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## Evaluation of Anti-asthmatic Activities of *Ixora coccinea* Linn (Rubiaceae)

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### ABSTRACT

*Ixora coccinea* L. (Rubiaceae) possess anti-inflammatory and antitussive properties. It is traditionally used for various respiratory ailments including catarrhal bronchitis cough and asthma. In the present study we investigated anti-asthmatic properties of an hydroalcoholic leaf extract of *I. coccinea* in an ovalbumin (OVA)-induced asthmatic rat model. We also evaluated the anti-allergic property of the extract by Abdominal Wall (AW) method and histamine-induced cutaneous reaction. Rats were sensitized with intraperitoneal (i.p.) ovalbumin and challenged by OVA intranasally to induce chronic airway inflammation. Randomized treatment groups of sensitized rats received *I. coccinea* extract or distilled water. *I. coccinea* extract at doses of 1000 and 1500 mg kg<sup>-1</sup> suppressed eosinophilia and significantly inhibited AHR in rat with OVA-induced asthma. Based on lung histopathological study using hematoxylin and eosin *I. coccinea* reduced inflammatory cell infiltration and repaired epithelial cells damaged. In addition the extract at the same doses significantly decreased the diameter of the blue spot (16 and 55%, respectively) compared with the controls and inhibited the skin reactions induced by histamine (23.55 and 53.36%, respectively). In conclusion our results provide evidence that *I. coccinea* has anti-asthmatic properties and then can support its use in folk medicine to treat asthma.

**Key words:** *Ixora coccinea*, airway inflammation, airway hyperresponsiveness, eosinophil, anti-allergic activity, abdominal wall method, histamine

### INTRODUCTION

Herbal medicines are widely used for the treatment and prevention of various diseases in Africa and other developing countries of the world (Diallo *et al.*, 2009, 2010). *Ixora coccinea* Linn (Rubiaceae) has been reported to have anti-inflammatory activity in the carrageenan-induced paw oedema test in rats and anti-mitotic activity (Ratnasooriya *et al.*, 2005a; Zachariah *et al.*, 1994). Its flower has shown anti-tumour activity (Latha and Panikkar, 1998), chemoprotective and hepatoprotective effects (Latha and Panikkar, 1999, 2001) and also are used in treatment of dysentery, leucorrhoea, dysmenorrhoea. Leaves are used to treat diarrhoea. The flowers and bark are used on reddened eyes and eruptions (Ratnasooriya *et al.*, 2005b). The plant is traditionally used for various respiratory ailments including catarrhal bronchitis (Ghani, 2003), cough and

asthma (Missebukpo *et al.*, 2007). Recently, Missebukpo *et al.* (2007) demonstrated antitussive effect of the hydroalcoholic extract of *I. coccinea* leaves. To our knowledge, no studies have been performed to evaluate its anti-asthmatic properties.

Allergic asthma is one of the most common disorders encountered in clinic and the mortality associated with allergic asthma has increased worldwide over the last two decades (Renauld, 2001). Asthma is a chronic inflammatory disease of the respiratory tract that is characterized by increased Airway Hyper-Reactivity (AHR), infiltration of leucocytes, especially eosinophils into airways, bronchial epithelial injury and mucus production that lead to episodes of wheezing, coughing and shortness of breath (Busse and Rosenwasser, 2003; Xiangping *et al.*, 2006). In allergic bronchial asthma, the stimulation of allergen-specific IgE production is believed to contribute to the development of AHR (Broide *et al.*, 1991; Fang *et al.*, 2005) which has been attributed to chronic airway inflammation (Henderson *et al.*, 1997). Mast cells are important in the pathogenesis of asthma : IgE, bound to its receptor Fc $\epsilon$ RI on mast cells and other cells, triggers the release of pre-formed and newly-mediated mediators (Kay, 2005) including histamine, leukotrienes and platelet activation factor which can potentially induce AHR (Kawada *et al.*, 2001).

Histamine is a primary amine released by mast cells and basophils after IgE cross-linking by allergen with effects such as vasodilation mucus hypersecretion, oedema and smooth muscle cell contraction (Marone *et al.*, 2003). It thought to be the most potent vasoactive mediator involved in the acute phase of immediate allergic reaction (Dai *et al.*, 2004). Thus it also has pro-inflammatory properties through its effect on macrophages, epithelial and endothelial cells. Cumulative evidence suggests that histamine release may also have a pathophysiologic role in allergic asthma (Gelfand, 2002). As most allergic and inflammatory effects of histamine are mediated by binding to H<sub>1</sub> receptors, for this purpose, H1 receptor antagonists are widely used to treat allergies (White, 1990).

Therefore, in the present study we investigated the therapeutic potential of the hydroalcoholic extract of *I. coccinea* leaves on allergic airway inflammation and AHR in a murine model of allergic asthma and its effect in histamine receptor.

## MATERIALS AND METHODS

**Plant material:** The leaves of *I. coccinea* were collected from Lomé not far from University of Lomé (Togo) in July 2007. The plant was authenticated by Professor Akpagana Koffi from Laboratory of Botanic and Plant Ecology (Faculty of Science/University of Lomé). A voucher specimen was deposited at the herbarium under the number TOGO 12671 of this Laboratory. The leaves were washed under running water, air-dried and cut into small pieces. The dried sample was extracted in water/ethanol mixture (1:1) for 72 h with manual discontinue agitation. The solution was filtered and evaporated using a rotary evaporator (Buchi R120) set at 45°C to obtain a dry extract which contained alkaloids, flavonoids and tannins as revealed by phytochemical screening previously (Missebukpo *et al.*, 2007).

**Animals:** Wistar rats (150-200 g) of either sex were housed in standard conditions temperature relative humidity and 12 h light/dark cycle. They were fed with standard diet and water *ad libitum* and were kept in the Animal House of the Faculty of Sciences of University of Lomé (Togo).

## Methods

**Sensitization and challenge procedure:** The rats were actively sensitized by intraperitoneal injections of 20 mg ovalbumin with 100 mg Al(OH)<sub>3</sub> (chicken OVA, grade V, Sigma Chemical Co., St Louis, MO) as described by Agbonon *et al.* (2005). Rats were challenged intranasally (i.n) (Henderson *et al.*, 1997) on days 24, 25, 26 and 27. Control animals received i.p saline with Al (OH)<sub>3</sub> on days 0, 3, 7 and 21 and (i.n) saline without Al(OH)<sub>3</sub> on days 24, 25, 26 and 27. Twenty-four hours after the last OVA challenge, the rats were prepared for the collection of Bronchoalveolar Lavage Fluid (BALF) or for the assessment of AHR to methacholine or for histological study.

**Treatment protocol:** The extract (1 and 1.5 g kg<sup>-1</sup>) was dissolved in distilled water and administered orally for 4 consecutive days 1 h before every intranasal challenge (from day 24 to day 27) and 1 h before BALF collection or MCh administration.

**BALF study:** To evaluate airway inflammation we examined the accumulation of inflammatory cells in BALF. Experiments were performed according to previously described methods (Agbonon *et al.*, 2005). The remaining samples of BALF were centrifuged at 2500 trs min<sup>-1</sup> for 10 min (Electric Centrifuge SPN-400 Japan). Cells were fixed and stained using May-Grünwald-Giemsa (RHONE-POULENC, France). Eosinophil count was performed, using standard morphologic criteria, by a naïve observer. The eosinophil % was expressed as a percentage of the total leukocytes.

**Histological study:** After BAL collection, bronchial and lung were removed from the chest cavity and fixed in 10% formaldehyde. Lobes were isolated, embedded in paraffin and sectioned at 5 µm. Tissue sections were stained with haematoxylin eosin (H and E) for general morphology (McManus and Mowry, 1965; McKay *et al.*, 2004; Rogerio *et al.*, 2007).

**Assessment of AHR to methacholine:** Measurement of bronchial responsiveness to methacholine (Sigma, Germany) was carried out according to Hannon *et al.* (2001). Pulmonary Inflation Pressure (PIP) was measured with pressure transducer (BIOPAC SYSTEM, MODEL MP100, HAVARD APPARATUS) with a computer (logiciel Acq Knowledge III). The response was measured as the peak increase above the baseline immediately after MCh administration.

**Inhibitory effect on anaphylactic reaction (abdominal wall method):** The experiments were carried out according to a method reported previously (Kataoka *et al.*, 1997, 2002). Extract (1 and 1.5 g kg<sup>-1</sup>) and Citirizine (Pharmacy) at 10 mg kg<sup>-1</sup> were administered orally to rats and control rats were administered distilled water alone. The anti-allergic activity (type I) was expressed as the percentage inhibition compared with the control group.

$$\% \text{ Inhibition} = (D_{\text{control}} - D_{\text{treated}}) / D_{\text{control}}$$

where, D is diameter of the bleu area.

**Histamine-induced cutaneous reactions:** Rats were given orally test drugs and were anesthetized by i.p. injection of 1 g kg<sup>-1</sup> urethane according to the previously described method (Dai *et al.*, 2004; Saito *et al.*, 2004).

The anti-histaminic activity was expressed by calculating the area (A) of the dye spot as follows:

$$A = (R^2 - r^2) \pi$$

where, R is the long radius and r is the short radius.

The inhibitory effects of the test drugs were expressed as the percent reduction of dye leakage compared with the control group.

**Statistical analysis:** Experimental data are presented as Means±S.E.M. Data were analyzed by one-way analysis of variance (ANOVA) followed by Fisher's test with multiple comparisons. The p-values <0.05 were regarded as significant. All statistical analysis were carried out using the Instat Statistical package (Graph Pad software, Inc. USA).

## RESULTS

**Effect of *I. coccinea* extract on airway inflammation:** To investigate the anti-asthmatic effect of *I. coccinea* extract in vivo, asthma was induced in rat using OVA. The total number of leukocytes and the alteration of the cellular component in BALF of rat were evaluated. OVA challenge in OVA-sensitized rat caused a marked infiltration of leukocytes recovered in BALF

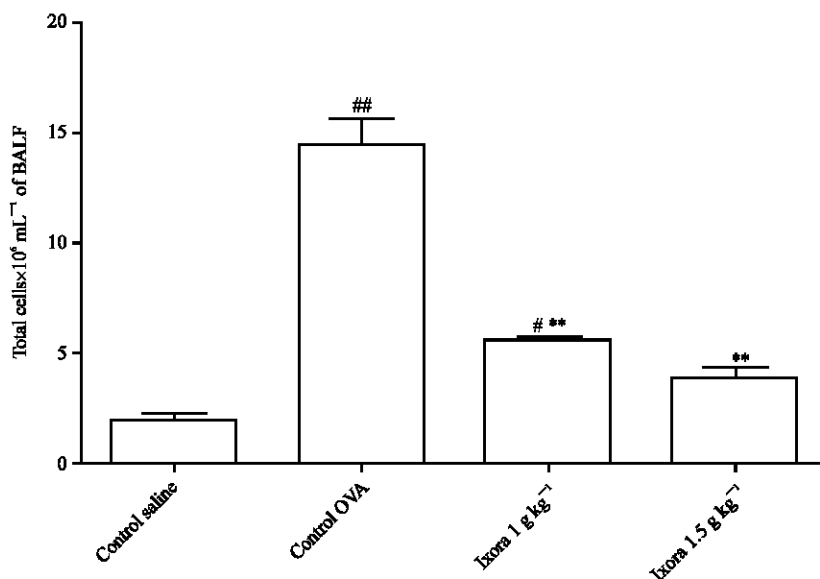


Fig. 1: Effect of *I. coccinea* extract on antigen-induced increases in leukocytes in BAL obtained from rats. The extract was given orally 4 consecutive days before antigen challenge and 1 h before animal was killed. BAL was obtained 24 h after the last challenge. Each value represents the Mean±S.E.M. (N = 6). \*\*p<0.001 treated vs control OVA; ##p<0.001 control saline vs control OVA

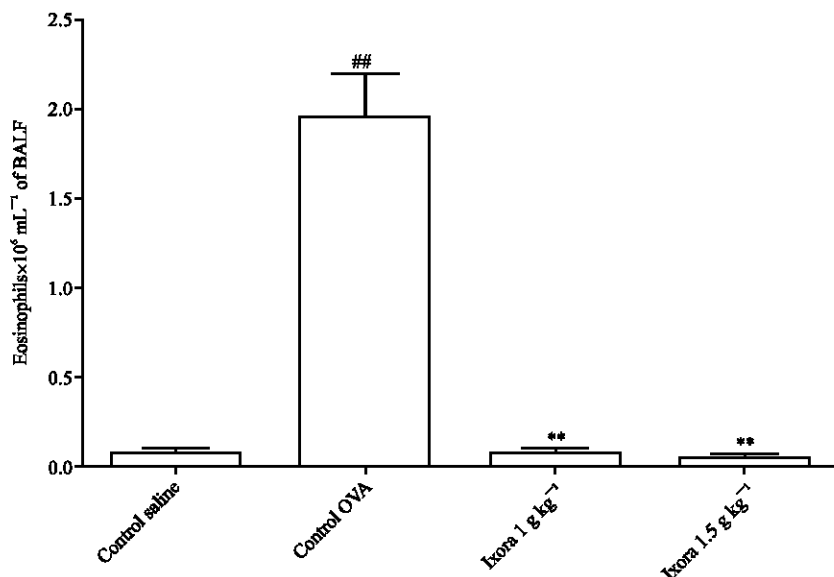


Fig. 2: Effect of *I. coccinea* extract on antigen-induced increases in eosinophils in BALF obtained from rats. The extract was given orally 4 consecutive days before antigen challenge and 1 h before animal was killed. BALF was obtained at 24 h after the last challenge. Eosinophils from BALF were counted using a hemacytometer and stained with May-Grünwald-Giemsa. The eosinophil % was expressed as a percentage in total leukocytes. Each value represents the Mean±S.E.M. (N = 6). \*\*p<0.001 treated vs control OVA; ##p<0.001 control saline vs control OVA

by 4.92-fold as compared with that in the control-saline group (Fig. 1). In the control OVA, there are in BALF 25.65-fold eosinophils, as compared that in the control-saline group. Treatment with *I. coccinea* extract by oral administration at doses of 1 and 1.5 g kg<sup>-1</sup> decreased the number of BALF leukocytes by 61.8 and 70.27% compared with the control-OVA group (p<0.001) (Fig. 1) and also reduced the number of eosinophils by 97.53 and 98.60%, respectively (p<0.001) (Fig. 2).

**Histological study:** Histological examination revealed that the lungs of control-saline rat were normal in appearance without inflammatory cells in the airways (Fig. 3a) whereas the control-OVA group showed a marked infiltration of inflammatory cells, in peribronchial and perivascular spaces and alveolar walls (Fig. 3b). *I. coccinea* treatment (1.5 g kg<sup>-1</sup>) markedly reduced the inflammatory changes in OVA-sensitized/challenged rat and repaired epithelium damage (Fig. 3c).

***I. coccinea* inhibits antigen-induced AHR:** We found that immunised rats challenged with OVA (control OVA) developed AHR to increasing doses of methacholine when compared with control saline (Fig. 4a, b). *I. coccinea* at 1.5 g kg<sup>-1</sup> suppressed airway hyperresponsiveness significantly when compared with control OVA (p<0.05) (Fig. 4a, c).

**Inhibitory effect on anaphylactic reaction (abdominal wall method):** As shown in Fig. 5, oral administration of *I. coccinea* extract significantly decreased the diameter of the blue spot

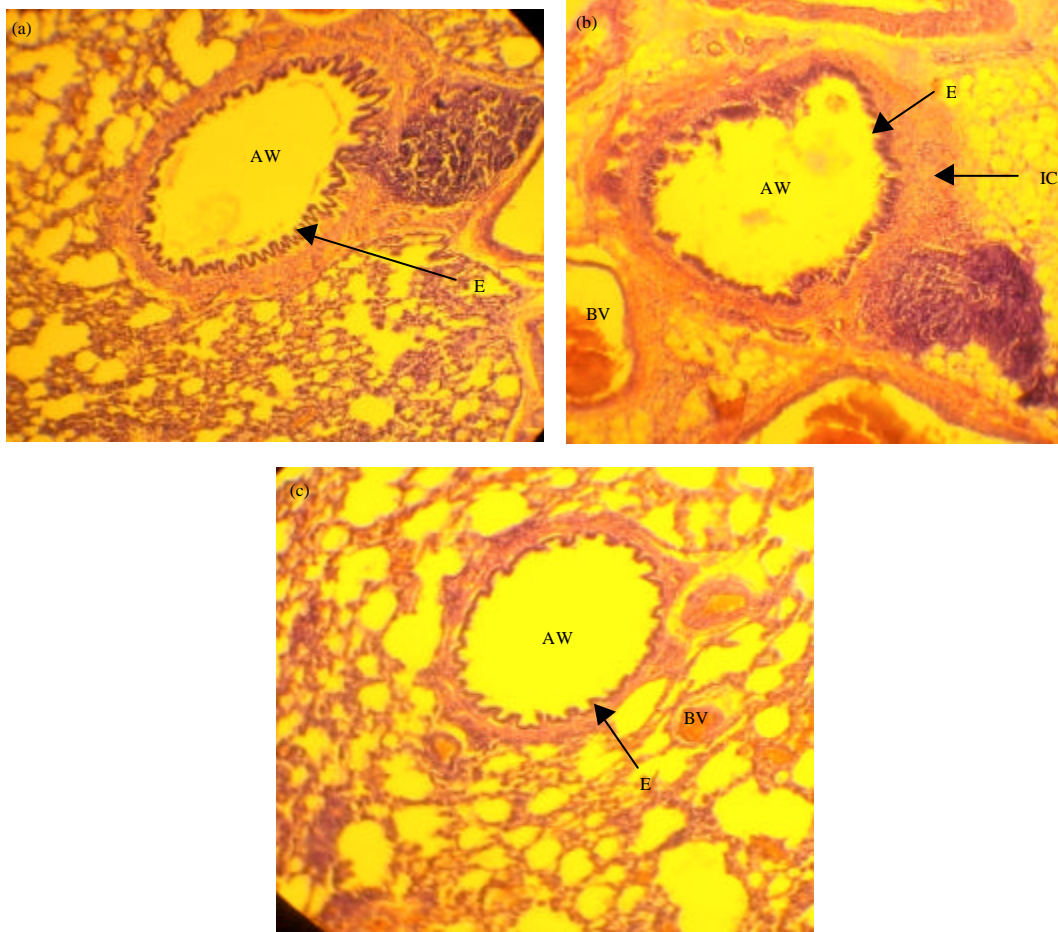


Fig. 3 (a-c): Histological evidence of decreased lung inflammation in rat treated with extract of *I. coccinea* ( $1.5 \text{ g kg}^{-1}$  orally). Paraffin-embedded. Hematoxylin and eosin-stained lung sections from each group of five rats is shown. (a) Non sensitised rat given saline challenge. (b) OVA-challenged rat; peribronchial and perivascular inflammatory cells (IC), epithelium (E) damaged, surrounding blood vessel (BV) structures and mucosal hyperplasia are seen. (c) OVA-challenged rat plus treatment with extract of *I. coccinea*; a reduction in inflammatory cells is seen, no mucosal hyperplasia and epithelium repaired

compared with the controls. Also, the anti-allergic effect of  $1500 \text{ mg kg}^{-1}$  body weight of *I. coccinea* was lightly superior than that of the  $10 \text{ mg kg}^{-1}$  body weight of citirizine (55 and 40.28%, respectively) which has been widely used against allergic disorder as histamine  $\text{H}_1$ -receptor antagonist.

**Histamine-induced cutaneous reactions:** Cutaneous reactions were elicited by an intradermal injection of histamine; *I. coccinea* ( $1$  and  $1.5 \text{ g kg}^{-1}$  p.o.) reduced the skin reaction induced by histamine at 23.55 and 53.36%, respectively (Fig. 6).

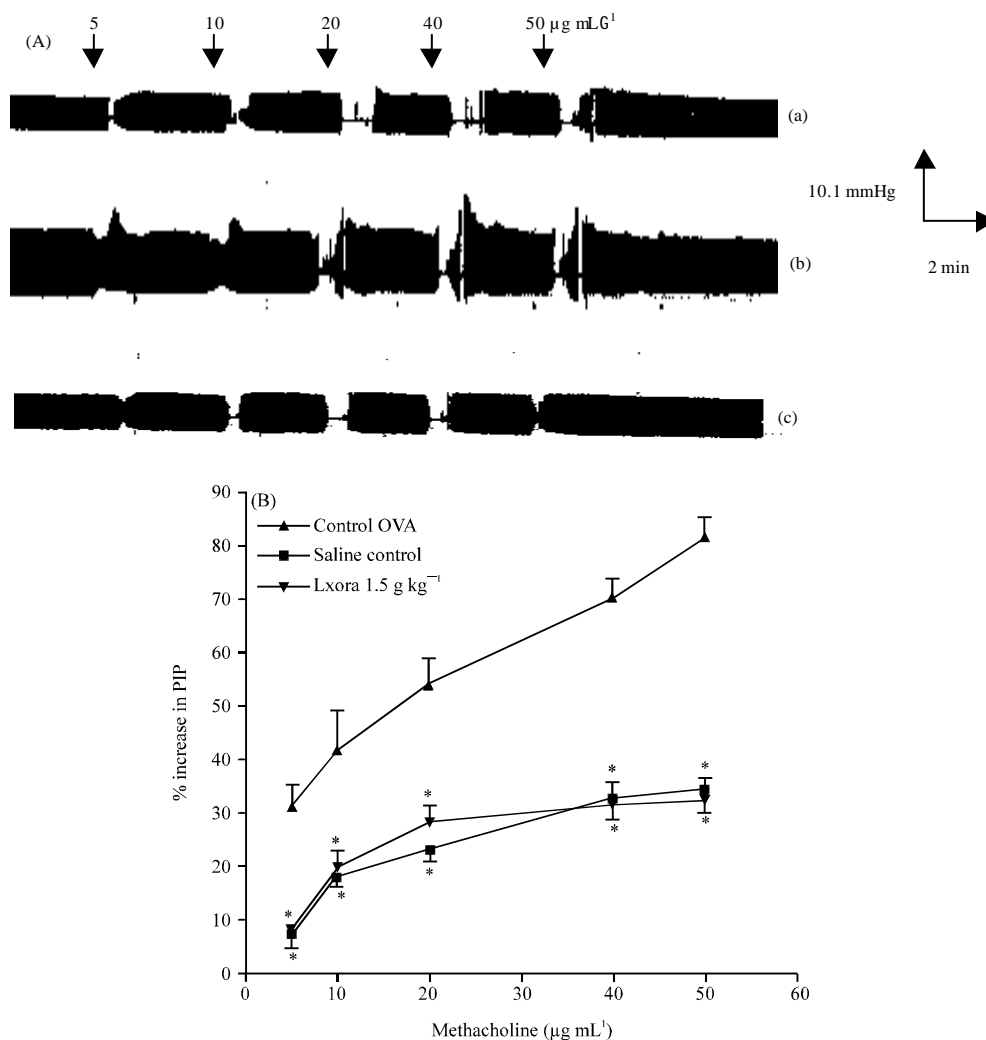


Fig. 4 (A-B): Effect of *I. coccinea* extract (1.5 g kg<sup>-1</sup>) on antigen-induced BHR in rats. The extract was given orally 4 consecutive days before antigen challenge and 1 h before MCh administration. AHR was measured 24 h after the final OVA challenge and increasing concentrations of MCh (5-50 µg mL<sup>-1</sup> i.v.) were administered with a 2 min interval between doses; (A) Representative traces showing the effects of extract (1.5 g kg<sup>-1</sup>) on MCh-induced increases in PIP, (a) saline control, (b) control OVA and (c) rat sensitized, challenged and treated with *I. coccinea* extract (1.5 g kg<sup>-1</sup>); (B) Graph showing inhibiting effect of *I. coccinea* extract (1.5 g kg<sup>-1</sup>) on MCh-induced increases in PIP. Airway hyperresponsiveness was assessed by percentage change from the baseline level of PIP. Each value represents the Mean±S.E.M. (N = 6-9) per treatment group. \*p<0.05 vs control OVA. They are analyzed by one-way ANOVA followed by Fisher's test

## DISCUSSION

A rat model of allergic asthma was used to evaluate the anti-asthmatic effects of *I. coccinea* *in vivo*. Airway hyperreactivity to direct or indirect stimuli is a cardinal feature of asthma



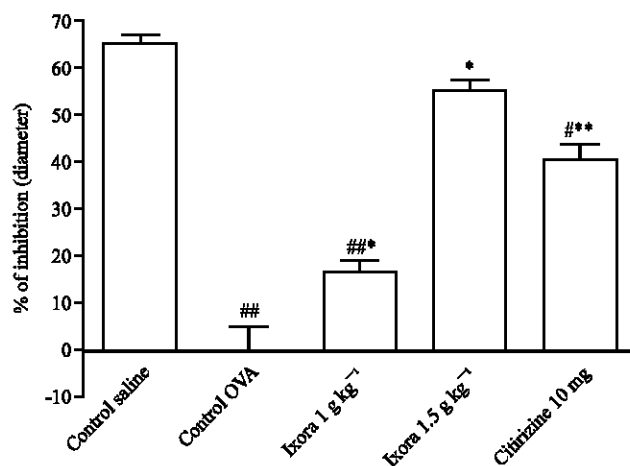


Fig. 5: Type I anti-allergic activities of the hydroalcoholic extract of *I. coccinea* leaves. Rats were sensitized intraperitoneally with OVA in alum (1 : 5) on day 0. On day 14 distilled water, extract (1000 and 1500 mg kg<sup>-1</sup>) and citirizine as reference compound (10 mg kg<sup>-1</sup>) were administered orally 1 h before to challenge on the abdominal wall with OVA (4 mg mL<sup>-1</sup>). Control saline was not sensitized and not challenged. The results are expressed as Means±S.E.M. (N = 5-9) \*p<0.05, \*\*p<0.001 as compared with control OVA group, #p<0.05, ##p<0.001 compared to the control saline group and analyzed by one-way ANOVA followed by Fisher's test

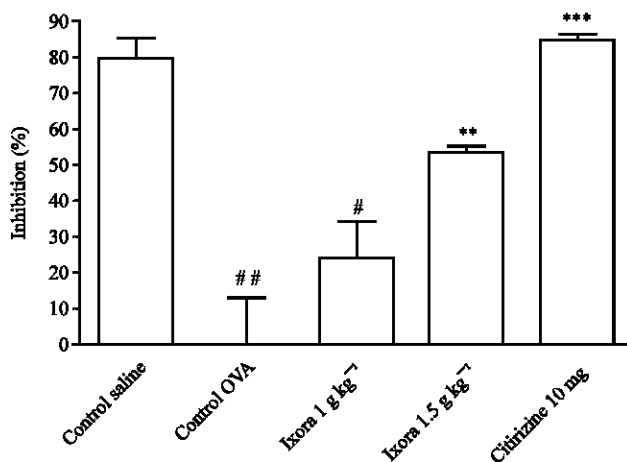


Fig. 6: Effect of the hydroalcoholic extract of *I. coccinea* leaves on the histamine-induced cutaneous reaction. Histamine (0.5 mg kg<sup>-1</sup>) was intradermally injected into spots on the dorsal skin of rats. There after, 1% of Evans blue was immediately injected in the tail vein. Extract and citirizine (reference compound) were orally administered 1 h before challenge. Control saline group was injected saline intradermally. Rats were sacrificed 30 min after the induction of reactions and the amount of dye that leaked into the spots was determined. The results are expressed as Means±S.E.M. (N = 5) \*\*p<0.01, \*\*\*p<0.001 as compared with control Histamine group; #p<0.05, ##p<0.001 compared to the control saline group and analyzed by one-way ANOVA followed by Fisher's test

(Wyss *et al.*, 2005). In the present study the extract clearly inhibited antigen-induced AHR. There is convincing evidence that AHR occurs as a consequence of epithelial damage resulting from the accumulation of large numbers of activated eosinophils and mast cells within the respiratory tract (Bradley *et al.*, 1991). Eosinophils that migrated to the site of inflammation have been shown to play a causative role in tissue damage by releasing cytotoxic granules, proteins and tissue-damaging superoxide (Wu *et al.*, 2000; Palmqvist *et al.*, 2007) they are the central effector cells that are responsible for ongoing airway inflammation (Kay, 2005; Shen, 2005), the main source of lipid mediators that play major roles in the pathogenesis of asthma and other forms of allergic inflammation (Bandeira-Melo and Weller, 2003). Thus we investigated the effect of *I. coccinea* extract on pulmonary eosinophilia. The result indicated a significant ( $p < 0.05$ ) inhibition of OVA-induced eosinophilia in rat. In addition to the decreased percentages of granulocytes in BALF we also observed less cellular infiltration around airways and blood vessels in lungs of rats treated with *I. coccinea* extract. Histological studies confirmed also airway protective effect of extract. This is in accordance with Shen *et al.* (2003) and Justice *et al.* (2003) who demonstrated that AHR is eosinophil dependant.

Since, histamine may be a dominant pathophysiologic factor of asthma (Kitamura, 2005) and inflammation and symptoms that ensue may be susceptible to H<sub>1</sub>-receptor antagonist blockade (Gelfand, 2002) we investigated on the one hand, the effect of the extract on immediate allergic response with AW method and on the other hand, its anti-histaminic effect with histamine-induced cutaneous reaction. The AW method is one of simplest techniques for inducing and detecting anaphylactic reaction *in vivo* models (Kataoka *et al.*, 1997, 2002). In sensitised rats, chemical mediators such as histamine and leukotrienes are released from mast cells upon allergen challenge, followed by an increase in the vascular permeability of the abdominal wall (Kataoka *et al.*, 1997). In present study oral administration of *I. coccinea* extract decreased significantly the diameter of the blue spot compared with the control groups. The anti-allergic effect of 1.5 g kg<sup>-1</sup> of *I. coccinea* extract was slightly higher than that of the 10 mg kg<sup>-1</sup> of citirizine which has widely used against allergic disorders as a histamine H<sub>1</sub>-receptor antagonist. However this effect is lower than that obtained with citirizine in histamine-induced vasodilatation in rat skin. Thus the inhibition of the diameter of the spot may be due to an effect of the *I. coccinea* extract: (1) as a histamine H<sub>1</sub> receptor blocker, since *I. coccinea* extract shows antihistaminic action in intradermal histamine-induced vasodilatation in rat skin. These results corroborates those obtain by Ratnasooriya *et al.* (2005a) who showed anti-histaminic activity of the aqueous leaf extract of *I. coccinea*. (2) as an inhibitor of mast cells degranulation since the increase in vascular permeability was dependent on the histamine concentration (Kataoka *et al.*, 1997).

Mast cells activation contributed to the induction of enhanced airway responsiveness and chronic inflammatory including eosinophil and lymphocyte infiltration (Nauta *et al.*, 2008; Yu *et al.*, 2006). According to Kawada *et al.* (2001), drugs which inhibited PCA in rat suppressed the mast cell activation. Therefore, the dose of 1500 mg kg<sup>-1</sup> of our extract was sufficient to suppress the mast cell activation, because it clearly inhibited antigen-specific anaphylaxis in rat using abdominal wall as a challenge site.

## CONCLUSION

These results are the first to provide experimental evidence demonstrating that *I. coccinea* has anti-asthmatic activities in *in vivo* on animal model. In the rat model of asthma used, *I. coccinea* extract effectively suppressed two important pathological characteristics of asthma such as airway

inflammation and airway hyperreactivity. In addition the extract has shown inhibitory effect on immediate allergic reactions which is probably mediated by reducing the release of mediators such as histamine from mast cells or acted as H<sub>1</sub>-receptor antagonist.

Even though the results obtained here are not sufficient to prove the mechanism of *I. coccinea* in the inhibition of allergic and inflammatory reactions. Further investigations are needed to clarify the active components and precise mechanism of *I. coccinea*. However, this study could confirm the traditional use of *I. coccinea* on the treatment of asthma.

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