

Antiallergic activity of *Aristolochia bracteolata* Lank in animal model

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Antiallergic activity of *Aristolochia bracteolata* was evaluated by using compound 48/80 induced anaphylaxis, dermatitis rhinitis and pruritis, as a preclinical model for acute phase of hypersensitivity reactions. The late phase hypersensitivity was evidenced by considering toluidine diisocyanate induced volume of bronchoalveolar fluid secretion and its inhibition. The possible antiallergic mechanism was evaluated by using compound 48/80 induced mast cell activation and estimated serum nitric oxide (NO), rat peritoneal fluid NO, bronchoalveolar fluid NO and blood histamine levels. The present study implied that the chloroform extract of *Aristolochia bracteolata* had potent and significant inhibitory effect on compound 48/80 induced pruritis and dermatitis activity in Swiss albino mice. It showed significant effect in toluidine diisocyanate induced rhinitis in swiss albino mice. Mast cell membrane stabilization activity was also observed in compound 48/80 induced mast cell activation. A significant reduction was observed in serum nitrate levels, rat peritoneal fluid nitrate levels and BAL nitrate levels. The extract was also found to possess significant inhibitory effect on blood histamine levels. It could be concluded that chloroform extract of *A. bracteata* possess potent antiallergic activity, possibly through mast cell membrane stabilization, inhibiting NO and histamine pathway.

Keywords: Antiallergic, Antianaphylactic, Antidermatitis, Antipruritis, Antirhinitis, *Aristolochia bracteolata*, Disodium chromoglycate, Toluene diisocyanate

Mast cells are central mediators of allergic diseases. Currently it has been proved that there is cross-link between intestinal mast cells and constitutive NO synthase¹⁻³. It has been also reported that activation of mast cells and subsequent release of cytokines are involved in stimulation of nitric oxide at relatively and sustained level⁴.

Pharmacological management of allergic diseases includes antiallergic medication and immunotherapy for specific allergens, most commonly antihistamines, anticholinergics and topical corticosteroids⁵. However, the use of these drugs also suffers adverse effects like sedation, dry mouth and immunosuppression⁶. The antihistamines and anticholinergic drugs are only effective for symptomatic relief, but fail to produce therapeutic and productive effect. Most commonly used immunotherapy is associated with general anaphylaxis during induction phase. However, effective treatment of these allergic diseases is not

only difficult task, but to some extent treatment becomes impossible⁷.

In the present study, antiallergic activity of *Aristolochia bracteolata* and its possible mechanism of action was studied by using a suitable amount of potent secretagogue, compound 48/80⁸⁻⁹. After activation of mast cells by compound 48/80 the signaling pathway leading to the degranulation of mast cells after engagement of FCεRI has been extensively characterized^{10,11}. Compound 48/80 has been found to cause about 90% release of histamine from mast cells¹².

Present study was based on the promising outcome of the previous *Aristolochia bracteolata* anti-inflammatory, anti-arthritis, and analgesic activities shown in our laboratory¹³. The present study was designed with an objective to evaluate its possible utility and applicability as an antiallergic agent using series of experiments. The methods selected for the study were considered to be the most promising and clinically reproducible preclinical models resembling to different types of allergy such as allergic rhinitis, asthma, and dermatitis. For studying antianaphylactic activity, compound 48/80 was used to induce anaphylaxis in Swiss albino mice¹⁴⁻¹⁵. Antipruritic activity was evaluated by compound 48/80 induced

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scratching behavior in Swiss albino mice¹⁶. Acute local anti-inflammatory activity was studied in compound 48/80 induced ear edema¹⁴ and toluidine diisocyanate induced bronchoalveolar fluid secretion in Swiss albino mice was used to study the late phase reaction. In order to determine the possible mechanism of action on rat peritoneal mast cells stabilizing activity and level of nitric oxide in serum, bronchoalveolar (BAL) fluid, peritoneal fluid and blood histamine concentrations were considered¹⁷.

Materials and Methods

Materials— Various compounds, compound 48/80 (C2313); SIGMA Chemicals, St. Louis USA, RPMI-1640 medium (AT028); HiMedia Laboratories Pvt. Ltd. Mumbai, Toluidine blue (02912ED-016); Toluene di-isocyanate; SIGMA Chemicals, St. Louis USA, were purchased from the respective sources. Other chemicals including Span 80 and organic solvents used were of AR grade. Griess reagent was purchased from (G4410-10G) SIGMA Chemicals (St. Louis USA), O-Ptholdehyde was purchased from HiMedia. Research microscope (Metzer), LTE Scientific Ltd. U.K. Mini LYOTRAP (0L3 7EN), Refrigerated centrifuge [Remi, C-24], Laminar flow system (Klenzaid), and BOD incubator (Thermolab), Petridishes (35mm), Tubes (15 ml), Shimadzu UV Spectrophotometer (1601), Digital fluorimeter (CL-53) ELICO and Borosil Soxhlet extractor were used in this study.

Preparation of plant extract—Whole plant of *Aristolochia bracteolata* Lamk was collected from Rajkot-Gujarat India and authenticated at Department of Taxonomy, BVVS Ayurvedic College, Bagalkot, India and the specimen was deposited for future reference. The collected plant was sterilized by washing in 2% potassium permanganate and dried under shade, until it was free from moisture. The dried plant was powdered and then passed through sieve no.44 to get fine powder. The sieved powder was stored in air tight, high-density polyethylene containers before extraction. For extraction, the powder was subjected to hot continuous, successive extraction (soxhlet) for 24 and defatted with petroleum ether (40°C) followed by chloroform (50°C) extraction¹³. The excess solvent was completely removed using rotary flash evaporator to get semisolid mass, and then dried at -40°C using lyophilizer. The percentage yield of petroleum ether and chloroform extracts were 4.73 and 3.66%,

respectively. The extracts, thus, obtained were subjected to preliminary screening for anti-anaphylactic activity. The chloroform extract was found to possess better protective effect than petroleum ether extract. Hence, chloroform extract of *A. bracteolata* (CEAB) was studied extensively in the present study¹³.

Experimental animals—Swiss albino mice of either sex weighing 18-25 g were used for anaphylactic reaction, level of nitric oxide in BAL fluid, bronchoalveolar fluid accumulation, antipruritis and antidermatitis activity. Sprague Dawley rats weighing 200-250 g were used for mast cell stabilizing activity and estimation of nitric oxide level in rat serum and rat peritoneal fluid. These animals were maintained under standard conditions in animal house of H. S. K. College of Pharmacy, B.V.V.S. campus Bagalkot, India. The animals were provided with standard diet and water *ad libitum*. The detailed selection of animals, numbers in each group and groupings have been described in detail in each method of study. All the experiments using animals were carried out as per guideline of institutional animal ethics committee (821/01/a/CPCSEA) of college, after seeking approval (HSK/IAEC.Clear/2004-2005) dated 27/12/2004.

Antianaphylactic activity—Compound 48/80-induced systemic anaphylactic reaction was examined as previously described¹⁴⁻¹⁵. Mice were given an injection (ip) of 8 mg/kg of the mast cell degranulator compound 48/80. *Aristolochia bracteolata* extract at 100, 200, 400 and 500 mg/kg were given orally 1 hr prior to compound 48/80. Mortality was monitored for 1 hr after induction of anaphylactic reaction in 5 groups of 10 animals each. On the basis of per cent protection, the extract was selected for further evaluation¹⁴⁻¹⁵.

Antipruritis activity—Antipruritic activity was measured using a previously reported method by examining the number of incidence of scratching behavior in 7 groups of 10 animals each¹⁸. Compound 48/80, (3 mg/kg) was injected (sc) into the base of the neck on the back of mice to provoke scratching behavior. In normal in place of compound 48/80, saline (50 µl) was injected. The extract (100, 200, 400 and 500 mg/kg) and disodium-chromoglycate (10 mg/kg; ip) were given orally at 1 hr prior to compound 48/80 injection. Incidences of scratching behavior on the whole body and the site injected with compound 48/80 were counted for 20 min¹⁶.

Antidermatitis activity—Compound 48/80 (5g/l) was freshly dissolved in saline and injected intradermally into the dorsal aspect of a mouse ear using a microsyringe with a 28 gauge hypodermic needle in 7 groups of 10 animals each. The ear swelling response was measured in mg by cutting treated ear of anesthetized mice. Ear swelling response represented the incremental increase in weight above baseline control values. Ear swelling response was determined 1 hr after the injection of compound 48/80 because ear swelling response evoked by physiologic saline, returned nearly to the baseline thickness within a 1 hr interval. The extract doses were given 1 hr prior to compound 48/80 administration¹⁴.

Antirhinitis activity—2, 4-toluene di-isocyanate (TDI) was used as an antigen. TDI was prepared in ethyl acetate to a concentration of 10% for sensitization. Under slight ether anesthesia, mice were sensitized by administration of 5 μ l of TDI (10%) into nostrils for seven days. Before 1 hr of TDI sensitization, the mice were treated with extract of doses 100, 200, 400, 500 mg/kg and disodium chromoglycate (10 mg/kg; ip) were given orally. Normal group received normal saline and sensitized by ethyl acetate, the control group received Span 20 suspension and sensitized by TDI solution. After seven days of treatment, the animals were anesthetized by thiopental sodium (50 mg/kg ip), the lung was removed and phosphate buffer solution (PBS; 1ml) injected in lung by using microsyringe, the injected fluid was recollected in microtest-tube, centrifuged at 500 rpm for 5 min. The supernatant was taken out and volume was measured in microsyringe¹⁹.

Mast cell stabilizing activity—The active fraction of doses 100, 200, 400 and 500 mg/kg and disodium-chromoglycate (10 mg/kg; ip) were given orally to rats daily 5 days prior to collection of mast cells. The animals were anesthetized by diethyl ether and injected normal saline (10 ml) into the peritoneal cavity of male Sprague Dawley rats (250-300 g). After gentle massage the peritoneal fluid was collected and transferred into siliconised test tubes containing RPMI-1640 (pH 7.2-7.4). Mast cells were washed three times by centrifugation at a low speed (400-500 rpm) discarding the supernatant and taking the pellet of mast cells into the medium. Mast cells from control groups and treated groups were incubated with compound 48/80 (1 μ g/ml) at 37°C for

10 min. After incubation, mast cells were stained with toluidine blue (0.1%) and per cent of protection against degranulation was counted under high-power microscope (45X)²⁰.

Measurement of serum nitric oxide level— The active doses 100, 200, 400 and 500 mg/kg and disodium chromoglycate (10 mg/kg; ip) were given orally to rats daily 5 days prior to collection of blood. The male Sprague Dawley rats (250-300 g) were anesthetized by ether and blood was collected from retro orbital, centrifuged at 500 rpm for 5 min then equal volume of serum, acidic griess reagent (pH 2) and 40 μ l of glycine buffer was added (100 μ M, glycine; 100 μ M, NaCl; and 40 μ M, HCl) and incubated for 15 min at 37°C. The normal group sample was incubated with normal saline, controlled and treated groups samples were incubated with compound 48/80 (1 μ g/ml), the absorbance was measured at 546 nm^{4,21}.

Measurement of rat peritoneal fluid nitric oxide level—Peritoneal fluid was collected similarly as stated above in mast cell stabilizing activity. Peritoneal fluid, (100 μ l) acidic griess reagent (pH 2; 100 μ l) and 40 μ l of glycine buffer was added (100 μ M, glycine; 100 μ M of NaCl and 40 μ M HCl) and incubated for 15 min at 37°C, then the incubated sample was centrifuged and the supernatant was used for measurement of absorbance. The normal group sample was incubated with normal saline, controlled and treated groups samples were incubated with compound 48/80 (1 μ g/ml). The absorbance was measured at 546 nm^{4,21}.

Measurement of BAL fluid nitric oxide level— BAL fluid was collected similarly as stated above in peritoneal fluid accumulation. The centrifuged samples of normal were incubated without adding compound 48/80, controlled and treated groups were incubated with acidic griess reagent (pH 2) and 40 μ l of glycine buffer was added (100 μ M, glycine; 100 μ M, NaCl; and 40 μ M, HCl) and compound 48/80 (1 μ g/ml) for 10 min at room temperature, then absorbance was measured at 546 nm^{19,21}.

Generation of standard curve using sodium nitrate as standard—The molecular weight of NaNO₂ is 69, therefore to prepare stock solution (1000 μ M), dissolved 6.9 mg of NaNO₂ in 10 ml of distilled water. From this stock solution, 100 μ l of NaNO₂ was added to 9.9 ml of distilled water to obtain 100 μ M solution of NaNO₂. To this solution, added sufficient amount of distilled water to obtain

different concentrations (50, 25, 6.25, 3.125, 1.565 μ M) of NaNO₂. Solution (1.5 ml) from each concentration was added to 1.5 ml of Griess reagent, placed in dark room for 10 min. Then, absorbance were measured in photometer at 546 nm. A standard curve was obtained by plotting the concentration of NaNO₂ on the X-axis and the absorbance at 546nm on Y-axis²¹.

Determination of blood histamine level—Five ml of oxalated blood (5 ml of blood and 6 mg of ammonium oxalate) was hemolyzed by addition of 4.5 ml of distilled water and 0.5 ml of concentrated (10-12) perchloric acid. The tube was then shaken for 10 min, homogenized in 9 volumes of 0.4 N perchloric acid, allowed to stand for 10 min and then centrifuged. Supernatant (4 ml), 0.5 ml of 5N NaOH, 1.5 g of solid NaCl and 10 ml of n-butanol were mixed, and shaken for 5 min to extract histamine in butanol, centrifuged to take organic phase. To this organic phase, added 5 ml of salt saturated 0.1 NaOH, and shaken for 1 min. Aliquot (butanol; 8 ml) was taken, added 4.5ml of 0.1 HCl and 15ml of n-heptane, shaken for 1 min and centrifuged. The histamine in aqueous phase was collected and assayed fluorimetrically¹⁷. Two ml of aliquot was taken, mixed 0.1 ml of OPT reagent and then added 0.2 ml of 3N HCl. The fluorescence of acidified solution was stable for atleast 90 min. The fluorescence intensity was proportional to histamine concentration over the range 0.005 to 0.5 μ g/ml.

Statistical analysis—Data was subjected to statistical analysis using Student's unpaired *t* test and *Chi-square* test was also used for antianaphylactic evaluation. Values are presented as mean $\pm$ SEM. *P* value less than 0.05 was considered as significant.

Results

Antianaphylactic activity—Compound 48/80 induced 100% mortality in mice. Disodium cromoglycate (DSCG) showed 40% protection from

mortality. Treatment with CEAB 100, 200, 400 and 500 mg/kg showed dose-dependent protection against compound 48/80 induced mortality. A significant protection (70%) was observed at 400 mg/kg of CEAB treatment (Table 1).

Antipruritic activity—Intradermal injection (3 mg/kg) of compound 48/80 significantly increased the number of scratching compared to normal group of animals. DSCG significantly reversed the number of scratching induced by compound 48/80. The dose dependent significant inhibitory effect was seen in all extract treated animals (Table 1).

Antidermatitis activity—Significant gain in mice ear weight was noted when the mice ear was injected with compound 48/80 (3 mg/kg; sc; Table 1) within 1 hr of injection as compared to saline injected group of mice. Intraperitoneal injection (10 mg/kg) of disodium-chromoglycate inhibited significantly the effect of compound 48/80 induced mice ear swelling. Significant inhibitory effect in compound 48/80 induced ear swelling was also noted when groups of animals were treated with CEAB (400 and 500 mg/kg; po; Table 1).

Antirhinitis activity—Hypersensitization by nostril administration of toluidine di-isocyanate (TDI) had significantly increased bronchoalveolar secretion as compared to normal (Table 2). Significant dose dependent inhibitory effect was observed in CEAB treated (400 mg/kg and 500 mg/kg) groups of animals. Volume of BAL fluid secretion was significantly reduced with CEAB (400 and 500 mg/kg) treatment as compared to TDI induced BAL fluid secretion. Similarly, the disodium-chromoglycate also showed significant inhibitory effect on TDI induced BAL fluid secretion.

Mast cell stabilizing activity—Compound 48/80 activated rat peritoneal mast cells (RPMCs) as compared to normal group (Table 1). Treatment of CEAB (100, 200, 400 and 500 mg/kg) significantly

Table 1— Effect of CEAB on compound 48/80-induced allergic reactions
[Values are mean $\pm$ SE of 6 animals]

Treatment/Conc. (mg/kg)	Number of Scratches	Ear Weight	Activated mast cells (%)	Anaphylactic reaction induced mortality
Normal (Saline)	40.83 $\pm$ 1.493	11.73 $\pm$ 0.5	59.67 $\pm$ 2.44	--
Control (Comp. 48/80)	291.7 $\pm$ 12.93***	14.95 $\pm$ 1.33*	82.17 $\pm$ 2.66***	90
Standard (DSCG)	172.5 $\pm$ 5.451***	10.35 $\pm$ 1.34**	31.83 $\pm$ 1.98***	80
CEAB 100	207.7 $\pm$ 21.67***	12.57 $\pm$ 0.62	59.67 $\pm$ 2.44***	30***
CEAB 200	167.3 $\pm$ 7.961***	12.53 $\pm$ 0.45	45.17 $\pm$ 3.005***	60**
CEAB 400	146.2 $\pm$ 7.705***	10.61 $\pm$ 0.39*	41.50 $\pm$ 4.22***	100
CEAB500	112.7 $\pm$ 7.329***	10.13 $\pm$ 0.18**	37.20 $\pm$ 1.98***	40***

CEAB— Chloroform extract of *Aristolochia bracteolata* significant at **P*<0.05, ***P*<0.01, ****P*<0.001.

reduced the number of activated mast cells in a dose depended manner. In the group treated with DSCG, the percentage of activated mast cells. was much significant.

Serum and rat peritoneal mast cells nitric oxide — Compound 48/80 treatment induced a significant increase in level of serum and rat peritoneal mast cell fluid nitrate levels (Table 3). Treatment with disodium chromoglycate significantly reduced compound 48/80 augmented serum nitrate. Results obtained with 100 mg/kg doses on serum nitrate and rat peritoneal mast cell fluid, respectively. Reduction in serum nitrate was observed when treated with CEAB (100 mg/kg; Table3).

Bronchioalveolar nitric oxide—Level of nitrate was significantly increased in BAL fluid after sensitization with TDI compared to normal (Table 3). Dose dependent significant inhibitory effect on BAL fluid nitrate level was observed with all doses of CEAB (100, 200 400, 500 mg/kg). Significant inhibitory effect was also noted in the group of animals treated with disodium chromoglycate.

Effect on blood histamine levels—CEAB decreased the levels of histamine concentrations in a dose dependent manner. The control group showed significantly increase of histamine in blood, compared

to control. The standard group treated with DSCG and CEAB treated groups showed significant decrease in level of histamine in serum in a dose-dependent manner (Table 3).

Discussion

CEAB significantly inhibited the compound 48/80 induced scratching, cutaneous inflammation and anaphylaxis. It also inhibited TDI induced bronchoalveolar fluid accumulation through mast cell membrane stabilization and inhibition of NO synthesis. Anaphylaxis is a severe and systemic allergic reaction caused by systemic release of histamine and other inflammatory chemical mediators^{6,17}. One of the newer methods of anaphylaxis treatment involves use of immunotherapeutic agent by decreasing production of IgE⁶⁻⁷. In the present study, CEAB, showed dose-dependent protection against compound 48/80 induced anaphylaxis upto 70%.

Antipruritis activity was evaluated^{7,16} by observing incidence of scratching behaviour by subcutaneous administration of compound 48/80. Compound 48/80 alone administered had significantly increase in number of scratching thought to be associated with release of histamine from mast cell degranulation¹⁶. However, the chloroform extract of *Aristolochia bracteolata* showed significant inhibition of scratching. The results indicated that chloroform extract of *Aristolochia bracteolata* was useful in treatment of most of allergic diseases.

Atopic dermatitis is a complex mixture of allergic and inflammatory reaction associated with severe pruritis¹⁶. Compound 48/80 induced passive cutaneous anaphylaxis in mice ear was used. Injection of compound 48/80 (sc) into mice ear induced increased in mice ear weight. This could be due to degranulation of mast cells in skin resulting in an increased plasma exudation due to the release of histamine¹⁶.

Table 2— Effect of CEAB on toluene di-isocyanate-induced bronchioalveolar fluid secretion in rats
[Values are mean $\pm$ SE of 6 animals]

Treatment	Volume of BAL fluid	Inhibition of BAL fluid secretion (%)
Normal (Saline)	0.68 $\pm$ 0.15	----
Control (Comp. 48/80)	1.32 $\pm$ 0.13**	----
Standard (DSCG)	0.87 $\pm$ 0.15*	33.71
CEAB 100 mg/kg	1.26 $\pm$ 0.06	5.3
CEAB 200 mg/kg	1.18 $\pm$ 0.1	10.45
CEAB 400 mg/kg	0.98 $\pm$ 0.05*	25.5
CEAB500 mg/kg	0.92 $\pm$ 0.1*	30.43
CEAB— Chloroform extract of <i>Aristolochia bracteolata</i> Significant at *P<0.05, **P<0.01		

Table 3—Effect of CEAB on nitrate and histamine levels in rats
[Values are mean $\pm$ SE for 6 animals]

Treatment	Serum NO	RPMC NO	BAL NO	Serum Histamine
Normal (Saline)	11.78 $\pm$ 0.44	6.02 $\pm$ 0.34	11.7 $\pm$ 0.65	0.052 $\pm$ 0.007
Control(Comp. 48/80)	31.62 $\pm$ 3.76***	17.15 $\pm$ 1.33***	41.08 $\pm$ 0.87***	0.11 $\pm$ 0.008***
Standard (10 mg/kg (ip) DSCG)	17.48 $\pm$ 1.05**	13.23 $\pm$ 0.76**	26.08 $\pm$ 1.92 ***	0.055 $\pm$ 0.012 **
CEAB 100 mg/kg (po)	21.62 $\pm$ 1.12*	18.33 $\pm$ 1.13	37.2 $\pm$ 1.27*	0.086 $\pm$ 0.01
CEAB 200 mg/kg (po)	19.37 $\pm$ 0.82**	17.53 $\pm$ 0.91	34.53 $\pm$ 1.7**	0.064 $\pm$ 0.014*
CEAB 400 mg/kg (po)	12.97 $\pm$ 0.73***	14.65 $\pm$ 0.88*	33.27 $\pm$ 1.13***	0.047 $\pm$ 0.013 **
CEAB 500 mg/kg (po)	10.68 $\pm$ 0.66***	12.67 $\pm$ 0.83 **	33.72 $\pm$ 1.07 ***	0.035 $\pm$ 0.007***
CEAB- Chloroform extract of <i>Aristolochia bracteolata</i> Significant at. *P<0.05, **P<0.01 and ***P<0.001				

The results obtained in the present study in antidermatitis activity indicated the chloroform extract of *Aristolochia bracteolata* had potent and significant effect on compound 48/80 induced passive cutaneous anaphylaxis. On the basis of its inhibitory effect on nitric oxide and mast cell stabilizing activity, it indicated that chloroform extract of *Aristolochia bracteolata* might act by these mechanisms and inhibited passive cutaneous anaphylaxis induced by compound 48/80.

With regard to antirhinitis activity TDI induced BAL fluid secretion was significantly reverted by seven days regular oral dose of CEAB in a dose-dependent manner. As nitric oxide is implicated in the pathophysiology of intestinal inflammation, intestinal mast cells may amplify inflammatory response and mucosal injury in the inflammatory bowel disease³. The present study also showed inhibitory effect on nitric oxide and mast cell activation; it indicated that might be useful in allergic diseases which resemble antianaphylaxis, antidermatitis activity.

Prophylactic treatment of Sprague Dowley rats with CEAB for 5 days earlier to exposure with compound 48/80 significantly inhibited the mast cell activation. The nitrate levels in serum, peritoneal fluid and BAL fluid were significantly reduced after treatment with CEAB. The drug also showed significant inhibitory effects on blood histamine levels in Sprague Dowley rats, that indicated that the drug had antiallergic activity.

Finally, the results of the present study demonstrated that CEAB had significant antianaphylactic, antidermatitis and antipruritis activities. In case of mast cell stabilizing activity the drug probably acted by stabilization of mast cell membrane. Possibly the nitric oxide synthase inhibitory activity was also responsible for the antiallergic activity. An antiallergic activity of *Aristolochia bracteolata* might be contributed by inflammatory mediator inhibitory pathway¹³.

It has been concluded that the potent antiallergic activity of chloroform extract of *Aristolochia bracteolata* may be due to its potent active constituents as their presence has been evidenced by the chemical tests carried out for the chloroform extract²². Possibly the mechanism may involve mast cell membrane stabilization and also histamine and nitrates secretion and release inhibition by avoiding degranulation. The characterization and purification of active principles from *Aristolochia bracteolata* and

molecular level investigation of activity is required for successful development of drug for clinical use.

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