Pharmacological evaluation of *Musa paradisiaca* (Linn.) on bronchial asthma

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Objective
The present study was conducted to investigate the antiasthmatic potential from the flowers of *Musa paradisiaca* Linn. to validate its traditional claims.

Materials and methods
The antiasthmatic activity of the hydroalcoholic extract of *M. paradisiaca* flower (HMPF) was evaluated by studying histamine or acetylcholine-induced bronchospasm in guinea pigs, compound 48/80-induced mast cell degranulation in albino rats, and histamine-induced constriction in isolated guinea pig trachea. The preconvulsion dyspnea time at the 0th and seventh day at a dose of 200 and 400 mg/kg in guinea pigs, the percentage of granulated and degranulated mast cells at doses of 500, 750, and 1000 μg/ml in rats, and muscular contraction at doses of 500, 750, and 1000 μg/ml in isolated guinea pig trachea were evaluated and compared with their respective control groups.

Results
Phytochemical studies revealed the presence of flavonoids, steroids, saponin, terpenoids, lignins, and phenolic compounds in the extract. In addition, treatment with HMPF significantly ($P < 0.001$) decreased the bronchospasm induced by histamine or acetylcholine in guinea pigs, the degranulation of mast cell in rats, and histamine-induced constriction in isolated guinea pig trachea, when compared with the inducer group. In addition, HMPF showed a dose-dependent antiasthmatic effect in the animals.

Conclusion
The present study concluded that the antiasthmatic activity of the HMPF may be due to the presence of the above-mentioned phytoconstituents causing membrane stabilization, suppression of antibody production, and inhibition of antigen-induced histamine and acetylcholine.

Keywords:
asthma, bronchospasm, histamine, *Musa paradisiaca*, mast cell degranulation

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Introduction

Bronchial asthma is one of the most common disabling syndromes among respiratory diseases affecting humans, with a worldwide incidence of 155 million. It is a disease with no boundaries in terms of age, race, and sex [1]. It is a common chronic inflammatory disorder of the airways, characterized by airway narrowing, episodic wheezing, breathlessness, chest tightness, and coughing, particularly at night and on awakening in the morning [2]. It is triggered by various factors like allergens, respiratory infection, dust, cold air, exercise, emotions, occupational stimuli, certain drugs/chemicals, and histamine and can also be hereditary. These trigger factors accelerate the activation of immunoglobulin-E (IgE)-mediated mast cells and release of interleukins (IL-4 and IL-5) and other inflammatory factors including eosinophils, neutrophils, β cells, cytokines, and chemokines, which leads to inflammation or obstruction in the throat, bronchial hyper-responsiveness, and mucosal hypersecretions [3]. Therefore, the disease statistics clearly necessitate the use of drugs targeting mast cell stabilizers, cytokine inhibitors, neutralizing antibodies directed at IgE, histamine and leukotriene blockers, etc. for the management of asthma. Despite the availability of a wide range of antiasthmatic drugs, the relief offered by them is mainly symptomatic and show a poor or absent response even at high doses with a few side effects. Thus, an ideal approach for the development of new, safe and effective remedial drugs to treat bronchial asthma is the use of herbal sources. In this regard, natural compounds having greater antioxidant, anti-inflammatory, and immunomodulatory activities were recognized as gold candidates [4].

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Musa paradisiaca Linn. (Musaceae) is locally known as Kela in Hindi, an indigenous herb grown in the tropics and subtropics. It is widely cultivated in Karnataka, Assam, Madhya Pradesh, Bihar, Gujarat, Andhra Pradesh, Maharashtra, West Bengal, Odisha, Tamil Nadu, and in other coastal parts of India [5]. Almost all parts of this plant have been traditionally used in India for several medicinal purposes. Traditionally, the plant was used for abscess, alopecia, anasarca, burns, cancer, cataplasm, diabetes, diarrhea, dog bites, dysentery, dyspepsia, cruptions, fractures, gangrene, headache, hematuria, hemiplegia, hemoptysis, hemorrhage, hypertension, lizard bites, mange, marasmus, migraine, nausea, otalgia, psoriasis, ringworm infection, scorpion sting, septicemia, shingles, smallpox, snake bite, sore, strain, syphilis, tuberculosis, warts and wound [6,7]. Earlier pharmacological investigations reported that the entire *M. paradisiaca* plant possesses antidiarrheal, antiulcerative, antimicrobial, hypoglycemic, antioxidant, diuretic, wound healing, antiallergic, antimalarial, hypocholesterolemic, anti-snake venom, analgesic, hair growth-promoting, and anticonvulsant activity [8,9]. The flower of this plant has received very little attention from the world of science, particularly with respect to its medicinal value in human health. As per ethnomedicinal surveys around the world and supported by limited bioactivities and clinical research, it should have tremendous pharmacological value. The flower extract of this plant has scientifically proven effects on diabetes mellitus, oxidative stress, and malaria [10]. It has been traditionally used in asthmatic patients by tribal people and by various Ayurvedic practitioners [10–12]. In fact, the whole plant is used to treat asthmatic or bronchitis patients by various traditional practitioners of India [12–20]. On the basis of the above traditional claims, the present study was undertaken to investigate the antiasthmatic potential of the flower extract of *M. paradisiaca* L. in various experimental animals.

Materials and methods

Chemicals and reagents

Histamine dihydrochloride, acetylcholine chloride, ketotifen, and compound 48/80 were purchased from Sigma-Aldrich Chemical Co. (USA). All other chemicals were of analytical grade.

Collection of plant material

The flower of *M. paradisiaca* was collected from a local farmland in Bhopal, Madhya Pradesh, India. The species was identified and authenticated at the Department of Botany, Dr H.S. Gour Central University, Sagar (MP), where a plant specimen was deposited.

Preparation of the extract

The flowers were separated into florets or bracts and allowed to dry in the oven for 7 days at 40°C. Then the samples were ground into powder using a grinder and weighed. The powdered samples were then stored in an air-tight container. Initially, the powdered sample (500 g) was defatted with petroleum ether and then subjected to continuous hot extraction in a soxhlet apparatus using ethanol and water (70 : 30) as solvent for 48 h. The extraction was continued until the solvent became colorless. After complete extraction, the extract was filtered and evaporated to dryness under vacuum using a rotary evaporator to obtain dried hydroalcoholic extract (extractive yield, 13.24% w/w).

Phytochemical screening

Preliminary phytochemical tests were performed on hydroalcoholic extract of *M. paradisiaca* flower (HMPF) to determine the presence of various phytoconstituents [21].

Experimental animals

Albino rats (175–200 g) and guinea pigs (400–600 g) of either sex housed in standard conditions of temperature (22 ± 2°C), relative humidity (55 ± 5%), and light (12 h light/dark cycles) were used. They were fed with standard pellet diet and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee as per the guidelines of CPCSEA, Ministry of Social Justice and Empowerment, Government of India.

Acute toxicity testing

The animals were fasted overnight before the experiment. Different graded doses (50–2000 mg/kg, orally) of HMPF were administered to groups of rats and they were observed continuously for 1 h and then at half-hourly intervals for 4 h for any gross behavioral changes and further up to 24 h for any mortality as per the OECD Guidelines 425 [22].

Histamine and acetylcholine-induced bronchospasm in guinea pigs

Guinea pigs of either sex were divided into two groups. Each group comprised six animals, which were exposed to 0.1% w/v of histamine dihydrochloride aerosol in a histamine chamber. Progressive dyspnea was observed in animals when exposed to histamine aerosol. The endpoint, preconvulsion dyspnea (PCD), was determined from the time of aerosol exposure to the onset of dyspnea leading to the appearance of convulsion. As soon as PCD commenced, the animals were removed from the chamber and placed in fresh air.

The PCD at this time point was taken as the value on day 0. Both groups of guinea pigs were given HMPF at doses of 200 and 400 mg/kg orally, respectively, once a day for 7 days. On the seventh day, 2 h after the last dose, the time of onset of PCD was recorded as on day 0. The same procedure was followed in another set of animals (n = 6) for acetylcholine-induced bronchospasm study, except that 0.5% acetylcholine chloride was used in place of histamine dihydrochloride [23]. The percentage increase in time of onset of PCD was calculated using the following formula:

Percentage increased in time of PCD = $(1-T_1 / T_2)\times 100$,

where T_1 is time for PCD onset on day 0, T_2 is time for PCD onset on day 7.

Mast cell degranulation study

Male albino rats were divided into six groups; each group contained six animals and they were killed by means of cervical dislocation. The animals were immediately injected with 15 ml of prewarmed (37°C) buffered salt solution (NaCl 137 mmol/l; KCl 2.7 mmol/l; $MgCl_2$ 1 mmol/l; $CaCl_2$ 0.5 mmol/l; NaH_2PO_4 0.4 mmol/l; glucose 5.6 mmol/l; HEPES 10 mmol/l) in the peritoneal cavity and massaged gently in this region for 90 s to facilitate cell recovery. A midline incision was made and the peritoneum was exposed. The pale fluid was aspirated using a blunted plastic Pasteur pipette and collected in a plastic centrifuge tube. The fluid was then centrifuged at 1000 rpm for 5 min, and the supernatant was discarded to reveal a pale cell pellet. The cell pellets were resuspended in fresh buffer and recentrifuged. The peritoneal cell suspension was divided into six parts: untreated control, positive control, reference standard (ketotifen 10 μg/ml), and HMPF at different concentrations of 500, 750, and 1000 μg/ml, each containing 0.1 ml of cell suspension and incubated at a constant temperature of 37°C in a water bath for 15 min. Then 0.1 ml of compound 48/80 was added to all samples except in untreated controls, and suspensions were further incubated for 10 min at 37°C. The cells were then stained with 10% of Toluidine blue solution and observed under higher magnification using a microscope. The proportion of granulated and degranulated mast cells was measured in each group [24].

Histamine-induced guinea pig tracheal chain contraction

For tracheal chain preparation, guinea pigs of body weight 200–500 g were selected and allowed to starve overnight with free access to water. The animals were killed by a blow on the head and exsanguinated. The trachea was isolated, cut into individual sections of 1 cm, and then divided into four groups; each group consisted of six tracheas. Group I was the control group (histamine 0.5 μg/ml only) and groups II, III, and IV were treated as test groups and were administered histamine with HMPF extract at 500, 750, and 1000 μg/ml, respectively. The isolated trachea was mounted in a 30 ml organ bath containing tyrode solution, maintained at 37 ± 1°C, and gassed with air. The tissue was equilibrated for 45 min during which the bath solution was replaced every 10 min. At the end of the equilibration period, histamine (0.5 μg/ml)-induced contraction as well as the effect of extract (500, 750, 1000 μg/ml) was recorded. A drug tissue contact time of 1 min was maintained. The percentage response of each tested group was calculated from the height of the peaks obtained and compared with histamine controls [25].

Statistical analysis

The results of various studies were expressed as mean ± SEM and analyzed statistically using one-way analysis of variance, followed by Dunnett's test to find out the level of significance. Data were considered statistically significant at P value less than 0.001 and P value less than 0.01.

Results

Phytochemical screening

Preliminary screening of the crude extract of *M. paradisiaca* (HMPF) showed the presence of flavonoids, phenols, alkaloids, steroids, tannins, terpenoids, saponins, lignins, amino acids, and carbohydrates.

Acute toxicity study

The HMPF was found to be safe up to 2000 mg/kg body weight when administered orally in albino rats. After 24 h, the animals were found to be well tolerated; there was no mortality and no signs of toxicity. The extract was found to be safe, and hence the doses of 200 and 400 mg/kg of body weight were selected for the present study.

Effect of hydroalcoholic extract of *M. paradisiaca* flower on histamine and acetylcholine aerosol-induced bronchospasm in guinea pigs

The HMPF significantly and dose-dependently increased the time to onset of PCD following exposure to histamine ($P < 0.001$) and acetylcholine ($P < 0.001$) aerosol-induced bronchospasm in guinea pigs (Table 1). The percentage increase in time to onset of PCD in the histamine-induced bronchospasm group

at doses of 200 and 400 mg/kg body weight was found to be 70.73 and 79.68%, respectively, whereas in case of acetylcholine-induced bronchospasm at the same dose the percentage increase was 59.39 and 62.2%, respectively. Thus, the percentage increase in the time to onset of PCD was higher in histamine-induced bronchospasm as compared with acetylcholine-induced bronchospasm by the administration of *M. paradisiaca*.

Effect of hydroalcoholic extract of *M. paradisiaca* flower on compound 48/80-induced mast cell degranulation in rats

The percentage of mast cell degranulation was observed as 11.84 ± 2.34, 78.52 ± 2.57, 26.42 ± 3.53, 62.58 ± 3.45, 44.32 ± 1.34, and 31.09 ± 2.57 in untreated controls, positive controls, the ketotifen group (standard), HMPF I (500 µg/ml), HMPF II (750 µg/ml), and HMPF III (1000 µg/ml), respectively, as shown in Fig. 1. In the groups treated with HMPF and in the standard group, significant ($P < 0.001$ and $P < 0.01$) inhibition of mast cell degranulation from rat peritoneal cells was observed. The groups treated with HMPF also revealed dose-dependent mast cell protection against compound 48/80, compared with baseline values of positive controls.

Effect of hydroalcoholic extract of *M. paradisiaca* flower on guinea pig tracheal chain

In isolated guinea pig tracheal studies, HMPF significantly ($P < 0.001$ and $P < 0.01$) inhibited the contraction of tracheal muscles induced by histamine as compared with histamine controls in a dose-dependent manner, as shown in Fig. 2. In the HMPF-treated groups, the percentage of inhibition was found to be 33.51, 47.19, and 71.39% as compared with the histamine-treated group.

Discussion

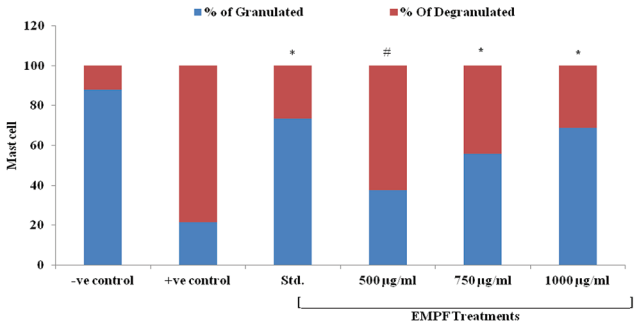
Bronchial asthma is characterized by airway reactivity to exposure to various spasmogens. The airway stimulation leads to the release of numerous mediators like histamine, acetylcholine, leukotrienes, and prostaglandins, which cause an acute attack of bronchoconstriction [3]. There is a very close resemblance in pulmonary responses to histamine challenge in both guinea pigs and human species, as well as in the anaphylactic sensitization, which made guinea pigs the model of choice. Inhalation of histamine and acetylcholine is a classic method of inducing bronchoconstriction, which results in intense smooth muscle contractions, hypoxia, and convulsions in guinea pigs. Bronchodilators can delay the occurrence of these symptoms [26]. In the present study, HMPF

Table 1 Effect of hydroalcoholic extract of *M. paradisiaca* flower on histamine-induced and acetylcholine-induced bronchospasm in guinea pigs

Treated group	Preconvulsive dyspnoea time (s)					
	Histamine-induced bronchospasm			Acetylcholine-induced bronchospasm		
	Before treatment (control)	After treatment	% of increase	Before treatment (control)	After treatment	% of increase
HMPF (200 mg/kg)	126.19 ± 1.53	431.24 ± 1.93*	70.73	147.86 ± 1.91	364.10 ± 1.07*	59.39
HMPF (400 mg/kg)	138.42 ± 1.82	681.32 ± 2.29*	79.68	156.37 ± 1.21	414.19 ± 1.85*	62.2

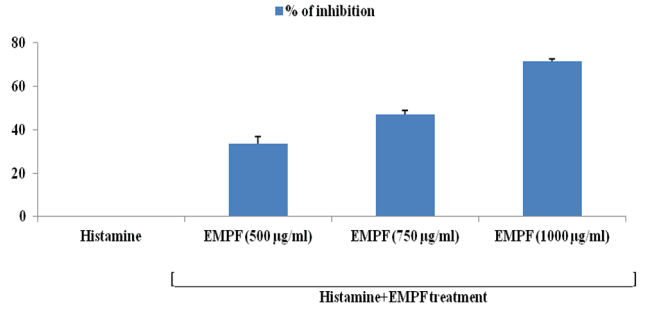
Each value is expressed as mean ± SEM, where $n = 6$ in each group; HMPF, hydroalcoholic extract of *M. paradisiaca* flower; *$P < 0.00$ as compared with control by one-way analysis of variance followed by Dunnett's test.

Figure 1



Effect of hydroalcoholic extract of *M. paradisiaca* flower (HMPF) on compound 48/80-induced mast cell degranulation in albino rats. Values are expressed as percentage of granulated and degranulated mast cell, where $n = 6$ in each group. *$P < 0.001$, #$P < 0.01$ when compared with positive control by one-way analysis of variance followed by Dunnett's test.

Figure 2



Effect of hydroalcoholic extract of *M. paradisiaca* flower (HMPF) on percentage inhibition in histamine-induced constriction on isolated guinea pig trachea. Values are expressed as mean ± SEM, where $n = 6$ in each group. *$P < 0.001$, #$P < 0.01$ when compared with histamine control by one-way analysis of variance followed by Dunnett's test.

showed a sustained inhibitory effect on preconvulsive breathing and prolonged latent period of convulsion in the guinea pigs exposed to aerosolized histamine and acetylcholine. The results of the study suggest that the extract significantly increased the time of occurrence of PCD through dilatation of the bronchial smooth muscles. Again, the extract showed a dose-dependent inhibitory effect on preconvulsive breathing in sensitized guinea pigs exposed to aerosolized spray in an enclosed chamber.

Mast cell degranulation plays a pivotal role in the pathogenesis of allergic disorders. Antigen challenge in sensitized animals results in degranulation of mast cells, which is an important feature of anaphylaxis [27]. The binding of allergen to IgE results in the release of inflammatory mediators like histamine, eosinophils, neutrophils, chemotactic factors, leukotrienes, prostaglandins, and platelet-activating factor, which are responsible for the development of airway inflammation and bronchoconstriction [25]. In our present investigation, the disruption of mast cells was due to exposure to compound 48/80 (an agent having histamine-releasing capacity). In our study, HMPF showed significant protection against compound 48/80-induced mast cell degranulation in a dose-dependent manner, which may prevent the release of various inflammatory mediators. The mast cell-stabilizing activity of HMPF may be due to the suppression of IgE antibody production, which is responsible for degranulation of mast cells.

Histamine contracts the tracheal-bronchial muscle of guinea pigs, goats, horses, dogs, and humans [28]. Antiasthmatic drugs act on the contraction of the trachea-bronchial muscle through several mechanisms, including stimulation of b-adrenergic receptors, inhibition of histamine (H_1) receptors, or through an anticholinergic property [25]. In the present study, in the isolated guinea pig tracheal chain preparation, a right-sided shift was observed in the dose–response curve of histamine in the presence of HMPF, indicating antiasthmatic activity. Antihistaminic activity of HMPF may be due to inhibition of H_1 receptor or by stimulation of b-adrenergic receptor.

Phytochemical screening of HMPF showed the presence of flavonoids, steroids, saponins, terpenoids, lignins, etc. Flavonoids are reported to possess smooth muscle relaxant and bronchodilator and spasmolytic property [29–31], whereas saponins are reported to have mast cell-stabilizing property and lignins are responsible for antibacterial, antioxidant, spasmolytic, and anti-inflammatory effects [32]. Steroids and terpenoids were also responsible for spasmolytic action by relaxing the tracheobronchial tree of lungs [33,34]. The antiasthmatic activity of HMPF may be due to the presence of the above-mentioned phytoconstituents.

Conclusion

The results of this study suggest that the HMPF possesses direct in-vivo antihistaminic and anticholinergic activity with moderate activity in active and mast cell protection against degranulation in animals. These pharmacological activities collectively constitute significant preventive action against asthma. However, further studies should be carried out to evaluate its mechanism of action and for identification of the compound responsible for antiasthmatic activity.

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Conflicts of interest

There are no conflicts of interest.

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