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Anti-asthmatic activity of Hydro Alcoholic Extract of *Ficus trichopoda* Bak. Leaves in Guinea Pig

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ABSTRACT

The anti-asthmatic activity of *Ficus trichopoda* was studied in guinea pigs *in vivo* and *in vitro* using the isolated guinea pig trachea. 33%, 66% and 100% of the animals treated with the hydroalcoholic extract of *Ficus trichopoda* at doses 100mg/kg, 200mg/kg and 300mg/kg respectively, were protected from spasm of the air way caused by pulverization of 5% histamine aerosol. *In vitro*, the isolated guinea pig trachea contracted with histamine at 10^{-4} M was relaxed with the extract with an EC_{50} of 65.75µl/ml. The pre-incubation of the isolated trachea in the bath containing the extract at concentrations of 100µl/ml and 200µl/ml increased the EC_{50} of histamine from (1.10. ± 0.025) 10^{-6} M to (5.95±0.05) 10^{-6} M and (1.49±0.01) 10^{-5}M respectively. The maximal contraction provoked by histamine, however, remained unchanged. These effects could be caused by the alkaloids and/or flavonoids present in the extract.

Key words: *Ficus trichopoda*, asthma, guinea pig

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INTRODUCTION

The aim of this study was to verify the anti-asthmatic activity of *Ficus trichopoda* leaves used by the local population in traditional medicine. Asthma is a chronic obstructive disease of the respiratory tract. It is caused by inflammation of the lower airways which narrows the bronchioles responsible for the dyspnea observed during asthma crisis[1]. The bronchoconstriction is consecutive to the airway smooth muscle contraction [2, 3], or the airways wall inflammatory edema [4, 5], or a hypersecretion of mucous. Asthma could be allergic (extrinsic) or not (intrinsic)[6, 7]. The allergic asthma results to allergen or repeated infections. Interaction between allergen and IgE on the mast cells' membrane and the basophil polynuclear induces their activation[8, 9]. Once activated, these cells release histamine, responsible for the immediate responses with a bronchial contraction and mucous hyper secretion, and inflammatory mediators such as prostaglandins and leukotrienes, responsible for inflammatory edema[10-13]. The management of asthma consists of the use of bronchodilators such as the β2mimetics, anti cholinergics, mast cells' antidegranulation and anti histaminic[14, 15] and on the other hand, anti inflammatory agents such as the corticoids, anti leukotriene, inhibition of IgE[16, 17]. Phytotherapy is also used in asthma management [18, 19]. *Ficus trichopoda* is one of the plants used for the management of asthma in Madagascar. This work was aimed to study of *Ficus tricopoda'*s anti-asthmatic activity *in vivo* and *in vitro* in guinea pigs. The experiments were conducted following the guidelines of the ethic committee of Sciences Faculty, University of Antananarivo, Madagascar (Ref: CE/Fac Sciences/Pharmacol./07, 08/18/2015).

MATERIALS AND METHODES

Extraction and phytochemical screening

The leaves of *Ficus trichopoda* were collected in Antananarivo, and dried in shade at room temperature. They were ground to powder and the powder was macerated in a hydro alcoholic mixture in the proportion of water/alcohol (60:40). After filtration, the macerate was evaporated to dryness, using a rotavapor (Evapotec ®) at the temperature of 80°C. The extract was screened, following[20] to detect major phytochemical entities.

Activity of *Ficus trichopoda* extract on respiratory difficulty induced by histamine *in vivo*

The bronchodilator activity of the extract of *Ficus trichopoda* leaves was studied in guinea pig using histamine to induce bronchoconstriction[21,19].

Guinea pigs of either sex, aged 4months and weighing between 300 to 350 g were used. The animals were kept under a cycle of light and dark (12/12 H), had free access to food and water *ad*

libidum. The animals used in the test were selected according to their sensitivity to histamine after they have been put in a closed chamber pulverized with 5% of histamine. Animals which presented signs of suffocation in less than 3minutes were considered to be sensitive to histamine and used for the test[22]. The selected animals were fasted for 12 hours prior to the test, and put into 4 groups of 5 animals per group: group one represented the control group; the rest were the experimental groups treated with the extract. The control group received distilled water, the second, third and fourth groups received the extract at the dose of 100mg/kg, 200mg/kg, 300mg/kg and 400mg/kg respectively. The different products were administered by oral route in **a** volume of 10ml/kg[23]. One hour after the administration of the different products, one animal per group was put in the 5% histamine chamber, the suffocation test was conducted, and the appearance of a sign of suffocation was recorded[24]. Animals which didn't present any sign of suffocation were considered as protected[25]. Animals were removed and placed in fresh air as soon as they presented any sign of suffocation. The effect of the extract was expressed in percentage of protected animals:

$$\% \text{ of protected animals} = \frac{(n \times 100)}{N}$$

Where:

n : number of protected animals

N : total number of animals treated with the same dosage

Activity of *Ficus trichopoda* extract *in vitro*

This test was conducted to complete the previous test, using isolated trachea of guinea pig, an organ rich in histaminic receptor[26].

a. Effect of the extract on the contraction induced by histamine

Guinea pig of both sexes, of age 5 weeks and weighing between 300g and 350g, were used. The animals were euthanized by injecting, intra muscularly, Phenobarbital 100mg/kg and exsanguinated by cutting the carotids. The trachea was isolated and plunged into Tyrode solution aerated with air (mM: NaCl: 137; KCl: 2.7; $CaCl_2$: 2.5; $MgCl_2$: 1.0; $NaHCO_3$: 11.9; NaH_2PO_4: 0.4; glucose: 11.1). The mesenteric tissue was removed and the trachea was cut into strips[27] of 1.5cm and mounted in an isolated organ bath containing 10ml of Tyrode solution, with a weight of 1.5g as a counterbalance. The bath temperature was maintained at 37°C and was aerated[28]. The organ was equilibrated for 90min, during which it was rinsed every 15min[25]. The contraction responses were recorded with an isotonic transducer (Harvard).

After the equilibration period, a viability test was carried out by injection of histamine into the bath at a final concentration of 5.10^{-3}M in the bath. The preparation was afterwards rinsed and left to

equilibrate until the tension of the organ got back to its initial value. During this equilibration period, the preparation was rinsed 3 times[29]. After the equilibration period, the organ was contracted with histamine injected in the bath cumulatively to get increasing concentration until the maximal contraction of the organ. At the pic contraction, the extract was injected cumulatively into the bath until the organ was completely relaxed. The total volume of product injected in the bath didn't exceed 10% of the bath volume[29].

The effect of the extract was expressed as percentage of relaxation of the trachea contracted by histamine:

$$\% \text{ relaxation} = 100 \text{ X } \frac{(AH-AE)}{AH}$$

AH: amplitude of the contraction induced by histamine

AE: amplitude of the contraction induced by histamine in the presence of the extract

The relaxation curve was traced on a semi logarithmic scale, the efficient concentration of the extract which provoked 50% of the relaxation was determined graphically.

b. Inhibitory effect of the extract on the contraction induced by histamine

To study the inhibitory effect of the extract on the contraction induced by histamine, the trachea was pre incubated in a bath containing the extract at different concentrations before injecting histamine into the bath[30,31].

A piece of isolated trachea in strip (1.5cm) was pre incubated 10min in a bath containing 100µl/ml. After that period, histamine was injected in the bath cumulatively to get maximum contraction of the trachea. Then the preparation was rinsed and left to equilibrate, and the organ was pre incubated in a bath containing 200µl/ml of the extract for 10min, and contracted with histamine injected cumulatively in the bath until the contraction is maximal.

The effect of histamine in the presence and absence of the extract was expressed:

$$\% \text{ contraction} = 100 \text{ X } \frac{A}{A_{max}}$$

Where:

A: amplitude of the contraction induced by histamine

A_{max}: amplitude of the contraction induced by histamine in the presence of the extract

The amplitude of the trachea contraction in the presence and absence of the extract was traced on semi logarithmic scale. The efficient concentration of histamine which gave 50% of the maximal effect (EC_{50}) was calculated using linear regression from the linear part of the curve.

Expression and analysis of results

The results were expressed as $\bar{m}\pm$ s.e.m. The means were compared using the Student 't' test with a signification degree $p<0.05$.

RESULTS AND DISCUSSION

Phytochemical results

The phytochemical screening of *Ficus trichopoda* leaves showed an abundance of polyphenols and saponins, low amounts of alkaloids, flavonoids, steroids and anthocyanins.

Bronchodilator activity of *Ficus trichopoda* extract *in vivo*

Pulverization of histamine in the chamber provokes respiratory distress in the sensitive animals. The animals of the control group showed respiratory distress from 0.27 ± 0.02 min as opposed to 2.57±0.23 min, 3.07±0.15 min and 3.97±0.15min ($p<0.05$) in the animals orally given the extract at doses of 100mg/kg, 200mg/kg and 400mg/kg respectively. These results show that during the 3minutes of observation, 33%, 66% and 100% of the animals that received the extract at the doses of 100mg/kg, 200mg/kg and 400 mg/kg respectively were protected from the respiratory distress induced by histamine pulverization. This means that either he extract contains molecules capable of inhibiting the contraction induced by histamine or capable of dilating the airways contracted by histamine. In the first case the molecule could be a competitive or non-competitive antagonist of histamine and in the second case it could be a β2 agonist[32]. That was the reason *in vitro* test was carried on.

Bronchorelaxation effect of *Ficus trichopoda* extract *in vitro*

a. Relaxation effect of *Ficus trichopoda* extract on trachea contracted by histamine

Injection of cumulative concentration of histamine in the isolated organ bath, from 10^{-7}M to 10^{-4}M, induced the maximal contraction of the trachea. This maximal contraction was used as 100% of contraction with EC_{50} of (1.10 ± 0.025) 10^{-6}M.

Injection of cumulative concentration of *Ficus trichopoda* extract in the bath, from 10µl/ml to 160µl/ml induced 17±0.21% to 99±0.18% of relaxation of the trachea contracted by histamine (Figure 1). Graphic determination of *Ficus trichopoda* extract EC_{50} gives a value of 65.75 µl/ml. These results show that it inhibits the histamine induced contraction of trachea. This inhibition might be a competitive or non-competitive, To clarify it the next test was carried out.

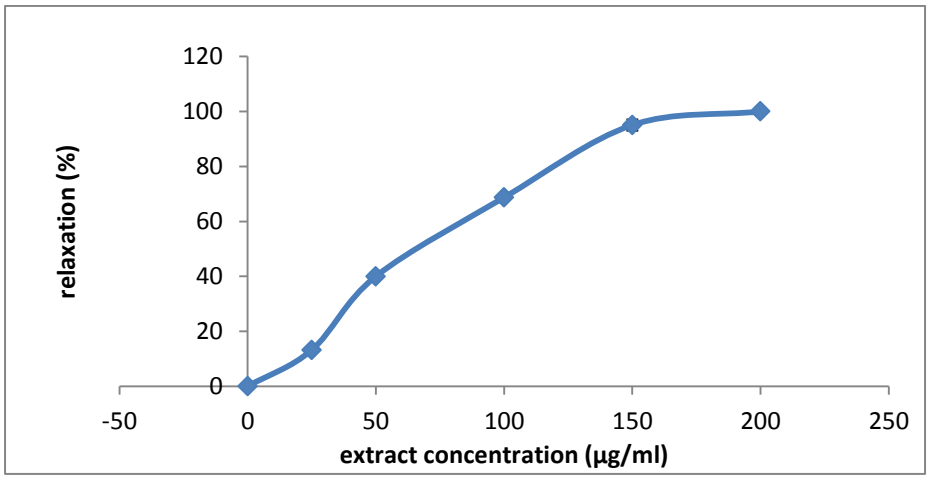

Figure 1. Relaxation of the trachea contracted by cumulative histamine by the Ficus *trichopoda* extract added cumulatively in the bath ($\bar{m}$±s.e.m., n= 5, p<0.05).

b. Inhibitory effect of *Ficus trichopoda* extract

Pre incubation of trachea in a bath containing *Ficus trichopoda* extract inhibits the contraction induced by histamine. EC_{50} values of histamine increased**,** but its maximal effect remained unchanged. In the presence of 100µl/ml and 200 µl/ml of the extract in the bath, EC_{50} values of histamine passed from (1.10 ±0.025) 10^{-6}M to (5.96 ± 0.06)10^{-6}M and (1.50 ± 0.01)10^{-5}M respectively ($p < 0.05$) (Figure 2).

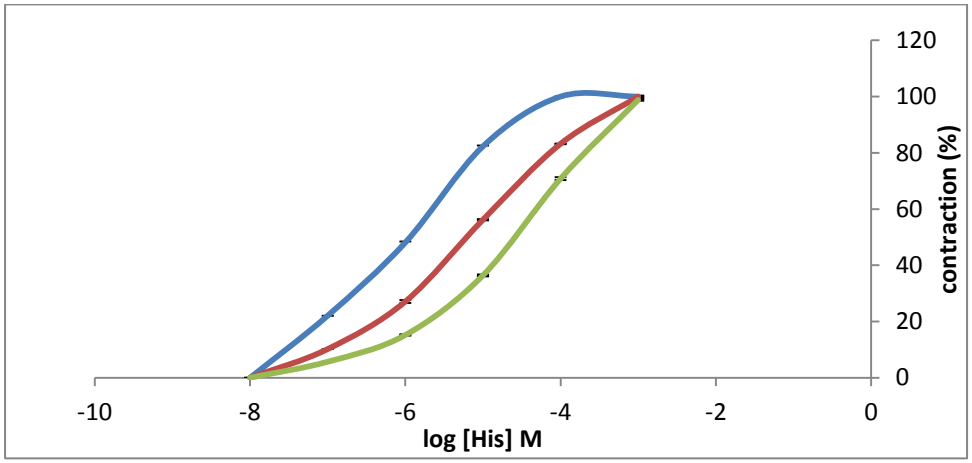

Figure 2. Histamine induced contraction of trachea pre incubated in a bath containing *Ficus trichopoda* extract at 100µg/ml ■ and ■200µg/ml and in its absence ■ ($\bar{m}$±s.e.m., n= 5, p<0.05).

These results show a competitive inhibition, because the maximal effect of histamine remained unchanged, but the EC_{50} value increased when the organ is pre incubated in a bath containing different concentrations of *Ficus trichopoda* leaves extract[33].

These results mean that one of the molecules in *Ficus trichopoda* extract interacts with the histamine receptor, most probably the alkaloids, like the case of *Ficus exaspeata* extract[34]. On the other hand, *in vivo* the protection of the animals against the histamine induced dyspnea confirms that hypothesis. But the anti asthmatic activity of *Ficus trichopoda* extract *in vivo* could also be due to the presence of polyphenols which have an anti allergen activity[35], or the inhibition of the liberation of histamine from the mast cells by flavonoids[36], like the anti-asthmatic activity of *Waltheria indica* L. extract[15].

CONCLUSION

Administered orally *Ficus trichopoda* leaves extract has an anti asthmatic activity. It protects the sensitive animals to the respiratory distress induced by histamine. *In vitro* it inhibits, in a competitive manner, the bronchoconstriction induced by histamine. Since the extract inhibits histamine on its receptors, it might be active against allergic asthma. This activity could be attributed to alkaloids and flavonoïds in the extract.

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