Vascular and Antioxidant Effects of an Aqueous *Mentha cordifolia* Extract in Experimental N^G-Nitro-L-arginine Methyl Ester-Induced Hypertension

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The effect of an aqueous *Mentha cordifolia* (MC) extract on the haemodynamic status, vascular remodeling, function, and oxidative status in N^G-nitro-L-arginine methyl ester (L-NAME)-induced hypertension was investigated. Male Sprague-Dawley rats were given L-NAME [50 mg/(kg body weight (BW) d)] in their drinking water for 5 weeks and were treated by intragastric administration with the MC extract [200 mg/(kg BW d)] for 2 consecutive weeks. Quercetin [25 mg/(kg BW d)] was used as a positive control. The effects of the MC extract on the haemodynamic status, thoracic aortic wall thickness, and oxidative stress markers were determined, and the vasorelaxant activity of the MC extract was tested in isolated mesenteric vascular beds in rats. Significant increases in the mean arterial pressure (MAP), heart rate (HR), hind limb vascular resistance (HVR), wall thickness, and cross-sectional area of the thoracic aorta, as well as oxidative stress markers were found in the L-NAME-treated group compared to the control ($P < 0.05$). MAP, HVR, wall thickness, cross-sectional area of the thoracic aorta, plasma malondialdehyde (MDA), and vascular superoxide anion production were significantly reduced in L-NAME hypersensitive rats treated with the MC extract or quercetin. Furthermore, the MC extract induced vasorelaxation in the pre-constricted mesenteric vascular bed with intact and denuded endothelium of normotensive and hypertensive rats. Our results suggest that the MC extract exhibits an antihypertensive effect via its antioxidant capacity, vasodilator property, and reduced vascular remodeling.

Key words: Hypertension, Vasodilator Property, *Mentha cordifolia*

Introduction

NO-deficient hypertension induced by chronic nitric oxide synthase inhibition in animals has been widely used as an animal model for hypertension (Jover *et al.*, 1993; Ribeiro *et al.*, 1992). In such animal models, cardiovascular alterations have been reported, including left ventricular hypertrophy, tachycardia, an increase in peripheral vascular resistance (Loeb and Longnecker, 1992), aortic stiffness, and vascular remodeling (Nakmareong *et al.*, 2012; Pereira *et al.*, 2004). Subsequently, an increase in oxidative stress with imbalance between antioxidants and reactive oxygen species (ROS) production was found in rats after N^G-nitro-L-arginine methyl ester (L-NAME) administration (Aventin *et al.*, 2002; Nakmareong *et al.*, 2012; Tsukahara *et al.*, 2000).

Nowadays, hypertension treatment is primarily a long-term drug therapy. Health promotion and disease prevention are encouraged by positive lifestyle behaviours and nutritional care. Dietary intake of traditional medicinal plants has been used in the treat-

ment of many diseases including arterial hypertension. Available evidence indicates that chronic oral administration of quercetin, a flavonoid with antioxidant properties, has preventive effects on the development of hypertension in L-NAME-induced hypertensive rats (Perez-Vizcaino *et al.*, 2009). Plants of the mint family (Lamiaceae) have been shown to have a high content of total polyphenols that have antioxidant properties (McKay and Blumberg, 2006; Pakdeechote *et al.*, 2011). It has been reported that *Mentha cordifolia* Opiz ex Fresen. contains menthalactone, a pain-relieving substance (Villasenor and Sanchez, 2009), and, furthermore, that it has antimutagenic activity (Villasenor *et al.*, 2002). A protective effect of *M. cordifolia* against the development of hypertension induced by L-NAME has also been demonstrated (Pakdeechote *et al.*, 2011), however, therapeutic effects of the aqueous *Mentha cordifolia* (MC) extract on L-NAME-induced hypertension have not been evaluated in detail. The aim of our present study was to investigate the effect of mint extract on the haemodynamic status in L-NAME-induced hypertension in a rat model. Vascular effects and antioxidant properties of *M. cordifolia* were also assessed.

Materials and Methods

Preparation of an aqueous extract from Mentha cordifolia

Fresh leaves of *M. cordifolia* were collected from a local farm in Khon Kaen province, Thailand, weighed, chopped into small pieces, and heated in distilled water to 95 °C for 30 min. The water extract was filtered, evaporated in a vacuum evaporator, and then lyophilized. The extract yield was approximately 2.4 % by the fresh weight of the leaves. The powdered MC extract was kept in a tight, light-protected container and stored at −20 °C until used. It was dissolved in distilled water before use. The mint extract was administered intragastrically to the rats at a volume of 0.15 ml/100 g body weight (BW).

Animals

Male Sprague-Dawley rats, weighing 220 – 225 g, were obtained from the Animal Care Unit, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand. All animals were maintained in a temperature-controlled room at 24 °C with a 12-h dark/12-h light cycle. The animals were given free access to standard chow diet (Chareon Pokapan, Bangkok, Thailand) and distilled water (DW) or L-NAME [50 mg/(kg BW d)] in DW. Hypertension was induced in rats by administering L-NAME [50 mg/(kg BW d)] in their drinking water for 5 weeks, whereas the control rats received only DW. All animal procedures were reviewed and approved by the Institutional Animal Ethics Committee of Khon Kaen University (AEKKU 20/2551). Animals were weighed throughout the study period.

Experimental design

Rats were randomly divided into five groups. In the control group, rats received DW for 5 weeks which was intragastrically administered as a vehicle at 0.15 ml/(100 g BW d) for 2 consecutive weeks. In the normotensive MC extract-treated group, rats received DW for 5 weeks and the MC extract [200 mg/(kg BW d)] was intragastrically administered for 2 consecutive weeks. In the hypertensive control group, rats received L-NAME [50 mg/(kg BW d)] in their drinking water for 5 weeks and DW was intragastrically administered as a vehicle at 0.15 ml/(100 g BW d) for 2 consecutive weeks. In the hypertensive MC extract-treated group, rats received L-NAME [50 mg/(kg BW d)] in their drinking water for 5 weeks and the MC extract [200 mg/(kg BW d)] was intragastrically administered for 2 consecutive weeks. In the hypertensive quercetin-treated group, L-NAME hypertensive rats were fed quercetin [25 mg/(kg BW d)] for 2 consecutive weeks, and this group served as a positive control. The dose of the MC extract used in this study is based on our previous study in which the MC extract at 200 mg/(kg BW d) partially inhibited the increase in blood pressure of L-NAME-treated rats (Pakdeechote *et al.*, 2011), while the quercetin dose is based on the report on its antihypertensive and antioxidant effects in hypertensive rats (Monteiro *et al.*, 2012). Systolic blood pressure (SP) of animals was measured weekly using non-invasive tail-cuff plethysmography (IITC/Life Science Instrument model 229 and model 179 amplifier; Woodland Hills, CA, USA).

Sample collection and haemodynamic assessments

Rats were anaesthetized by peritoneal injection of sodium pentobarbital (60 mg/kg BW) and placed on a heating pad. Subsequently, a tracheotomy was made to assist respiration. The femoral artery was cannulated with a polyethylene tube. SP, diastolic blood pressure (DP), mean arterial pressure (MAP), and heart rate

(HR) were continuously monitored by way of pressure transducers and recorded using the Acknowledge Data Acquisition and Analysis Software (Biopac Systems, Santa Barbara, CA, USA). The abdominal aorta was carefully separated from the abdominal vein, cleaned of connective tissue, and fitted with a flow probe to detect hind limb blood flow [HBF; in ml/(min 100 g tissue)] with an electromagnetic flowmeter (Carolina Medical Electronics, Carolina, NC, USA). Hind limb vascular resistance [HVR; in mmHg/(ml min 100 g tissue)] was calculated as MAP divided by HBF. Blood samples were collected via the abdominal aorta for biochemical assays. Carotid arteries (about 2 cm in length) were cut out rapidly from animals for assessing superoxide production.

Morphometric measurement

The thoracic aorta was perfused with 4% (w/v) paraformaldehyde, cleaned of surrounding adipose and connective tissue, and fixed in Bouin's solution. The material was then processed routinely in paraffin, and serial 5-μm thick sections (8 sections per animal) were stained with hematoxylin and eosin. Sections were captured with a Digital sight DS-2MV instrument (Nikon, Tokyo, Japan). For morphometric evaluation, wall thickness, cross-sectional area, and luminal diameter were determined using ImageJ, NIH image analysis software (the National Institutes of Health, Bethesda, MD, USA).

Vasorelaxant effects

The vasorelaxant activity of the MC extract was determined using the perfused mesenteric vascular bed of normotensive and L-NAME hypertensive rats. The mesenteric circulation, which is the largest vascular bed, influences the peripheral resistance. Rats treated with L-NAME [50 mg/(kg BW d)] for 3 weeks and having an SP higher than 150 mmHg, were considered to be hypertensive and were used in this test. Hypertensive rats were anaesthetized with sodium pentobarbital [60 mg/kg BW, intraperitoneal injection] followed by exsanguination. The abdominal cavity was opened, and the main branch of the superior mesenteric artery was identified, cleaned of connective tissue, and cannulated with a blunted hypodermic needle (no. 21). The superior mesenteric vein was cut, and preparations were flushed gently with Kreb's solution (0.5 ml) (Luangaram *et al.*, 2007). Subsequently, the mesenteric vascular bed was separated from the gut by carefully cutting close to the intestinal wall. The mesenteric bed preparation was placed on a stainless steel grid (7 cm $\times$ 5 cm) in a warm humid chamber (37 °C) and perfused at a constant flow rate of 5 ml/min, using a peristaltic pump (07534-04; Cole-Palmer, Vernon Hills, IL, USA). Kreb's solution is composed as follows (mM): NaCl (118), $NaHCO_3$ (25), KCl (4.8), KH_2PO_4 (1.2), $MgSO_4 \cdot 7H_2O$ (1.2), $CaCl_2$ (1.25), and glucose (11.1). The solution was maintained at 37 °C and continually bubbled with a 95% O_2/5% CO_2 gas mixture. Mesenteric vascular responses were detected as changes in the perfusion pressure (in mmHg). Mean perfusion pressure was monitored using a pressure transducer and data recorded using the software from Biopac systems. The preparation was allowed to equilibrate for 30 min before experimentation. Vascular endothelium was chemically removed using 1.8 mg/ml sodium deoxycholate (SD) in saline for 30 s (Iwatani *et al.*, 2008). SD produces a transient increase in perfusion pressure (20 – 30 mmHg) (Takenaga and Kawasaki, 1999). The preparation was subjected to a 30-min washout period and the tone raised with 5 – 7 μM methoxamine, an α_1-adrenoceptor agonist. A bolus injection of 1 nmol acetylcholine through rubber tubing proximal to the tissue was performed in order to ensure the normal endothelial function. Under conditions of raised tone, high vascular tone was maintained for 3 h providing time control data. Under conditions of methoxamine-raised tone, the vasorelaxant effect of the MC extract was tested in normal and hypertensive rat mesenteric beds with or without endothelium. MC extract was added to the perfusate in a cumulative manner, with doses of 0.01, 0.03, 0.1, 0.3, and 1 mg/ml. Thereafter, the percentage of vasorelaxation was calculated.

Biochemical assays

The level of plasma malondialdehyde (MDA), a lipid peroxidation indicator, was examined by measuring the thiobarbituric acid-reactive substance using a spectrometric method described previously (Luangaram *et al.*, 2007). In brief, plasma samples were reacted with 10% (w/v) trichloroacetic acid, 5 mM ethylenediamine tetraacetic acid, 8% sodium dodecylsulfate, 0.5 μg/ml of butylated hydroxytoluene, and 0.6% thiobarbituric acid, and the mixture was boiled for 30 min. After cooling to room temperature, the absorbance of the supernatant was measured at 532 nm. Results were expressed according to the standard curve obtained with 1,1,3,3-tetraethoxypropane

(0.3 – 10 μmol/l). The production of superoxide anion in carotid arteries was determined by a lucigenin-enhanced chemiluminescence method described previously (Luangaram *et al.*, 2007; Sompamit *et al.*, 2009). In brief, the arterial segment was carefully cleaned and incubated in 1 ml oxygenated Krebs-Ringer bicarbonate solution at 37 °C for 30 min. The chemiluminescence signal was measured after the addition of lucigenin (30 μM) and counted in a luminometer (Turner Biosystems, Sunnyvale, CA, USA). The photon counts were integrated every 15 s for 5 min and averaged. The vessels were then dried for 24 h at 45 °C and weighed. Superoxide anion production in the arterial tissues is expressed as relative light unit counts/(mg dry weight min).

Statistical analysis

Data are presented as mean ± SEM. Statistical comparisons between groups were made using one-way analysis of variance (ANOVA) with a post-hoc Duncan's multiple range test. The vasorelaxant effect of the MC extract at each concentration was determined by repeatedly applied ANOVA with Duncan's test. A value of ($P < 0.05$) was taken to indicate statistical significance.

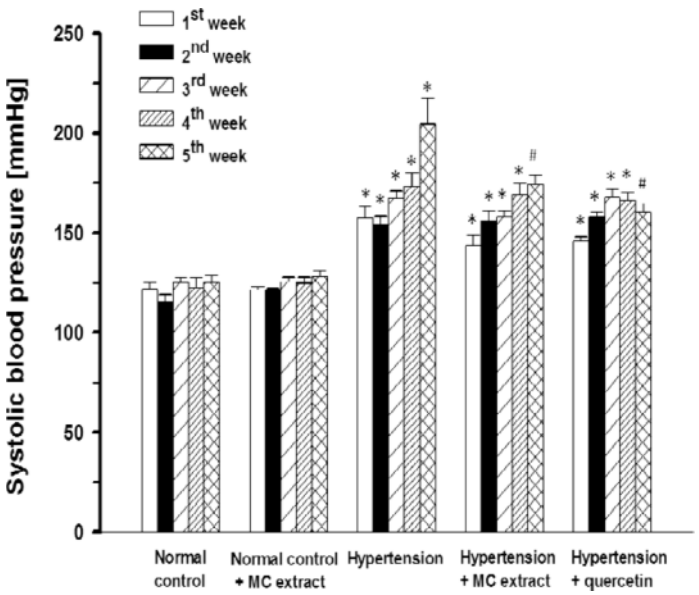

Fig. 1. Effects of MC extract and quercetin on systolic blood pressure in control rats, normal rats with MC extract, L-NAME-treated rats (hypertension), L-NAME-treated rats with MC extract (hypertension + MC extract), and L-NAME-treated rats with quercetin (hypertension + quercetin). * $P < 0.05$ *vs.* normal control; # $P < 0.05$ *vs.* hypertension; † $P < 0.05$ *vs.* hypertension + MC extract ($n = 8 – 10$/group).

Table I. Effects of MC extract and quercetin on blood pressure, HBF, HVR, and HR in all experimental groups ($n = 8 – 10$).

Parameter	Normal control	Normal control + MC extract	Hypertension + vehicle	Hypertension + MC extract	Hypertension + quercetin
SP [mmHg]	133.2 ± 1.8	129.1 ± 2.7	231.5 ± 6.9*	190.4 ± 6.7*#	170.6 ± 4.0*#†
DP [mmHg]	83.4 ± 5.5	75.0 ± 5.6	152.0 ± 9.8*	125.7 ± 9.1*#	117.7 ± 3.81*#
MAP [mmHg]	102.8 ± 2.7	96.4 ± 3.9	184.3 ± 8.7*	158.6 ± 6.4*#	135.3 ± 3.8*#†
HBF [ml/(100 g tissue min)]	8.3 ± 0.8	7.7 ± 0.7	3.7 ± 1.0*	6.2 ± 1.0*#	7.47 ± 0.4*#
HVR [mmHg/(min 100 g ml)]	13.1 ± 1.7	13.3 ± 1.1	47.7 ± 9.9*	24.4 ± 6.4*#	18.43 ± 1.1*#
HR [beat/min]	321.0 ± 7.7	349.8 ± 9.9	403.6 ± 12.5*	382.0 ± 9.9*	358.0 ± 4.5#

* $P < 0.05$ *vs.* normal control; # $P < 0.05$ *vs.* hypertension; † $P < 0.05$ *vs.* hypertension + MC extract.

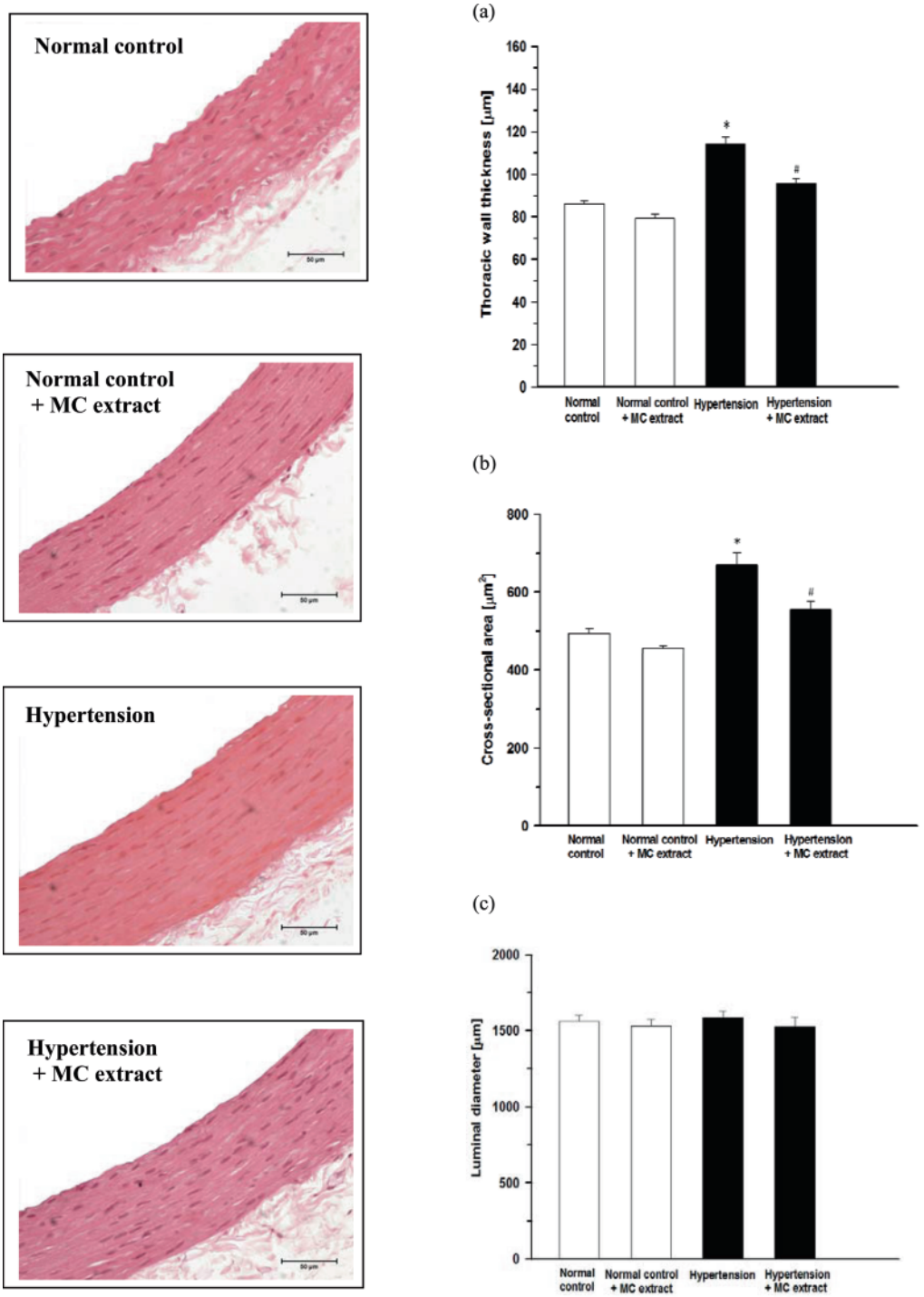

Fig. 2. Effects of MC extract on structural modification in the aorta of L-NAME-induced hypertensive rats. Left panel, representative micrographs of thoracic aortic samples (scale bar, 50 μm); right panel, (a) thoracic wall thickness, (b) cross-sectional area, and (c) luminal diameter. * $P < 0.05$ *vs*. normal control; $^{\#}$ $P < 0.05$ *vs*. hypertension ($n = 7$/group).

Results

Effects of L-NAME, MC extract, and quercetin on body weight

L-NAME treatment for 5 weeks resulted in a significant reduction in the body weight [(293.4 ± 9.4) g] compared to that of control rats [(334.9 ± 6.1) g] ($P < 0.05$). MC extract increased the body weight in the hypertensive group [(330.1 ± 5.9) g] but not in the normal control group [(317.8 ± 4.7) g]. Moreover, quercetin significantly increased the body weight in hypertensive rats [(359.4 ± 6.7) g] ($P < 0.05$).

Lowering hypertension in L-NAME-induced hypertensive rats (conscious rats) by MC extract and quercetin

Daily oral administration of L-NAME resulted in a progressive increase in SP measured by tail-cuff plethysmography. After 5 weeks of L-NAME treatment, SP was significantly higher than in control rats [(204.3 ± 4.7) *vs.* (125.1 ± 3.5) mmHg] ($P < 0.05$). Treatment with the MC extract for 2 consecutive weeks significantly decreased SP of L-NAME-induced hypertensive rats [(174.3 ± 4.7) mmHg] compared to the hypertensive group ($P < 0.05$). Moreover, MC extract had no effect on SP of normal control rats [(126.3 ± 4.3) mmHg]. A significant reduction in SP [(160.3 ± 4.5) mmHg] in hypertensive rats treated with quercetin was also observed (Fig. 1).

Effects of MC extract and quercetin on haemodynamic changes in L-NAME-induced hypertension

SP, DP, and MAP were significantly increased following 5 weeks of L-NAME treatment compared to the respective values of the normal control group ($P < 0.001$). Concomitant treatment with the MC extract for 2 consecutive weeks of L-NAME-treated rats resulted in significant decreases of SP, DP, and MAP compared to the respective values of the hypertensive group ($P < 0.001$). HBF of hypertensive rats was lower than that of normal rats ($P < 0.05$) due to a significant increase in HVR. Daily treatment with the MC extract for 2 weeks also markedly reduced HVR and hence increased HBF in L-NAME-induced hypertensive rats ($P < 0.05$). Besides, a significant increase in HR was not changed by the MC extract in L-NAME-treated hypertensive rats. Our results confirm that the MC extract had no hypotensive effect. Interestingly, quercetin markedly improved all haemodynamic alterations found in L-NAME hypertensive rats ($P < 0.05$) (Table I).

Effects of MC extract on the thoracic wall in L-NAME-induced hypertensive rats

Thoracic wall thickness and cross-sectional area (CSA) were significantly increased in the hyperten-

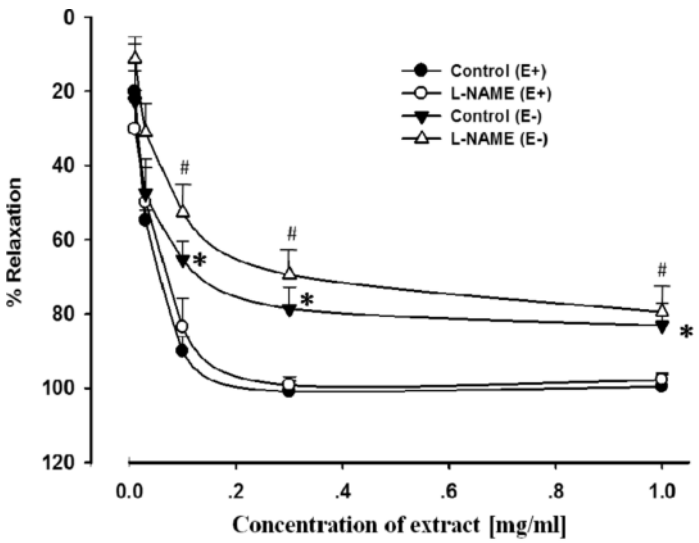

Fig. 3. Dose-response curves of the relaxation effect of MC extract (0.01, 0.03, 0.1, 0.3, and 1 mg/ml) in rat mesenteric vascular beds with (E+) and without endothelium (E−), prepared from normal control and L-NAME-induced hypertensive rats, respectively. * $P < 0.05$ *vs.* normal rats (E+); # $P < 0.05$ *vs.* L-NAME (E+) ($n = 7$/group).

sive group compared to the respective values of normal controls ($P < 0.05$). MC extract decreased the thickness of the thoracic wall as well as their CSA ($P < 0.05$) (Figs. 2a and 2b). However, there were no significant differences in lumen diameter among the groups (Fig. 2c).

Vasodilator effect of MC extract on rat perfused mesenteric vascular beds

After methoxamine had raised the tone conditions, mesenteric vascular tone was stable for over 3 hours. This observation provided the time control data. MC

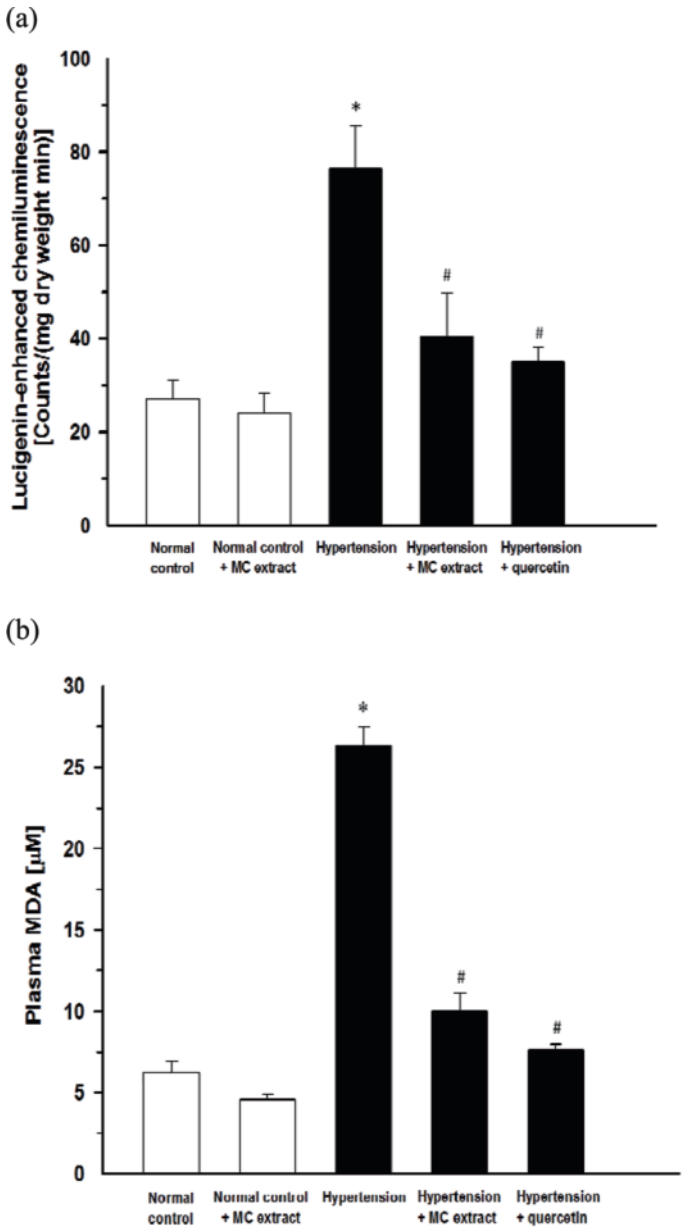

Fig. 4. Effects of MC extract and quercetin on (a) superoxide anion production in carotid arteries and (b) plasma MDA levels in all groups of rats. * $P < 0.05$ *vs.* control; # $P < 0.05$ *vs.* hypertension ($n = 8 - 10$/group).

extract caused vasodilation in the rat perfused mesenteric vascular bed of both animal groups at doses of 0.1, 0.3, and 1 mg/ml ($P < 0.05$). Endothelium removal partially inhibited the vasorelaxant effect of the MC extract, and this inhibitory effect was more prominent in the hypertensive group than in the control group (Fig. 3).

Effects of MC extract and quercetin against oxidative stress in L-NAME-induced hypertension

In L-NAME-induced hypertensive rats, significant increases in superoxide anion production in carotid arteries [(74.3 ± 6.7) counts/(mg dry weight min)] and plasma MDA level [$(26.3 \pm 1.1)\,\mu$M] were observed in comparison to values found in the control group [(29.4 ± 2.3) counts/(mg dry weight min) and $(6.2 \pm 0.6)\,\mu$M, respectively] ($P < 0.001$). In the MC extract-treated hypertensive group, the elevation of superoxide anion production and plasma MDA levels was significantly reduced towards the values of normal rats [superoxide anion production, (44.2 ± 5.8) counts/(mg dry weight min); MDA, $(10.0 \pm 1.1)\,\mu$M] ($P < 0.001$). Moreover, quercetin treatment significantly lowered the levels of superoxide anion production [(35.0 ± 2.5) counts/(mg dry weight min)] and plasma MDA [$(7.6 \pm 0.3)\,\mu$M] compared to the values of the hypertensive group ($P < 0.001$) (Figs. 4a and 4b).

Discussion

In the present study, we examined therapeutic effects of the MC extract against alterations of haemodynamics, vascular remodeling as well as oxidative stress profiles in L-NAME-induced hypertension in rats. Furthermore, the vasorelaxant activity of the MC extract on the perfused mesenteric bed under raised tone conditions was examined. Consistent with a previous report (Hu *et al.*, 2005), L-NAME treatment for 5 weeks caused a reduction in rat body weight, which was counteracted by the MC extract. Daily administration of L-NAME for 5 weeks caused a sustained high blood pressure associated with increases in HR and HVR. MC extract reduced BP and HVR in these hypertensive rats. Thoracic aorta remodeling was found in L-NAME hypertension which was normalized by the MC extract. The results of biochemical assays showed the antioxidant capacity of the MC extract by reducing plasma MDA content and vascular tissue superoxide production in L-NAME-induced hypertensive rats. In the perfused mesenteric vascular bed prepared from hypertensive and control rats, MC extract caused vasorelaxation in all preparations. Additionally, quercetin exhibited an antihypertensive effect that was associated with decreases in HVR and HR in hypertensive rats, and furthermore, quercetin reduced the levels of oxidative stress markers in these rats.

Two main factors affecting blood pressure are cardiac output and total peripheral resistance. Under the conditions of this study, these two factors were raised, since there was an elevation of blood pressure with an increase in HVR in rats that had received L-NAME. NO-dependent endothelium vasodilation to control the vascular tone has been proposed (Furchgott and Zawadzki, 1980). In fact, blockade of NO release with L-NAME, a NO synthase inhibitor, produced systemic vasoconstriction, an increase in vascular resistance and arterial blood pressure (Arnal *et al.*, 1992; Gardiner *et al.*, 1990; Hu *et al.*, 2005; Rees *et al.*, 1989). In addition, an increased HR, which determines cardiac output, was also observed in rats treated with L-NAME. It has been suggested that an increase in sympathetic outflow has an important role in mediating the chronotropic effect in response to chronic administration of L-NAME in rats (Vasquez *et al.*, 1994). Daily administration of L-NAME to rats for six days induced an increase in both blood pressure and HR. These effects were associated with the overactivity of the central sympathetic tone (Cunha *et al.*, 1993). Autonomic nerve blockage abolished L-NAME-induced rat tachycardia (Souza *et al.*, 2001). Moreover, the increase in blood pressure after L-NAME administration was accompanied by the hypertropic-outward remodeling of thoracic aorta. Hypertension induced by chronic inhibition of L-NAME was characterized by vascular remodeling, judging by an increase in wall thickness, cross-sectional area of vascular wall, and wall to lumen ratio (Pereira *et al.*, 2004). An increased ROS production in hypertension has been proposed as the possible mechanism mediating vascular remodeling (Xu and Touyz, 2006).

MC extract significantly reduced SP, DP, and MAP in L-NAME-treated rats, suggesting that this extract could lower the vascular resistance. This finding was supported by an improvement of HBF as well as HVR in the hypertensive rats treated with the MC extract. This study provided evidence that the MC extract improve vascular remodeling, which might be related to its beneficial effect of reducing the severity of oxidative stress under L-NAME-induced hypertension. The vasorelaxant effect of the MC extract

was confirmed in this study using the rat perfused mesenteric vascular bed under conditions of raised tone. Changes in the mesenteric vascular bed diameters obviously determine peripheral vascular resistance (Mulvany and Aalkjaer, 1990). The vasorelaxant effect of the MC extract in the preparation with the intact endothelium was greater than in that without, suggesting that the MC extract caused vasodilatation in the mesenteric vascular bed by affecting both vascular endothelium-dependent and -independent pathways. The precise mode of the extract's vasorelaxant action remains unclear. Other studies with plants of the mint family showed that leaf extracts from *M. arvensis* exhibited the vasorelaxing effect in the aortic ring, and that this effect was partially inhibited in the ring without endothelium. However, in mesenteric preparations, inhibition of NO and prostanoid production abolished the vasorelaxant activity of *M. arvensis* (Runnie *et al.*, 2004). Furthermore, the hypotensive and vasodilator effects of *Mentha* x *villosa* were reported to be mediated by both endothelium-dependent and endothelium-independent pathways (Guedes *et al.*, 2004).

The flavonoid quercetin, a potent antioxidant agent, is found in a variety of fruits and vegetables, such as onions, broccoli, apples, berries, and red grapes. Reduction of high blood pressure by quercetin supplementation has been proven in various animal models of hypertension. Such an effect of quercetin has been related to the prevention of cardiac and renal hypertrophy, endothelium-dependent dysfunction, and a reduction of oxidative stress in L-NAME-induced hypertensive rats (Duarte *et al.*, 2002). Furthermore, in spontaneously hypertensive rats, quercetin treatment reduced the blood pressure and oxidative stress markers and improved the baroreflex sensitivity (Monteiro *et al.*, 2012).

Increased oxidative stress leading to vascular endothelial dysfunction, vascular remodeling, and hypertension has been observed in L-NAME-induced hypertensive rats (Torok, 2008; Xu and Touyz, 2006), and we also observed an increase in oxidative stress markers, plasma MDA, and vascular tissue superoxide production in the L-NAME-induced hypertensive rats. It is well established that increased superoxide levels contribute to the development of hypertension by affecting the NO bioavailability, since NO reacts with superoxide to produce peroxynitrite. Nakmareong *et al.* (2012) found that the plasma nitrate/nitrite concentration was reduced in L-NAME-treated hypertensive rats, and this was accompanied by the downregulation of eNOS expression in aortic tissues. MC extract treatment restored the plasma MDA level and carotid tissue superoxide production in L-NAME-induced hypertensive rats. This finding is consistent with our previous study on the protective effect of the MC extract in L-NAME-induced hypertension. The extracts of *M. cordifolia* and other species of the mint family are rich in antioxidant phenolic compounds (McKay and Blumberg, 2006; Pakdeechote *et al.*, 2011) which increase the NO availability and thus improve the vascular function. Quercetin is a therapeutic agent against oxidative stress in L-NAME-induced hypertensive rats, as demonstrated in the present study and in agreement with our previous finding that this compound suppresses plasma MDA and superoxide anion production in phenylhydrazine-induced oxidative stress in rats (Luangaram *et al.*, 2007). Recently, a vascular protective effect of quercetin in lipopolysaccharide-induced endotoxaemia and vascular dysfunction in mice has been shown to be associated with its antioxidant activity (Kukongviriyapan *et al.*, 2012).

In conclusion, the present study demonstrates that the MC extract has an antihypertensive effect in L-NAME-treated rats by reducing the vascular resistance. This action may involve a reduction in vascular remodeling and vasorelaxation of the mesenteric vascular bed. This cardiovascular improvement brought about by the MC extract appears to be related to its antioxidant properties, since quercetin, a positive control agent, likewise exerts antihypertensive and antioxidant effects. Thus the antioxidant capacity of the MC extract may provide a non-therapeutic treatment option for hypertension.

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Arnal J. F., Warin L., and Michel J. B. (1992), Determinants of aortic cyclic guanosine monophosphate in hypertension induced by chronic inhibition of nitric oxide synthase. J. Clin. Invest. **90**, 647 – 652.

Aventin A., Espadaler M., Casas S., Duarte J., Nomdedeu J., and Sierra J. (2002), Chromosome 16 inversion-associated translocations in acute myeloid leukemia elucidated using a dual-color CBFB DNA probe. Cancer Genet. Cytogenet. **134**, 142 – 144.

Cunha R. S., Cabral A. M., and Vasquez E. C. (1993), Evidence that the autonomic nervous system plays a major role in the L-NAME-induced hypertension in conscious rats. Am. J. Hypertens. **6**, 806 – 809.

Duarte J., Jimenez R., O'Valle F., Galisteo M., Perez-Palencia R., Vargas F., Perez-Vizcaino F., Zarzuelo A., and Tamargo J. (2002), Protective effects of the flavonoid quercetin in chronic nitric oxide deficient rats. J. Hypertens. **20**, 1843 – 1854.

Furchgott R. F. and Zawadzki J. V. (1980), The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature **288**, 373 – 376.

Gardiner S. M., Compton A. M., Bennett T., Palmer R. M., and Moncada S. (1990), Control of regional blood flow by endothelium-derived nitric oxide. Hypertension **15**, 486 – 492.

Guedes D. N., Silva D. F., Barbosa-Filho J. M., and de Medeiros I. A. (2004), Endothelium-dependent hypotensive and vasorelaxant effects of the essential oil from aerial parts of *Mentha* x *villosa* in rats. Phytomedicine **11**, 490 – 497.

Hu C. T., Chang H. R., Hsu Y. H., Liu C. J., and Chen H. I. (2005), Ventricular hypertrophy and arterial hemodynamics following deprivation of nitric oxide in rats. Life Sci. **78**, 164 – 173.

Iwatani Y., Kosugi K., Isobe-Oku S., Atagi S., Kitamura Y., and Kawasaki H. (2008), Endothelium removal augments endothelium-independent vasodilatation in rat mesenteric vascular bed. Br. J. Pharmacol. **154**, 32 – 40.

Jover B., Herizi A., Ventre F., Dupont M., and Mimran A. (1993), Sodium and angiotensin in hypertension induced by long-term nitric oxide blockade. Hypertension **21**, 944 – 948.

Kukongviriyapan U., Sompamit K., Pannangpetch P., Kukongviriyapan V., and Donpunha W. (2012), Preventive and therapeutic effects of quercetin on lipopolysaccharide-induced oxidative stress and vascular dysfunction in mice. Can. J. Physiol. Pharmacol. **90**, 1345 – 1353.

Loeb A. L. and Longnecker D. E. (1992), Inhibition of endothelium-derived relaxing factor-dependent circulatory control in intact rats. Am. J. Physiol. **262**, H1494 – 1500.

Luangaram S., Kukongviriyapan U., Pakdeechote P., Kukongviriyapan V., and Pannangpetch P. (2007), Protective effects of quercetin against phenylhydrazine-induced vascular dysfunction and oxidative stress in rats. Food Chem. Toxicol. **45**, 448 – 455.

McKay D. L. and Blumberg J. B. (2006), A review of the bioactivity and potential health benefits of peppermint tea (*Mentha piperita* L.). Phytother. Res. **20**, 619 – 633.

Monteiro M. M., Franca-Silva M. S., Alves N. F., Porpino S. K., and Braga V. A. (2012), Quercetin improves baroreflex sensitivity in spontaneously hypertensive rats. Molecules **17**, 12997 – 13008.

Mulvany M. J. and Aalkjaer C. (1990), Structure and function of small arteries. Physiol. Rev. **70**, 921 – 961.

Nakmareong S., Kukongviriyapan U., Pakdeechote P., Kukongviriyapan V., Kongyingyoes B., Donpunha W., Prachaney P., and Phisalaphong C. (2012), Tetrahydrocurcumin alleviates hypertension, aortic *Mentha cordifolia* stiffening and oxidative stress in rats with nitric oxide deficiency. Hypertens. Res. **35**, 418 – 425.

Pakdeechote P., Kukongviriyapan U., Berkban W., Prachaney P., Kukongviriyapan V., and Nakmareong S. (2011), *Mentha cordifolia* extract inhibits the development of hypertension in L-NAME-induced hypertensive rats. J. Med. Plant Res. **5**, 1175 – 1183.

Pereira L. M., Bezerra D. G., and Mandarim-de-Lacerda C. A. (2004), Aortic wall remodeling in rats with nitric oxide deficiency treated by enalapril or verapamil. Pathol. Res. Pract. **200**, 211 – 217.

Perez-Vizcaino F., Duarte J., Jimenez R., Santos-Buelga C., and Osuna A. (2009), Antihypertensive effects of the flavonoid quercetin. Pharmacol. Rep. **61**, 67 – 75.

Rees D. D., Palmer R. M., and Moncada S. (1989), Role of endothelium-derived nitric oxide in the regulation of blood pressure. Proc. Natl. Acad. Sci. USA **86**, 3375 – 3378.

Ribeiro M. O., Antunes E., de Nucci G., Lovisolo S. M., and Zatz R. (1992), Chronic inhibition of nitric oxide synthesis. A new model of arterial hypertension. Hypertension **20**, 298 – 303.

Runnie I., Salleh M. N., Mohamed S., Head R. J., and Abeywardena M. Y. (2004), Vasorelaxation induced by common edible tropical plant extracts in isolated rataorta and mesenteric vascular bed. J. Ethnopharmacol. **92**, 311 – 316.

Sompamit K., Kukongviriyapan U., Nakmareong S., Pannangpetch P., and Kukongviriyapan V. (2009), Curcumin improves vascular function and alleviates oxidative stress in non-lethal lipopolysaccharide-induced endotoxaemia in mice. Eur. J. Pharmacol. **616**, 192 – 199.

Souza H. C., Ballejo G., Salgado M. C., Da Silva V. J., and Salgado H. C. (2001), Cardiac sympathetic overactivity and decreased baroreflex sensitivity in L-NAME hypertensive rats. Am. J. Physiol. Heart Circ. Physiol. **280**, H844 – 850.

Takenaga M. and Kawasaki H. (1999), Endogenous calcitonin gene-related peptide suppresses vasoconstriction mediated by adrenergic nerves in rat mesenteric resistance blood vessels. Eur. J. Pharmacol. **367**, 239 – 245.

Torok J. (2008), Participation of nitric oxide in different models of experimental hypertension. Physiol. Res. **57**, 813 – 825.

Tsukahara H., Hiraoka M., Kobata R., Hata I., Ohshima Y., Jiang M. Z., Noiri E., and Mayumi M. (2000), Increased oxidative stress in rats with chronic nitric oxide depletion: measurement of urinary 8-hydroxy-2′-deoxyguanosine excretion. Redox Rep. **5**, 23 – 28.

Vasquez E. C., Cunha R. S., and Cabral A. M. (1994), Baroreceptor reflex function in rats submitted to chronic inhibition of nitric oxide synthesis. Braz. J. Med. Biol. Res. **27**, 767 – 774.

Villasenor I. M. and Sanchez A. C. (2009), Menthalactone, a new analgesic from *Mentha cordifolia* Opiz. Leaves. Z. Naturforsch. **64c**, 809 – 812.

Villasenor I. M., Echegoyen D. E., and Angelada J. S. (2002), A new antimutagen from *Mentha cordifolia* Opiz. Mutat. Res. **515**, 141 – 146.

Xu S., and Touyz R. M. (2006), Reactive oxygen species and vascular remodelling in hypertension: still alive. Can. J. Cardiol. **22**, 947 – 951.