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Original Article

Antiallergic potential of a pseudo-stem powder of *Musa paradisiaca* L. (banana)

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Abstract

Introduction: The use of *Musa paradisiaca* L. (banana) pseudo-stem powder and juice for the treatment of cold and asthma is part of Cuban traditional medicine. There is not information about the influence of banana stem products on allergic reactions. Therefore, this work was aimed to assess the antiallergic potential of a banana pseudo-stem powder (BPSP) by the use of an experimental model with mice.

Methods: Banana stems were collected at Marianao Municipality in Havana. The plant material was ground and filtered and the resulting powder was suspended with acacia 10 mg/mL for experiments. Acute oral toxicity was determined in both sex rats. Female BALB/c mice were sensitized against ovalbumin. Single doses of the powder (60 mg/kg b.w.) or ketotifen (3 mg/kg b.w.) were administered 1 hour before the induction systemic anaphylaxis by i.v. injection of ovalbumin. Moreover, the test product, (0,6; 2; 6 and 20 mg/kg b.w. x day) was given to mice during the immunization period, followed by the induction of systemic anaphylaxis or the measurement of passive cutaneous anaphylaxis titers of mice antisera in rats.

Results: There were neither deaths nor any sign of toxicity among the rats treated with banana powder. Unlike ketotifen, a single oral dose of this product was unable to inhibit systemic anaphylaxis in mice. However, a daily oral treatment produced the significant reduction of active and passive anaphylaxis.

Conclusion: This work has given the first experimental evidence that a pseudo-stem powder of *M. paradisiaca* grown in Cuba has an antiallergic potential.

Keywords: *Musa paradisiaca* L., Pseudo-stem powder, Medicinal plant, Mast cells, Immunoglobulin E, Anaphylaxis, Histamine, 5-Hydroxytryptamine, Mouse, Ovalbumin

Introduction

Rhinitis and bronchial asthma are very frequent allergic diseases. Despite the availability of a wide range of antiallergic and antiasthmatic drugs, the relief offered by them is mainly symptomatic.[1] Consequently, many patients with chronic allergic conditions seek for medicinal plants as natural, costless and safe therapeutic alternatives.[2, 3] For instance, a descriptive, cross-sectional study among asthmatic patients attending a polyclinics of Camagüey, a Cuban city, showed that 23,5 % of participants used Phytotherapy in the inter-crisis phase,[4] being some nutraceuticals containing *Musa paradisiaca* L. (banana) pseudo-stem powder or juice[5] among the most appreciated by allergic patients[6] that may be related with the traditional use of this part of the plant for the treatment of cold and asthma in Cuba.[7-13]

The anti-inflammatory effect of a *M. paradisiaca* stem juice methanol extract has been reported.[14] It could contribute to alleviate the symptoms of respiratory complaints. However, there is not information about the influence of banana stem products on allergic reactions. Therefore, this work was aimed to assess the antiallergic potential of a banana pseudo-stem powder (BPSP) by the use of an experimental model with mice. It fit in a research strategy that deals with the scientific validation of plant preparations used in Cuban traditional medicine and the identification of plants as natural sources for the development of therapeutic options.

Materials and Methods

Plant Material

Musa paradisiaca stems were collected at Marianao Municipality in Havana. Its identity was demonstrated by comparison with the sample registered at Havana Botanic Garden (Voucher ROIG 4779). They were cut into small pieces, washed twice with tap water, followed by drying in an oven at 110°C for 2 hours. Afterwards the plant material was ground into powder and filtered to obtain about of 53 µm of banana stem powder particle size and also to remove any foreign matter and large banana stem

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powder particles and stored in closed dark bottles at 4 to 8°C until use. It was suspended with acacia 10 mg/mL for experiments just before administration to animals.

Laboratory Animals

Sprague Dawley (SD) rats and BALB/c mice were purchased from the National Center for the Production of Laboratory Animals (Havana, Cuba). The animals were placed into polyurethane cages and housed at controlled environment (23°C and 50%-60% relative humidity) with free access to food (ALYCO® CMO 1000) and water.

Acute Oral Toxicity

BPSP acute oral toxicity was evaluated in Sprague Dawley rats (both sex, 200 ± 20 g b.w.) according to 423 OECD guideline.[15] A single intragastric administration (1 mL/100 g b.w.) of BPSP at a maximal allowable dose[16] (2000 mg/kg b.w.) was given to rats. They were observed for a 14-day period in order to detect the occurrence of deaths or any sign of toxicity.

Induction of Systemic Anaphylaxis in Mice

Sensitization Scheme

An association between the dose of ovalbumin (OVA) administered as antigen with $Al(OH)_3$ gel as an adjuvant and asthma-like changes (circulating IgE antibodies, interleukins and others) was demonstrated in female 6-8-week-old BALB/c mice.[17] In addition different sensitization schemes with this combination administered in a fourteen-day period have been used.[17-20] Accordingly, a preliminary experiment was done to select an appropriate scheme for our experimental conditions. The one described earlier[17] was selected for this purpose and two OVA doses (10 and 50 µg) were tested. The animals were sensitized by i.p. injections of a mixture of OVA and $Al(OH)_3$ gel (4 mg) in 200 µL of PBS pH 7.4 at 0; 7th and 14th days.

The mice were challenged by the i.v. injection (through the ocular plexus) of 0.1 mL of PBS containing 70 µg OVA at 15th day. The negative control group received PBS without OVA. Mortality was monitored for 1 h and expressed as follows:

$$\%\ of\ Mortality = \frac{Number\ of\ death\ mice(1h)}{Number\ of\ experimental\ mice} \times 100$$

Since the maximum OVA dose (50 µg) provoked the highest anaphylactic mortality rate (87% vs 17 and 0% in 10 µg and negative control groups respectively) it was selected for the following experiments.

Treatments

Mice were randomly assigned to each treatment group (n = 10/group) for the first set of experiments as follows:

A single dose of BPSP (60 mg/kg b.w.), ketotifen 3 mg/kg b.w. (the positive control), acacia (10 mg/mL) or saline (negative controls for BPSP and ketotifen respectively) were administered to the animals 1 hour before the antigenic challenge.

For the second set of experiments, mice were distributed at random in the following treatment groups:

BPSP (0.6; 2; 6 and 20 mg/kg b.w. x day) or acacia 10 mg/kg (negative control) during the immunization period, starting at 0 day.

Dosing (0.1 mL/20 g b.w.) was given to mice by using an intragastric cannula.

Identification of the Presence of IgE-Like Antibodies in Mice Antisera

Intragastric administrations of BPSP (0.6; 2; 6 and 20 mg/kg b.w. x day) or acacia 10 mg/mL (negative control) were given to groups of mice (n = 10) during the period of sensitization. On the 15th day the blood of the mice from all treatment groups were drawn from their ocular plexus and collected into Eppendorf tubes and the antisera obtained and stored as described in the literature.[21] After 1 hour of incubation at room temperature, the samples were centrifuged at 10 000 g for 1 minute and the sera obtained from animals of the same treatment group were pooled and stored at −80°C until use to identify the presence of IgE-like antibodies in them by means of the method of passive cutaneous anaphylaxis (PCA) in rats. Five replicates of this experiment were done.

PCA consists of the passive sensitization of rat skin mast cells by intradermal administration of autologous or heterologous antisera containing IgE antibodies. It is followed by the intravenous injection of a mixture of Evan´s blue dye and the antigen. In consequence, a local antigen-antibody reaction occurs, leading to blue spots that are proportional to the increase of vascular permeability triggered by chemical mediators released from mast cells.[22,23]

Heterologous PCA reaction against each pool of mice antisera was tested as originally described.[22,23] The backs of two SD rats were shaved with an electric hair clipper, care being taken to avoid irritation. One intradermal injection of 0.1 mL of antiserum (serial dilutions from 1/20 to 1/640 with saline) was made on each side of the dorsal skin, about 1-5 cm from the midline, with a sharp gauge short bevel hypodermic needle. Twenty-four hours later, the animals received intravenous injections of 1 mL of saline solution containing OVA (1 mg) and Evans blue (10 mg). One hour after antigenic challenge, the rats were sacrificed, the skin of their backs was inverted, and the diameter of the blue spots at the sites of injection was measured on the inner surface of the skin with a transparent ruler. The PCA titer was the inverse of the highest serum dilution giving a positive reaction (at least 5 mm diameter).

Statistical Analysis

The PCA titers of mice antisera were converted to its $\log_2$ value for statistical analysis.[23] The experimental results

were expressed as the mean ± S.D. of each group of treatment. Normal distribution of data and homogeneity of variance were assessed by Shapiro-Wilk's and Bartlett's tests, respectively. The statistical comparison between each group of treatment with the negative control group in each experiment was performed by Student's t-test and Mann-Whitney U test. Differences lower than 5% were considered significant. The GraphPad Prism 5 statistical program was used for this purpose.

Ethical Statement

All procedures described were carried out using a protocol approved by the Institutional Research Ethics Committee of Salvador Allende Faculty of Medicine. The guidelines for good practices for the management of laboratory animals[24,25] were followed.

Results and Discussion

Oral Acute Toxicity

The evaluation of acute toxicity in experimental animals is a primary stage of non-clinical estimation of a product safety. It consists of the evaluation of quantitative and qualitative changes, associated to the administration of single or repeated doses of the substance in a 24-hour period of time.[18] This information offers a basis to calculate the doses of a product for its pharmacological characterization. In the present study the acute intragastric administration of BPSP 2000 mg/kg b.w. to rats caused neither deaths nor signs of toxicity among these animals during the 14-day period of observation following the treatment, suggesting that this product could be safe after a single dose treatment. However, other toxicological studies should be done for comprehensive information about its security.

Effect of BSPS on Systemic Active Anaphylaxis in Mice

The allergic process has an important inflammatory component in which mast cell activation and degranulation are the first phenomena observed. The interaction of immunoglobulin E (IgE) antibodies with specific antigens on mast cell surface induces the release of several inflammatory mediators like histamine, serotonin (5-HT), platelet-activating factor (PAF), leukotrienes, and a variety of cytokines leading to local or systemic clinical manifestations, according to the via of access and the diffusion of the allergen into the body. Rhinitis and bronchial asthma are two examples.[1]

It has been demonstrated that like in humans, IgE antibodies, as well as histamine and 5-HT are involved in the immunological reaction in mice and that systemic anaphylaxis in these animals is a consequence of the increase of hematocrit percentage, due to the extravasation of intravascular liquid, induced by histamine and 5-HT, that are released from mast cells in the first five minutes after antigenic stimulation. In consequence, the animal dies in a short time.[17,21 26,27] Thus, it is among the experimental models used to evaluate the antiallergic potential of a given product.[28] Therefore, this model was first used to assess the potentials of BPSP against IgE-mediated immediate-type allergic reactions.

As expected, a single dose of ketotifen, a known antiallergic drug with antihistamine and mast cell stabilizing activity, significantly reduced the rate of fatal anaphylactic shock in mice. However, the acute treatment with BPSP (60 mg/kg) was ineffective (Table 1), thus suggesting a lack of influence on immunological activation of mast cells and/or on the biological effects of chemical mediators released from them. Otherwise, a repeated dosing could be necessary. Consequently, the effect of the treatment with BPSP during the sensitization period on the percentage of mortality was evaluated.

There was not a statistical difference between the groups of animals treated with BPSP at doses 0.6; 2; 6; 20 mg/kg x day. However, all of them showed statistically lower values with respect to the negative control group, as a demonstration of a preventing action. In contrast, the lower BPSP tested (0.6 mg/kg x day was ineffective (Table 2).

The clear dose-response relationships that pure substances usually show are frequently difficult to demonstrate when plant preparations are the test products. This fact could be a consequence of their heterogeneous compositions. It could explain the difference of the effects in a narrow interval of doses (between 0.6 and 2 mg/kg) and a maintained inhibitory action between 2 and 20 mg/kg, indicating that the lowest inhibitory dose is about 2 mg/kg x day and (Table 2).

Effect of BSPS on PCA Titers of Mice Antisera

The relevance of this data is that it is the first evidence of the antiallergic potential of daily oral treatments with

Table 1. Effect of a Single Oral Dose of BSPS on Mortality by Active Systemic Anaphylaxis in Mice

Treatment Groups (N=10/Group)	Mortality (%)
Control (saline)	89.4 ± 7.0
Control (acacia)	94.6 ± 9..4
BPSP 60 mg/kg b.w.	90.1 ± 8.2
Ketotifem 3 mg/kg b.w.	25.2 ± 6.6[a]

The data are the mean ± SD of five independent experiments.
[a] $P<0.05$: significant different from control value.

Table 2. Effect of Daily Oral Doses of BSPS on Mortality by Active Systemic Anaphylaxis in Mice

Treatment Groups (N=10/Group)	Mortality (%)
Control	83.3 ± 6.9
BPSP 0.6 mg/kg b.w	79.2 ± 7.8
BPSP 2.0 mg/kg b.w.	42.9 ± 4.6[a]
BPSP 6.0 mg/kg b.w.	37.5 ± 6.4[a]
BPSP 20 mg/kg b.w.	44.4 ± 5.7[a]

The data are the mean ± SD of five independent experiments.
[a] $P<0.05$: significant different from control value.

BPSP. According to the knowledge about the immediate allergic process,[1] the inhibition of mast cells activation, antagonism of chemical mediators release from them or reduction of IgE levels are among the main mechanisms possibly involved in this result. Hence, a similar experimental design was used in order to evaluate the third mechanism. Future studies will be dedicated to assess the two first options.

PCA is one of the most used experimental models for screening of antiallergic activity.[28] Accordingly, we used this method to assess the effect of a fourteen-day period of oral treatment with BPSP on the presence of IgE antibodies in the mice antisera.

As shown in Table 3, there was a decay of PCA titers of mice treated with BPSP between 0.6 and 2 mg/kg x day. However, it remained at a similar level even though higher doses were given to animals, thus suggesting that the minimum inhibitory dose was around 2 mg/kg (Table 3). The similarities between PCA and active systemic anaphylaxis behaviors (Table 2) suggest that BPSP could have an antiallergic action through the inhibition of IgE production.

Since PCA offers indirect semi-quantitative information on antisera IgE levels, this apparent BPSP immunomodulatory ability should be further characterized by measuring its influence on serum concentrations of IgE and other immunoglobulins, interleukins and cytokines involved in IgE production, as well as on B cells and the process of antigen-antibody reaction. Moreover, it could be interesting to evaluate its effect on the secondary response of immune reaction, not only on the primary one. Also, the identification of possible bioactive compounds responsible for this effect should be another objective of research.

Table 3. Effect of Daily Oral Doses of BSPS on PCA Titers of Mice Antisera

Treatment Groups (N=10/Group)	Log_2 PCA Titer	PCA Titer[b]
Control	8.84 ± 0.64	1/471
BPSP 0.6 mg/kg b.w.	8.25 ± 0.58	1/320
BPSP 2.0 mg/kg b.w.	4.94 ± 0.75[a]	1/31
BPSP 6.0 mg/kg b.w.	4.25 ± 0.84[a]	1/20
BPSP 20 mg/kg b.w.	4.25 ± 0.66[a]	1/20

The data are the mean ± SD of five independent experiments.
[a] $P<0.05$: significant different from control value.
[b] Antilog$_2$ of the mean of Log_2 PCA titer.

Conclusions

The present work has given the first experimental evidence that a pseudo-stem powder of *M. paradisiaca* grown in Cuba has an antiallergic potential, a scientific support to the popular believes about the utility of this part of the plant to control the symptoms of bronchial asthma. It did not show oral acute toxicity in rats, but other toxicological studies should be done for comprehensive information about its security. This product was able to inhibit the antigen-induced active systemic anaphylaxis and reduce serum PCA titer (a sign of the decay of IgE levels) in mice after the administration of daily oral doses during the immunization period. Consequently, it is recommendable to do more studies in order to assess the possibility of developing natural antiallergic options from this product.

Competing Interests

None.

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