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Anti-asthmatic activity of hydro alcoholic extract of *Indigofera tinctoria* L. *l*eaves in guinea pig

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Abstract

This work aimed to evaluate the anti-asthmatic activity of the hydro alcoholic extract of *Indigofera tinctoria* L. leaves in guinea pig. It was administered orally at doses 75, 150 and 300 mg/kg. The animals were exposed to histamine 0.5 % aerosol and latent period of dyspnoea onset of animal was recorded. Its mechanism of action was investigated in vitro using isolated trachea. Results of these tests indicate that the extract increases the latent period of dyspnoea onset from 38±1.4 sec in control group to 47.5 ± 2.04, 77.5 ± 2.20 and 138 ± 3.14 sec in animals treated with the extract at doses 75, 150 and 300 mg/kg respectively ($p<0.05$). It completely relaxes the isolated trachea contracted with histamine at 10-5 M with EC50 equal to 0.036 mg/ml. Pre incubating the organ in bath containing the extract at 0.04, 0.05 and 0.06 mg/ml increases histamine EC50 from 4.6 10-8 M to 6.12 10-7, 6.8 10-6 and 10.21 10-5 M and reduces its maximal effect from 100 % to 90, 82 and 52 % respectively. These results indicate that the extract possesses bronchodilation activity which justifies the traditional use of Indigofera tinctoria as anti-asthmatic.

Keywords: Anti-asthmatic; bronchodilation; dyspnoea; Guinea pig; *Indigofera tinctoria*

1. Introduction

Asthma is a multifactorial respiratory disease that is rising in prevalence worldwide. It affects about 300 million people worldwide and it has been estimated that a further 100 million will be affected by 2025 [1]. Its management is a long-term process, and due to side effects that the medicines provoke and for other reasons including financial problem, patients are seeking complementary and alternative medicine to treat their asthma [2]. Medicinal plants are also used for the treatment of asthma because of their anti-inflammatory, immunomodulatory, antihistaminic, smooth-muscle relaxants, and anti-allergic activities [3]. Now a days, herbal drugs have become a subject of global importance, with both medicinal and economic implications. Meanwhile, some people are concerned over their quality, safety, and efficacy. Thus, a proper scientific assessment has become the criteria for acceptance of traditional health claims, therefore research on medicinal plants keep increasing, including on plants used in asthma management [4].

According to the results of an ethnobotanical survey that we have conducted in the northeastern part of Madagascar, the aerial part of *Indigofera tinctoria* L. is used to take care of dyspnoea that we have considered as asthma crisis. Many asthma attacks are triggered by allergens, such as dust, mould spores….[5]. Since histamine plays a major role in this pathology, we examined the effect of the hydro alcoholic extract of aerial the leaves of this plant *in-vivo* model using histamine-induced dyspnoea in guinea pig and *in-vitro* on the isolated guinea pig trachea contracted by histamine.

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2. Material and methods

2.1. Collection and identification of plant material

Aerial parts of *Indigofera tinctoria* were collected from wild source from Antalaha, in the northeastern part of Madagascar, and were authenticated at the botany department of Botanical and Zoological Park of Tsimbazaza, Antananarivo, Madagascar.

2.2. Extract preparation

The leaves were shade dried, at room temperature and ground to powder. Powder was macerated in ethanol-water (60:40) at room temperature, for 3 days. Macerate was filtered on cotton and Whatman filter n°2. The filtrate obtained was dried in rotary vacuum evaporator at 60°C.

2.3. Animals

Guinea pigs of either sex, weighing 350 - 500 g were used in this work. They were bred in the animal house of the laboratory of pharmacology and cosmetology, the sciences faculty of the University of Antananarivo, Antananarivo, Madagascar, under standard environmental conditions of temperature and humidity, 12/12 h dark and light cycle, fed with animal feed and had water *ad libitum*. They were starved 12 hours prior to tests.

2.4. Anti-asthmatic activity *in vivo*

Animals starved for 12 hours were used in this work. They were divided into 4 groups: 1 control and 3 treated with the extract. Distilled water was administered orally to the control group and extract at doses 75, 150 and 300 mg/kg were respectively administered to the tested groups, by oral route in a volume of 10 ml/kg [6]. After 30 minutes of administration, one animal per group was put in a hermetic enclosure where 0.5 % of histamine was pulverised. Time for onset of dyspnoea was noted for each animal. As soon as animal presented respiratory problem, it was removed from the enclosure and placed in fresh air to recover [7].

2.5. Bronchodilation activity *in vitro*

Isolated trachea tissue was obtained immediately after slaughter of the animal. Trachea was cut into rings and cartilage was cut across. Trachea was suspended in bath of Krebs solution (composition (g/l): NaCl (6.5), KCl (0.33), $CaCl_2$ (0.26), $MgSO_4 7H2O$ (0.28), $NaHCO_3$ (2.5), KH_2PO_4 (0.19), glucose 5.0, and distilled water q.s. 1 liter) [8], which was continuously aerated and maintained at 37 ± 1ºC. One end of the cartilage was attached to the base of the organ bath, and the other end was attached to an isometric transducer STATHAM GOULD ©. Tissue was allowed to equilibrate for 45 min, under a tension of 500 mg. A concentration response curve for histamine was taken from 10^{-11} M until maximal contraction. Afterwards, *Indigofera tinctoria* extract was added into the bath in a cumulative manner until total relaxation. Graph of percentage of relaxation response on ordinate and concentration of extract on abscissa was plotted to record its concentration response curve.

To evaluate its action vis-à-vis of histamine, the organ was pre incubated in bath containing different concentrations of the extract before injecting histamine in the bath in a cumulative manner. Maximal contraction induced by histamine and its EC_{50} in the presence of different concentration of the extract were evaluated.

2.6. Statistical analysis

The statistical analysis was performed by using one-way analysis-of-variance (ANOVA) followed by Student's "t" test for individual comparison of groups with control; $p < 0.05$ is considered as significant.

3. Results and discussion

Histamine was used to induce bronchoconstriction because most often, asthma crisis is triggered by allergen, and histamine liberation as result of mast cells degranulation [5]. When inhaled, histamine induces bronchoconstriction by direct H1-receptor activation and by a naturally mediated bronchoconstrictor effect via vagal reflexes [9]. The onset of dyspnoea was used as parameter to evaluate the capacity of the extract to protect the animals against the histamine induced dyspnoea. The guinea pigs when exposed to 0.5 % histamine aerosol, showed signs of progressive dyspnoea. The extract significantly prolonged the latent period of dyspnoea in animals treated with it as compared to control group. Administered orally at doses 75, 150 and 300 mg/kg, the hydro alcoholic extract of *Indigofera tinctoria* leaves expressly extended the latent period of dyspnoea for animals exposed to histamine aerosol from 38 ± 1.4 sec in control

group to 47.5 ± 2.04, 77.5 ± 2.20 and 138 ± 314 sec for the groups treated with the extract ($p<0.05$) respectively (Figure 1). These results show the protection activity of the hydro alcoholic extract of *Indigofera tinctoria* leaves from histamine induced dyspnoea. This may be due to inhibition of mast cell degranulation or histamine induced bronchoconstriction or by the extract's proper bronchodilation activity [10, 11].

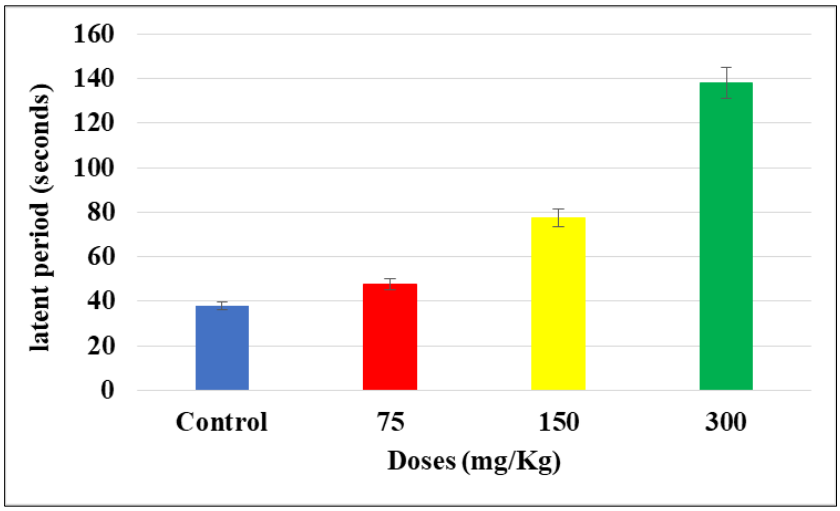

Figure 1 Latent period of histamine 0.5 % induced dyspnoea of control group ( ) and animals treated with hydro alcoholic extract of *Indigofera tinctoria*, administered orally, at doses 75 ( )150 ( ) and 300 mg/kg ( ) ($\bar{x}$ ± s. e. m; n=6; $p<0.05$)

3.1. Effect of hydro alcoholic extract of *I. tinctoria* on histamine induced contraction

Histamine contracts the tracheo-bronchial muscle of guinea pig by stimulation of H1 receptors [12]. To investigate the effect of the extract on histamine induced bronchoconstriction, its effect on isolated trachea pre contracted with histamine was evaluated *in vitro*. Injected in a bath containing trachea pre contracted with histamine at concentration of 10^{-5} M, hydro alcoholic extract of *Indigofera tinctoria* relaxes the organ in concentration dependent manner. Maximal relaxation (100 %) is obtained with the concentration of 0.06 mg/ml with EC_{50} of 0.036 mg/ml (Figure 2). These results indicate the bronchodilation activity of *Indigofera tinctoria* leaves hydro alcoholic extract. This activity may be due to its competitive or non-competitive effect with histamine on H1 receptor [13].

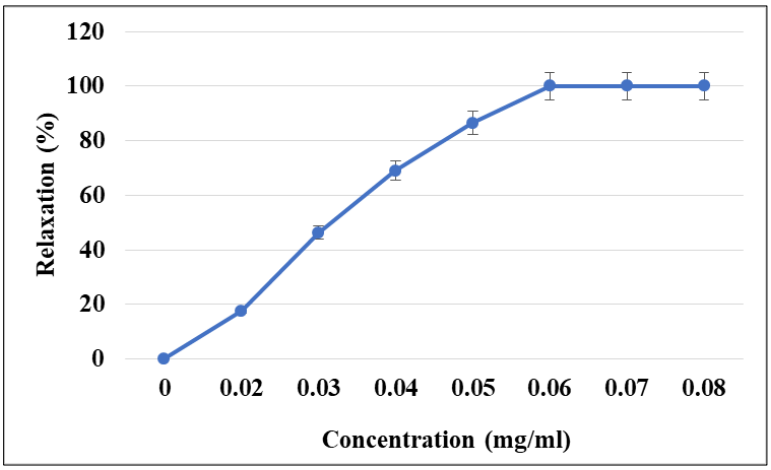

Figure 2 Effect of *Indigofera tinctoria* leaves hydro alcoholic extract, injected in a cumulative manner in the isolated organ bath, on isolated trachea pre contracted with histamine at 10^{-5} M ($\bar{x}$ ± s. e. m; n=6; $p<0.05$)

To determine the receptor involved in the bronchodilation activity of the hydro alcoholic extract of *Indigofera tinctoria* leaves, the isolated trachea was pre incubated in organ bath containing increased concentrations of the extract before contracting it with histamine. The results of this test indicated that, in the presence of the extract at 0.04, 0.05 and 0.06 mg/ml, the maximal effect of histamine is depressed from 100 % to 90, 82 and 52 % (Figure 3). There is also right-side

shift of concentration response curve of histamine in the presence of the hydro alcoholic extract of *Indigofera tinctoria* leaves with EC_{50} of histamine increasing from 4.6 10^{-8} M to 6.12 10^{-7}, 6.8 10^{-6} and 10.21 10^{-5} M indicating a non-competitive action of the extract on H1 receptor of histamine [14]. These results indicate that this extract is not H1 antagonist, this means the dyspnoea protection might be due to its bronchodilation activity which passes through another mechanism. Since β2 adrenergic agonist induces bronchodilation, we suggest that *Indigofera tinctoria* might act through this receptor [15].

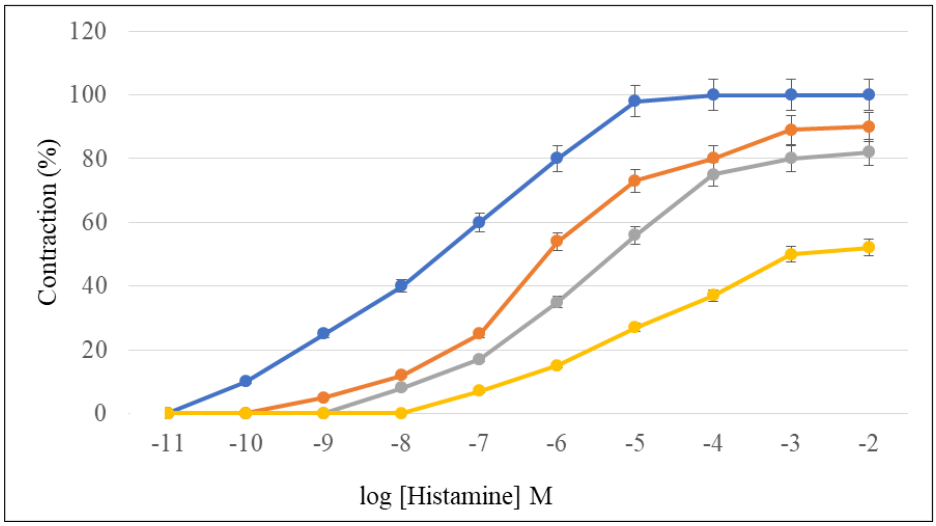

Figure 3 Bronchoconstriction effect of histamine in the absence ( ) and presence of *Indigofera tinctoria* hydro alcoholic extract, at concentrations 0.04 ( ), 0.05 ( ) and 0.06 mg/ml ( ) ($\bar{x}$ ± s. e. m; n=6; p<0.05)

4. Conclusion

This work aimed to investigate the anti-asthmatic activity of the leaves of *Indigofera tinctoria.* According to our results, it protects experimental animals from histamine induced dyspnoea and shows significant dose-dependent bronchodilation activity. Therefore, the result of present study indicates the utility of the hydro alcoholic extract of *Indigofera tinctoria* in the treatment of asthma by virtue of its ability to oppose dyspnoea induced by histamine. This activity was proven by the results of *in vitro* test, which revealed its bronchodilation activity. The results of this test exhibited that the hydro alcoholic extract of *Indigofera tinctoria* leaves is a non-competitive antagonist of histamine, which suggests its probable beta 2-adrenergic activity.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declared no conflict of interest.

Statement of ethical approval

The protocol of the study was approved by the Science Faculty of University of Antananarivo Animal Ethical committee under registration n° ECFS-0219/16 on February 25, 2019

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