Chinese Journal of Physiology 55(1): 22-30, 2012
DOI: 10.4077/CJP.2012.AMM104

Attenuation of the Extract from *Moringa Oleifera* on Monocrotaline-Induced Pulmonary Hypertension in Rats

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Abstract

The purpose of this study was to determine the effects of an extract from *Moringa oleifera* (MO) on the development of monocrotaline (MCT)-induced pulmonary hypertension (PH) in Wistar rats. An ethanol extraction was performed on dried MO leaves, and HPLC analysis identified niaziridin and niazirin in the extract. PH was induced with a single subcutaneous injection of MCT (60 mg/kg) which resulted in increases in pulmonary arterial blood pressure (Ppa) and thickening of the pulmonary arterial medial layer in the rats. Three weeks after induction, acute administration of the MO extract to the rats decreased Ppa in a dose-dependent manner that reached statistical significance at a dose of 4.5 mg of freeze-dried extract per kg body weight. The reduction in Ppa suggested that the extract directly relaxed the pulmonary arteries. To assay the effects of chronic administration of the MO extract on PH, control, MCT and MCT+MO groups were designated. Rats in the control group received a saline injection; the MCT and MCT+MO groups received MCT to induce PH. During the third week after MCT treatment, the MCT+MO group received daily i.p. injections of the MO extract (4.5 mg of freeze-dried extract/kg of body weight). Compared to the control group, the MCT group had higher Ppa and thicker medial layers in the pulmonary arteries. Chronic treatments with the MO extract reversed the MCT-induced changes. Additionally, the MCT group had a significant elevation in superoxide dismutase activity when normalized by the MO extract treatments. In conclusion, the MO extract successfully attenuated the development of PH *via* direct vasodilatation and a potential increase in antioxidant activity.

Key Words: pulmonary hypertension, *Moringa oleifera*, monocrotaline, superoxide dismutase

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Received: November 3, 2010; Revised: January 31, 2011; Accepted: February 17, 2011.
 ISSN : 0304-4920. http://www.cps.org.tw

Introduction

Moringa oleifera (MO) belongs to the species Moringaceae. MO contains several phytochemicals, some of which are of high interest for their medicinal value. The leaves of MO contain nitrile glycosides such as niazirin and niazirinin, and mustard oil glycosides such as 4-[(4'-O-acetyl-α-L-rhamnosyloxy) benzyl] isothiocyanate, niaziminin A, and niaziminin B (6). These glycosides are reported to have hypotensive (6) and antioxidant activities (9). Animal studies have shown that administration of an extract from MO for two weeks elevates the level of glutathione (GSH) in the liver (7) and prevents acetaminophen-induced liver injury (7). Niaziridin, a nitrile glycoside, is a bioenhancer for drugs and nutrients, including vitamins A and C (15). Clinically, co-administration of niaziridin is useful for reducing drug toxicities. Therefore, in this study we sought to determine the medicinal properties of the MO extract.

Pulmonary hypertension (PH), a severe and life-threatening disease in humans, is characterized by a significant increase in pulmonary arterial blood pressure (Ppa). To investigate this ailment, animal models of PH induced by chronic hypoxia exposure (23) or monocrotaline (MCT) administration (20) have been established. MCT is a pyrrolizidine alkaloid that is metabolized in the liver producing a toxic metabolite. Pulmonary endothelial injury is evident within one week of MCT administration (11, 12). This pathologic lesion induces proliferation of smooth muscle cells in the pulmonary arterial tree (12). Remodeling is the critical step in the development of PH (12) as smooth muscle proliferation increases the resistance in the pulmonary circulation to elevate Ppa (12). Ppa is significantly increased two weeks after MCT treatment and is more severe three weeks post-MCT (2). Prolonged elevation of Ppa leads to compensatory right ventricular hypertrophy (16).

Analysis of lung tissue homogenate has shown that reactive oxygen species contribute to the development of PH (2, 3). Reducing the production of reactive oxygen species, either in the early or late period of MCT-induced PH, successfully attenuates the development of PH (2, 3). This effect reflects the fact that oxygen radical scavengers are effective either in the prevention or in the treatment of PH. The major consideration of the clinical treatment of PH shall be in vasodilation. Many of the vasodilators that are useful for systemic hypertension work poorly for PH. Inhalation of nitric oxide gas is a common clinical therapy for PH, but tolerance by patients is poor (19). PH is not well understood and is not easily detected or treated. Therefore, one of the purposes of this study is to find a useful treatment for PH.

Considering the role of reactive oxygen species in enhancing PH and both the antioxidant and vasodilatory properties of the MO extract (7, 9), we hypothesized that the MO extract could reverse the development of PH. In this study, we examined the effects of an MO extract on MCT-induced PH. Our results suggest that the MO extract attenuates PH in rats *via* an elevation in antioxidant activity. As far as we are aware this report is the first to demonstrate the pharmacological benefits of the MO extract in PH.

Materials and Methods

MO Extract

The extract was prepared by Dr. Chao-HsunYang of Providence University in Taichung, Taiwan. In brief, fresh leaves of MO were collected from Nantou, Taiwan. Soon after collection, the leaves were freeze-dried, ground into powder, and stored at -18°C until further use. The bioactive components of the herbal powder were then isolated by hexane extraction (powder:solvent = 1:2 w/v) with constant shaking (50 rpm) at room temperature overnight. The extract was filtered, and the residue was suspended in hexane to repeat the extraction process. The filtered fluids were mixed well and were concentrated under vacuum at 50°C. The concentrate was then freeze-dried as a powder form and stored at -18°C. Before usage, the extract was dissolved in ethanol (stock solution was 3 g freeze-dried MO extract/100 ml ethanol). The working solution was further diluted with double-distilled H_2O.

High Performance Liquid Chromatography (HPLC)

The compounds in the extract from MO were determined by HPLC (Agilent 1100 series, USA) using a pre-packed RT 250-4.6 MIGHTYSIL RP-18 GP 5 mm column (4.6 × 250 nm, Kanto Chemical, Tokyo, Japan) and a 220-nm UV detector. The mobile phase, composed of methanol:sodium dihydrogen phosphate-acetic acid buffer (0.1 M, pH 3.8; 20:80), was used as the eluant at a flow rate of 1.0 ml/min.

Animal Preparation

All the animals were cared for in accordance with the *Guide for the Care and Use of Laboratory Animals* (1996, published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, U.S.A.), and experiments were approved by the Laboratory Animal Care Committee of the School of Health, Ming Chuan University, and by the Taiwan Council on Animal Care. We obtained the experimental animals from the animal center of National

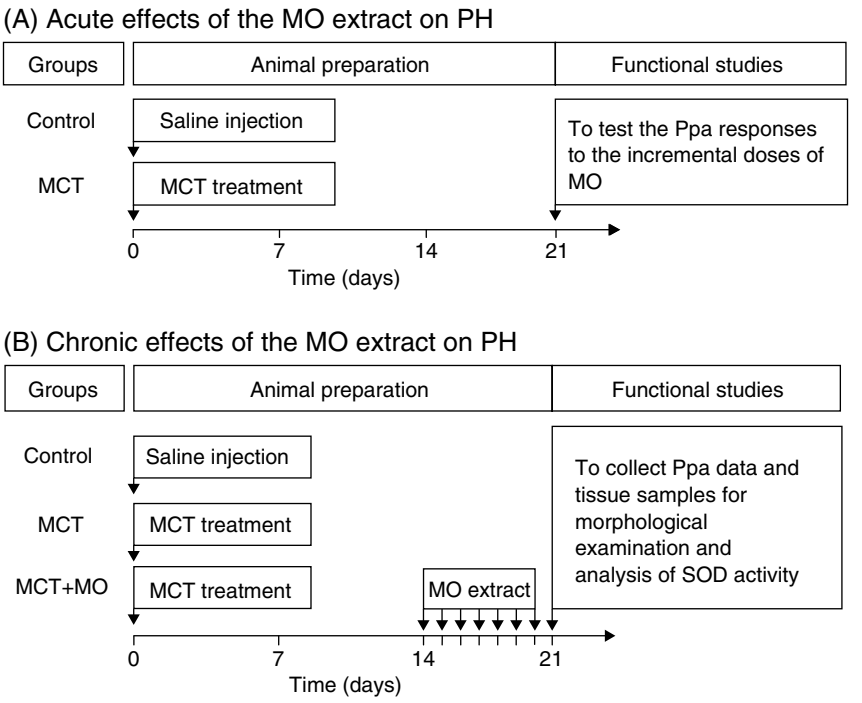

Fig. 1. Time schedules of treatments with the MO extract for analyzing acute and chronic effects of the extract on monocrotaline (MCT)-induced pulmonary hypertension (PH). (A) 6-week-old rats in the control and MCT groups received saline or an MCT injection on day 0. Functional studies to test the vasodilatory effects of an extract from *Moringa olifera* (MO) were carried out on day 21. (B) The control, MCT and MCT+MO groups were designed to analyze the chronic effects of the MO extract on MCT-induced PH. The injection of saline in the control group, or MCT in the MCT and MCT+MO groups, was carried out on day 0. The MO extract was given to the MCT+MO group from day 14 to day 20.

Taiwan University.

The schedule of acute treatments and functional assays is depicted in Fig. 1A. To understand the acute vasodilatory effects of the MO extract on PH, 6-week-old male Wistar rats were divided into two groups: control (n = 26) and MCT (n = 22). Rats in the control group received a saline injection. For the MCT group, one bolus of MCT (60 mg/kg) was given subcutaneously to each rat to induce PH. Neither activity nor food was restricted in either group of rats. Various doses of the MO extract were administered 3 weeks following MCT treatment to determine its acute effects on Ppa.

The schedule of chronic animal treatments and functional evaluation is depicted in Fig. 1B. To examine the chronic effects of the MO extract on the development of PH, 6-week-old male Wistar rats were divided into three groups: control (n = 7), MCT (n = 7) and MCT+MO (n = 8). Rats in the control group each received a saline injection. Rats in the MCT and MCT+MO groups each received a subcutaneous MCT injection (60 mg/kg). Rats in the MCT+MO group received daily i.p. administrations of the MO extract (4.5 mg of freeze-dried MO extraction/kg) on days 14-20 following MCT injection. All rats were freely mobile and fed *ad libitum*. Functional studies were carried out when the rats were 9 weeks old.

Functional Studies

Acute Vasodilatory Effects of the MO Extract on PH

Ppa was used as the index for PH. Measurements of Ppa and systemic hemodynamics were carried out 3 weeks after the MCT or saline treatment. Rats in the control group were monitored for the same period of time as the MCT group. On the day of the measurements, rats were anesthetized with urethane (1.2 g/kg, i.p.). After insertion of an endotracheal cannula and a femoral arterial catheter, a saline-filled catheter was introduced into the right jugular vein and then advanced to the right atrium, right ventricle, and finally to the pulmonary artery. The catheter monitored acute changes in Ppa. Ppa was measured with a Grass pressure transducer (P23XL, Grass technologies, Rhode Island, USA), with its diaphragm located at the level of the heart, and Ppa was recorded on a Biopac System (MP35, Biopac, Goleta, CA, USA) (Fig. 2). Mean Ppa was further calculated according to the following formula: diastolic pressure + 1/3 pulse pressure. The systemic mean blood pressure and heart rate were measured through the right femoral

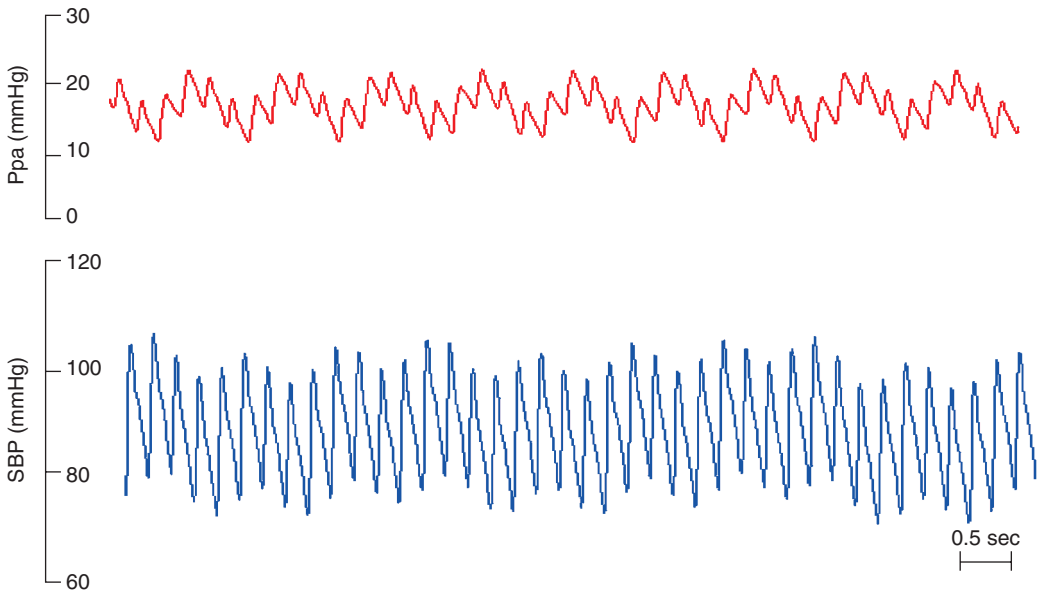

Fig. 2. Tracing curves of pulmonary arterial blood pressure (Ppa) and systemic blood pressure (SBP) in the control rats.

arterial catheter with a Grass pressure transducer and Biopac MP35 System recorder, as described for Ppa measurement and recording. The measurements were collected when values stabilized after the surgical procedures. After recording the baseline hemodynamic parameters and the mean Ppa, the effects of incremental doses of the MO extract (1.5, 4.5 and 15.0 mg of freeze-dried MO extract/kg, i.v.) were then studied. Each animal received two different doses. The second dose was always higher than the first and was administered at least one hour after all parameters had recovered to their initial level.

Chronic Effects of the MO Extract on PH

To evaluate the chronic effects of the MO extract on PH, three weeks after the MCT treatment, we measured Ppa, conducted morphologic examinations, and determined superoxide dismutase (SOD) activity in the control, MCT and MCT+MO groups. On the day of the measurement, rats were anesthetized with urethane (1.2 g/kg, i.p.). After insertion of an endotracheal cannula and a right femoral arterial catheter, artificial ventilation with a respirator at a rate of 60 breaths/min and a tidal volume of 6-8 ml/kg was applied. The chest of the rat was then opened *via* a midline sternotomy. A 22-G needle filled with heparinized saline was inserted through the wall of the right ventricle and advanced into the pulmonary artery (15). Ppa, systemic mean blood pressure and heart rate were measured, and mean Ppa was calculated as the sum of diastolic pressure and 1/3 pulse pressure. After the measurements were obtained, the heart was removed and the right ventricle (RV) was separated from the left ventricle and septum (LV+S). Both portions were weighed, and the weight ratio RV/(LV+S) was calculated and used as the index of right ventricular hypertrophy. Finally, the right lung tissue was dissected and frozen at -80°C for subsequent determination of SOD activity. The left lung was dissected for morphological examinations.

Morphological Examinations

Lung samples were taken from each experimental group for morphological examinations. The left lung was excised after the functional study and was inflated for 30 min with 4% formaldehyde to maintain a pressure of 25 cmH_2O. Then, the trachea was tied, and the lung lobe was immersed in a 4% formaldehyde solution. Tissue blocks were taken from rostral, middle and caudal portions of the formaldehyde-immersed lung. These blocks were washed, fixed and vacuum-embedded in paraffin. From each block, at least three nonconsecutive 2-µm sections were cut, stained with hematoxylin and eosin, and examined by light microscopy for the thickness of pulmonary arteries according to the method of Zhou and Lai (24).

SOD Activity Assay

The endogenous SOD activity in the lung tissue was measured by an SOD assay kit (Cat. 7500-100K; Trevigen Int, Gaithersburg, MD, USA). Aliquots of supernatants from the homogenized lung tissues were used for determining SOD activity by both the xanthine/xanthine oxidase (X/XOD) (8) and the NitroBlue

Tetrazolium (NBT) assays. In brief, homogenized lung tissue (0.05 g) was added to 60 µl of cold PBS for 5 min. The insoluble components were separated by centrifugation. The supernatant was then removed into a clean cuvette and deionized water was added to 42.5 µl. The 25× Reaction Buffer (60 µl), X solution (7.5 µl) and NBT Solution (30 µl) were then mixed well with the homogenized tissue extract. At the absorbance at 550 nm, each sample was measured for 330 seconds immediately after addition of 10 µl XOD. Displacement of tissue extract by deionized water was used as a negative control, and SOD was used as a positive control. Superoxide anion, generated from the conversion of X/XOD, converted NBT to NBT-diformazan which absorbed light at 550 nm (A550). SOD reduced superoxide anion concentrations and thereby lowered the rate of NBT-diformazan formation. Determinations of the rate of increase in absorbance units (A) per minute for the negative control and samples were calculated as $(A550_{330\ sec.} - A550_{30\ sec.})/5$ min = ΔA550/min. Thus, the % inhibition of SOD in the lung tissue was calculated as $[(\Delta A550/min)_{negative\ control} - (\Delta A550/min)_{sample}]/(\Delta A550/min)_{negative\ control} \times 100$ = % inhibition. This value increased with the concentration of SOD in the lung tissue. All measurements were performed in duplicates.

Statistical Analysis

Data are presented as means ± SEM. Using Student's *t*-test, the acute Ppa responses to the MO extract were analyzed by comparison to baseline data before MO extract administration. To evaluate the chronic effects of the MO extract, the systemic hemodynamic parameters, Ppa, pulmonary arterial medium thickness, RV/(LV+S) and % inhibition of SOD were analyzed as follows: a one-way analysis of variance was used to establish the statistical significance of differences among groups; if there was a significant difference among groups, statistical differences between any two groups were analyzed with Newman-Keuls multiple group comparisons. Differences were considered significant if $P < 0.05$.

Results

The HPLC fingerprint of the crude 3% MO extract is shown in Fig. 3. The fingerprint was similar to that reported by Shanker *et al.* (17). Two peaks representing niaziridin and niazirin were identified in the spectrum from the MO extract (Fig. 3).

The acute responses of Ppa to the MO extract are summarized in Fig. 4. In the control rats, there was no significant difference in Ppa at any dose used. In addition, vehicle did not alter Ppa either in the control or MCT-treated rats. Ppa decreased immediately

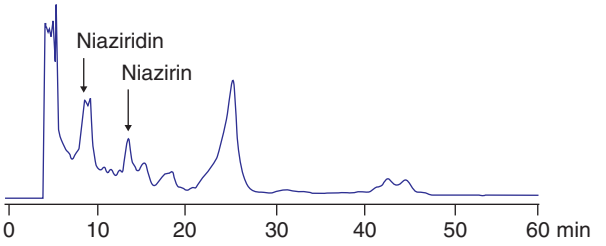

Fig. 3. Analysis of the 3% *Moringa oleifera* (MO) crude extract by HPLC identified two components, niazirin and niaziridin.

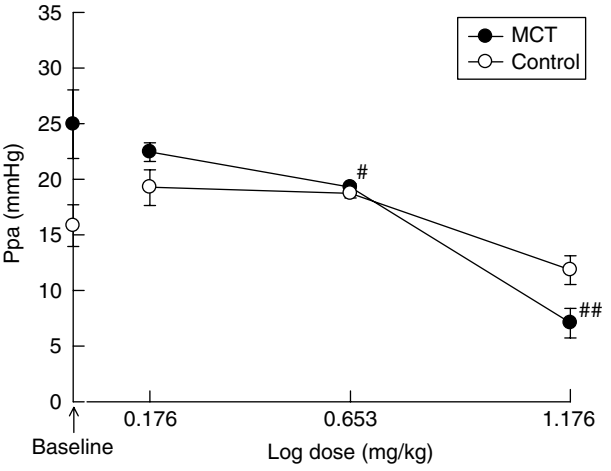

Fig. 4. Pulmonary arterial pressure (Ppa) in rats. Abscissa: log of dose (mg of freeze-dried MO extract per kg of body weight). Open circles indicate the Ppa changes caused by the acute *Moringa oleifera* (MO) extract in the control group. Solid circles indicate the Ppa changes affected by acute treatment with the MO extract in the monocrotaline (MCT)-treated group. Bars indicate 1 SE. The MO extract caused a significant decrease in Ppa at the dose of 4.5 ($^{\#}P < 0.05$) and 15.0 ($^{\#\#}P < 0.01$) mg of freeze-dried MO extract/kg.

after the administration of the MO extract to MCT-treated rats (data not shown). The MO extract caused a dose-dependent decrease in Ppa in the MCT groups. Low doses of the MO extract (*e.g.* 1.5 mg/kg) caused a small non-significant decrease in Ppa. Administration of 4.5 mg/kg of the MO extract reduced Ppa to approximately 80% of that of the MCT control and reached statistical significance. The highest dose, at 15.0 mg/kg, significantly reduced Ppa to 51.4% of that of the MCT control.

The effects of chronic administrations of the MO extract to MCT-treated rats are summarized in Table 1 and Figs. 5-9. There were no significant differences in either body weight or systemic blood pressure among the groups (Table 1). Notably, the MO extract increased the heart rate of the rats (Table 1). The chronic effects of treatment with the MO ex-

Table 1. Body weight (BW) and systemic hemodynamic parameters in three groups of rats

Group	Control (n = 7)	MCT (n = 7)	MCT+MO (n = 8)
BW (g)	259.3 ± 3.5	247.9 ± 3.5	245.6 ± 7.7
SBP (mmHg)	89.0 ± 5.1	79.1 ± 4.1	84.3 ± 4.9
DBP (mmHg)	55.7 ± 5.2	60.9 ± 6.0	65.9 ± 3.8
MBP (mmHg)	66.8 ± 4.8	67.0 ± 5.3	72.0 ± 3.8
HR (beats/min)	313.5 ± 14.0	315.1 ± 12.8	387.6 ± 12.8$^{*,\#}$

Values are means ± SE. n, number of rats; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; HR, heart rate; MCT, monocrotaline; MO, *Moringa oleifera* (MO) extract was injected once daily during the third week post MCT. MO extract caused an increase in HR when compared to control group ($^{*}P < 0.05$) or MCT group ($^{\#}P < 0.05$).

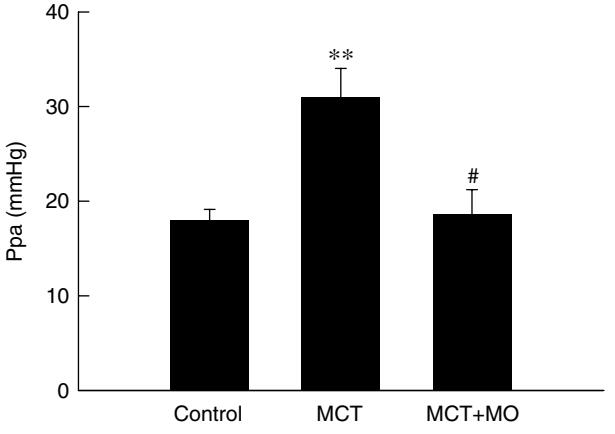

Fig. 5. Pulmonary arterial pressure (Ppa) in three groups of rats. MCT, monocrotaline. MO, *Moringa oleifera* extract administered at 4.5 mg/kg once daily during the third week post MCT. Bars indicate 1 SE. MCT administration caused a significant increase in Ppa ($^{**}P < 0.01$) that was attenuated by the MO extract ($^{\#}P < 0.05$).

tract on Ppa are shown in Fig. 5. In comparison to the control group, MCT administration increased Ppa indicative of PH (Fig. 5). Repeated administrations of the MO extract during the latter stages (third week) significantly reversed the MCT-induced increase in Ppa to a level similar to that of the control group (Fig. 5). This finding reflected treatment, not prevention, of progression of MCT-induced PH by the MO extract.

Photomicrographs of histological sections also supported the results from measurements of Ppa (Fig. 6). Compared to the control group, MCT administration induced thickening of vessel walls with luminal narrowing (Figs. 6A and 6B). Administration of the MO extract for one week reduced the MCT-induced thickening of vessel walls (Figs. 6B and 6C). The statistical results of pulmonary arterial medium thickness are summarized in Fig. 7. Consistent to the Ppa results (Fig. 5), the MCT-induced morphologic change was attenuated by administration of the MO extract (Fig. 7).

The effects of the MO extract on right ventricular hypertrophy are summarized in Fig. 8. MCT administration significantly elevated the weight ratio of RV/(LV+S) indicating the right ventricular hypertrophy. The MCT and MCT+MO groups had a similar degree of right ventricular hypertrophy (Fig. 8).

The results from the SOD activity assay on the tissue samples from the three groups are summarized in Fig. 9. There was no significant difference in the SOD activity between the control and the MCT groups. The MO extract treatments caused a significant increase in SOD activity (Fig. 9).

Discussion

We sought to determine the acute and chronic effects of an extract from MO on MCT-induced PH. Acute administration of the extract reduced Ppa in a dose-dependent manner (Fig. 4). To determine whether the MO extract is able to treat MCT-induced PH, we administered the extract in the third week after MCT treatment. Chronic administrations of the MO extract significantly attenuated the MCT-induced increase in Ppa (Fig. 5) and thickening of pulmonary arterial walls (Figs. 6 and 7). The extract also increased SOD activities (Fig. 9). These effects are further discussed below.

Two important components, niaziridin and niazirin, were identified by HPLC analysis of the MO extract in ethanol (Fig. 3). The HPLC pattern analysis, the so-called "fingerprint" method, provides a useful means of identifying crude drugs and preparatory batches of pharmacologically active standardized extract. Our results were consistent with those of Shanker *et al*. (17): two peaks representing niaziridin and niazirin were identified in the spectrum from the MO extract (Fig. 3). Niaziridin has been demonstrated to be a bioenhancer for antioxidative nutrients such as

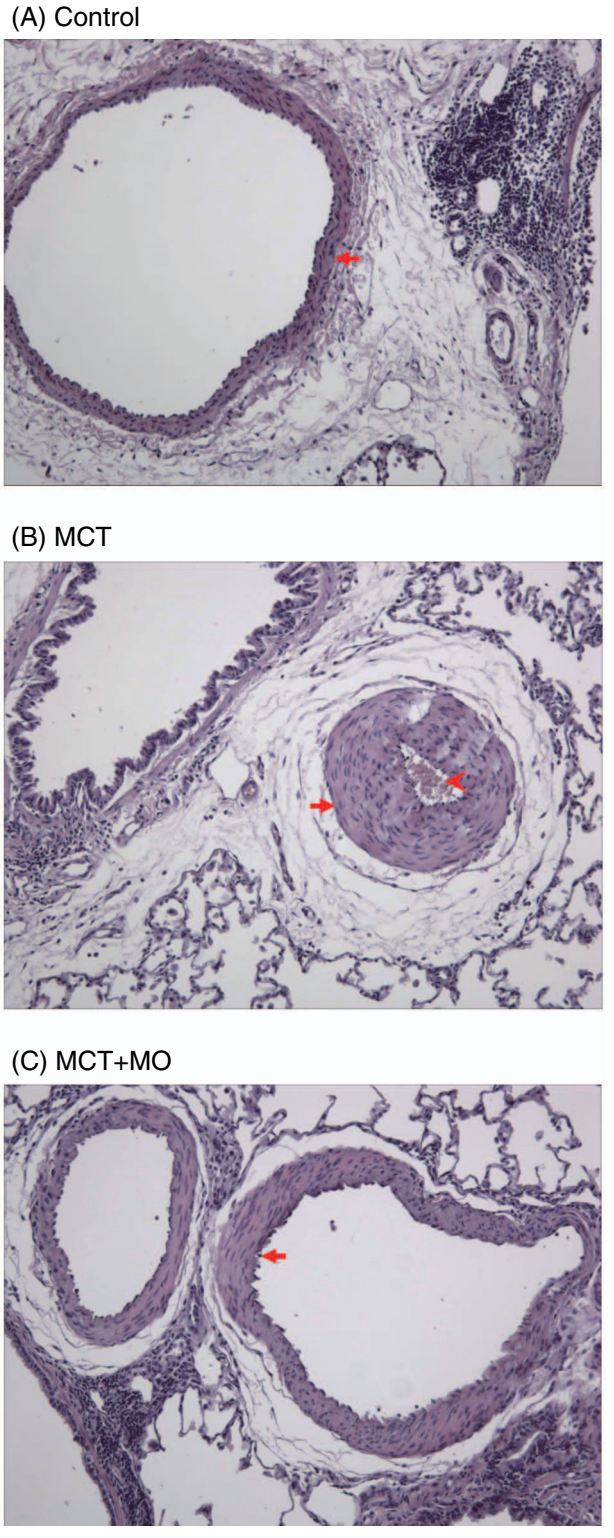

Fig. 6. Photomicrograph of histologic sections from the three groups of rats. MCT, monocrotaline; MCT+MO, monocrotaline treatment followed by MO extraction injections within the third week post MCT. Arrows indicate the smooth muscular layers in pulmonary arterioles. Arrow head in (B) indicates red blood cells. All the imaged fields are amplified 100-fold.

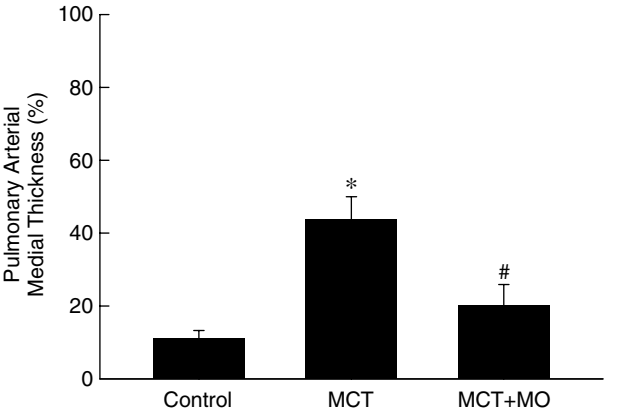

Fig. 7. Differences in pulmonary arterial medium thickness among different experimental groups. MCT, monocrotaline; MCT+MO, monocrotaline treatment followed by the MO extract injections within the third week post MCT. Bars indicate 1 SE. MCT administration caused a significant increase in the muscularization ($^{*}P < 0.05$) that was attenuated by the MO extract ($^{\#}P < 0.05$).

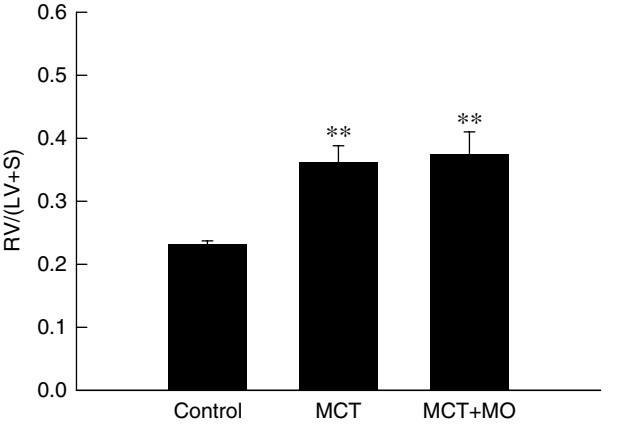

Fig. 8. Weight ratio of the right ventricle (RV) to the sum of the left ventricle and septum (LV+S) in three groups of rats. MCT, monocrotaline; MO, *Moringa oleifera* extract (MO) administered at 4.5 mg/kg once daily during the third week post MCT. Bars indicate 1 SE. MCT administration caused a significant increase in this ratio ($^{**}P < 0.01$).

vitamin A and vitamin C (15), and hypotensive effects of niazirin on spontaneous hypertension in rats have also been documented (5). The hypotensive activity of the MO extract on the systemic circulation has been demonstrated in several studies (5, 6). Bioactive components extracted from the leaves of plants such as thiocarbamates (6) and niazirin (5) have shown hypotensive activity. However, there are many differences between the systemic and pulmonary circulations. The hypotensive agents used in the clinic to control systemic hypertension often do not induce similar reductions in PH thereby limiting the treatment and worsening

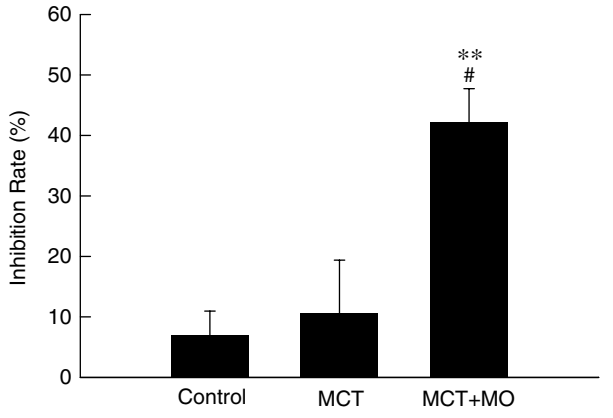

Fig. 9. The SOD activity in three groups of rats. MCT, monocrotaline; MO, *Moringa oleifera extract* (MO) administered at 4.5 mg/kg once daily during the third week post MCT. Bars indicate 1 SE. The MO extract caused a significant increase in SOD activity when compared with the control group ($^{**}P < 0.01$) and the MCT group ($^{\#}P < 0.05$).

the severity of PH. In this study, the hypotensive activity of the extract from MO was evaluated by measurements of Ppa (Fig. 4). The extract we used for this study was from the leaves of MO, similar to that used by Faizi *et al*. (6). We also identified the active component, niazin (Fig. 3). Pulmonary hypotensive activity was observed only in MCT-treated rats immediately after injection of the MO extract, and this activity was dose-dependent (Fig. 4). According to our results, the acute hypotensive effect is attributable to the bioactive component, niazin. Moreover, the MO extract caused a slight, but not statistically significant, increase in Ppa in control rats (Fig. 4), consistent with previous findings (4, 18) that tonic pressure plays an important role in vasodilation and contraction in pulmonary circulation. The only difference between our results and those of Faizi *et al*. (5) was the lack of systemic hypotensive effects. We used the low-dose MO treatment (4.5 mg/kg) in contrast with the high dose (30 mg/kg) used in Faizi's study (5). This most likely explains the absence of systemic hypotension in our study (Table 1). Many reagents have acted as systemic vasodilators, but few have affected pulmonary circulation. To our knowledge, our study is the first to demonstrate hypotensive activity of the MO extract on the pulmonary circulation.

We suggest that the effects of the prolonged MO extract treatment in attenuating MCT-induced PH occur *via* its anti-oxidant properties. Pharmacologically-induced PH in rats is due to inflammatory damage of the pulmonary endothelium (11, 12) followed by remodeling of the pulmonary arterial tree and subsequent elevation of Ppa (12, 14). This progression has been associated with the appearance of reactive oxygen species (ROS) (2). Administrations of antioxidants, dimethylthiourea and hexa (sulfobutyl)fullerenes during the late phase of vascular injury reduced the MCT-induced increases in Ppa and ROS, indicative of the ability of ROS scavengers to treat MCT-induced PH (2). Furthermore, inducing SOD expression *via* gene transfer increased oxygen radical scavenging in MCT-treated rats and successfully prevented the development of MCT-related PH (13). Notably, the MO extract has previously been reported to have antioxidant properties (13). Our data, which show that treatment with the MO extract during the third week following MCT successfully attenuated both the MCT-induced increase in Ppa (Fig. 5) and the thickening of pulmonary arterial walls (Fig. 6), support this claim. Additionally, the MO extract increased SOD activity (Fig. 9). Accordingly, we hypothesize that modulation of PH by the MO extract is due to its antioxidant effects.

The MCT-induced right ventricular hypertrophy was not improved by treatment with the MO extract (Fig. 8). Two possibilities might explain its ineffectiveness. First, the length of administration of the extract was long enough to reduce Ppa but not long enough to decrease right ventricular hypertrophy, which is a compensatory mechanism occurring subsequently to high Ppa. Second, the MO extract might affect cardiac tissue directly. The bioactive components of the MO extract are purported cardiac stimulants (1). Time-course studies in MCT-induced PH suggest the occurrence of right ventricular hypertrophy in as soon as 10 days post-MCT treatment (10), with severity increasing for 3 weeks (21, 22). We administered the MO extract only in the third week by which point the right ventricular hypertrophy might have already manifested. Although both the MCT-induced increase in Ppa and the thickening of the pulmonary arterial walls were reduced by treatment with the MO extract (Figs. 5, 6 and 7), evidence revealed that MCT-induced right ventricular hypertrophy was not affected by this extract (Fig. 8). Additionally, the heart rate was also significantly accelerated by the MO extract in comparison to the MCT group (Table 1). Given these results, we attributed the appearance of right ventricular hypertrophy following treatment with the MO extract to our second hypothesis, a direct stimulatory effect of the MO extract.

There are limited clinical agents to treat PH; thus, it is necessary to develop a new source of pharmacological agents. We demonstrated that the MCT-induced increase in Ppa was reduced by treatment with an extract from MO acting through the antioxidant properties and vasodilatory effect of the extract. The MO extract is readily available and might be a novel drug candidate. More studies are needed, however, to examine therapeutic applications of the

MO extract in the future.

Acknowledgments

This study was supported by grants from the National Science Council (NSC93-2320-B254-001) and from Keelung Hospital.

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