Effect of Mamao Pomace on the Reduction of Blood Pressure in L-NAME- Induced Hypertensive Rats

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Background and Objective: Mamao juice and wine are the commercial products produced from mamao fruits. It has been demonstrated that mamao pomace (MP), a by-product generated from mamao fruits, contains a large amount of antioxidant polyphenolic compounds. *Hypertension* is one of the major risk factors of *cardiovascular disease (CVD) and its development has* a close link with oxidative stress. The aim of this study was to evaluate whether MP could protect against N(G)-Nitro-L-arginine-methyl ester (L-NAME)-induced hypertension and *oxidative stress* in rats.

Methods: Male Sprague–Dawley rats were administered with L-NAME (a nitric oxide synthase inhibitor) at a dose of 50 mg/kg/day in drinking water for three weeks. MP (100 mg/kg) was administered once daily to animals simultaneously with L-NAME. Rats in normal control group served as normotensive animals.

Results: Marked increases in blood pressure, peripheral vascular resistance, and oxidative stress were found in rats after L-NAME administration. MP significantly reduced these deleterious effects, by reducing blood pressure and improving hemodynamic status concomitant with reduction in vascular superoxide ($O_2^{\cdot-}$) production when compared with L-NAME control group ($p<0.05$).

Conclusion: The present study suggests that MP might be used as a dietary supplement to protect against hypertension and oxidative stress in NO (nitric oxide) deficient condition.

Key words: Mamao pomace, hypertension, oxidative stress

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Proceeding

Introduction

Hypertension is one of the risk factors related to *CVD*[1,2]. It is a widely accepted that nitric oxide (NO) plays a central role in protecting the cardiovascular system against hypertension and injury. L-NAME, an L-arginine analogue, is the most frequently used NO synthase inhibitor in experimental animals. Administration of L-NAME has been reported to induce hypertension which is associated with oxidative stress in rats[3]. Previous studies have been demonstrated that hypertension is attenuated by antioxidants [4]. Therefore, supplementation of antioxidant should be one of the important components of an effective treatment and prevention L-NAME-induced hypertension.

Mamao (Family *Stilaginaceae,* Genus *Antidesma*) is an evergreen tree grown in various parts of Thailand. Currently, the product from mamao juice and wine are very popular. A large number of by-products in the form of mamao seed and mamao pomace (MP) have been generated and recently reported to possess strong antioxidant of polyphenolic compounds [5].

Objective

The present study was carried out to investigate whether MP could reduce blood pressure and improve hemodynamic disturbance in associations with oxidative stress in rats with L-NAME-induced hypertension.

Methods

Animals

The procedures and experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee of Khon Kaen University. Male Sprague–Dawley rats weighing 200–220 g were obtained from the National Laboratory Animal Center, Mahidol University. All study animals were housed under constant temperature and exposed to a 12-h light–dark cycle at North Eastern Laboratory Animal Center, Khon Kaen University. They were fed with a standard chow diet (Chareon Pokapan Co. Ltd., Thailand). After one week of acclimatization, rats were randomly divided into four groups of 4 animals, consisting of (1) normal control, (2) normal control + MP 100 mg/kg, (3) L-NAME control and, (4) L-NAME + MP 100 mg/kg. Rats in the normal control group received tap water, whereas rats in the L-NAME-treated group received L-NAME (50 mg/kg/day) in their drinking water for three weeks.

Hemodynamic measurements

Systolic blood pressure (SBP) was measured in conscious rats by using the indirect method of tail-cuff plethysmography (Blood pressure analyzer, model 29; IITC, Woodland Hills, California, USA). SBP measurement was performed before and after three weeks of the treatment periods. On the last day of the experiment, rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg). A tracheotomy was performed for spontaneous breathing, and left femoral artery was cannulated with polyethylene catheter connected to a pressure transducer for continuous monitoring of blood pressure (BP), using the Acqknowledge data acquisition analysis software (BIOPAC Systems Inc., California, USA). Baseline values of BP and heart rate were monitored for 10 min, and a second polyethylene catheter was inserted into the femoral vein to allow i.v. drug delivery. Subsequently, hindlimb blood flow (HBF) was continuously measured by opening the abdominal cavity below the kidneys and placing an electromagnetic flow probe around the abdominal aorta connected to an electromagnetic flowmeter (Carolina Medical Electronics Inc., East Bend, NC, USA). Hindlimb weight was obtained by cutting the hindlimb of the rat and weighing. Hindlimb vascular resistance (HVR) was calculated from mean arterial pressure an hindlimb blood flow in 100mg tissue units. At the end of study, rats were sacrificed by over dosage of the anesthetic drug. The carotid arteries were rapidly excised from the animal and used for analysis of $O_2^{\cdot-}$ production. Vascular $O_2^{\cdot-}$ production was measured using lucigeninenhanced chemiluminescence method as described previously [6].

Results

Effect of MP on hemodynamic measurements

Effects of MP on indirect and direct blood pressure are shown in Figure 1 and 2. At the beginning of the experiments, there were no significant differences in average baseline values of SBP among all experimental groups (Figure 1). Administration of L-NAME caused a progressive increase in SBP when compared with normal controls. The increased SBP was already significant after the first week of L-NAME administration. Treatment with MP (100 mg /kg per day) at the second and third weeks reduced the increase in SBP induced by L-NAME. Moreover, a significant increase in SBP, diastolic blood pressure (DBP), and mean arterial blood pressure (MAP) were found in rats after receiving L-NAME ($p<0.05$; Figure 2A, B and C).

The increase in arterial blood pressure was accompanied by decreased HBF and increased HVR

(Figure 3A and B). Rats receiving MP (100 mg/kg/day) together with L-NAME showed a significant decrease in arterial blood pressure ($p<0.05$; Figure 2A, B and C) and prevented decreased HBF and increased HVR indicating maintenance of normal vasomotor tone and improved hemodynamic status in these animals when compared to those treated with L-NAME alone.

Effect of MP on vascular $O_2^{\cdot-}$ production

$O_2^{\cdot-}$ is a reactive oxygen species that is generated under physiological and pathological conditions. Exposure to L-NAME for 3 weeks induced a marked production of $O_2^{\cdot-}$ as shown in Figure 4, that rats treated with L-NAME had a higher amount of $O_2^{\cdot-}$ production in the carotid arteries by 3-fold when compared with those found in untreated rats (Figure 4). MP significantly reduced the rate of $O_2^{\cdot-}$ production as compared to rats treated with L-NAME alone ($p< 0.05$).

Conclusion

The major novel finding of our study is that MP partially reduces high BP in L-NAME-induced hypertensive rats. This was associated with an improvement of hemodynamic status. The underlying mechanism might be partly due to the strong antioxidant property of MP, including the suppression of $O_2^{\cdot-}$ production. These findings suggest that dietary supplementation of MP may be useful to prevent oxidative stress and hypertension.

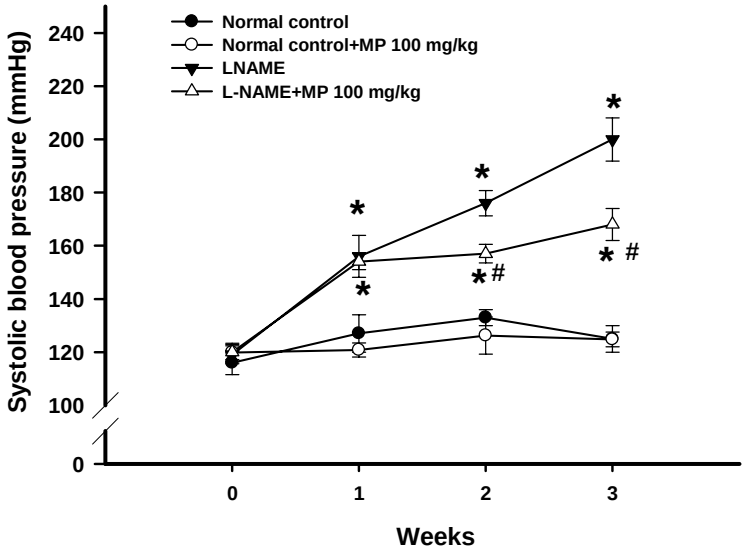

Figure 1 Effect of MP on systolic blood pressure during LNAME administration for three weeks. Results are expressed as mean ± SE of five animals/group. * $p<0.05$ vs. normal control group, # $p<0.05$ vs. L-NAME control group

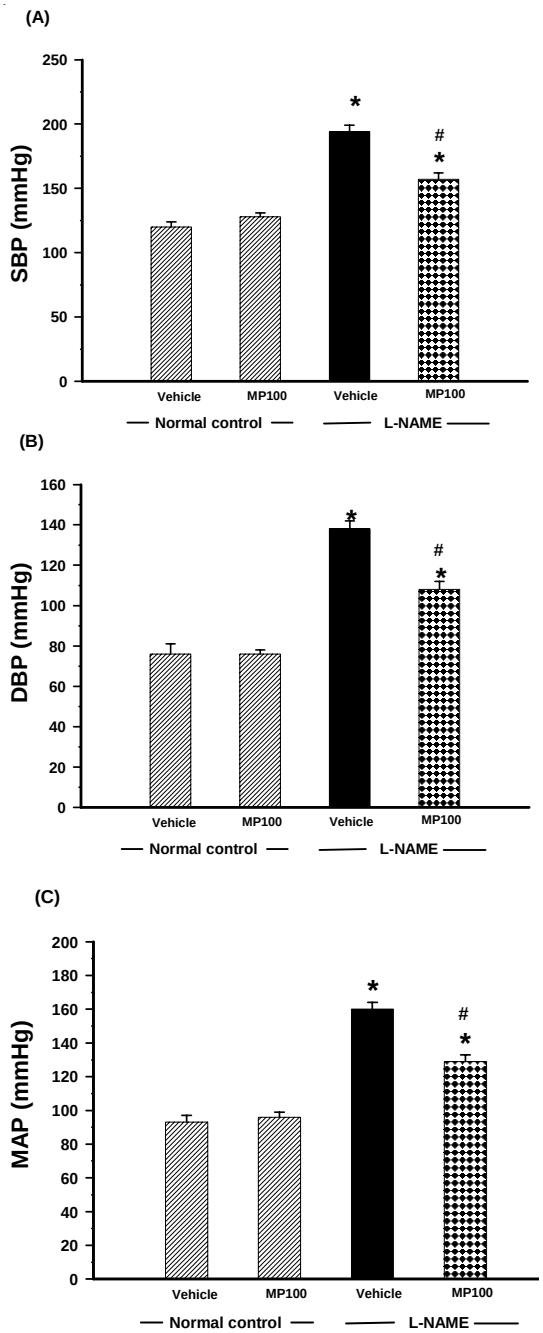

Figure 2 Effect of MP on arterial blood pressure of rats in all experimental groups. Values are mean ± SE. Each group contains five animals. *SBP*; Systolic blood pressure, *DBP;* diastolic blood pressure, *MAP*; mean arterial pressure. * p <0.05 vs. normal control group; # p <0.05 vs. L-NAME control group

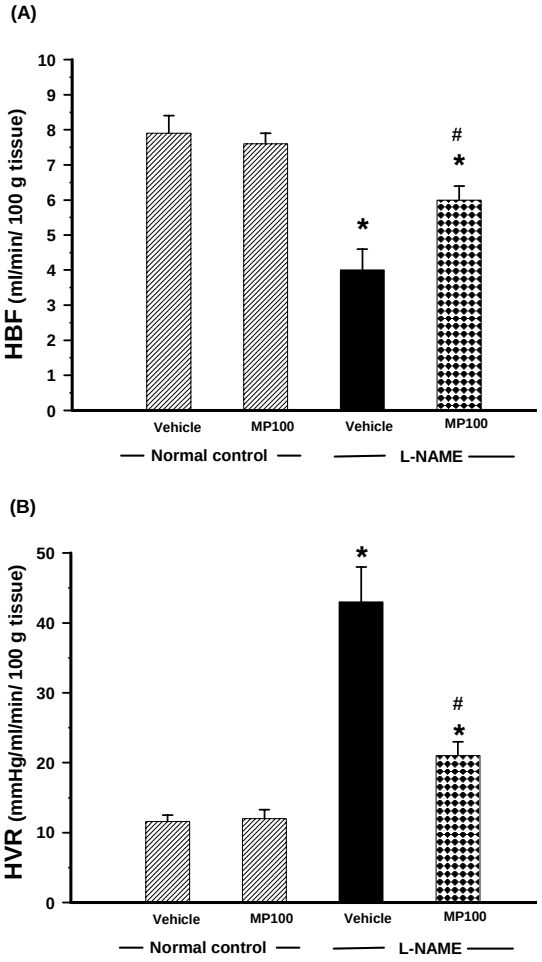

Figure 3 Effect of MP on hemodynamic status of rats in all experimental groups. Values are mean ± SE. Each group contains five animals. *HBF*; hindlimb blood flow, *HVR*; hindlimb vascular resistance. * p<0.05 vs. normal control group; # p<0.05 vs. L-NAME control group.

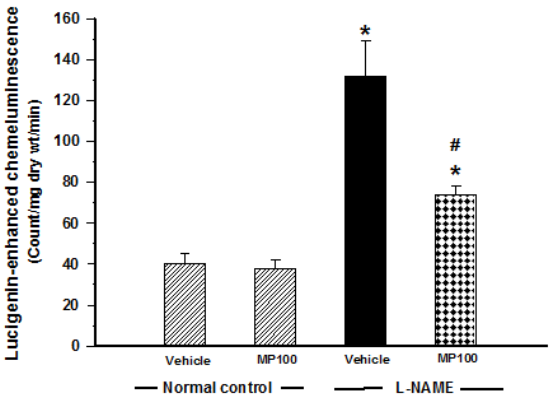

Figure 4 Effect of MP on superoxide production in the carotid arteries. Results are expressed as mean ± SE of five animals/group. * p<0.05 vs. normal control group, # p<0.05 vs. L-NAME control group.

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