et al., 2015; Senaphan et al., 2016). However, studies on the biological activities of rice bra-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 (Yawadio et al., 2007). Meanwhile, rice with red color consists high amount of polyphenols, phenolics and flavonoids (Gunaratne et al., 2013; Sompong et al., 2011).

It is well established that nitric oxide (NO) plays a crucial role in regulating a wide spectrum of functions in the cardiovascular system (Forstermann, 2008; Forstermann and Sessa, 2012). 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 (Kitamoto et al., 2000; Nakmareong et al., 2011; Priviero et al., 2007). Interestingly, there are several evidences supported that oxidative stress plays an important role in pathogenesis of hypertension (Heitzer et al., 2001; Yamashita et al., 2007). 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 hypertensive rats.

Methodology

Tubtim Chum Phae rice (RD69) 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. 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%,

Hypertension was induced in rats by administering L-NAME at dose of 50 mg/kg/day in drinking water for 3 weeks. 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.

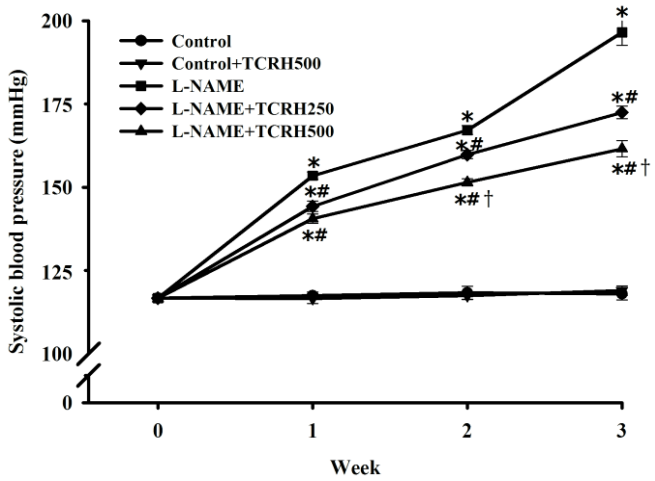

Figure 1 Systolic blood pressure measured by tail-cuff method throughout the study period in all experimental groups. Data are shown as mean ± S. E. M. (n= 8 / group). * $P<0.05$ compared with normal control group, # $P<0.05$ compared with L-NAME group, † $P<0.05$ compared with L-NAME+TCRH 250 group.

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).

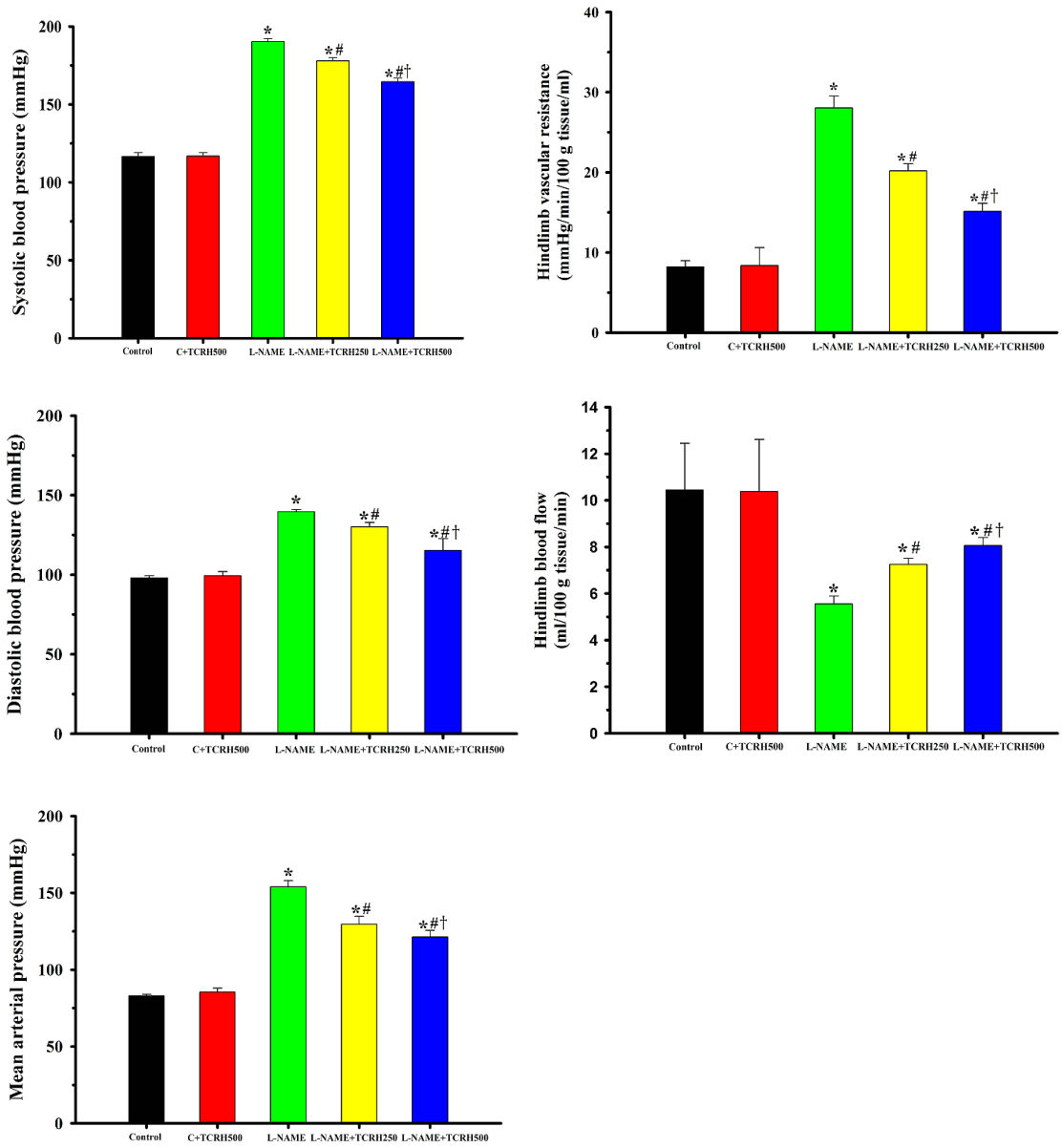

Figure 2 Effect of TRBH on hemodynamic status. Data are shown as mean ± S.E.M. (n= 8 /group). * $P<0.05$ compared with normal control group, # $P<0.05$ compared with L-NAME group, † $P<0.05$ compared with L-NAME+TCRH 250 group.

Biochemical assay

Assay of superoxide production

Vascular superoxide ($O_2^{\cdot -}$) 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 (Kukongviriyapan et al., 2014). The absorbance of the supernatant was measured at 532 nm by spectrophotometer. 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 (Kukongviriyapan *et al.*, 2007; Luangaram *et al.*, 2007). In brief, plasma samples were deprotenizated by ultrafiltration using centrifugal concentrators (NANOSEP^{TM}, 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 $NaNO_2$.

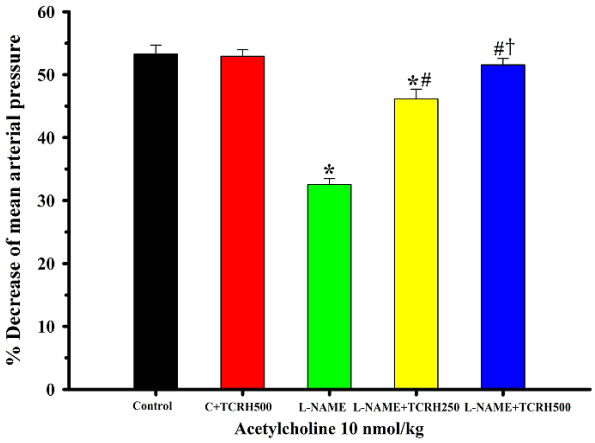

Figure 3 Effect of TCRH on endothelium-dependent relaxation induced by acetylcholine in all experimental groups. Data are shown as mean ± S. E. M. (n= 8 / group) . * P<0. 05 compared with normal control group, # P<0. 05 compared with L-NAME group, † P <0.05 compared with L-NAME+TCRH 250 group.

Data analysis

Results were expressed as mean ± S.E.M., and indicated the number of animals. The differences among experimental groups were analyzed by one-way analysis of variance (ANOVA) followed by post-hoc Duncan's multiple range test. Statistical significance was defined as a P value of less than 0.05.

Results

TCRH attenuated blood pressure of L -NAME hypertensive rats.

At the beginning of experiments, the baseline values of systolic blood pressure (SBP) were similar in all experimental groups (Fig. 1; $P<0.05$). The SBP of L-NAME hypertensive rats were progressively increased throughout the study period of 3 weeks. A significant reduction of SBP was found in L-NAME hypertensive rats-treated with TCRH in dose-dependent manner ($P<0.05$, 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 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. Since 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).

TCRH improved endothelium-dependent relaxation by ACh

A significant blunted response to ACh was found in L-NAME 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 dose of 250 and 500 mg/kg (Fig. 3; $P<0.05$). There were no differences in endothelium-independent relaxation induced by SNP in all study groups (data not shown).

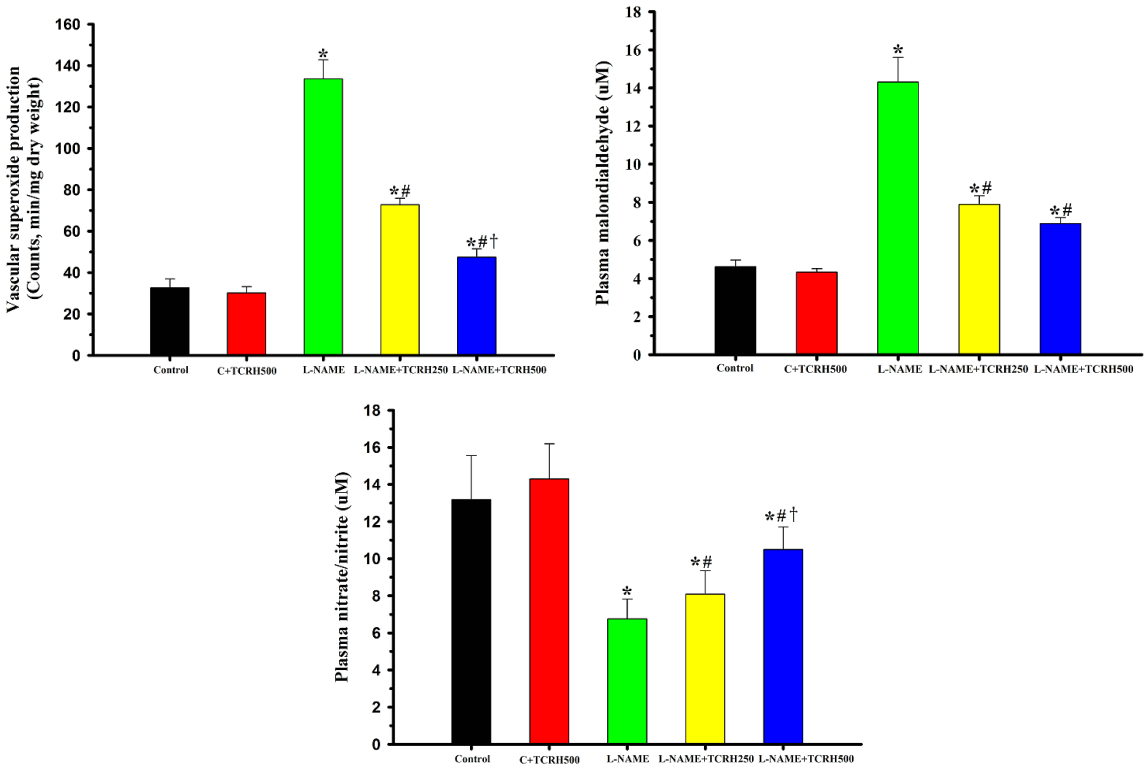

Figure 4 Effects of TCRH on oxidant and antioxidant status. Data are shown as mean ± S. E. M. (n= 8/group).* $P<0.05$ compared with normal control group, $^{\#}P<0.05$ compared with L-NAME group, $^{\dagger}P<0.05$ compared with L-NAME+TCRH 250 group.

TCRH alleviated oxidative stress in L-NAME hypertensive rats

The levels of $O_2^{\bullet -}$ production in carotid arteries and plasma MDA were significantly increased, whereas the concentration of plasma NOx was dramatically decreased in L-NAME hypertensive rats when compared with normal controls (Fig. 4; $P<0.05$), suggesting the occurrence of oxidative stress in L-NAME hypertensive rats. TCRH in a dose-dependent manner alleviated oxidative stress by reducing $O_2^{\bullet -}$ production, decreasing plasma MDA and increasing plasma NOx levels (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.

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 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 $O_2^{\bullet -}$. Several lines of evidences support the concept that increased vascular $O_2^{\bullet -}$ production is associated with increased expression of NADPH oxidase, a major source of $O_2^{\bullet -}$ generation in hypertensive rats (Gonzalez et al., 2000; Sarr et al., 2006). It has been demonstrated that excessive production of $O_2^{\bullet -}$ can rapidly scavenge NO, resulting in formation of peroxynitrite ($ONOO^-$), which thereby reduced NO bioavailability and causing oxidative damage and endothelial dysfunction (Torok, 2008). As TCRH reduced $O_2^{\bullet -}$ 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 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 (Tavares et al., 2012), peptide derived from rice grain (Li et al., 2007), rice bran (Kokaew and Thawornchinsombut, 2011) and rice bran protein hydrolysates (Boonla et al., 2015; Senaphan et al., 2016; Tuangpolkrung, 2012). Therefore, the ACE-inhibiting activity and antioxidant properties might explain the antihypertensive effect of TCRH in this animal model.

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.

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