จะออกฤทธิ์ต่อกล้ามเนื้อเรียบของหลอดเลือดโดยตรงโดย ยังต้องทำการศึกษาต่อไปเนื่องจากการทดลองนี้จัดเป็น preliminary study

สรุปผลการทดลอง จากการทดลองนี้แสดงให้เห็นว่าสารสกัดสะระแหน่สามารถออกฤทธิ์ทั้งการป้องกันและการรักษาภาวะความดันเลือดสูงเนื่องจากการให้สาร L-NAME และปรับพลศาสตร์การไหลเวียนเลือดให้ดีขึ้น รวมทั้งช่วยให้การทำงานของหลอดเลือดที่ตอบสนองต่อสารที่ออกฤทธิ์ต่อหลอดเลือดได้ดีขึ้น สำหรับกลไกที่เกี่ยวข้องอาจเป็นผลจากคุณสมบัติของการเป็นสารที่มีฤทธิ์ขยายหลอดเลือดและต้านอนุมูลอิสระของสารสกัดสะระแหน่

เอกสารอ้างอิง

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ภาคผนวก

Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. P. Pakdeechote[1], U. Kukongviriyapan[1], S. Nakmareong[1], P. Prachaney[2], W. Berkban[1], S. Luangaram[3].
Antioxidant and antihypertensive effects of *Mentha Cordifolia* extract in L-NAME-induced hypertensive rats
SFRR-Europe meeting 2009 in Rome (poster presentation)

2. P. Pakdeechote[1], U. Kukongviriyapan[1], S. Nakmareong[1], P. Prachaney[2], W. Berkban[1], S. Luangaram[3].
Antioxidant and antihypertensive effects of *Mentha Cordifolia* extract in L-NAME-induced hypertensive rats. Free radicals, health and Lifestyle; Europe Meeting of Society for Radical Research, Rome (Italy), August 26-29, 2009; 105-108.International proceeding.

3. อยู่ในช่วงดำเนินการส่งตีพิมพ์ในวารสารวิชาการนานาชาติ (ดัง manuscript ที่แนบ)

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(50 mg/kg/day) in drinking water for 3 weeks. Curcumin (100 mg/kg/day) or polyethylene glycol (a vehicle) was orally administered to the animals, either before or after induction of hypertension by L-NAME. It was found that treatment with curcumin significantly reduced mean arterial pressure by 15% and 18% in both protectively- and therapeutically-treated regimens when compared with the vehicle-treated L-NAME hypertensive rats. The attenuation of endothelial-dependent vasodilation to acetylcholine in L-NAME-treated rats was also improved by curcumin. Moreover, curcumin increased plasma nitrate/nitrite levels, reduced superoxide anion production in vascular tissues, and decreased lipid and protein oxidations in both protective- and therapeutic-treated regimens, indicating the effect of curcumin on restoration of NO/ROS balance. This study provides the first evidence for the beneficial effect of curcumin on prevention and treatment of oxidative stress and endothelial dysfunction in L-NAME treated rats.

PIII-21

Different roles of mitochondrial and cytosolic Glutaredoxin2 isoforms

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Glutaredoxin 2 (Grx2) from Saccharomyces cerevisiae is subjected to postranslational processing leading to different subcellular localizations. The topological distribution of the isoforms is finely tuned by a sophisticated mechanism based in part on the efficiency of the translocation through the mitochondrial membrane. The aim of the present study is to define whether this evolutionary achievement is matched by a distribution of functions among the Grx2 isoforms.

We have prepared Grx2 mutants affected on key residues within the presequence to direct the protein to a single cellular compartment, the cytosol, the mitochondrial membrane or the matrix and have analyzed their functional phenotypes. Grx2 could not be targeted preferentially to the mitochondrial membrane, but substitutions of charged residues by hydrophobic ones in the presequence produced an unstable translation product which is rapidly degraded by the proteolytic machinery. On the other hand, strains expressing Grx2 only in the cytosol are equally sensitive to H_2O_2 than strains lacking the gene, whereas those expressing Grx2 exclusively in the mitochondrial matrix are more resistant. No differences were found towards diamide treatment among the whole set of mutants.

We conclude that the antioxidant functions of Grx2 so far described rely only on the isoform present in the mitochondrial compartment. The functions of the cytosolic counterpart remains to be elucidated.

PIII-22

Anthocyanins inhibit peroxynitrite-triggered endothelial cells toxicity by up-regulating cellular nitric oxide and down-regulating NF-kB

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Anthocyanins (Ac), a major dietary flavonoid group widely distributed in fruits, vegetables and red wine, have received increasing attention as natural anti-atherogenic factors. Besides its antioxidant activity, recent evidences point out other relevant action mechanisms by interfering with cellular signaling pathways. Atherosclerosis, the main cause of cardiovascular disease, is a chronic inflammatory condition associated with an overproduction of oxidant species, namely peroxynitrite. Thus, the endothelium damage mediated by such species and the NF-kB activation are potential therapeutic targets to be explored. The aim of this work was to study the protection afforded by some Ac, malvidin-(Mv3glc), cyanidin- delphinidin- and pelargonidin-3-glucoside, against peroxynitrite-promoted endothelial cells toxicity and underlying mechanisms. Pre-incubation of bovine aortic endothelial cells with Ac, at very low micromolar concentrations, protects cells from death induced by authentic peroxynitrite but there is no correlation between such effects and antioxidant activities. On the other hand, as illustrated with Mv3glc, our results suggest that Ac prevent peroxynitrite-induced IKBα degradation and thereby the NF-kB activation. In similar damage conditions, higher NO levels have been detected in pre-incubated cells with Ac, as compared with controls without Ac, as measured by the Griess reaction. Considering that NO may play a role in NF-kB activation, research is under way to ascertain the relationship between both events and thereby the role of Ac on NF-kB inflammatory response in endothelial cells.

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PIII-23

Antioxidant and antihypertensive effects of Mentha Cordifolia extract in L-NAME-induced hypertensive rats

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Mentha Cordifolia Opiz. (Family Lamiaceae) has been known as marsh mint, pepper mint or kitchen mint. The biological activities of Mentha Cordifolia (MC) involve analgesic, local anesthetic, antimutagenic and vascular relaxation effects. The present study was aimed to investigate the antioxidant and antihypertensive effects of the aqueous extract of MC leaves in L-NAME-induced hypertensive rats. Male Sprague-Dawley rats were induced hypertension by giving N[omega]-nitro-arginine methyl ester (L-NAME) at dose of 50 mg/kg/day in drinking water for 3 weeks. The hypertensive rats were intragastrically administered with MC extract (200 mg/kg/day) or vehicles for 2 weeks. It was found that L-NAME significantly increased mean arterial pressure (MAP) from $107.05+/-4.13$ to $207.56+/-9.44$ mmHg ($P<0.001$). Subsequently, the significant reduction in MAP by 12% was found in L-NAME hypertensive rats treated with MC extract ($P<0.05$). There was no significant difference of heart rate between groups. The levels of plasma malondialdehyde, plasma protein carbonyl and superoxide production in vascular tissues were significantly increased in L-NAME hypertensive rats, suggesting that oxidative stress occurred after L-NAME administration. However, MC extract significantly reduced superoxide production and decreased lipid peroxidation in those hypertensive rats. In conclusion, the results indicate that MC extract exhibited antihypertensive effect in the L-NAME induced-hypertensive rats. The mechanisms involved appear to be mediated by the antioxidant properties of the MC extract.

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PIII-24

Protective effect of pyrrolidine dithiocarbamate on monocrotaline induced liver injury

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The aim of the present study was to examine the efficacy of the pyrrolidine dithiocarbamate (PDTC), an antioxidant and inhibitor of the NF-kappa B activation, in monocrotaline (MCT) induced liver injury. Adult male rats randomized into three groups: 1. Control group, 2. MCT-injected rats (60 mg/kg MCT i.p) 3. MCT-injected rats treated with NF-kappa B inhibitor PDTC (100 mg/kg once daily i.p on days 3 to 28 (the MCT/PDTC group). At the time of sacrifice, livers were collected for biochemical and histopathological examination. Lipid

Antioxidant and antihypertensive effects of Mentha Cordifolia extract in L-NAME-induced hypertensive rats

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Summary

The antioxidant and antihypertensive effects of *Mentha Cordifolia* Opiz. (MC) extract were tested in L-NAME-induced hypertensive rats. Male Sprague-Dawley rats were given L-NAME (50 mg/kg/day) in drinking water for 3 weeks to induce hypertension. Subsequently, the hypertensive rats were intragastrically administered with MC extract (200 mg/kg/day) or vehicles for 2 weeks. Results showed that MC extract significantly decreased mean arterial pressure in L-NAME-induced hypertensive rats ($P<0.05$). The levels of plasma malondialdehyde (MDA), plasma protein carbonyl and superoxide production in vascular tissues were significantly increased in hypertensive rats. These oxidative markers were decreased in MC extract-treated group ($P<0.05$). Results of this study indicate that MC extract exhibited the antioxidant and antihypertensive effects in the L-NAME induced-hypertensive rats.

Introduction

Acute or chronic inhibition of nitric oxide synthesis using L-arginine analogues, N^{ω}-monomethyl L-arginine (L-NMMA) and N^{ω}-nitro-L-arginine-methyl ester (L-NAME), has been commonly used as an effective model of animal hypertension (1). Previous study demonstrated that L-NAME-induced hypertension is associated with increased oxidative stress. Consequently, supplementation with antioxidants have been observed to decrease blood pressure and reactive oxygen species production in L-NAME hypertension (2).

Mentha Cordifolia Opiz. (Family Lamiaceae) has been known as marsh mint, peppermint or kitchen mint. It was widely used as herb and named in Thai "sa-ra-nae". The biological activities of mint family in term of analgesic, local anesthetic (3), antioxidant (4) and vascular relaxation effects (5) have been reported. However, evidence of antihypertensive effects of *Mentha Cordifolia* has not been reported.

Therefore, the aim of the present study was to evaluate whether *Mentha Cordifolia* can decrease blood pressure in L-NAME-induced hypertension.

Materials and Methods

Preparations of plant extractions

The plant leaves were collected from Khon Kaen, Thailand, chopped into small pieces and boiled in distilled water at 95 °C for 30 minutes. The water extract was filtered and lyophilized. The powder extract was dissolved in distilled water before used.

Animals and experimental protocols

Male Sprague-Dawley rats (weighing 220-225 g) were randomly divided into 3 groups. The first group was normotensive group, received vehicle. The second group, rats were given L-NAME (50mg/kg) in drinking water for 5 weeks served as hypertensive group. The last group was received L-NAME (50mg/kg) in drinking water for 5 weeks and orally administrated with MC extract (200 mg/kg) for 2 weeks. All animals were fed on normal diet and maintained following the guide for Care and Use of Laboratory Animals, Khon Kaen University Animal Ethic Committee. After 5 weeks of treatment, animals were anesthetized by peritoneal injection of ketamine:xylazine (100:2.5 mg/kg). Subsequently, a tracheotomy was made to assist respiration. Femoral artery was identified, cleaned of connective tissue and cannulated with polyethylene tube. Mean arterial blood pressure and heart rate were continuously monitored by a way of pressure transducer and recorded using the acknowledge data acquisition and analysis software (Biopac System Inc., California, USA). At the end of experimentation, blood collection was performed for biochemical assays.

Biochemical assays

Plasma MDA, lipid peroxidation indicator, was examined by measuring the thio-barbiturate acid-reactive substance. The superoxide production in carotid arteries was assessed by using lucigenin-enhanced chemiluminescence methods as previously described (6). Plasma protein carbonyl, protein oxidation marker, was determined by measuring the reaction of carbonyl group with DNPH (7) with modifications.

Statistical analysis

Data are presented as mean ± S.E.M. Statistical comparisons between groups were made using one-way analysis of variance (ANOVA) with a post-hoc Duncan' s multiple range test. A value of $P < 0.05$ was taken to indicate statistical significance.

Results

1. Effect of MC extract on mean arterial blood pressure in L-NAME-induced hypertensive rats

Mean arterial blood pressure of rat receiving L-NAME (204.18 ± 8.05 mmH)

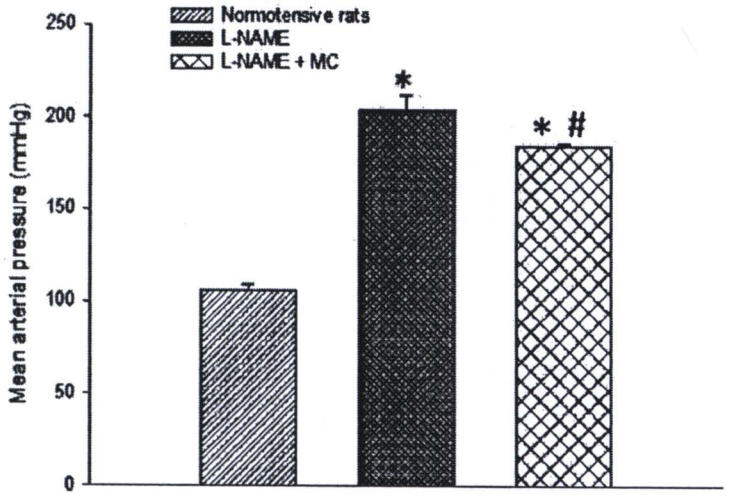

*Figure 1 Mean arterial pressure in normotensive rats (n=5), L-NAME-induced hypertensive rats (n=5), and L-NAME-induced hypertensive rats with MC extract (n=5). * P< 0.001 vs normotensive rats, # P< 0.05 vs L-NAME-induced hypertensive rats (ANOVA).*

Parameters	Normotensive rats	Hypertensive rats	Hypertensive rats with MC extract
Plasma MDA (μM)	6.69±0.93	24.44±2.09*	9.28±0.62#
Superoxide production in carotid arteries (Count/mg dry wt/min)	40.27±21.85	281.04±17.94*	200.40±21.24*#
Plasma protein carbonyl (nmol/mgprotein)	1.98±0.17	2.58±0.29*	2.69±0.31*

*Table 1 Oxidative stress markers in plasma and tissues in normotensive rats (n=5), L-NAME-induced hypertensive rats (n=5), and L-NAME-induced hypertensive rats with MC extract (n=5). * P< 0.001 vs normotensive rats, # P< 0.05 vs L-NAME-induced hypertensive rats (ANOVA).*

was significantly higher than those of normal rat (105.7 ± 3.47 mmHg) (P<0.01, n = 5, ANOVA). Oral administration of MC extract (200 mg/kg/day) for 2 weeks significantly reduced mean arterial blood pressure by 12% in L-NAME-induced hypertensive rats (Figure 1) when compared with the hypertensive group. There was no significant difference of heart rate between groups.

2. Effect of MC extract on oxidative stress marker in L-NAME-induced hypertensive rats

A significant increase in levels of plasma MDA, plasma protein carbonyl and superoxide production in vascular tissues was found in L-NAME-induced hypertensive

rats. However, there was a significant reduction of plasma MDA and superoxide production in the hypertensive rats after MC treatment ($P<0.05$) (Table 1).

Conclusions

We found that MC extract can reduce mean arterial blood pressure in L-NAME-induced hypertensive rats. The mechanisms involved appear to be mediated by the antioxidant properties of MC extract. Further study is required to test hemodynamic and vasorelaxant effects of MC extract.

Acknowledgements

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Abstract: This study was to test the inhibitory action of Mentha Cordifolia (MC) extract on the development of hypertension in L-NAME hypertensive rats. Male Sprague-Dawley rats received L-NAME (50 mg/kg/day) in drinking water and were either administered with MC extract (200 mg/kg/day) or deionized water for 3 weeks. The MC extract markedly reduced mean arterial pressure (about 16.7 %), heart rate and hindlimb vascular resistance in L-NAME-treated rats. The extract improved vascular response to acetylcholine and decreased oxidative stress of hypertensive rats. The ability of MC extract to inhibit progression of L-NAME hypertension might be due to its antioxidant capacity.

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Letter

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Editors-in-Chief
Fitoterapia

3rd August 2010

Dear Professor G. Appendino, Editors-in-Chief, Fitoterapia

We would like to submit our manuscript entitled "***Mentha Cordifolia* extract inhibits the development of hypertension in L-NAME-induced hypertensive rats"** to be considered for publication in *Fitoterapia.*

This work describes the antihypertensive activity of our indigenous plants, which are naturally grown and consumed as dietary supplements and claimed for health promotion in the Southeast Asian traditional medicine. In this study, we found that the antioxidant property of the extract from *Mentha Cordifolia* has the ability to prevent development of hypertension in rats treated with L-NAME. We believe that "*Fitoterapia*" is a proper media for communicating this scientific information.

We affirm that this manuscript has not been previously published and is not under consideration by any other publication. The study was approved and all procedures were in accordance with the guideline of the Institutional Animal Care and Use Committee of Khon Kaen University, Thailand. The authors have authorized me to act as corresponding author during the review process. Each author has made a valuable contribution to the preparation of this manuscript and there are no conflicts of interest.

Thank you very much for considering our manuscript and look forward to hearing from your in soon.

With kind regards,

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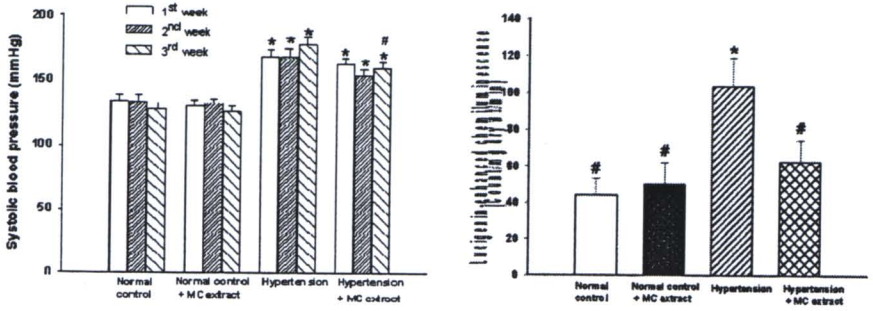
***Mentha cordifolia* has an ability to prevent the development of L-NAME-induced hypertension which was associated with a reduction in oxidative stress**



Mentha Cordifolia extract inhibits the development of hypertension in L-NAME-induced hypertensive rats

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Abstract

This study was to test the inhibitory action of *Mentha Cordifolia* (MC) extract on the development of hypertension in L-NAME hypertensive rats. Male Sprague-Dawley rats received L-NAME (50 mg/kg/day) in drinking water and were either administered with MC extract (200 mg/kg/day) or deionized water for 3 weeks. The MC extract markedly reduced mean arterial pressure (about 16.7 %), heart rate and hindlimb vascular resistance in L-NAME-treated rats. The extract improved vascular response to acetylcholine and decreased oxidative stress of hypertensive rats. The ability of MC extract to inhibit progression of L-NAME hypertension might be due to its antioxidant capacity.

Keywords: *Mentha Cordifolia* Opiz., L-NAME, hypertension, antioxidant, nitric oxide

1. Introduction

A most common contributory to the pathogenesis hypertension is endothelial dysfunction. The imbalance between vasodilator agents (nitric oxide (NO), endothelium-dependent hyperpolarizing factor (EDHF)) and vasoconstrictor agents (endothelin, thromboxane A2, endoperoxides) released from endothelial cells as well as an increase in free radical production appear to be the most important cause of endothelial dysfunction in hypertension [1]. As Furchgoott and Zawadzki showed nitric oxide released from vascular endothelium mediating vascular smooth muscle cell relaxation plays a key role in the maintenance of vascular tone [2]. Subsequently, induction of hypertension by chronic administration of nitric oxide synthase (NOS) inhibitors has been widely accepted as a model of animal hypertension [3,4]. Many studies supported that N^G-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor, produces a sustained elevation of blood pressure [5-8] and heart rate [9,10]. It is well established that this model of hypertension is likely to be associated with an increase in peripheral vascular resistance [11]. This includes vascular functional, structural changes [12-14] and cardiac hypertrophy [15]. Other possible causes of systemic vasoconstriction in NO-deficient rats were evidently found including, enhancements of sympathetic nerve activation in conscious rats [16], alterations of the renin-angiotensin system [17], and an increase in oxidative stress markers [1,18]. It has been suggested that superoxide production is raised and suppressed NO availability leads to endothelial dysfunction and finally hypertension [19,20]. Consequently, there is evidence in animals supporting the notion that supplementation of antioxidant substances may ameliorate hypertension and restore endothelial function [21,22].

Mentha Cordifolia Opiz. (Family Lamiaceae) is easily grown throughout Thailand and in many Southeast Asian countries. Its common name is kitchen or marsh mint. It is a popular flavoring herb of Thai food and herbal tea. This mint is generally used in traditional medicine to relieve gastrointestinal problem, asthma, muscle spasm and inflammation. Evidence supporting the biological effect of *Mentha Corlifolia* has been reported; such as anti-mutagenicity [23], analgesic [24], anti-nociceptive [25], anti-inflammatory [26] and antioxidant activities [27]. A vasorelaxing effect of mint leaf extract has been shown in the rat mensenteric bed [28]. Many studies showed that the biological activity of this Mentha species is due to the presence of the phenolic compounds especially flavanoids [28-30].

The effect of the MC extract on the vascular responsiveness and antioxidative properties has not been elucidated. Therefore, the aim of this study was to explore the protective effect of MC extract on blood pressure and vascular functions.

2. Materials and methods

2.1 Plant and extraction

Fresh plant leaves were collected from a local farm in Khon Kaen city, Thailand, weighed, chopped into small pieces and boiled in distilled water at 95 °C for 30 minutes. The water extract was filtered, evaporated in vacuum evaporator and then lyophilized to get the dry extract. The yield was about 2.4 % by weight from the fresh leaves. The powdered MC extract was kept in an airtight container at -20 °C and dissolved in distilled water before use.

2.2 Animals

Male Sprague-Dawley rats (220-225 g) were obtained from the Animal Care Unit of the Faculty of Medicine, Khon Kaen University (Khon Kaen, Thailand). All animals were maintained in a temperature controlled room at 24 °C with a 12-hour dark/ light cycle. The animals were given free access to standard chow diet (Chareon Pokapan Co. Ltd., Thailand) and distilled water (DW) or L-NAME (50 mg/kg/day) in DW. All animal procedures were reviewed and approved by the Institutional Animal Ethics Committee of Khon Kaen University (AEKKU 20/2551).

2.3 Experimental design

Rats were randomly divided into 4 groups with 6-8 in each group. Group I- control group, normal rats received vehicle DW; group II-normal control treated group, were normal rats treated with MC extract (200 mg/kg); group III-hypertension group, comprised rats given L-NAME (50 mg/kg/day) in drinking water and vehicle; group IV hypertension treated group, were rats given L-NAME (50 mg/kg/day) in drinking water and MC extract. Animals received L-NAME and MC extract for 3 weeks. Vehicle and MC extract were orally administrated using a feeding tube. The choice of MC extract dosage used in this study was determined by a preliminary study which showed that MC extract at 200 mg/kg/day prevents the increase

in blood pressure of rats treated with L-NAME. To assess the onset and progression of hypertension, systolic blood pressure (SBP) was measured weekly using non-invasive tail-cuff plethysmography (IITC/life science instrument model 229 and model179 amplifier, Woodland Hills, California, USA).

2.4 Measurement of hemodynamic status and vascular reactivity

For direct blood pressure measurement, after three weeks of treatment, animals were anesthetized by peritoneal injection of pentobarbital-sodium (50 mg/kg) and placed on a heating pad. Subsequently, a tracheotomy was made to assist respiration. The femoral artery was identified, cleaned of connective tissue and cannulated with a polyethylene tube. SBP, diastolic blood pressure (DBP), mean arterial blood pressure (MAP) and heart rate were continuously monitored by a way of pressure transducers and recorded using the acknowledge data acquisition and analysis software (Biopac Systems Inc., California, USA). The abdominal aorta was carefully separated from the abdominal vein, cleaned of connective tissue and fitted with a flow probe to detect hindlimb blood flow (HBF) with an electromagnetic Flowmeter (Carolina Medical Electronics, Inc., North Carolina, USA). Hindlimb vascular resistance (HVR) (mmHg/min/100 g tissue/ml) was calculated as mean arterial blood pressure divided by HBF. Following equilibration, vascular responses to vasoactive agents, acetylcholine (ACh 3, 10 and 30 nmol/kg); an endothelium-dependent vasodilator, sodium nitroprusside (SNP 1, 3 and 10 nmol/kg); an endothelium-independent vasodilator, and phenylephrine (0.1, 0.3 and 1 μmol/kg); an α_1 adrenoceptor agonist, were tested to evaluate vascular function via intravenous injection in a stepwise fashion at 5-minute intervals. In separate experiments, after obtaining a stable baseline of hemodynamic measurement, the animals were sacrificed and blood samples were collected via the abdominal aorta for biochemical assays. Carotid arteries (about 2 cm in length) were cut out rapidly from animals to assess superoxide production.

2.5 Biochemical assays

Plasma Malondialdehyde (MDA), a lipid peroxidation indicator, was examined by measuring the thiobarbiturate acid-reactive substance by a spectrometric method as previously described [31]. In brief, plasma samples were reacted with 10% Trichloroacetic acid, 5 mM ethylenediamine tetraacetic acid, 8%

sodium dodecylsulfate, 0.5 μg/ml of butylatedhydroxyluene and 0.6% thiobarbituric acid. The mixture was boiled for 30 min. After cooling to room temperature, the absorbance of the supernatant was measured at 532 nm by spectrophotometer. Results were expressed according to a standard curve of 1,1,3,3-tetraethoxypropane (0.3–10 μmol/l).

The production of superoxide in carotid arteries was determined by the lucigenin-enhanced chemiluminescence method as described previously [31,32]. In brief, the vessel 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, 23 CA, USA). The photon counts were integrated every 15 s for 5 min and averaged. The vessels were then dried for 24 hours at 45 °C and weighed. Superoxide production in vessel tissues was expressed as relative light unit count/mg dry wt/min.

2.6 Assay for total phenolic compounds

The determination of total phenolics followed the method previously described with some modifications by Zheng and Wang [33]. The MC extract or vitamin C dilution was mixed with 0.2 N Folin-Ciocalteu reagents and the reaction was neutralized with saturated sodium carbonate 10% w/v. Total phenolic compounds were measured using spectrophotometry and expressed as gram of Gallic acid equivalent (GAE) per 100 g of MC extract.

2.7 Chemicals

All chemicals used were of analytical grade quality.

2.8 Statistical analysis

Data are presented as mean ± S.E.M. Statistical comparisons between groups were made using one-way analysis of variance (ANOVA) with a post-hoc Duncan' s multiple range test. A value of $P < 0.05$ was taken to indicate statistical significance.

3. Results

3.1 Effect of MC extract on hemodynamic changes in L-NAME-induced hypertension

Indirect measurement of blood pressure showed that chronic administration of L-NAME markedly elevated rat SBP (168.7 ± 5.8, 168.5 ± 6.7 and 178.2 ± 4.6 mmHg in the 1st, 2nd and 3rd week, respectively) when compared with the control group (134.1± 5.0, 133.2 ± 5.5 and 127.8 ± 4.7 mmHg in the 1st, 2nd and 3rd week, respectively) (Fig. 1). A concomitant administration of MC extract with L-NAME for 3 weeks had a significant effect in inhibiting the development of hypertension as shown by the reduction in SBP in the 3rd week (160.2 ± 4.2 mmHg) as compared to that of L-NAME hypertensive group. Daily treatment of MC extract had no effect on SBP of normal rats (Fig.1).

Similarly, the results obtained from direct measurement showed that there was a significant increase of SBP, DBP and MAP after three weeks of L-NAME treatment (199.1 ± 3.2, 129.1 ± 3.5 and 162.3 ± 2.7 mmHg, respectively) comparing to those of control group (Table 1). In L-NAME treated rats a decrease of HBF (3.6 ± 0.2 ml/100 g tissue/min) was found when compared to the control group (6.8 ± 0.3 ml/100 g tissue/min). This hemodynamic alteration was related to increased resistance which was determined by the increase in HVR (L-NAME treated rats = 39.1 ± 3.8 mmHg/min/100 g tissue/ml; control group = 12.6 ± 0.6 mmHg/min/100 g tissue/ml) as shown in Table 1.

The effect of MC extract on SBP illustrated by direct measurement showed similar results as those obtained from indirect SBP measurement. Thus, the elevation of SBP, DBP and MAP was significantly attenuated (about 16%) in L-NAME-induced hypertensive rats simultaneously receiving MC extract for three weeks (Table 1). The alterations of HBF and HVR were also markedly prevented in L-NAME-treated rats receiving MC extract. Moreover, a significant increase in heart rate was observed in L-NAME-induced hypertensive rats comparing to those of the control group ($P < 0.05$). The tachycardic effect of L-NAME was clearly prevented by MC extract ($P < 0.05$) (Table 1). Heart rate value was restored about to a normal level in these animals (Table 1).

3.2 Effect of MC extract on vascular reactivity in L-NAME-induced hypertension

The decrease in MAP in response to acetylcholine in the L-NAME-induced hypertensive group was significantly attenuated when compared to that of the control group ($P < 0.05$). Interestingly, the

vasodilatation response to SNP in all groups was similar. In addition, a significant reduction of the increase in MAP in response to phenylephrine was found in hypertensive rats. The treatment of MC extract markedly improved the blunted vascular activity to acetylcholine in L-NAME hypertensive rats (Fig. 2a) but did not affect the vascular response to SNP (data not shown). Nevertheless, the impairment of vascular function in contractile responses to phenylephrine was not recovered in the hypertensive group treated with MC extract (Fig. 2b).

3.3 Effect of MC extract on oxidative stress markers

It was found that there was a significant increase in plasma MDA content and vascular superoxide production in rats receiving L-NAME for three weeks ($P < 0.05$) (Fig. 3). The concomitant administration of MC extract improved the oxidative stress status in hypertensive rats but had no effect on the level of plasma MDA in normal rats (Fig 3a, 3b). These results indicated that MC extract exhibited a potential antioxidant capacity in the L-NAME hypertensive rat model.

3.4 Total phenolic contents in MC extract

To evaluate the potential antioxidant effect of MC extract used in this study, the total phenolic content in MC extract was evaluated. It was found that MC extract contains a total phenolic compound that was expressed as 13.93 ± 0.09 g of GEA/100 g of MC extract. This amount of total phenols is approximately three times less than that of vitamin C (43.31 ± 0.5 g of GEA/100 g of vitamin C).

4. Discussion

This study examined the protective effect of MC extract on hemodynamic status, vascular responsiveness and oxidative stress markers in L-NAME-induced hypertension. These hypertensive animals had a sustained high blood pressure and an associated increase in heart rate, HVR and alterations of vascular functions. The main findings of this study are that MC extract partially inhibited the development of hypertension, and restored heart rate and vascular reactivity close to normal values. A significant increase in HBF and a reduction of HVR was found in L-NAME treated with MC extract, suggesting an improvement of hemodynamic status after MC extract administration. The results of

biochemical assays showed the antioxidant effect of MC extract by reducing plasma MDA level and vascular tissue superoxide production in L-NAME-induced hypertension. This was supported by the finding of the phenolic compounds in the MC extract.

As blood pressure is determined primarily by cardiac output and total peripheral resistance, an increase in one of these factors can cause hypertension. Lack of NO in the circulatory system induces vasoconstriction, an increase in peripheral resistance and eventually leads to hypertension [34]. This study has shown that daily administration of L-NAME for three weeks prevented the development of hypertension and that this was associated with an increase in HVR. This finding is consistent with the observation in rats that daily L-NAME treatment for four weeks produced a sustained elevation of blood pressure and regional vascular resistance [34,35]. An increased heart rate was also found in rats chronically treated with L-NAME. There is accumulating evidence that nitric oxide modulates sympathetic nervous activity by suppressing sympathetic outflow [36,37] and leading to hypertension. Cunha and coworkers (1993) demonstrated that treatment of L-NAME in rats for six days induced an increase in blood pressure and heart rate and this was correlated with the overactivity of central sympathetic tone [38]. It was demonstrated that, sympathetic drive to the heart in rats treated with L-NAME was abolished by autonomic nerve blockers [10]. Therefore, an increase in sympathetic outflow is likely to have an important role in mediating the chronotropic effect in response to chronic administration of L-NAME in rats [9]. Although, the increase in heart rate was observed in this study, this was not sufficient to increase cardiac output in the hypertensive animals. On the other hand several studies reported that hypertensive rats with nitric oxide synthase inhibition showed a lower cardiac output and that this was possibly due to a decrease in stroke volume [35,39] as well as cardiac hypertrophy [15,40].

The present findings show that MC extract significantly inhibited the increase of SBP, DBP and MAP in rats treated with L-NAME. These results suggest that the effect of MC extract against hypertension involved the reduction of total peripheral resistance. We found an improvement in HBF and HVR in L-NAME induced hypertensive rats-treated with MC extract. The vascular relaxation effect of the extract from *Mentha arvensis* has been previously reported in both large conductance and resistance arteries where nitric oxide was a mediating factor [28]. In addition, this study demonstrated that there was a decrease in heart rate of nitric oxide deficiency-hypertensive rats simultaneously received MC

extract. The mechanism of heart rate alleviation by MC extract in this hypertensive model remains unclear. It could be explained by the antioxidant effect of MC extract leading to an increase in NO bioavailability, and this might subsequently suppress the sympathetic nerve outflow.

It is known that NO is formed and released by vascular endothelial cells in response to acetylcholine to mediate vasodilatation [2]. Later, vascular endothelial cell dysfunction was found in L-NAME- induced hypertensive rats and the impairment of vascular responses to endothelium-dependent vasodilator has been shown in both in vitro and in vivo studies [41]. In the in vivo study, alterations in the vascular activity of L-NAME hypertensive rats was seen, indicated by a blunted vascular response to acetylcholine, endothelium-dependent vasodilator [42]. A decrease in vasodilatation response to acetylcholine in the aorta of chronic L-NAME-treated rats has been suggested to be due to a reduction of cGMP content [43]. Interestingly, the treatment of L-NAME hypertensive rats with MC extract restored this vascular impairment. Since the response to SNP, an endothelium-independent vasodilator, did not alter in this animal model, it has been suggested that the production of cGMP in response to NO donors in vascular tissues did not change in chronic L-NAME induced hypertension [43]. These results could imply that there was only an impairment of endothelium dependent vasorelaxation in L-NAME hypertensive rats. An alleviation of oxidative stress after MC extract administration might enhance NO bioavailability in the vessels, and contribute to the improvement of vascular responses to acetylcholine in L-NAME hypertensive rats. The vasoconstriction response to phenylephrine in this model of hypertension was also impaired. The decrease in vascular responses to phenylephrine, α_1-adrenoceptor agonist, in L-NAME hypertensive rats was not recovered by MC extract. These observations were associated with a reduction of contractile response to exogenously administered phenylephrine in isolated aortic rings of L-NAME-hypertensive rats. Henrion and coworkers (1996) suggested that this appear to be a downregulation of the contractile signaling pathways induced by chronic nitric oxide inhibition and associated with a drop in the extracellular Ca^{2+}content of smooth muscle cells [44].

The excessive production of reactive oxygen species was proposed to be a major factor to mediate hypertension [45]. In the L-NAME-induced hypertension model, it was suggested that a large quantity of superoxide production suppressed nitric oxide bioavailability [19,46]. In addition, Duarte and

co-workers (2002) found an increase in plasma and liver MDA levels in L-NAME hypertensive rats, indicating the involvement of oxidative stress in this animal model [47]. Our study was carried out to analyze oxidative stress markers in plasma and vascular tissues and evaluate the antioxidant property of MC extract in L-NAME-induced hypertension. The presence of oxidative stress was indicated by an increase in superoxide production in vascular tissues and plasma MDA in L-NAME hypertension. These findings are consistent with the previous studies as mentioned above. A reduction of oxidative stress was found in the hypertensive rats receiving MC extract. We also found that L-NAME-induced hypertension was partially prevented by MC extract, and this preventive effect might be connected with its antioxidant properties. The antioxidant properties of MC extract used in this study were confirmed by the large amount of total phenolic compounds it contained. Moreover, most of phytochemical studies of plant in the mint family, *Mentha piperita* L., showed the potential antioxidant capacity to exert beneficial biological effects [48]. Several lines of evidence have shown that administration of antioxidant substances significantly inhibits the development of L-NAME-induced hypertension. For example, administration of the antioxidant flavonoid, quercetin, prevented the development of L-NAME-induced hypertension which was related to the reduction of oxidative stress status in this animal model. Penchanova and co-workers (2004) found that red wine polyphenols reduced the increase in blood pressure in rats treated with L-NAME, and this was accompanied by a reduction of oxidative stress and an increase of nitric oxide synthase activity [18].

In conclusion, the present study demonstrates that MC extract inhibited the development of hypertension in nitric oxide deficient rats. This was associated with a reduction in total peripheral resistance and improvement of vascular functions. The underlying mechanisms are likely to be related to the antioxidant properties of MC extract. This finding supports the beneficial effect on the cardiovascular system of a plant in the mint family, which is widely consumed in many countries.

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Figure captions

Fig. 1 Effect of MC extract on systolic blood pressure in control rats, normal rats with MC extract, L-NAME-treated rats (hypertension), and L-NAME-treated rats (hypertension) with MC extract (n=8-10/group) at 1st, 2nd, and 3rd week. * $P< 0.001$ vs normal control, # $P< 0.05$ vs hypertension (ANOVA).

Fig. 2 Effects of MC extract on the changes in MAP in response to acetylcholine 3, 10, 30 nmol/kg (a) and phenylephrine 0.01, 0.03, 1 mol/kg (b) in control rats, normal rats with MC extract, L-NAME-treated rats (hypertension), and L-NAME-treated rats (hypertension) with MC extract (n=8-10/group) * $P< 0.05$ vs control, # $P< 0.05$ vs hypertension (ANOVA).

Fig. 3 Effect of MC extract on plasma MDA (a) and superoxide production in carotid arteries (b) in control rats, normal rats with MC extract, L-NAME-treated rats (hypertension), and L-NAME-treated rats (hypertension) with MC extract (n=8-10/group) (n=8-10) * $P< 0.05$ vs control, # $P< 0.05$ vs hypertension (ANOVA).

1 **Table 1** Effect of MC extract on SBP, DBP, MAP, heart rate, HBF and HVR in all experimental groups.

Parameters	Normal control	Normal control + MC extract	Hypertension + vehicle	Hypertension + MC extract
SBP (mmHg)	129.6 ± 5.1	136 ± 4.3	199.1 ± 3.2*	176.4 ± 4.8*#
DBP (mmHg)	76.1 ± 7.7	74.1 ± 4.8	129.1 ± 3.5*	98.8 ± 6.3*#
MAP (mmHg)	92.9 ± 5.4	102.5 ± 4.7	162.3 ± 2.7*	135.1 ± 5.8*#
HBF (ml/100 g tissue/min)	6.8 ± 0.3	7.4 ± 0.6	3.6 ± 0.2*	5.4 ± 0.3*#
HVR(mmHg/min/100 g tissue/ml)	12.6 ± 0.6	15.3 ± 1.8	39.1 ± 3.8*	28.5 ± 2.2*#
Heart rate (beat/min)	331.7 ± 16.2	368.3 ± 8.8	414.7 ± 13.1*	363.5 ± 9.3#

2 * $P< 0.001$ vs normal control, # $P< 0.05$ vs hypertension (ANOVA) (n = 8-10/group).

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Fig.1

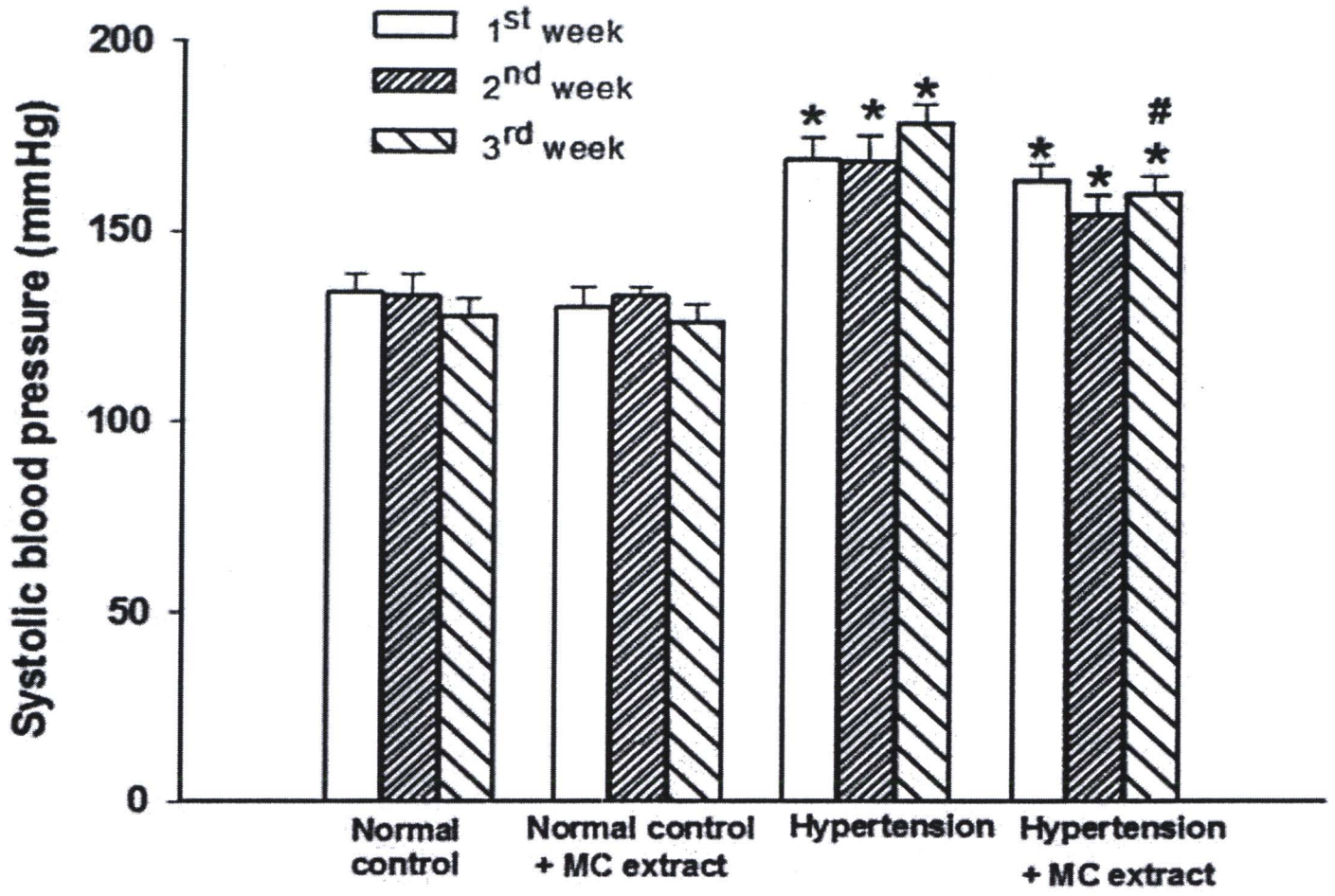

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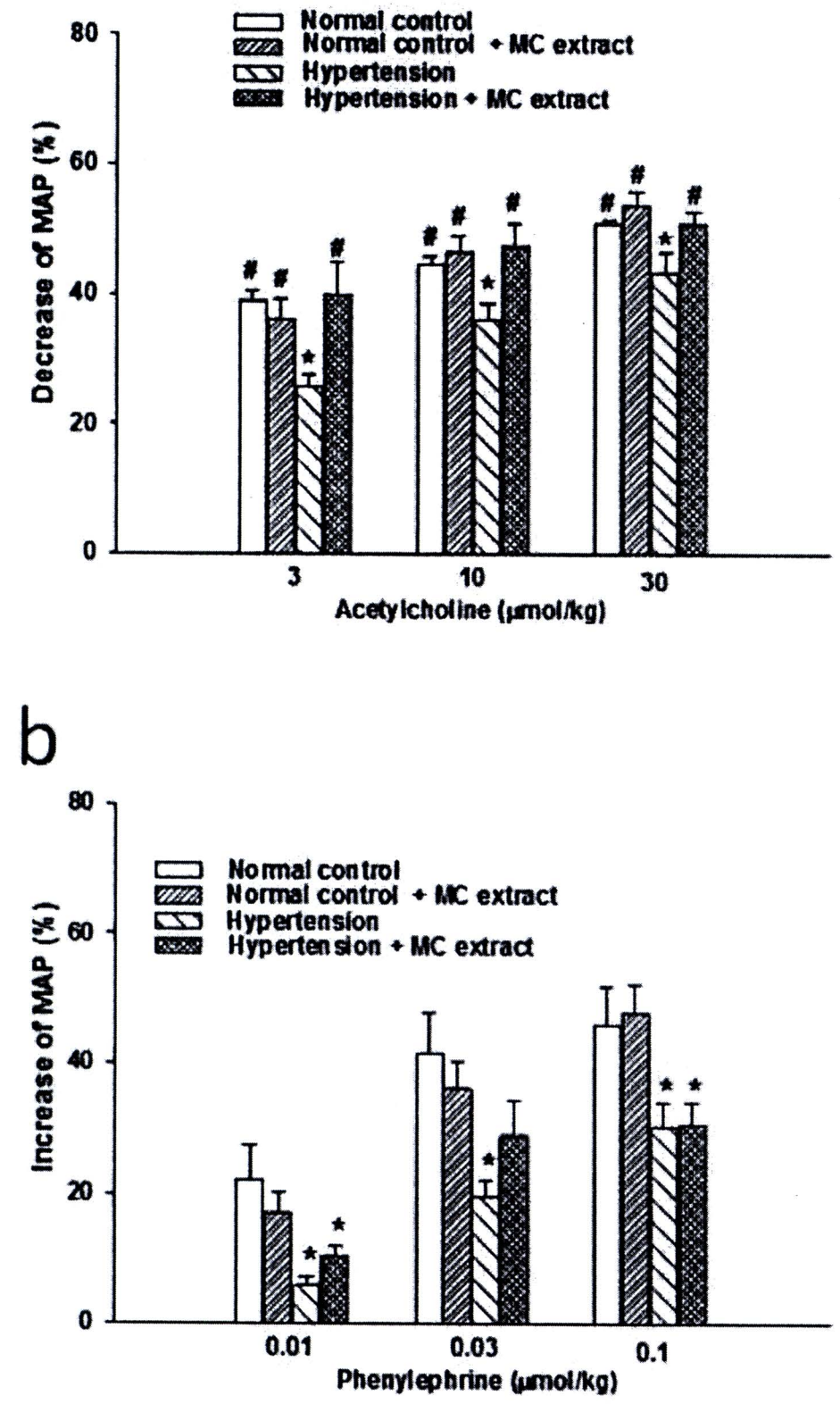
Fig. 2



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