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Studies on the anti-asthmatic and antitussive properties of aqueous leaf extract of *Bryophyllum pinnatum* in rodent species

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ABSTRACT

Objective: To evaluate the antiasthmatic and antitussive properties of the aqueous leaf extract of *Bryophyllum pinnatum* (*B. pinnatum*) (BP) Lam. **Methods:** Ovalbumin-sensitized guinea pigs which were treated with BP for 21 consecutive days were exposed to 0.2% histamine aerosol in a glass chamber. Mucus viscosity, white blood cell and lymphocyte counts and tracheal wall morphometry were measured. Bouts of cough were counted pre and post acute exposure of extract-treated (×7 d) guinea pigs to 7.5% citric acid aerosol in a chamber. Phenol red expectoration was estimated in mice after 7 d of daily administration of BP. **Results:** Doses of 200 and 400 mg/kg/day (×21 d) BP significantly increased the time for guinea pigs to experience preconvulsive dyspnoea. BP and salbutamol (0.5 mg/kg/day × 21 d) reduced mucus viscosity in the sensitized group to values comparable with controls. White blood cell, lymphocyte counts and tracheal morphometry were not significantly altered. Both doses of BP also significantly reduced the bouts of cough but only 400 mg/kg/day significantly inhibited the amount of phenol red secreted. **Conclusions:** BP has demonstrated antiasthmatic and antitussive properties in these rodent models. These properties may underscore its use in Nigerian ethnomedicine.

1. Introduction

Herbal remedies have become popular for the treatment of various airway diseases[1-3]. Herbalists in Nigeria use the aqueous leaf extract of *Bryophyllum pinnatum (B. pinnatum)* (Lam.) Oken (= *Kalanchoe pinnata* Pers) for the prophylaxis of asthma and treatment of cough. It belongs to the family Crassulaceae and has many common names including air plant, Canterbury bells, cathedral bells, life plant, Mexican love plant, miracle plant and resurrection plant. It is found in many parts of the world perhaps because of its ease of cultivation. Phytoconstituents of the leaves include flavonoids, saponins, tannins and alkaloids[4,5]. Vitamins including ascorbic acid, riboflavin, thiamine, niacin and minerals such as calcium, zinc and phosphorus are also present in the leaves[4].

The various scientifically validated medicinal properties have been listed in our previous reports[6,7]. These properties include antimicrobial, antifungal, antiulcer, antihypertensive, tocolytic, antidiabetic, hepatoprotective, anti-inflammatory, analgesic, and wound healing. Other reported properties include anti-tumour, sedative, muscle relaxant and effectiveness in the treatment of leishmaniasis. The aqueous leaf extract has been shown to attenuate responses of isolated tracheal smooth muscles to spasmogens such as histamine and carbachol[6]. Acute and sub-acute toxicity assessments have been carried out on the plant[7].

The aqueous leaf extract of the plant is used by herbal practitioners in Nigeria and other parts of Africa for the treatment of cough and as a prophylactic medicine for asthma. In Benin City, Nigeria, the leaves are boiled, filtered through a clean white cloth and the yield reconstituted for daily oral use by asthmatic patients. The leaves are also warmed over fire and the juice expressed from them is taken for the treatment of cough. The present study was designed to evaluate the anti-asthmatic and antitussive properties of the extract in rodent species.

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2. Materials and methods

2.1. Plant material

Leaves of *B. pinnatum* not exposed to herbicides were collected from a suburb of Benin City, Nigeria, in August 2010. A herbarium specimen of the plant with voucher number FHI 107762 exists at the Forest Research Institute of Nigeria. As reported previously[6,7] adulterants were picked out and the leaves were thoroughly rinsed in tap water. The leaves (2 kg) were boiled for one hour in 4 L of distilled water, allowed to cool and then filtered two times with a clean white cloth. The resulting extract was concentrated in a rotary evaporator before drying in an oven at 40 ℃ over 24 h (yield = 3.6% w/w). The extract (BP) was then stored in amber-coloured bottles at 4 ℃.

2.2. Animals

Adult guinea pigs of either sex weighing (350-400 g) were obtained from the Animal Unit, Ambrose Alli University, Ekpoma, Nigeria. Mice of either sex weighing 22-30 g were in-bred in the Animal House, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. The animals were housed in standard plastic cages (males separate from females) and allowed free access to pellets (Bendel Feeds and Flour Mill Ltd, Ewu, Nigeria) and tap water. They were exposed to day-night light cycle and room temperature (27.0 ± 2.0) ℃. The animals were handled according to standard protocols for the use of laboratory animals[8]. The study was approved by institutional ethical committee on the use of animals for experiments.

2.3. Anti-asthmatic experiments

2.3.1. Exposure to histamine aerosol

Guinea pigs were randomly allotted to five groups: A -non-sensitized + 2 mL/kg/day distilled water; B- sensitized + 2 mL/kg/day distilled water; C-sensitized + 200 mg/kg/day BP; D-sensitized + 400 mg/kg/day BP; and E-sensitized + 0.5 mg/kg/day salbutamol. Animals were sensitized by single administration of 100 mg/kg (*i.p.*) ovalbumin on the first day and another 50 mg/kg (*i.m.*) the following day[9]. Treatment with water, extract or salbutamol was per os for 21 consecutive days.

One hour after the last dose (on the 21st day), response to an allergen was measured in the guinea pigs[10]. The animals were exposed to 0.2% histamine aerosol using Omron compressor nebulizer (USA) at a rate of 0.4 mL/min and particle size 5 μm, in a glass chamber (60 cm × 36 cm× 60 cm) until preconvulsive dyspnoea was observed.

2.3.2. Measurement of white blood cell and lymphocyte counts

After exposure to histamine aerosol the animals were allowed 1 h of full recovery before they were anaesthetized with chloroform vapour and 0.5 mL blood samples were withdrawn from the abdominal aorta. Total white blood cells (WBC) and the lymphocytes (LYM) were analyzed by use of an automated blood analyzer (QBC Autoread Plus, UK). The blood samples were first pipetted into QBC capillary tubes and spun in a parafuge centrifuge (Becton Dickson, UK) for 5 min and read by means of the autoread analyzer[11].

2.3.3. Measurement of mucus viscosity

Mucus viscosity was measured by flushing 2 cm length of trachea (from the point of thoracic bifurcation towards the pharynx). The pieces of trachea were held upright and flushed each with 2 mL of normal saline over 5 min. The effluent fluid was then subjected to viscosity test. In brief, the effluent fluid was shaken thoroughly and 1 mL was withdrawn into a 1 mL syringe which was then held in place with a retort stand. The plunger of the syringe was carefully withdrawn and the time it took for the whole fluid to drain was noted[11]. Viscosity was expressed as mL/s.

2.3.4. Tissue histology and morphometry

The remaining parts of each trachea and the lungs were stored in 10% formosaline. The tissues were later dehydrated, embedded in paraffin wax and sectioned (5 μm thickness) with a microtome. They were stained with hematoxylin and eosin, and viewed under an Olympus CH10 optical microscope at ×100 or ×400 magnification. Photomicrographs of the tissues were obtained at × 400 magnification by use of an Olympus CH10 optical microscope fitted with a 12.2 megapixels Samsung ES17 digital camera. Micrographs of the tracheae (under uniform magnification) were printed out and the thickness of the tracheal wall and cartilage measured with a ruler.

2.4. Antitussive experiments

2.4.1. Exposure to citric acid aerosol

Guinea pigs were exposed to 7.5% citric acid aeroso[10,12] using the Omron compressor nebulizer (rate 0.4 mL/min and particle size 5 μm) in the glass chamber (described above) for 5 min. Animals with cough bouts of (15 ± 5) were randomly allotted into three groups and administered 200, 400 mg/kg/day BP and 25 mg/kg/day codeine phosphate orally respectively. The animals were re-exposed to 7.5% citric acid aerosol after 1 h and the bouts of cough recorded.

2.4.2. Phenol red expectoration

In the phenol red expectorant method[13], mice were randomly allotted into five groups: A-distilled water control; B-200 mg/kg/day BP; C-400 mg/kg/day BP; D-sodium cromoglycate (50 mg/kg/day); and E-bromhexine (15 mg/kg). Except for sodium cromoglycate which was administered *i.p.*, other drugs and BP were administered per os. Treatment was for seven consecutive days except for bromhexine (15 mg/kg) which was administered to the fifth group on the 7th day. NH_4Cl (5 mg/kg *p.o.*) was administered 1 h after the 7th-day dose and then followed with phenol red dye (0.5 g/

kg *i.p.*) after 30 min. Each trachea (2 cm) was excised after cervical dislocation of each mouse and then placed in a solution of 1 mL normal saline + 0.1 mL 1 N NaOH to stabilize the pH. Absorbance of dye secreted from the trachea was measured at 460 nm with a UV-Visible spectrophotometer (Cecil Instrument Limited, Milton Technical Centre, England).

2.5. Statistics

Data are presented as mean ± SEM (standard error of the mean) and n represents the number of guinea pigs or mice per group. Data from experimental groups were compared by use of one-way ANOVA with Tukey post hoc. All data were analyzed using GraphPad Prism software (UK). $P<0.05$ indicates statistically significant difference.

3. Results

3.1. Anti-asthmatic effects

Table 1 shows that 400 mg/kg/day × 21 BP significantly (40.8%, $P<0.005$) inhibited histamine-induced bronchospasm as indicated by the time taken for the animals to experience preconvulsive dyspnoea, compared with sensitized non-extract treated group but not when compared with control (distilled water treated) group. The time taken to experience preconvulsive dyspnoea was also prolonged in 200 mg/kg/day × 21 BP (23.2%, $P<0.05$) and 0.5 mg/kg/day × 21 salbutamol (33%, $P<0.05$) treated groups when compared with sensitized non-extract treated group.

The total WBC and lymphocytes counts were not significantly altered across the groups. The mean WBC count was highest in the ovalbumin sensitized group and lowest in the control (distilled water treated) group. Lymphocyte counts paralleled WBC counts but values were also not significantly different across the groups. Mucus viscosity was significantly ($P<0.05$) reduced by both doses of extract when compared with the sensitized non-extract treated group (Table 2). The mean value for the extract treated group was comparable with that of distilled water control group. Salbutamol significantly reduced mucus viscosity ($P<0.05$) when compared with sensitized only group.

Morphometry of the tracheal rings shows that mean values for tracheal wall and cartilage thickness were highest in the sensitized group that were not given the extract but lowest in the salbutamol treated group. Values are however not significantly different (Table 3).

Representative photomicrographs of the lungs are shown in Figure 1. The lungs from control group contained patent bronchioles and clear airways (plate A) while the lungs from ovalbumin sensitized guinea pigs contained mucus plugs and interstitial infiltrates of eosinophils (plate B). The lungs from 400 mg/kg/day × 21 BP treated group and 0.5 mg/kg/day × 21 salbutamol treated group all showed varying degrees of dilated bronchioles and inflated alveoli (plates C and D respectively).

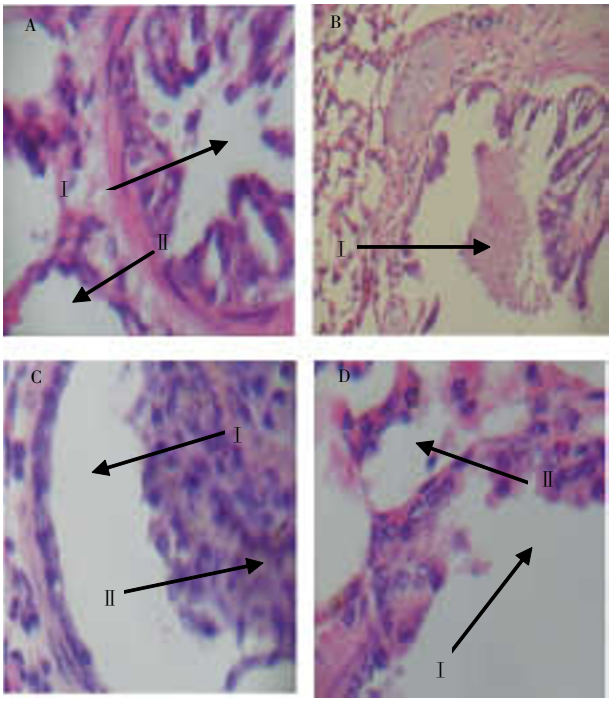

Figure 1. Photomicrographs of lungs of guinea pigs treated for 21 consecutive days.
(A) Distilled water control showing patent bronchiole (Ⅰ), well inflated alveoli (Ⅱ) and interstitial infiltrates of inflammatory cells. (B) Ovalbumin-sensitized but distilled water treated showing bronchiolar mucus plug (Ⅰ) and interstitial infiltrates of eosinophils. (C) *B. pinnatum* (400 mg/kg/day) treated showing markedly dilated bronchiole (Ⅰ) with luminal necrotic debris and mild infiltrates of inflammatory cells (Ⅱ). (D) Salbutamol (0.5 mg/kg/day) treated showing dilated bronchiole (Ⅰ), well inflated alveoli (Ⅱ) and mild infiltrates of inflammatory cells. A, C, D (H & E, × 400); B, (H & E, × 100).

3.2. Anti-tussive effects

Table 4 shows that BP at dose of 200 mg/k/day × 7 reduced cough bouts from (12.4±1.3) to (4.6±0.7) (63% inhibition, $P<0.05$) and 400 mg/kg/day × 7 reduced cough bouts from (14.2 ±1.8) to (7.2±1.2) (49.3%, $P<0.05$). The standard drug (codeine phosphate) administered for 7 days reduced the bouts of cough from (14.8±1.6) to (3.0±0.4) (80% inhibition, $P<0.05$). There was no significant difference in the bouts of cough by the distilled water control group.

Compared with distilled water control, BP doses of 200 and 400 mg/kg/day × 7 significantly ($P<0.05$) reduced phenol red secretion in mice tracheae by 31.94% and 38.89% respectively. Sodium cromoglycate reduced dye secretion by 29.01% when compared to distilled water group. Bromhexine, a known mucolytic and bronchitis drug increased dye concentration by 8.03% compared to distilled water group (Table 5).

Table 1
Effect of *B. pinnatum* extract administered for 21 consecutive days on time taken for occurrence of preconvulsive dyspnoea in guinea pigs.

Groups	Dose(Quantity/kg/day)	Preconvulsion time(s)	Protection(%)
Control	2 mL (DW)	135.40±4.85	–
Sens + BP	200 mg	176.30±27.24*	23.20
Sens + BP	400 mg	228.70±23.53*	40.80
Sens	2 mL (DW)	86.50±6.68	−56.53
Sens +Sal	0.5 mg	202.20±55.45*	33.04

*P<0.05 *vs.* Sens. DW: distilled water; Sens: sensitized; BP: aqueous leaf extract of *B. pinnatum*; Sal: salbutamol, n = 5−7 per group.

Table 2
WBC lymphocyte and mucus viscosity in guinea pigs treated for 21 consecutive days with aqueous leaf extract of *B. pinnatum*.

Groups	Dose(Quantity/kg/day)	WBC counts(× 10^3/ μ L)	Lymphocyte counts(× 10^3/ μ L)	Mucus viscosity(× 10^{-3}mL/s)
Control	2 mL (DW)	8.76±0.93	7.04±0.83	8.34±0.40*
Sens + BP	200 mg	10.92±3.19	8.03±2.14	8.37±0.39*
Sens + BP	400 mg	9.58±1.79	6.87±1.09	8.41±0.37*
Sens	2 mL (DW)	12.52±3.74	9.64±2.92	6.53±0.45
Sens +Sal	0.5 mg	11.28±1.15	8.02±0.70	8.83±0.11*

*P<0.05 compared with sens. DW: distilled water; Sens: sensitized; BP: aqueous leaf extract of *B. pinnatum*; Sal: salbutamol, n = 5−7 per group.

Table 3
Tracheal morphometry after 21 consecutive days of administering aqueous extract of *B. pinnatum* to guinea pigs.

Groups	Dose (Quantity/kg/day)	Tracheal wall thickness (cm)	Cartilage thickness (cm)
Control	2 mL (DW)	18.67±2.40	13.83±1.88
Sens + BP	200 mg	19.08±1.24	14.50±0.85
Sens + BP	400 mg	21.00±0.58	11.25±1.93
Sensitized	2 mL (DW)	21.67±1.67	15.17±2.80
Sens +Sal	0.5 mg	17.58±1.57	13.00±1.29

Values in columns are not significantly different. DW: distilled water; Sens: sensitized; BP: aqueous leaf extract of *B. pinnatum*; Sal: salbutamol, n = 5 per group (× 400 magnification).

Table 4
Effect of oral daily (× 7) administration of *B. pinnatum* extract on citric acid−induced acute cough in guinea pigs.

Groups	Dose(Quantity/kg/day)	Cough bouts before	Cough bouts after	Inhibition of cough bouts (%)
Control	2 mL (DW)	11.50±0.87	10.00±1.68	13.04
BP	200 mg	12.40±1.25	4.60±0.68*	62.90
BP	400 mg	14.20±1.83	7.20±1.24*	49.30
Codeine PO4	25 mg	14.75±1.60	3.00±0.41*	79.66

**P*<0.05 compared with values before treatment. DW: distilled water; BP: aqueous leaf extract of *B. pinnatum*. n = 5 per group.

Table 5
Effect of oral daily (× 7) administration of *B. pinnatum* extract on phenol red dye secretion in mice.

Groups	Dose(Quantity/kg/day)	Conc. of dye (μ g/mL)	Inhibition of dye secretion (%)
Control	2 mL (DW)	12.96±2.72	–
BP	200 mg	8.82±1.46	31.94
BP	400 mg	7.92±1.74*	38.89
Na Cromoglycate	50 mg (*i.p.*)#	9.20±1.07	29.01
Bromhexine HCl	15 mg	14.00±2.22	−8.03

**P*<0.05 compared with control. DW: distilled water; BP: aqueous leaf extract of *B. pinnatum*. #Administered on the 7th day. n = 5 per group.

4. Discussion

The data from this study suggest that the aqueous leaf extract of *B. pinnatum* (BP) may prevent acute asthmatic attacks in human. The dose of 400 mg/kg/day for 21 d was comparable with 0.5 mg/kg/day of salbutamol in protecting guinea pigs from histamine−induced preconvulsive dypsnoea. A previous study has shown that BP does not possess direct tracheal smooth muscle relaxant property but attenuates responses of the isolated guinea tracheal rings to spasmogens such as histamine and acetylcholine[6]. It therefore seems that the protection offered the sensitized animals may have been by prevention of airway smooth muscle spasms. While the mechanisms involved are not certain, BP is known to possess antioxidant properties.

BP contains flavonoids among others[4,5]. Flavonoids as secondary plant metabolites have been associated with potent antioxidant properties that underlie their use in the management of chronic obstructive airway disorders such as asthma[14−17]. Flavonoids also inhibit Ca^{2+} release and utilization mechanisms in smooth muscles[18,19]. Reduced mucus viscosity is valuable in the therapy of asthma since increased secretion and viscosity impede airflow in the respiratory tree[20,21]. This property has been demonstrated

by BP in the sensitized group.

Sensitization with ovalbumin is a model of obstructive airway disease in which the airways become inflamed and hyper-responsive[22]. Ability of agents to inhibit responses by ovalbumin-sensitized guinea pigs often suggests usefulness in obstructive airway diseases. However sensitization with ovalbumin did not significantly increase tracheal wall/cartilage thickness or the white blood cell/lymphocyte counts at the time of sacrifice. Although BP possesses anti-inflammatory activity[6], the present data indicate that the protection of the guinea pigs did not depend on this as tracheal wall/cartilage thickness was comparable across the groups. Histological findings reveal that the indices are better in guinea pigs which were administered the extracts compared with those which were sensitized but not treated with either extract or salbutamol. Such positive indices include inflated alveolar sacs and absence of mucus in the airway.

Antitussive data from the present study indicate that BP reduces the number of bouts of cough and quantity of dye secreted by the trachea. The mechanisms which underlie cough reflex are complex and difficult to understand but are either peripheral or central[23,24]. With reduction in the mucus viscosity and secretion, airflow resistance is consequently reduced and irritation which would trigger cough is also reduced. While this effect is peripheral, the involvement of central mechanisms in the inhibition of the bouts of cough cannot be excluded since agents such as codeine act centrally[25,26]. Also, the involvement of peripheral sensory airway neuronal inhibition in the antitussive action of BP is not clear. These neurons have been the focus of new search for antitussive agents since the centrally acting ones that have been the gold standard for decades have serious adverse effects[23,27,28]. The findings from study suggest that BP may be helpful for irritant coughs due to hypersecretion by airway pathologies such as asthma and infections.

In conclusion this study has shown that BP possesses some anti-asthmatic and antitussive properties in rodent species which may underscore its use in ethnomedicine.

Conflict of interest statement

We declare that we have no conflict of interest.

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