World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
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Available online at: http://www.wjpsonline.org/
Original Article

Bidens pilosa attenuates monocrotaline-induced pulmonary arterial hypertension in rats

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Received: 12-02-2017 / Revised: 27-05-2017 / Accepted: 28-05-2017 / Published: 28-05-2017

ABSTRACT

Bidens pilosa is an Asteraceae plant commonly used as a herb and as an ingredient in teas or herbal medicines. In the present study, we investigated the effects of an ethyl acetate extract of *Bidens pilosa* leaves on pulmonary hypertension in rats induced by intraperitoneal injection of monocrotaline (50 mg/kg) in Wistar rats. One week later, the animals were treated orally with the extract (100 or 200 mg/kg) or sildenafil (1.7 mg/kg) used as control drug for fourteen days. Three weeks after monocrotaline injection, pulmonary arterial pressure and lung weight was significantly increased in the vehicle-treated group, whereas they were attenuated by the extract treatment at the highest dose studied (200 mg/kg). The Fulton index (ratios of right ventricle weight to left ventricle + septum weight) was increased ($P<0.01$) in monocrotaline-treated rats, suggesting the occurrence of right ventricular hypertrophy. Both the extract (200 mg/kg) and sildenafil significantly ($P<0.01$) reduced the Fulton index. Compared to the control group, monocrotaline induced thickening of lung vessel walls with luminal narrowing. The monocrotaline-induced morphologic change was attenuated by administration of the extract. These overall results suggest that the ethyl acetate extract of *Bidens pilosa* might have a promising therapeutic potential for pulmonary arterial hypertension.

Keywords: *Bidens pilosa*, monocrotaline, pulmonary hypertension, cardiac hypertrophy, lung morphology

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a progressive disease which causes an increase in pulmonary vascular resistance and pulmonary arterial pressure and leads to right ventricular dysfunction, failure and ultimately death [1]. The right ventricle (RV) of the heart initially adapts by hypertrophy to maintain cardiac output, but eventually fails [2]. PAH is a chronic progressive and devastating disease in which means pulmonary arterial pressure increases by more than 25 mmHg in the resting state and finally leads to right ventricular failure [3]. The epidemiology of pulmonary hypertension in sub-Saharan Africa is not well-known, but the incidence could be higher than that reported from developed countries, owing to the pattern of diseases prevalent in the region [4]. Many known factors causing pulmonary hypertension are hyperendemic including human immunodeficiency virus (HIV) Acquired Immune Deficiency Syndrome (AIDS), chronic hepatitis B and C, schistosomiasis and sickle cell disease [5].

The treatment of pulmonary hypertension includes lifestyle modifications, conventional treatments such as diuretics, warfarin anticoagulation, and disease-specific treatments such as calcium channel blockers, prostacyclins (intravenous epoprostenol, subcutaneous treprostinil, aerosolized iloprost and oral beraprost), endothelin (ET) receptor antagonists (bosentan, sitaxsentan and ambrisentan) and the known potent and highly specific phosphodiesterase type 5 inhibitor, sildenafil [6]. Although prostacyclin and endothelin receptor antagonists are suggested as first line of therapeutic

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strategy of PAH [7] there are still several potential limitations in these modalities, including side effects of drugs, complexity of administration methods, high medical costs and the development of additional novel therapeutic approaches focusing on the components of multifactorial pulmonary vascular pathobiology is necessary [8,9]. Although current therapies could improve the quality of life of PAH patients, PAH cannot be cured completely by the drugs used in current clinic therapy [10]. Some reports have suggested increased oxidative stress as a mediator in the pathogenesis and the development of PH [11]. Accordingly, antioxidant therapy has been effective in the treatment of right ventricle (RV) dysfunction in PAH [12]. Monocrotaline (MCT)-induced PH is widely recognized as an experimental model for studying right ventricular (RV) hypertrophy [13].

Monocrotaline injures the endothelial cells of the pulmonary blood vessels, causing pulmonary hypertension and interstitial pulmonary fibrosis [14]. This substance also induces the proliferation of muscular intimal cells in arterioles and fibroblasts in alveolar walls at the capillary level [15]. It is now believed that medicinal plants can be a valuable source of assistance for prescription medicines and can be taken to aid recovery from serious disease such as heart failure.

Bidens pilosa L. (Asteraceae) is a plant widely found in the tropical and subtropical regions of the world [16,17]. It is well known in African folk medicinal practices, especially in the western region of Cameroon and parts of Central America, as a potent hypotensive agent. Previous studies have shown that *B. pilosa* exerts a dose-dependent decrease in systolic blood pressure in hypertensive rats [18]. Its roots, leaves, and seeds are reported to have antibacterial, anti-dysenteric, anti-inflammatory and antimicrobial, antimalarial, diuretic, hepatoprotective and hypotensive properties [17,19-22]. Although more and more evidence suggests that *B. pilosa* shows a number of beneficial cardiovascular actions in experimental settings, its effect on PAH has not yet been clarified. On this basis, the present study was carried out to evaluate the efficacy of an ethylacetate extract of *B. pilosa* leaves in an experimental rat PAH model induced by monocrotaline. The study was undertaken to determine the therapeutic effects of an ethyl acetate extract of *B. pilosa* (EAEBP) by measuring not only pulmonary arterial pressure and right ventricular hypertrophy but also histopathological changes and transaminases activity in the lung and liver.

MATERIALS AND METHODS

Extract preparation: Fresh leaves of *B. pilosa* were collected early in the morning from the fields at Messassi-Yaoundé (Center Region-Cameroon) in December 2012. They were identified at the National herbarium and compared to the voucher specimen deposited by Dr Longo Frida under the number 65112/HNC. Powdered leaves (1000 g) were extracted by maceration in 5 L of methylene chloride-methanol (1/1) at room temperature for 2 days. The methylene chloride–methanol extract were concentrated in rotavapor (HEIDOLPH W2000) to yield 60 g dried extract. 500 mL of ethyl acetate was added to this extract and the fraction soluble in ethyl acetate was concentrated to yield 12.7 g of dried extract. This fraction soluble in ethyl acetate was kept in refrigerator for all experiments.

Animals: Male *Wistar* rats (200-240 g; 8 weeks old) were used in this study. They were housed in the Animal House of the Laboratory of Physiology of the Higher Teachers' Training College, University of Yaoundé I at controlled ambient temperature of 23 ± 2 ° C with 12 h of light and 12 h of darkness. The animals were given food and water *ad libitum*. This study was performed in accordance with the procedures approved by the Cameroon National Ethical Commitee (Reg. No. FWA-IRD 0001954).

Study design: Animals were randomized into five groups of 10 rats in each. Group 1 (normal control) received an intraperitoneal (i.p) injection of physiological saline (0.5mL) and remained untreated for the whole period of the experiment (3 weeks). Groups 2 to 5 were injected (i.p) a single dose of monocrotaline (MCT, 50 mg/kg) and one week later they were given respectively dimethylsulfoxide 1% (10 mL/kg), sildenafil citrate (1.7 mg/kg), 100 or 200 mg/kg of the extract of *B. pilosa*, daily, continued for 2 weeks. The extract were suspended in dimethylsulfoxide (1%). At the end of the experiments, pulmonary arterial pressure, blood pressure and heart rate were measured. Then the animals were sacrificed under anesthesia, and their hearts, lungs and liver were removed.

Assessment of hemodynamics: Pulmonary arterial pressure (PAP) was used as the index for pulmonary hypertension (PH). Measurement of Ppa and was carried out three weeks after the MCT or saline treatment. The animals were anaesthetized with urethane (1.5g/kg; i.p). After insertion of an endotracheal cannula for artificial respiration, 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 PAP. All parameters were continuously recorded using grass pressure transducer and a Biopac MP35 System recorder (MP 35, Biopac, USA). The measurements were collected when values stabilized after the surgical procedures.

Measurement of organ weight: Following hemodynamic measurement, the hearts and lungs, were removed and weighed. The heart was dissected, and the ratio of the right ventricle to left ventricle plus septum weight (RV/LV+S) was calculated as an index of right ventricular hypertrophy. The right lung tissue and the liver were dissected and frozen at -20°C for subsequent biochemical analysis. Finally, the left lung, was dissected for histological examinations.

Paraffin embedding and microscopy: Histological specimens of lung were fixed in 10% buffered formalin. Representative cross-sections of the lungs which include the peripheral and the central pulmonary arteries were sampled and embedded in paraffin blocks. Serial sections (5-μm-thick) were prepared and stained with hematoxylin and eosin for the assessment of vascular pathology and examined by light microscopy Olympus. Transversely cut pulmonary arterioles, randomly distributed over the lung were measured, using ImageJ image analysis software.

Biochemical assays: The liver and lungs were dissected out and homogenized in Tris–HCl 50mM buffer solution (20%, m/v). The enzyme activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) were determined respectively in liver and lung homogenate by using spectrophotometric assay kits (SGM, Italia).

Data analysis: Data were presented as mean ± SE. The Sigma Stat 3.5 Software were used for statistical analysis. Comparisons between groups were made with one-way ANOVA. If ANOVA analysis indicated significant differences, a Bonferroni multiple-comparison post test was performed to compare mean values between groups. A P- value less than 0.05 was considered statistically significant.

RESULTS

Effects of EAEBP on development of monocrotaline-induced pulmonary arterial hypertension: Figure 1 shows tracing curves of the measured PAP in a rat 15 days after a single injection of MCT (50 mg / kg) (Figure 1A) and in a rat receiving the EAEBP (200 mg/kg) once daily during the two weeks post MCT (Figure 1B). The PAP of the rat of the MCT group is around 32 mmHg. This value is well above the normal value (<25 mmHg), characteristic of pulmonary hypertension. Meanwhile, the treatment with the extract (200 mg/kg) prevented the elevation of the PAP induced by MCT. As shown in Figure 1B, the PAP is stably maintained around 21 mmHg, a normal value of PAP.

Morphometric evaluation: As shown in Table 1, body weight was significantly decreased in the MCT-treated group after 14 days of administration. Moreover, the ratios of RV weight to LV+ S weight was increased ($P<0.01$) in MCT-treated rats, suggesting the occurrence of right ventricular hypertrophy. This value was significantly ($P<0.01$) reversed by the EAEBP treatment at the dose of 200 mg/kg and by sildenafil citrate (1.7 mg/kg; $P<0.01$). Compared to the control group, MCT administration caused a significant increase ($P<0.05$) in lung/body weight. The MCT-induced increase in lung/body weight was significantly attenuated by the EABP at 200 mg/kg ($P < 0.05$).

Activities of liver and pulmonary transaminases: A non-significant increase in transaminases activities was observed in the liver and lung of rats subjected to monocrotaline compared to the rats in the control group (Figure 2). Treatment with the extract decreased the activity of transaminases in a dose-dependent and non-significant manner for the liver ALT (Figure 2A.); and significantly ($P <0.01$) at a dose of 200 mg / kg for the lung AST (Figure 2B).

Histomorphometric examination of lung: Figure 3A shows the histological analysis of the pulmonary vasculature. Compared to the control group, MCT administration induced thickening of vessel walls with luminal narrowing (Figure 3A). Administration of the BP extract (200 mg/kg) for two weeks reduced the MCT-induced thickening of vessel walls (Figure 3A). The results of mean pulmonary arterial lumen are summarized in Figure 3B. Consistent to the PAP results (Figure 1), the MCT-induced morphologic change was attenuated by administration of the BP extract (200 mg/kg).

DISCUSSION

In the present study, the beneficial effects of the ethyl acetate extract of *Bidens pilosa* (EAEBP) on pulmonary hypertension (PH) progression were investigated in MCT-induced PH rats, employing the sildenafil citrate as a positive control. The EAEBP was given by intragastric administration for a period from the 8th day to 21st day after MCT injection, which is the aggressive phase of progression of MCT-PH model and is usually

chosen as the therapeutic time window by some investigators [23,24]. At the end of the 3rd week after MCT injection, a series of pathological changes in rats were found, such as elevated PAP, right ventricular hypertrophy, inflammatory status of liver and lung and pulmonary artery remodeling. These findings suggests that the model was successfully established. MCT is metabolized by hepatic cytochrome P450 3A and changed to monocrotaline pyrrole, an active form, which injures pulmonary vascular endothelium and causes PH [25]. PH is characterized by gradually increasing pulmonary arterial pressures and secondary vascular remodeling in 1 to 2 weeks after MCT single subcutaneous injection [26]. In the present study, monocrotaline (50 mg/kg) produced a large increase in pulmonary arterial pressure. It is known that enhancement of reactivity of pulmonary artery to vasoconstrictive substances and pulmonary artery remodeling caused by monocrotaline pyrrole toxicity result in increase of mean PAP and compensatory hypertrophy of the right ventricle due to overload in MCT-PH rat model [27]. In this study, the reduction in pulmonary arterial pressure with oral treatment was associated with a reduction in right ventricular mass at the highest dose studied (200 mg/kg). The extract has a similar effect as Sildenafil, the phosphodiesterease type 5 inhibitor used as reference drug in our study. This result is consistent with other studies [15,28]. Sildenafil may upregulate expression NO, then induces the effects of vasodilatation. It is not excluded that our plant acts like sildenafil, since its neutral extract was found to exert vasorelaxant activity [29]. Furthermore, the authors found that *Bidens pilosa* acts as a calcium antagonist. The beneficial effect of calcium antagonists on pulmonary hypertension is well known [30,31]. In our study, MCT caused a significant increase in lung weight. This increase was prevented by the extract at the highest dose studied (200 mg/kg). This result is consistent with previous results [32-34]. The early period of pneumotoxicity due to MCT is characterized by increases in vascular permeability and subsequent pulmonary edema [35,36]. As we observed, edema in pulmonary histological sections could be correlated with the increase in lung weight observed in the MCT-treated group. We also noticed a significant weight loss in the MCT-treated group as compared to the control group. This result is similar with those obtained by other studies [33,34] and could be explained by the general loss of apetite observed throughout the MCT-post injection period. We observed an increase of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity respectively in liver and lung of MCT treated rats, as compared to the control rats. Monocrotaline a pyrrolizidine alkaloid phytotoxin, has well-documented hepatic and cardiopulmonary toxicity for animals, including ruminants and humans [36-39]. It has been reported that its toxicity depends on cytochrome P-450 mediated bioactivation to the reactive pyrrolic metabolite dehydromonocrotaline (DHM) [36]. This metabolite, despite having a half-life of only a few seconds in aqueous media, is a powerful alkylating agent that binds to DNA and proteins [40]. Some authors have demonstrated that DHM, but not MCT, is toxic to hepatocytes by mechanisms involving mitochondrial respiration dysfunction [41]. Measurements of liver enzyme activities including ALT and AST are critical in the diagnosis and assessment of liver disease [42]. Despite the fact that this increase was not significant as in other studies [43,44], it nevertheless reveals the detrimental effect of MCT on these organs. Similarly, a non-significant decrease in ALT activity was observed in liver, while the extract at the dose of 200 mg/kg dose significantly decreased pulmonary AST activity indicating the protective effect of the extract on the damage induced by MCT.

To the best of our knowledge, our study is the first to demonstrate hypotensive activity of the *B. pilosa* extract on the pulmonary circulation. Pharmacologically- induced PH in rats is due to inflammatory damage of the pulmonary endothelium [35,45] followed by remodeling of the pulmonary arterial tree and subsequent elevation of pulmonary arterial pressure (PAP) [45,46]. This progression has been associated with the appearance of reactive oxygen species (ROS) [47,48]. Administrations of ROS scavengers such as dimethylthiourea and hexa (sulfobutyl) fullerenes were found to reduce the MCT-induced increases in PAP and reactive oxygen species. Notably, *B. pilosa* has previously been reported to have antioxidant properties [49-52]. Our data showing that treatment with the *B. pilosa* extract one week following MCT-administration successfully attenuated both the MCT-induced increase in PAP and the thickening of pulmonary arterial walls support this claim.

CONCLUSION

This study shows that the ethyl acetate extract of *Bidens pilosa* treatment ameliorates the progression of pulmonary arterial hypertension induced by MCT in rats. Our findings suggest that *B. pilosa* may be beneficial for the treatment of pulmonary hypertension. Future studies regarding the effects of *B. pilosa* treatment on antioxidative defense function in pulmonary arterial hypertension are clearly needed.

Table 1: Morphometric parameters of control and monocrotaline-injected male rats.

	MCT-induced pulmonary hypertension groups. EAEBP or Si (mg/kg/day)				
Parameters	Control	0	100	200	Si
BW gain	15.41 ± 1.40	1.33 ± 2.55**	7.12 ± 2.30	4.13 ± 2.78**	1.90 ± 3.17**
RV/LV+S	0.33 ± 0.01	0.43 ± 0.02**	0.41±0.03	0.34 ± 0.01$^{\#\#}$	0.30 ± 0.02$^{\#\#}$
Lung/BW	0.66 ± 0.04	0.84 ± 0.05*	0.82 ± 0.04*	0.61 ± 0.06$^{\#}$	0.89 ± 0.04**

Results are expressed as mean ± S.E.M. n=5. *P<0.05; **P<0.01, significantly different from the control group. $^{\#}$P<0.05; $^{\#\#}$P<0.01, significantly different from the monocrotaline group. BW: body weight RV/ LV + S: ratio of right ventricle to left ventricle plus septum. EAEBP: ethyl acetate extract of *Bidens pilosa*; Si: sildenafil citrate (1.7 mg/kg).

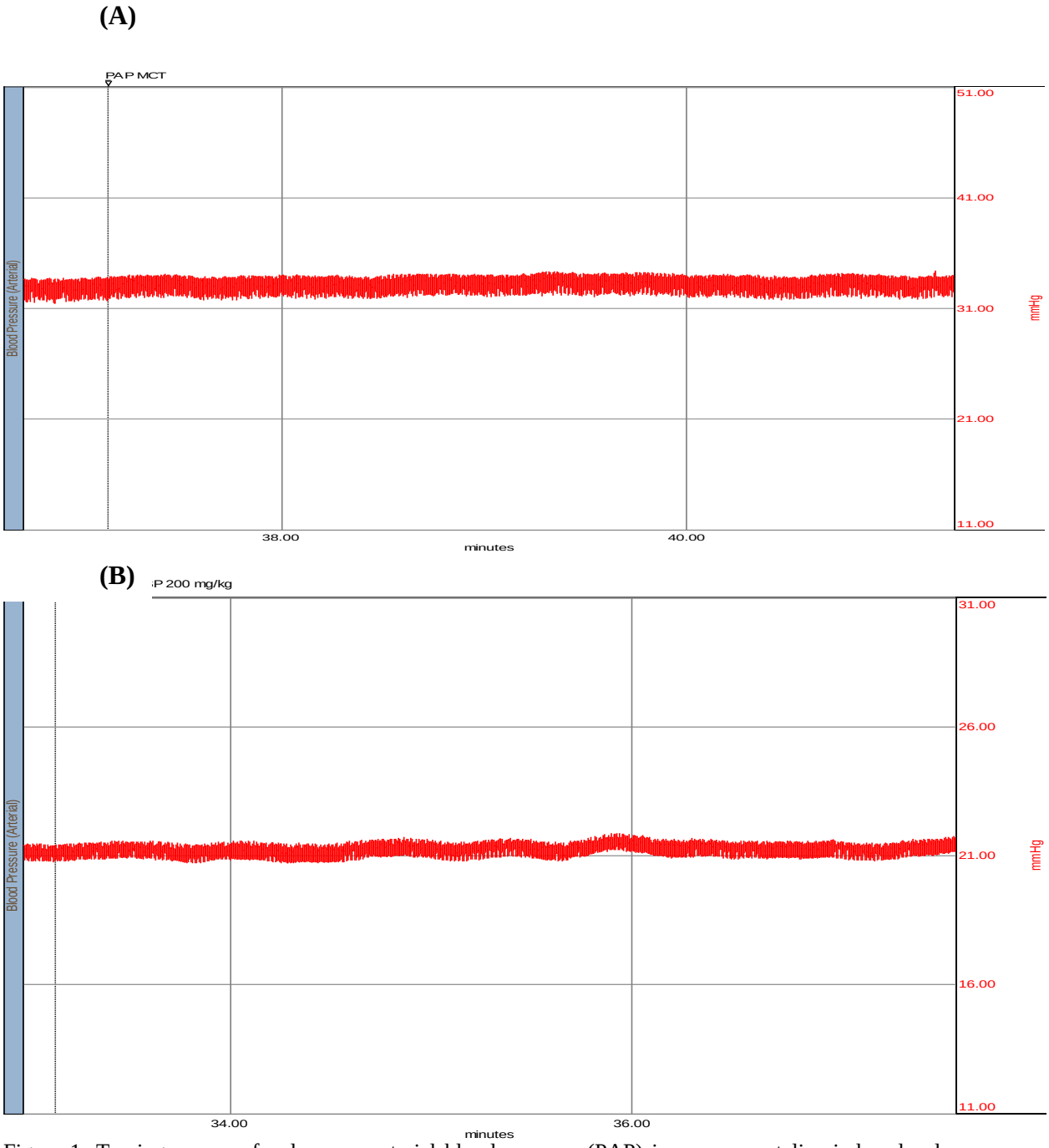

Figure 1: Tracing curves of pulmonary arterial blood pressure (PAP) in a monocrotaline-induced pulmonary hypertensive rat (A) and in rat receiving the EAEBP (200 mg/kg) once daily during the two weeks post MCT.

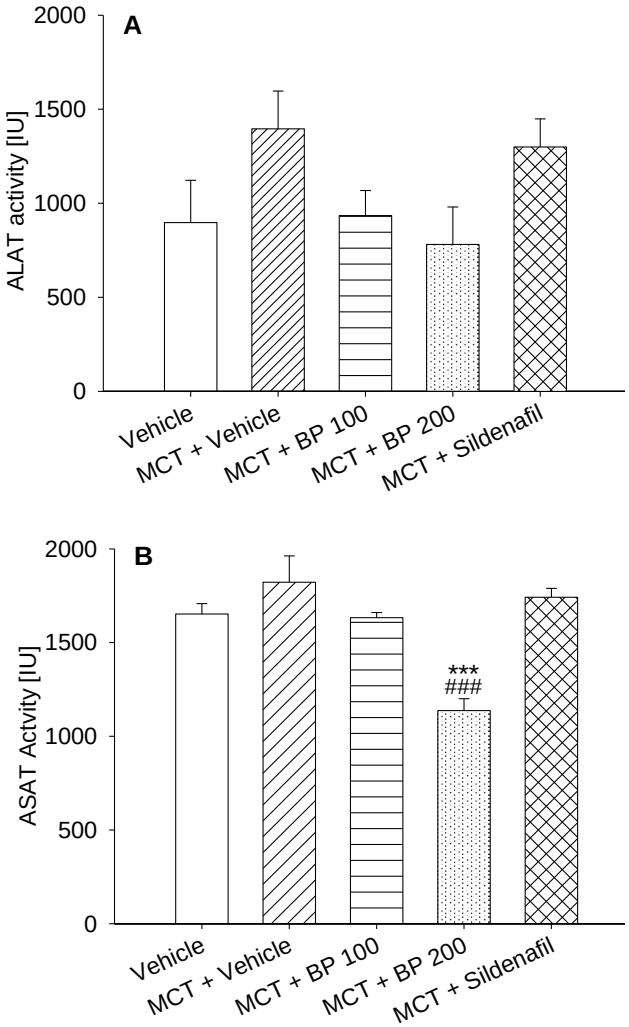

Figure 2: Activities of alanine-amino-transferase (ALAT) and aspartate-amino-transferase (ASAT) respectively in liver and lung of rats. Each bar indicate mean ± S.E.M (n=5). ***P<0.001, significantly different from the control group. ###P<0.001, significantly different from the monocrotaline group. MCT + Vehicle: monocrotaline group; MCT + BP 100 and MCT + BP 200: Groups of rats receiving the *Bidens pilosa* ethyl acetate extract respectively at the dose of 100 and 200 mg/kg daily during two weeks post monocrotaline injection; MCT + Sildenafil: Group of rats receiving Sildenafil (1.7 mg/kg) daily during two weeks post monocrotaline injection.

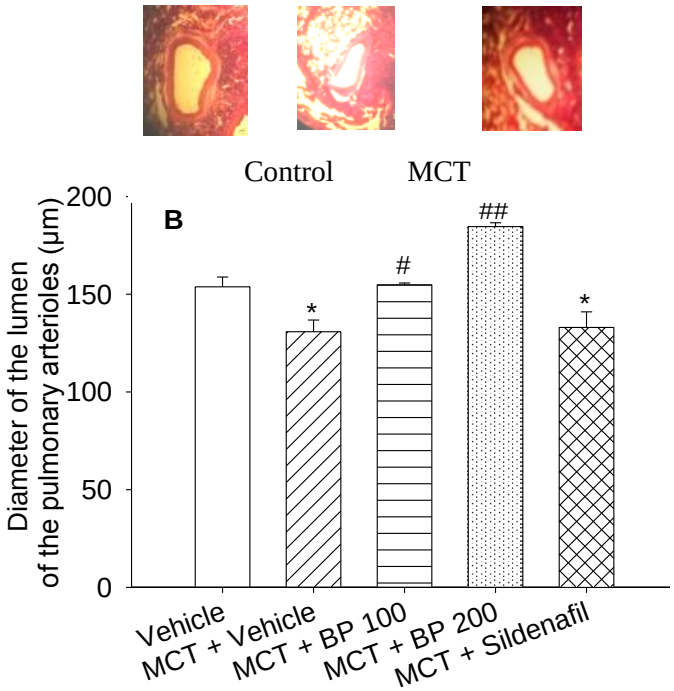

Figure 3: Photomicrograph of histologic sections from three groups of rats (A) and mean pulmonary arterial lumen among different experimental groups (B). All the imaged fields are amplified 100-fold. *P<0.05; **P<0.01, significantly different from the control group. #P<0.05; ##P<0.01, significantly different from the monocrotaline group. MCT + Vehicle: monocrotaline group; MCT + BP 100 and MCT + BP 200: Groups of rats receiving the *Bidens pilosa* ethyl acetate extract respectively at the dose of 100 and 200 mg/kg daily during two weeks post monocrotaline injection; MCT + Sildenafil: Group of rats receiving Sildenafil (1.7 mg/kg) daily during two weeks post monocrotaline injection.

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