

(RESEARCH ARTICLE)



Anti-asthmatic activity of hydro alcoholic extract of *Buddleja madagascariensis* (Lam.) leaves in Guinea pig

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Abstract

The anti-asthmatic activity of the hydro alcoholic extract of *Buddleja madagascariensis* (Lam.) leaves was evaluated in vivo in guinea pigs and in vitro using the isolated guinea pig trachea. Administered orally at doses 50, 100, 200 and 400 mg/kg it protects animals from spasm of the air way caused by histamine aerosol 0.5 % pulverization. Dyspnoea starts at 68 ± 1.20 sec for control group animals, versus 120.25 ± 1.03 , 156 ± 2.04 , 240 ± 2.07 and 279 ± 1.68 sec for the animals treated with the extract at doses 50, 100, 200 and 400 mg/kg respectively ($p < 0.05$). In vitro, the hydro alcoholic extract of *B. madagascariensis* leaves relaxes isolated guinea pig trachea pre contracted with histamine at 10^{-4} M with an EC₅₀ of 0.037 mg/ml. These results indicate the bronchodilation activity of this plant. The pre-incubation of the isolated trachea in the bath containing the extract at concentrations of 40, 50 and 60 μ l/ml increased the EC₅₀ of histamine from $4.61 \cdot 10^{-8}$ M to $5.58 \cdot 10^{-7}$ M, $6.42 \cdot 10^{-6}$ M and $8.32 \cdot 10^{-5}$ M respectively ($p < 0.05$). The maximal contraction induced by histamine decreases from 100 % to 92 ± 3 , 83 ± 2 and 62 ± 3 %. This effect could be caused by the alkaloids and/or flavonoids present in the extract.

Keywords: Asthma; dyspnoea; bronchodilation; *Buddleja madagascariensis*; Guinea pig

1. Introduction

Asthma is a chronic obstructive pulmonary disease with different aetiologies. It is characterized by episodic or persistent respiratory symptoms and airflow limitation due to bronchoconstriction, airway wall thickening and increased mucus. Asthma control medications reduce airway inflammation or rescue medicines quickly ease symptoms that may arise acutely, such as bronchodilators [1]. Despite the progress in western medical research, many people in developing countries still depend on traditional medicines, due to their geographical situation, lack of medical structure of finances [2]. These traditional medicines come from forests. However, few of the phytomedicinal products have clear documentation. Searching for their mechanism of action helps for the security of their users and may result in creating new ideas for producing more effective pharmaceutical drugs for those who do not have access to medicinal plants. The result of an ethnobotanical survey that we have conducted in the north-eastern part of Madagascar showed that *Buddleja madagascariensis* is used for the management of allergic respiratory cases. It is mainly indicated in allergic conditions such as allergic rhinitis, allergic asthma. According to the people interviewed it is remarkably effective in respiratory tract disorders. Analysing that information, we thought that this plant might have a bronchodilation activity. The present study aimed to justify the traditional use of *Buddleja madagascariensis* in the management of asthma and explore its pharmacodynamic properties.

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2. Material and methods

2.1. Plant material

The leaves of *Buddleja madagascariensis* were collected from Antalaha, in the north-eastern part of Madagascar and identified at the Botany Department of Botanical and Zoological Park of Tsimbazaza, Antananarivo.

2.2. Preparation of extract

The leaves of *Buddleja madagascariensis* were dried in shade at room temperature and ground to powder. The powder was macerated in 60 % ethanol at room temperature for 3 days and filtered. The ethanolic filtrate was concentrated under reduced pressure to get the dry extract used in this work.

2.3. Experimental animals

Guinea pigs of either sex, weighing 350 - 500 g were selected and randomly divided into four groups of four animals per group. They were lodged at the animal house of the Pharmacology Laboratory, Faculty of Sciences, University of Antananarivo, under standard environmental conditions of temperature and humidity, 12/12 h dark and light cycle, fed with animal feed and had water *ad libitum*.

2.4. In-vivo assessment

The animals used in this test were fastened 12 h prior to test with free access to water. Histamine was dissolved in distilled water to prepare 0.5 % solution. Extract was dissolved in distilled water and administered orally. Single dose treatment was given half an hour before the study. The control group was given distilled water, while the others received the extract at doses 50, 100, 200 and 400 mg/kg in 10 ml/kg [3]. Half an hour later, one animal from each group were put together in a tightly closed enclosure made of plexiglass and exposed to 0.5 % histamine aerosol, and the time for onset of dyspnoea was noted for each animal. As soon as the sign of dyspnoea happened, the animal was removed from the chamber and placed in fresh air to recover [4].

2.5. In-vitro assessment

Animals were sacrificed by cervical dislocation and carotid bleeding. The trachea was dissected out and transferred to a dish containing Krebs solution (composition (g/l): NaCl (6.5), KCl (0.33), CaCl₂ (0.26), MgSO₄·7H₂O (0.28), NaHCO₃ (2.5), KH₂PO₄ (0.19), glucose 5.0) [5] and cut crosswise between the sections of the cartilage of the trachea and continuously ventilated and maintained at 37 ± 1 °C. The isolated trachea was mounted in isolated organ bath under basal tension of 500 mg and allowed to equilibrate for at least 40 minutes. During this time, the bath was supplied with fresh Krebs solution every 15 minutes. The cumulative concentration response to histamine in the absence and presence of ethanolic extract of *Buddleja madagascariensis* leaves were recorded. To assess its mechanism of action, the isolated trachea was pre incubated in a bath containing extract at concentrations of 40, 50 and 60 µg/ml before adding an increasing concentration of histamine in the bath [6].

2.6. Statistical analysis

All the values of *in vitro* and *in vivo* anti-asthmatic activity were expressed as mean ± Standard error of mean (S.E.M) and was examined for significance by ANOVA, and groups were compared with control using Student's "t" test. Difference was considered as significant for p<0.05.

3. Results

3.1. Effect of hydro alcoholic extract of *Buddleja madagascariensis* leaves on histamine induced dyspnoea

Buddleja madagascariensis leaves extract administered orally at doses 50, 100, 200 and 400 mg/kg prior to histamine 0.5 % pulverisation increases the latency time of dyspnoea onset. Dyspnoea starts at 68 ± 1.20 sec for control group, versus 120.25 ± 1.03, 156 ± 2.04, 240 ± 2.07 and 279 ± 1.68 sec respectively for the animals treated with the extract (p<0.05) (Figure 1). These results indicate that this extract protects the animals from histamine induced dyspnoea. This effect might be due to its bronchodilation activity.

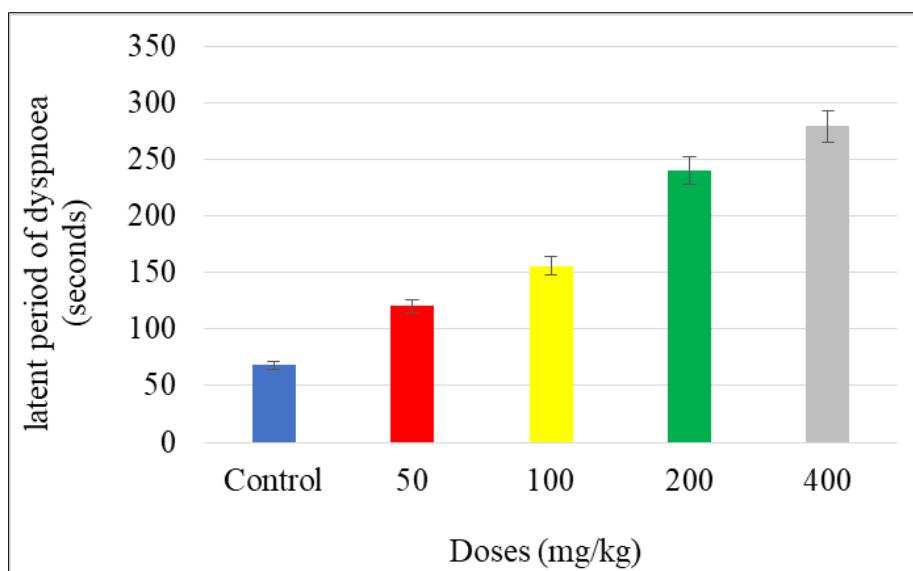


Figure 1 Latent period of histamine 0.5% induced dyspnoea of control group (■) and animals treated with the hydro alcoholic extract of *Buddleja madagascariensis* leaves, administered orally, at doses 50 (■), 100 (■), 200 (■) and 400 mg/kg (■) ($\bar{x} \pm s. e. m$; $n=6$; $p<0.05$)

3.2. Effect of hydro alcoholic extract of *Buddleja madagascariensis* leaves on histamine induced contraction

Injected, in a cumulative manner from 0.01 to 0.11 mg/ml, in the bath containing the isolated trachea contracted with histamine at concentration 10^{-4} M; the result shows significant broncho dilatory activity in the precontracted tracheal preparation. This is indicated by the attenuation of the histamine-induced contractions in concentration dependent manner. It produces 100 % bronchodilation (complete bronchorelaxation) against histamine induced contraction at 0.07mg/ml with EC_{50} of 0.037 mg/ml (Figure 2). Thus, the extract exhibits significant broncho dilatory activity in guinea pig pre-contracted tracheal preparation by counteracting histamine induced contraction.

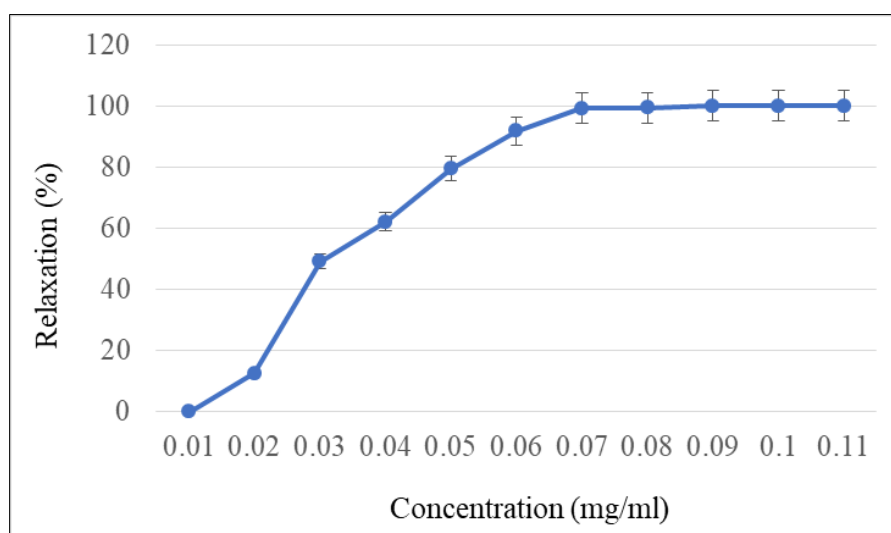


Figure 2 Effect of *Buddleja madagascariensis* leaves hydro alcoholic extract, injected in a cumulative manner in the isolated organ bath, on histamine induced contraction of isolated trachea ($\bar{x} \pm s. e. m$; $n=6$; $p<0.05$)

Pre incubating trachea in a bath containing *Buddleja madagascariensis* leaves hydro alcoholic extract at increasing concentrations inhibited contractile effect of histamine. There was a depression of maximal effect and a right-side shift of concentration response curve of histamine in the presence of the extract. Maximal effect of histamine reduces from 100 % in the absence of extract to 92 ± 3 , 83 ± 2 and 62 ± 3 % in the presence of extract at concentrations of 40, 50 and 60 $\mu\text{g/ml}$ respectively, while EC_{50} increases from $4.61 \cdot 10^{-8}$ M in the absence of extract to $5.58 \cdot 10^{-7}$, $6.42 \cdot 10^{-6}$ and 8.32

10^{-5} in the presence of the extract at concentrations 40, 50 and 60 $\mu\text{g}/\text{ml}$ respectively ($p < 0.05$) (Figure 3). These results indicate non-competitive antagonism between the active principle in the extract and histamine.

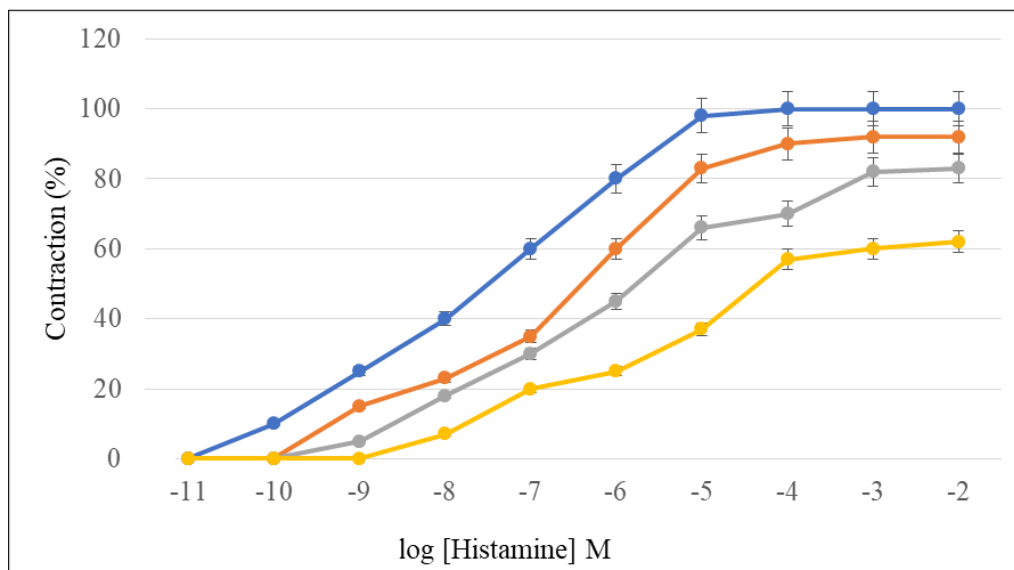


Figure 3 Bronchoconstriction effect of histamine in the absence (■) and presence of *Buddleja madagascariensis* hydro alcoholic extract, at concentrations 40 (■), 50 (■) and 60 $\mu\text{l}/\text{ml}$ (■) ($\bar{x} \pm \text{s. e. m}$; $n=6$; $p < 0.05$)

4. Discussion

Asthma is a multifactorial disease which involves different mediators triggering multiple biochemical reactions. Thus, the present study was undertaken to evaluate the action of *Buddleja madagascariensis* leaves hydro alcoholic extract on bronchoconstriction since bronchodilators quickly ease symptoms that may arise during crisis.

Allergens have been known as most important trigger of asthma [7]. It is recognized that exposure to an allergen initiates allergic immune response through the generation of IgE antibodies by B cells against allergen. Fixation of these IgE antibodies on their receptor on the mast cells and basophils membrane, leads to the release of mediators such as histamine, causing bronchoconstriction, inflammatory reaction, increases in airway hyperresponsiveness, and mucus production [8]. Our results indicate that *Buddleja madagascariensis* leaves hydro alcoholic extract delays the onset time of induced histamine aerosol dyspnoea, in a dose dependent manner, indicating its protective effect from bronchoconstriction induced by histamine [9]. In line with this notion, it may be due to inhibition of mast cell degranulation, since it is important in the initiation of immediate responses following exposure to allergens. Degranulated cells liberate mediators of inflammation such as histamine, leukotrienes, platelet activating factors and chemotactic factors for eosinophils, neutrophils etc. which play a significant role in airway inflammatory response such as airway eosinophilia, late asthmatic response, and airway hyperresponsiveness as well as in immediate hypersensitivity reaction like bronchial contraction [10]. On the other hand, *in vitro* results indicate that this extract relaxes the isolated trachea contracted with histamine, which suggests that it opposes the histamine induced bronchoconstriction [11]. This partly explains the increase of the latent period of histamine induced dyspnoea observed in the animals treated with the extract compared to the control group. Our results also revealed that preincubating the trachea in bath containing the extract at increasing concentrations depresses the maximal effect of histamine and shifts to the right the concentration response curve of histamine. This is characteristic of a non-competitive antagonism, which indicates that it is not an antihistaminic H1 and suggests it may act as β_2 memetic [12]. Beta-adrenergic agonists have emerged as the most potent class of bronchodilator available, and the inhalational route of administration has proven to be the most effective and least toxic method of delivery except among apnoeic or highly uncooperative patients [13,14]. Or this extract is still effective administered orally. Thus, the results confirmed bronchodilator activity of this extract and justified the use of *Buddleja madagascariensis* in the treatment of asthma as claimed in traditional medicine use in the area where we have conducted the ethnobotanical survey. It may be emphasized that *Buddleja madagascariensis* has a promising target for the control as well as management of complications arising due to bronchoconstriction in asthma.

5. Conclusion

From the results of the present investigation, it can be concluded that *Buddleja madagascariensis* leaves hydro alcoholic extract possesses significant anti-asthmatic activity which may be ascribed to anti-allergic, broncho dilating potential, confirming the traditional claim about this plant. These actions may be due the synergistic action of flavonoids, alkaloids and other phenolic constituents present in *Buddleja madagascariensis* leaves extract. Further, thorough investigation about active phytoconstituent responsible for its anti-asthmatic activity needs to be explored.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declared no conflict of interest.

Statement of ethical approval

All experiments conducted in this study were approved by the Sciences Faculty of Antananarivo Animal Ethics Committee with the approval number ECFS-0219/15 on February 25, 2019.

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