Garcinia mangostana Pericarp Extract Alleviates Hypertension via Oxidative Stress Reduction and Normalizes Cardiac Hypertrophy in L-NAME-induced Hypertensive Rats

สารสกัดจากเปลือกมังคุดช่วยลดภาวะความดันเลือดสูง โดยการลดภาวะเครียดออกซิเดชัน และภาวะหัวใจโตในหนูที่ทำให้เกิดความดันเลือดสูงด้วยสารแอลเนม

Pattanapong Boonprom (พัฒนพงษ์ บุญพรม)* Thewarid Berkban (เทวฤทธิ์ เบิกบาน)*
Rarinthorn Samrid (รรินธร สัมฤทธิ์)* Sarawoot Bunbupha (สราวุธ บรรบุผา)**
Dr.Poungrat Pakdeechote (ดร.พวงรัตน์ ภักดีโชติ)*** Dr.Upa Kukongviriyapan (ดร.ยุพา คู่คงวิริยะพันธุ์)****
Dr.Jariya Umka Welbat (ดร.จริยา อำคา เวลบาท)***** Dr.Parichat Prachaney (ดร.ปาริฉัตร ประจะเนย์)*****

ABSTRACT

Garcinia mangostana (mangosteen), a xanthone derivative, one class of polyphenolic compounds which were shown to be a potent benefit for antioxidant property. The aim of this study was to investigate the preventive effect of mangosteen pericarp extract on systolic blood pressure (SBP), oxidative stress markers and cardiac wall dimension in nitric oxide-deficient hypertensive rats. Male Sprague-Dawley rats were given N-nitro-L-arginine methyl ester (L-NAME) in drinking water to induce hypertension, and simultaneously treated with mangosteen pericarp extract (L-NAME+Mag group). Age-matched rats served as a control group and normotensive treated with mangosteen pericarp extract (Control+Mag group). We found that SBP, superoxide production, and plasma malondialdehyde level in L-NAME+Mag rats was significantly lower than those in L-NAME which is corresponding to an increase in ventricular luminal area (p<0.05). In conclusion, mangosteen pericarp extract has antihypertensive property and improved cardiac wall remodeling in L-NAME-induced hypertensive model.

บทคัดย่อ

เปลือกมังคุดคือสารประกอบฟีโนลิคซึ่งมีประสิทธิภาพดีในการต้านอนุมูลอิสระ วัตถุประสงค์ของการศึกษาในครั้งนี้เพื่อศึกษาผลการป้องกันของเปลือกมังคุดต่อความดันเลือด ภาวะเครียดออกซิเดชันและพารามิเตอร์ของหัวใจในหนูที่ทำให้เกิดความดันเลือดสูงจากภาวะพร่องไนตริกออกไซด์ หนูขาวเพศผู้ถูกเหนี่ยวนำให้เกิดความดันเลือดสูงโดยได้รับสารแอลเนมในน้ำดื่ม (กลุ่ม L-NAME) ร่วมกับเปลือกมังคุด (กลุ่ม L-NAME+Mag) กลุ่มควบคุมและกลุ่มควบคุมที่ได้รับเปลือกมังคุด (กลุ่ม Control+Mag) โดยวัดค่าความดันเลือด ภาวะเครียดออกซิเดชัน ขนาด น้ำหนักรวมทั้งศึกษาความหนา พื้นที่หน้าตัดและช่องว่างภายในหัวใจห้องล่างซ้าย พบว่า SBP การสร้างซุปเปอร์ออกไซด์และระดับ MDA ในพลาสมาในหนูกลุ่ม L-NAME+Mag ต่ำกว่าในหนูกลุ่ม L-NAME อย่างมีนัยสำคัญ ซึ่งสัมพันธ์กับการเพิ่มขึ้นของช่องว่างภายในหัวใจ (p<0.05) โดยสรุปสารสกัดเปลือกมังคุดมีฤทธิ์ต้านความดันเลือดสูงและมีผลต่อการปรับเปลี่ยนโครงสร้างของผนังหัวใจให้ดีขึ้นในภาวะความดันเลือดสูงที่ถูกเหนี่ยวนำด้วยสารแอลเนม

Key Words: L-NAME, Oxidative stress, *Garcinia mangostana*

คำสำคัญ: แอลเนม ภาวะเครียดออกซิเดชัน มังคุด

* *Student, Doctor of Philosophy Program in Anatomy, Faculty of Medicine, Khon Kaen University*
** *Student, Doctor of Philosophy in Medical Physiology, Faculty of Medicine, Khon Kaen University*
*** *Assistant Professor, Department of Physiology, Faculty of Medicine, Khon Kaen University*
**** *Associate Professor, Department of Physiology, Faculty of Medicine, Khon Kaen University*
***** *Assistant Professor, Department of Anatomy, Faculty of Medicine, Khon Kaen University*

Introduction

Hypertension is one of the most important public health problems worldwide and it is a common cause of cardiovascular disease including ischemic heart disease and finally cardiac failure (Black and Elliott, 2007; Cheung and Cheung, 2012). Common to these processes is an increased bioavailability of reactive oxygen species (ROS) (termed oxidative stress) due to excess ROS generation, decreased nitric oxide (NO) levels and reduced antioxidant capacity in the cardiovascular systems. In the cardiovascular system, oxidative stress and NO play a physiological role in controlling endothelial function, regulation of vascular tone, cardiac function, inflammation, proliferation of vascular smooth muscle cells (VSMCs), apoptosis, hypertrophy and fibrosis, all of which are important processes contributing to endothelial dysfunction and cardiovascular remodeling in hypertension (Montezano and Touyz, 2012; Napoli and Ignarro, 2009).

N-nitro-L-arginine methyl ester (L-NAME) is a nonspecific inhibitor of all three NO synthase (NOS) isoforms (neuronal, inducible, and endothelial NOS) (Hlavackova et al., 2011; Shimokawa and Tsutsui, 2010). It has been reported that L-NAME administration can cause increased in blood pressure, increased wall thickness and cross-sectional area of the aorta and decrease of elastin, collagen, actin content in rat model (Hlavackova et al., 2011) and left ventricular fibrosis resulting in cardiac hypertrophy (Paulis et al., 2008). Moreover, the increase in oxidative stress, such as vascular superoxide ($O_2^{\bullet -}$) production and malondialdehyde (MDA) were also reported in L-NAME-induced hypertensive rats (Ferroni et al., 2006; Usui et al., 1999). Oxidative stress promoted VSMCs proliferation, hypertrophy and collagen deposition, leading to thickening of the vascular media and narrowing of the vascular lumen (Grossman, 2008).

In recent years, polyphenols have attracted considerable attention as agents that protect cells or molecules from oxidative myocardial injury. *Garcinia mangostana* Linn., commonly known as "mangosteen" that contains high amounts of xanthones, a class of polyphenolic compounds which were shown to be effective for antioxidant, anti-inflammatory, anti-tumoral, antiviral and anti-obesity properties (Chomnawang et al., 2007; Devi Sampath and Vijayaraghavan, 2007; Jiang et al., 2010; Shibata et al., 2011). However, there is no evidence to support the preventive effects of *Garcinia mangostana* pericarp extract on the structural remodeling of the heart in the hypertensive rat induced by nitric oxide deficiency. Therefore, the aim of this study was to investigate the preventive effect of *Garcinia mangostana* pericarp extract on blood pressure, oxidative stress markers and cardiac hypertrophy in the L-NAME-induced hypertensive rats.

Materials and methods

Preparation of *Garcinia mangostana* (mangosteen)

The pericarp extract of mangosteen was supported by Assistant Professor Dr. Kanokporn Chayaburakul, Department of Anatomy, Faculty of Science, Rangsit University, Thailand. The process of extraction was followed by; the fresh pericarps of mangosteen (1 kg) were blended and boiled with water 1 liter at 70°C for 2 hours, then the supernatant was filtered and dried using spray dry machine. By this procedure the yield is 0.67% (w/w). Dried mangosteen pericarp extract was then packed in tight containers and kept at 4 °C.

Animal and Experimental protocols

Male Sprague-Dawley rats weighing 220-240 g were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom. Animals were housed in stainless steel cages and maintained in an air-conditioned room (25.1±1°C) with 12:12 h light/dark cycle. They were fed with a standard chow diet (Chareon Pokapan Co. Ltd., Thailand) and tap water ad libitum at Northeast Laboratory Animal Center of the Faculty of Medicine, Khon Kaen University. All procedures and experimental protocols were reviewed approved by the Institutional Animal Ethics Committee of Khon Kaen University (AEKKU-NELAC 4/2557).

After one week of acclimatization, rats were randomly divided into 4 groups with 8 animals in each group: (1) Control group receiving tap water, (2) Control+Mag, normotensive group received mangosteen pericarp extract 200 mg/kg/day, (3) L-NAME, hypertensive group received L-NAME (Sigma Chemical Co.) 40 mg/kg/day in their drinking water, and (4) L-NAME+Mag, hypertensive-treated group received L-NAME 40 mg/kg/day and treated with mangosteen pericarp extract 200 mg/kg/day. The pericarp extract of mangosteen was dissolved in distilled water (DW) 1.5 mg/kg/day and administered orally using intragastric tube daily for 5 weeks. The choice of mangosteen pericarp extract dosage used in this study was selected on the basis of previous study in experimental model of animals which shown to be a potent benefit for cardioprotective properties of mangosteen pericarp extract (Devi Sampath and Vijayaraghavan, 2007).

Body weight and general cardiac parameters measurement

The body weight was recorded every week until the end of experiment. After the end of experiment, the animals were sacrificed, their body weight (BW), weight of the heart (HW), left ventricular weight (LVW) were determined, and the LVW/BW ratio was calculated.

Blood pressure measurement

Systolic blood pressure (SBP) was measured non-invasively in conscious animals by using tail-cuff plethysmography (Blood pressure analyzer, model 29; IITC, Woodland Hills, California, USA) every week to assess the development of the L-NAME-induced hypertension during mangosteen treatment. Rats were placed in a heated chamber at an ambient temperature of 30–34 ˚C and restrained inside the cage for 10-15 minutes before blood pressure measurement. The rat tail was placed inside the tail cuff, and the cuff was automatically inflated and released. Then, blood pressure values were detected and recorded. The mean values of three successive measurements were obtained from each rat.

Biochemical analysis

Assay of $O_2^{\bullet-}$ production

Vascular $O_2^{\bullet-}$ production was measured using lucigenin enhanced chemiluminescence method as described previously (Nakmareong et al., 2011). Concisely, the carotid arteries were quickly dissected and cleaned of adherent fat and connective tissue on ice. The vessel segments 3-5 mm were placed in Krebs-KCl buffer and allowed to equilibrate at 37 ˚C for 30 min. Lucigenin was added in sample tube and placed in luminometer (Turner Biosystems, 23 CA, USA). The photon counts were integrated every 30 s for 5 min and averaged. The vessels were dried at 45 ˚C for 24 h, for determination of dry weight. $O_2^{\bullet-}$

production in vascular tissue was expressed as relative light unit counts per minute per milligram of dry tissue weight.

Assay of malondialdehyde

The concentration of plasma MDA was measured as thiobarbituric acid reactive substances by a spectrophotometric method as previously described (Nakmareong et al., 2011). Briefly, 150 µl of plasma samples were reacted with 10 % TCA, 125 µl of 5 mM EDTA, 125 µl of 8 % SDS and 10 µl of 0.5 µl/ml of BHT. The mixture was left for 10 min, then 0.6 % TBA was added in an equal volume and the mixture was heated for 30 min in a boiling water bath. After cooling to room temperature, the mixture was centrifuged 10,000 g for 5 minutes at 25 °C. The absorbance of the supernatant was measured at the wavelength of 532 nm by spectrophotomer. (Amersham Bioscience, Arlington, MA, USA). A standard curve was generated at different concentrations from 0.3 to 10 µmol/L using 1,1,3,3-tetraethoxypropane.

Cardiac morphometry

The hearts were rapidly removed. The atria, right ventricle and clotted blood in the heart chamber were removed. The left ventricle was isolated and bisected coronally at the midventricular position, equidistant between base and apex. Then, the heart was fixed 24 h in 4% paraformaldehyde and dehydrated in graded series of ethanol. The clearing processes were performed with xylene. After clearing with xylene, the specimens were infiltrated in the mixture between xylene and paraffin, finally with pure paraffin and embedded in paraffin block. The specimens from each block were serially cut at 5 µm thick slides from the midventricular surface, either to the base or to the apex. The sections were stained with Hematoxylin and Eosin (H&E) to investigate the general appearance in the heart ventricle. Morphometric evaluation of the heart ventricle was performed by capturing heart images with stereoscope at 1x objective lens (Olympus SZH-ILLD with NIS elements software). The values of left ventricular wall thickness, cross section area and luminal area were evaluated with Image-J NIH image analysis software for cardiac morphometry as follows:

1. The left ventricular wall thickness was measured every 45° interval around the cardiac circumference. The average value was calculated for each section.

2. Cross sectional area was calculated by using the difference between the value of the external circumferential area of the heart and the chamber area.

3. The ventricular luminal area was measured by using the value of the chamber area of the heart.

Statistical analysis

Data was expressed as mean ± S.E.M. 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. The p values<0.05 were considered statistically significant.

Results

Effect of mangosteen pericarp extract on BW

The body weight was observed every week during the experimental period. The values of body weights were not significantly different among experimental groups (Figure 1).

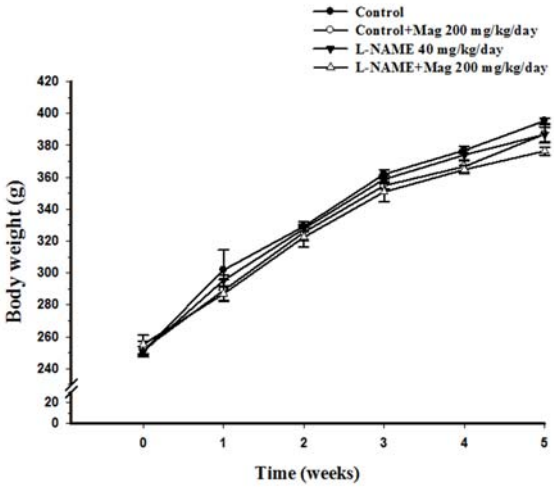

Figure 1 Effect of 5-week L-NAME and mangosteen pericarp extract on body weight. Results are expressed as mean±SEM (n = 5-8/group).

Effect of mangosteen pericarp extract on SBP

At the beginning of the experiment (week 0) SBP were not significantly different among the groups. Administration of L-NAME caused a significantly progressive increase in SBP during 5 weeks of its administration when compared with the control group (p<0.05). SBP in L-NAME-treated with mangosteen pericarp extract group was significantly lower than those in L-NAME group since the first week until the end of the experiment (p<0.05). However, blood pressure of mangosteen-treated group remained higher when compare to the control group (Figure 2).

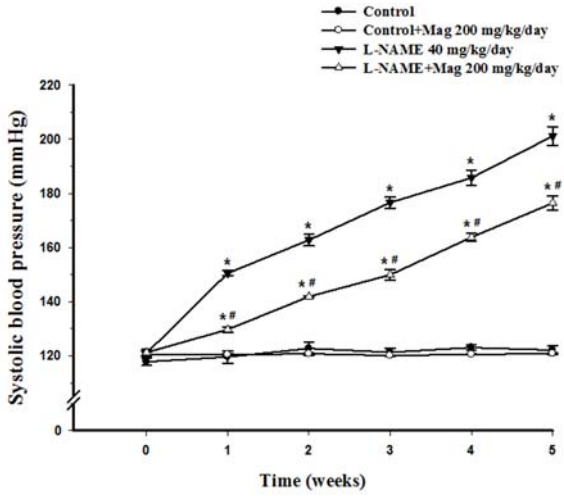

Figure 2 Effect of 5-week L-NAME and mangosteen pericarp extract on systolic blood pressure. Results are expressed as mean±SEM. *p<0.05 vs. control group, $^{\#}p$<0.05 vs. L-NAME group (n = 5-8/group).

Effect of mangosteen on oxidative stress markers

A change of vascular $O_2^{\bullet-}$ production

The vascular $O_2^{\bullet-}$ production in L-NAME-induced hypertensive rats was significantly higher than control rats (p<0.05). Treatment with mangosteen at dose 200 mg/kg/day for 5 weeks significantly attenuated the vascular $O_2^{\bullet-}$ production when compared to hypertensive rats (p<0.05) (Figure 3).

A change of plasma malondialdehyde level

Plasma MDA level was higher in L-NAME-induced hypertensive rats comparing to that of control rats (p<0.05). Administration of mangosteen pericarp extract at dose 200 mg/kg/day significantly decreased the oxidative stress by reducing the level of plasma MDA when compared to hypertensive rats (p<0.05) (Figure 4).

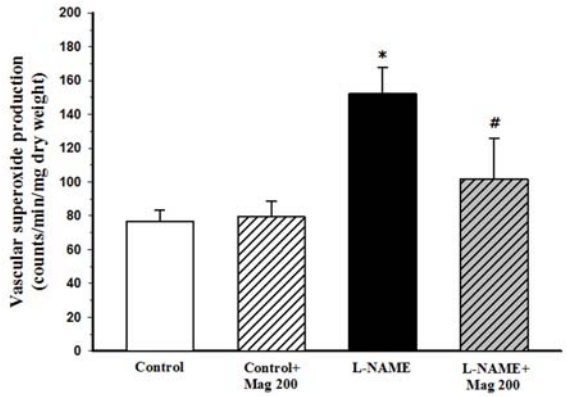

Figure 3 Effect of 5-week L-NAME and mangosteen pericarp extract on superoxide production in the carotid arteries. Results are expressed as mean±SEM. *p<0.05 vs. control group, $^{\#}p$<0.05 vs. L-NAME group (n =5-8/group).

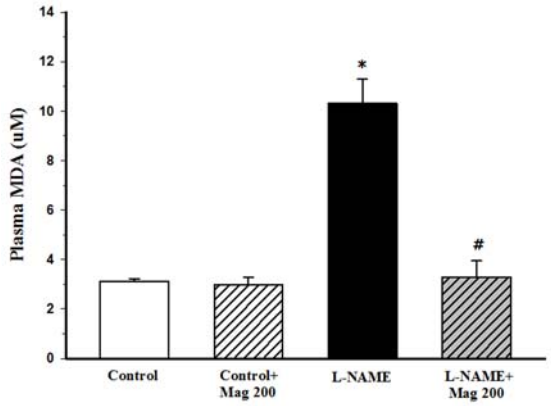

Figure 4 Effect of 5-week L-NAME and mangosteen pericarp extract on plasma MDA. Results are expressed as mean±SEM. *p<0.05 vs. control group, $^{\#}p$<0.05 vs. L-NAME group (n = 5-7/group)

Effect of mangosteen pericarp extract on body weight and general cardiac parameters

There was no significant difference in body weight among all experimental groups. After 5 weeks of L-NAME administration, HW, LVW and LVW/BW ratio were significantly increased when compare to those in the control groups. Administration of L-NAME and treated with mangosteen pericarp extract at dose 200 mg/kg/day caused significant decrease of these parameters when compared to the L-NAME-induced hypertensive group (Table 1).

Effect of mangosteen pericarp extract on wall thickness, cross-sectional area and luminal area of left ventricle

Administration of L-NAME only caused a significant increase in the left ventricular wall thickness and cross-sectional area when compare to the control group. The left ventricular wall thickness and cross-sectional area of L-NAME treated with mangosteen pericarp extract at dose 200 mg/kg/day were significantly lower than those in L-NAME rats. Moreover, after 5 weeks of L-NAME administration, the ventricular luminal area was significantly decreased when compared to the control group. L-NAME-treated with mangosteen pericarp extract at dose 200 mg/kg/day was able to improve the ventricular luminal area when compared to the NO-deficient hypertensive group (Table 2).

Table 1 Effect of mangosteen pericarp extract on general biological parameters of heart

	BW (g)	HW (g)	LVW (g)	LVW/BW (mg/g)
Control	398.25±1.59	1.31±0.01	0.88±0.01	2.22±0.03
Control+Mag	398.14±4.83	1.26±0.02	0.86±0.02	2.16±0.02
L-NAME	386.88±4.58	1.44±0.02*	1.06±0.02*	2.74±0.16*
L-NAME+Mag	378.40±2.34	1.29±0.05$^{\#}$	0.94±0.04$^{\#}$	2.48±0.09*$^{\#}$

Values are mean±SEM. *p<0.05 vs. control group, $^{\#}p$<0.05 vs. L-NAME group (n = 5-8/group)

Table 2 Effect of mangosteen pericarp extract on wall thickness, cross-sectional area and luminal area of left ventricle

	Wall thickness (mm)	CSA (mm^2)	Ventricular luminal area (mm^2)
Control	2.79±0.06	59.50±0.92	9.91±0.61
Control+Mag	2.84±0.05	55.55±1.70	8.25±0.30
L-NAME	3.45±0.12*	72.91±4.31*	6.27±0.39*
L-NAME+Mag	2.90±0.05$^{\#}$	62.81±1.50$^{\#}$	8.31±0.73$^{\#}$

Values are mean±SEM. *p<0.05 vs. control group, $^{\#}p$<0.05 vs. L-NAME group (n = 5-8/group)

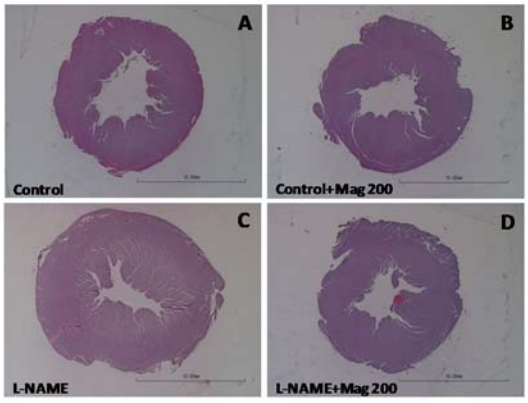

Figure 5 Morphology of left ventricle stained with H&E. Control (A), Control+Mag (B), L-NAME (C) and L-NAME+Mag (D). The heart sections were captured by stereoscope at 1x objective lens. Scale bar = 10 mm.

Discussion

This study examined the preventive effect of mangosteen pericarp extract on blood pressure, oxidative stress profiles and cardiac wall changes in L-NAME 5-week induced hypertension. We found that the animal received L-NAME had an increase of SBP since the first week until the end of the experiment. Moreover, the L-NAME not only did exaggerate the $O_2^{\bullet -}$ production in the blood vessel but also enhanced plasma MDA levels. This finding is consistent with the other observations (Kumar et al., 2012; Nakmareong et al., 2011; Paulis et al., 2008)

It has been known that blood pressure is determined by total peripheral resistance and cardiac output, an increase in one of these factors can cause hypertension. It was well known that NO is a major endothelium derived relaxing factor that induces relaxation of vascular smooth muscle cells (Vanhoutte, 2009). Thus, changes in arterial blood pressure or total peripheral resistance after acute L-NAME administration are connected with keeping balance between vasoconstriction and vasodilatation (Arnal et al., 1999).

In this study, the preventive effect of mangosteen pericarp extract on blood pressure, oxidative stress status and cardiac wall dimension were investigated. Administration of mangosteen pericarp extract 200 mg/kg/day for 5 weeks showed to be a potent benefit to inhibited progression of SBP from the first week until the end of the experiment. This would be the effect of an anti-hypertensive property of its phenolic compound because there is accumulating evidence reported that a phenolic compound provides an anti-hypertensive property (Kumar et al., 2012; Nakmareong et al., 2011; Yilmaz and Usta, 2012). In addition, we found that mangosteen supplementation suppressed oxidative stress biomarkers by reducing vascular $O_2^{\bullet -}$ production and attenuated plasma MDA. These

results indicated that antihypertensive effect of mangosteen pericarp extract may involve its antioxidant capacity because several lines of evidence have shown that antioxidant substances significantly decrease superoxide generation (Kumar et al., 2012; Nakmareong et al., 2011; Yilmaz and Usta, 2012). Therefore, antioxidant properties of mangosteen pericarp extract may decrease vascular tone by enhancing NO bioavailability and contributing to decrease blood pressure. However, in the present study, I did not investigate the effect of mangosteen pericarp extract directly on NO or NO synthesis. Therefore, the further study in this point should be done.

The wall thickness, CSA and luminal area of left ventricle were examined. It revealed that not only did the wall thickness and CSA of left ventricle increased, but also ventricular luminal area in the L-NAME-induced hypertensive rats did decrease. Regarding HW and the relative HW, there was significant difference among groups. These results showed that administration L-NAME at the dose of 40 mg/kg/day for 5 weeks causes an increased in the HW, LVW and the relative LVW. The structural alterations of heart ventricle are concordance with the elevation of blood pressure. These results indicated that the left ventricle of L-NAME-induced hypertensive rats have been remodeled by hypertrophy as cardiac adaptation to maintain the normal cardiac output. The remodeling which was observed in this study could be classified as an inward hypertrophic (Feihl et al., 2008). Similarly to previous reported, hypertension causes cardiovascular remodeling and hypertrophy. This alteration helps to normalized left ventricular and arterial wall stress and compensated for a reduction in myocardial function to preserve a normal cardiac output (Mayet and Hughes, 2003). Interestingly, Mangosteen pericarp extract supplementation has shown to be improve cardiac wall remodeling in NO-deficient rat.

Conclusion

In conclusion, pericarp extract of mangosteen showed to be an antihypertensive effect by attenuated the elevation of blood pressure, improves oxidative stress status and reverse the alterations of cardiac remodeling by decrease ventricular wall thickness and CSA together with improve ventricular luminal area leading to decrease cardiac hypertrophy. This could be its antioxidant property that related to the alleviation of oxidative stress. However, a specific mechanism of the myocardial remodeling need to be further investigated.

Acknowledgements

Pattanapong Boonprom holds a scholarship from Graduate School (571H220), Khon Kaen University, Thailand. The authors thank Assistant Professor Dr. Kanokporn Chayaburakul, Department of Anatomy, Faculty of Science, Rangsit University, for supporting the pericarp extract of mangosteen.

References

Arnal JF, Dinh-Xuan AT, Pueyo M, Darbladea B, Rami J: Endothelium-derived nitric oxide and vascular physiology and pathology. Cell Mol Life Sci 1999; 55: 1078-1087.

Black HR, Elliott WJ. Hypertension a companion to Braunwald's heart disease, Third edition 2007.

Cheung BMY, Cheung TT. Challenges in the management of hypertension in Asia. In:

European Heart Journal Supplements; 2012. p. A37-A38.

Chomnawang MT, Surassmo S, Nukoolkarn VS, Gritsanapan W. Effect of Garcinia mangostana on inflammation caused by Propionibacterium acnes. Fitoterapia 2007; 78: 401-8.

Devi Sampath P, Vijayaraghavan K. Cardioprotective effect of alpha-mangostin, a xanthone derivative from mangosteen on tissue defense system against isoproterenol-induced myocardial infarction in rats. J Biochem Mol Toxicol 2007; 21: 336-9.

Feihl F, Liaudet L, Levy BI, Waeber B. Hypertension and microvascular remodelling. Cardiovasc Res 2008; 78: 274-85.

Ferroni P, Basili S, Paoletti V, Davi G. Endothelial dysfunction and oxidative stress in arterial hypertension. Nutr Metab Cardiovasc Dis 2006; 16: 222-33.

Grossman E. Does increased oxidative stress cause hypertension? Diabetes Care 2008; 31 Suppl 2: S185-9.

Hlavackova L, Janegova A, Ulicna O, Janega P, Cerna A, Babal P. Spice up the hypertension diet-curcumin and piperine prevent remodeling of aorta in experimental L-NAME induced hypertension. Nutr Metab (Lond) 2011; 8: 72.

Hlavackova L, Vrankova S, Janega P, Pechanova O, Babal P. The effect of indapamide on development of myocardial hypertrophy and fibrosis in L-NAME-induced hypertension in rat. Physiol Res 2011; 60: 845-52.

Jiang HZ, Quan XF, Tian WX, Hu JM, Wang PC, Huang SZ, et al. Fatty acid synthase inhibitors of phenolic constituents isolated from Garcinia mangostana. Bioorg Med Chem Lett 2010; 20: 6045-7.

Kumar S, Prahalathan P, Raja B. Syringic acid ameliorates L-NAME-induced hypertension by reducing oxidative stress. Naunyn Schmiedebergs Arch Pharmacol 2012; 385(12): 1175-84.

Mayet J, Hughes A. Cardiac and vascular pathophysiology in hypertension. Heart 2003; 89: 1104-1109.

Montezano AC, Touyz RM. Molecular mechanisms of hypertension-reactive oxygen species and antioxidants: a basic science update for the clinician. Can J Cardiol 2012; 28: 288-95.

Nakmareong S, Kukongviriyapan U, Pakdeechote P, Donpunha W, Kukongviriyapan V, Kongyingyoes B, et al. Antioxidant and vascular protective effects of curcumin and tetrahydrocurcumin in rats with L-NAME-induced hypertension. Naunyn Schmiedebergs Arch Pharmacol 2011; 383: 519-29.

Napoli C, Ignarro LJ. Nitric oxide and pathogenic mechanisms involved in the development of vascular diseases. Arch Pharm Res 2009; 32: 1103-8.

Paulis L, Matuskova J, Adamcova M, Pelouch V, Simko J, Krajcirovicova K, et al. Regression of left ventricular hypertrophy and aortic remodelling in NO-deficient hypertensive rats: effect of L-arginine and spironolactone. Acta Physiol (Oxf) 2008; 194: 45-55.

Shibata M, Linuma M, Morimoto J, Kurose H, Akamatsu K, Okuno Y, et al. a-Mangostin extracted from the pericarp of the mangosteen (Garcinia mangostana Linn) reduces tumor growth and lymph node metastasis in an immunocompetent xenograft model of metastatic mammary cancer carrying a p53 mutation. BMC Medicine 2011; 69.

Shimokawa H, Tsutsui M. Nitric oxide synthases in the pathogenesis of cardiovascular disease: lessons from genetically modified mice. Pflugers Arch 2010; 459: 959-67.

Usui M, Egashira K, Kitamoto S, Koyanagi M, Katoh M, Kataoka C, et al. Pathogenic role of oxidative stress in vascular angiotensin-converting enzyme activation in long-term blockade of nitric oxide synthesis in rats. Hypertension 1999; 34: 546-51.

Vanhoutte PM. Endothelial dysfunction: the first step toward coronary arteriosclerosis. Circ J 2009; 73: 595-601.

Yilmaz B, Usta C. Ellagic Acid-Induced Endothelium-Dependent and Endothelium-Independent Vasorelaxation in Rat Thoracic Aortic Rings and the Underlying Mechanism. Phytother Res 2012