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Investigation into the Mechanism of Action of *Abutilon indicum* in the Treatment of Bronchial Asthma

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Abstract: There is a high prevalence of usage of complementary and alternative medicines for the treatment of bronchial asthma which is considered as a chronic inflammatory disorder of the airways. *Abutilon indicum* G. Don. (*Fam: Malvaceae*) is abundantly found as a weed throughout the tropical parts of India. It is reported to have anti-inflammatory action and is used internally for inflammation of the bladder. Our previous clinical studies done on Indian asthmatic patients have proved the effectiveness of *Abutilon indicum (A. indicum*) in bronchial asthma. The objective of our present investigation was to determine the mechanism of action of *A. indicum* in the treatment of bronchial asthma by carrying out various experimental studies. In the present study, methanolic extract of aerial parts of *A. indicum* did not show bronchodilating activity against histamine and acetylcholine induced bronchospasm in guinea pigs. *A. indicum* also did not show any inhibitory effect on agonist induced contractions of guinea pig ileum and tracheal chain preparations. However it was found to have significant mast cell stabilizing effect ($p<0.05$, Student's t test) against compound 48/80 and egg albumin induced rat peritoneal mast cell degranulation. Also *A. indicum* showed significant anti-inflammatory activity ($p<0.05$, Student's t test) when estimated using carageenan induced rat paw edema model. The results of the present study indicate that possible mechanism of action of *A. indicum* in the treatment of bronchial asthma is its mast cell stabilizing and anti-inflammatory activity.

Key words: *Abutilon indicum* · Bronchial asthma · Anti-inflammatory

INTRODUCTION

Bronchial asthma is a complex disease with several clinically well-defined pathogenic components, including recurrent reversible airway obstruction, chronic airway inflammation and development of airway hyperresponsiveness [1]. Currently available inhaled bronchodilators and anti-inflammatory drugs are effective in most asthmatics, but this palliative therapy requires long term daily administration and is associated with serious toxicities [2, 3]. As a result, there is high prevalence of usage of alternative and complementary medicines in the treatment of bronchial asthma [4]. Several medicinal plants having spasmolytic or anti-inflammatory activity are being found to be effective in the treatment of bronchial asthma. *Abutilon indicum* G. Don. (*Fam: Malvaceae*) is reported to have anti-inflammatory action and is used internally for inflammation of the bladder [5]. It is reported to be used in the treatment of asthma in several southern parts of India [6]. It is abundantly found as a weed throughout the tropical parts of India. Preliminary pharmacological screening of *Abutilon indicum (A. indicum*) has proven its analgesic and antimicrobial properties [7, 8]. In some recent studies, *A. indicum* is reported to have hepatoprotective, hypoglycemic, antidiarrhoeal, diuretic and antifertility activity [9-13]. *A. indicum* is reported to contain β sitosterol, phenolic acids such as vanillic acid, p-coumaric acid, p-hydroxybenzoic acid and caffeic acids, glucovanilloyl glucose and Gallic acid [14, 15]. It is one of the ingredients of the Ayurvedic syrup 'Madhuyashtyadi syrup' which is used for prevention of upper respiratory tract infections [16]. Seeds of this plant are expectorant and are useful in coughs. Our previous clinical studies done on Indian asthmatic patients have proved the effectiveness of *A. indicum* in decreasing the severity of asthmatic symptoms and in improving various lung function parameters [17]. The objective of our present

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investigation was to determine the mechanism of action of *A. indicum* in the treatment of bronchial asthma by carrying out various experimental studies.

MATERIALS AND METHODS

Collection and identification of plant material: Aerial parts of plant of *A. indicum* were collected from Gujarat (India) in September October 2003 at the end of flowering season. The plant was identified and authenticated by Dr. M. Daniel, Dept. of Botany, M.S.University, Vadodara, Gujarat, India and a voucher specimen was deposited in 'BARO' the herbarium of department of Botany, M.S. University, Vadodara. Aerial parts were collectively cleaned, dried under shade, powdered and used for the study.

Preparation of extract: Coarse powder of dried aerial parts of *A. indicum* was extracted by methanol using soxhlet apparatus. Each time 20 g of powder was extracted with 500 ml of methanol for 24 hours. Extract was dried under reduced pressure to yield a syrupy mass. Extractive value was found to be 9.255 % (w/w). The dried extract obtained from each batch was collectively pooled and stored in the refrigerator till use. The dried extract dissolved in distilled water was used for various studies.

Animals: Guinea pigs and wistar albino rats were housed at ambient temperature (21±1°C) and relative humidity (55±5%) with fixed 12 h light/dark cycles and free access to food and water. The experimental protocols were approved by Institutional Animal Ethical Committee as per the guidance of committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Studies on histamine and acetylcholine (Ach) induced bronchospasm in guinea pigs: Guinea pigs were selected and randomly divided into 4 groups each containing six animals. The drugs were administered orally in 0.5% sodium carboxymethyl cellulose (CMC) 2 hours before exposure to histamine or Ach aerosol. First group served as control and received 0.5% CMC. Second group served as standard and received Ketotifen (1 mg/kg). The third and fourth groups were given methanolic extract of aerial parts of *A. indicum* (250 mg/kg and 500 mg/kg respectively) suspended in 0.5% CMC. After 2 hours of drug administration, animals were exposed to an aerosol of 0.5% of histamine dihydrochloride and time for preconvulsion state was noted for each animal as described by Sheth *et al*. [18]. After about 15 days of wash out period the same animals were given the above treatments and preconvulsion time was noted for 0.5% of acetylcholine bromide aerosol spray.

Studies on isolated smooth muscle preparations:

1) Isolated guinea pig Ileum: Overnight fasted guinea pigs of either sex weighing 400-600 g were sacrificed using cervical dislocation method. Ileum was quickly dissected out and suspended in organ baths containing 20 ml Tyrode's solution maintained at 37±1°C under basal tension of 500 mg. The composition of solution in mM was NaCl, 137; $CaCl_2$, 1.8; KCl, 2.7; glucose, 5.55; $NaHCO_3$, 11.9; $MgCl_2$, 1; NaH_2PO_4, 0.4. The solution was continuously bubbled with air. The responses to drugs were recorded on a student physiograph (BioDevices) using isotonic transducer, which exerted a basal tension equivalent to 500 mg load on tissue. The tissue was allowed to equilibrate for 30 mins., during which the bathing solution was changed at every 10 mins. The contractile responses of ileum to various agonists (acetylcholine, histamine, 5HT and $BaCl_2$) were recorded. The effect of varying doses of *A. indicum* extract and its interaction with contractile responses of abovementioned agonists on ileum were studied [18].

2) Isolated tracheal chain preparation: Guinea pigs of either sex weighing 400-600 g were sacrificed using cervical dislocation method. The trachea was rapidly dissected free of surrounding tissues and placed in Petri dishes containing oxygenated Kreb's solution. Tracheal strips were prepared by cutting the trachea spirally. Tracheal strips were suspended in organ tubes filled with 20 ml of Kreb's solution and equilibrated under a uniform tension of 1.5 g. The bathing solution was bubbled with 95% O_2 and 5% CO_2 at 37±1°C. The tissues were equilibrated for a period of 90 mins. The responses of the trachea were measured using a student physiograph. The composition of Kreb's solution in mM was NaCl, 114.0; $CaCl_2$, 2.5; KCl, 4.7; glucose, 11.7; $NaHCO_3$, 25; $MgCl_2$, 1.2; KH_2PO_4, 1.2. Effect of varying doses of *A. Indicum* extract was observed on contractile responses of tracheal chain preparations to 5HT (5.678×10^{-3} mM) [18].

***In vitro* mast cell degranulation studies:**

Isolation of rat peritoneal mast cells: Normal saline containing 5 units/ml of heparin was injected in the peritoneal cavity of male rats lightly anaesthetized with

ether. After a gentle abdominal massage, the peritoneal fluid containing mast cells was collected in centrifuge tubes placed over ice. Peritoneal fluid of 4-5 rats was collected, pooled and centrifuged at 2000 rpm for 5 mins. Supernatent solution was discarded and the cells were washed twice with saline and resuspended in 1 ml of saline.

Degranulation studies: Degranulation of rat peritoneal mast cells was induced in vitro by two different stimuli: (1) Non-Immunological (Compound 48/80 induced) and (2) Immunological (Egg albumin induced). Effect of drug extracts was studied on mast cell degranulation induced by both the stimulus.

(1) Non-immunological (Compound 48/80 induced): To 0.1 ml of the total peritoneal cell suspension, 0.1 ml of the test agent in saline (*A. indicum* extract) or reference standard (Ketotifen 10 µg/ml) was added and incubated in a constant temperature water bath (37°C) for 15 min. Then 0.1 ml of compound 48/80 (10 µg/ml) was added and the suspension was further incubated for 10 min. The cells were then stained with 0.1% of Toluidine blue solution made in distilled water and observed under the high power (45X) of light microscope. The percentage of protection of the mast cells in the control groups and the treated groups was calculated by counting the number of degranulated cells from total of at least 100 mast cells counted. Control groups consisted of a positive control group in which compound 48/80 was added without addition of test agent and a negative control group in which neither compound 48/80 nor the test agent was added to correct for spontaneous degranulation of mast cells without any degranulating agent.

(2) Immunological (Egg albumin induced): Male rats were first sensitized with egg albumin by giving 3 subcutaneous injections (on 1st, 3rd and 5th day) of 350 µg of egg albumin adsorbed on 60 mg of aluminium hydroxide gel. On 10th day peritoneal mast cells were collected by the method described above. Mast cells were degranulated by 0.1 ml of egg albumin (10 mg/ml) and the effect of the test agent on degranulation was studied in same way as described in Compound 48/80 induced degranulation. The percentage of protection of the mast cells in the control groups and the treated groups was calculated by counting the number of degranulated cells from total of at least 100 mast cells counted. Control groups consisted of a positive control group in which egg albumin was added without addition of test agent and a negative control group in which neither egg albumin nor the test agent was added to correct for spontaneous degranulation of mast cells without any degranulating agent.

***In vivo* Mast cell degranulation studies:**

Compd. 48/80 induced rat paw edema: Albino wistar rats of either sex weighing 200-250 g were divided into 4 groups of 6 animals each. Group I served as a negative control (0.5% C.M.C.), Groups II and III were given *A. indicum* extract (250 and 500 mg/kg p.o.) and Group IV was given a reference standard (Ketotifen 1 mg/kg p.o.) Animals of different groups were treated with respective drugs and subsequently 1 hr after treatment, 0.1 ml solution of compd. 48/80 (10 µg/paw) was injected subcutaneously into the plantar region of right hind paw to induce edema. The paw volume was measured initially and at 10, 20, 30, 60, 120 and 180 mins. after compd. 48/80 injection using plethysmographic method of West [19] in order to observe the effect of *A. indicum* on inflammation produced by various mediators released by mast cell degranulation induced by compd. 48/80. Percentage of increase in paw volume from baseline was calculated and compared with control.

Anti-inflammatory studies:

Carageenan induced rat paw edema: Albino wistar rats of either sex weighing 200-250 g were divided into 4 groups of 6 animals each. Group I served as a negative control (0.5% C.M.C.), Groups II and III were given *A. indicum* extract (250 and 500 mg/kg p.o.) and Group IV was given a reference standard (Diclofenac sodium 20 mg/kg p.o.) Animals of different groups were treated with respective drugs and subsequently 1 hr after treatment, 0.1 ml of 1% carageenan was injected subcutaneously into the plantar region of right hind paw to induce edema. The paw volume was measured initially and at 3 and 5 hrs after carageenan injection using plethysmographic method of Harris and Spencer [20] in order to observe the effect of *A. indicum* on inflammation produced by various mediators. Percentage of increase in paw volume from baseline was calculated and compared with control.

Neutrophil adhesion study: The study was carried out by method of Wilkinson [21]. Albino rats of either sex and Wistar strain were used for the study. The animals were randomly divided into 3 groups of 6 animals each. One of the groups served as control, which received vehicle (10 ml/kg p.o.) Second and third group received test drug extract suspended in 0.5% CMC at the doses of 100 and 200 mg/kg/day p.o. for 8 days. On day 8, blood samples were collected from the retro-orbital plexus into heparinized vials and analysed for Total Leukocyte

Count (TLC). The Differential Leukocyte Count (DLC) was performed by fixing the blood smears and staining with leucofine and percentage of neutrophils in each sample was determined. After the initial counts, blood samples were incubated with 80 mg/ml of nylon fibers for 10 mins. at 37°C. The incubated blood samples were again analysed for TLC and DLC. The product of TLC and percentage of neutrophils, gave the neutrophil index of blood sample. Percentage of neutrophil adhesion was calculated from the following formula:

Neutrophil adhesion (%) = (NIu - NIt)/NIu

Where: NIu = neutrophil index of untreated blood sample; and NIt = neutrophil index of treated blood sample.

Statistical analysis: Results are expressed as the means±S.E.M. All data were evaluated by student's unpaired t test. P values of 0.05 or less were considered significant.

RESULTS

Antispasmodic activity of A. indicum on histamine and ach induced bronchospasm in guinea pigs (n = 6): Pretreatment with methanolic extract of *A. indicum* (250 mg/kg and 500 mg/kg) to guinea pigs did not cause any significant increase in preconvulsion time as compared to control when exposed to either histamine (0.5%) or Ach (0.5%) aerosol (Fig. 1).

Antispasmodic activity of A. indicum on agonist induced contractions of guinea pig ileum and tracheal chain preparations (n = 6): In the present study methanolic extract of aerial parts of *A. indicum* (250-1000 µg/ml) did not cause any inhibition of guinea pig ileal contractions induced by histamine ($5.407X\ 10^{-7}$ mM), Ach ($9.172\ X\ 10^{-4}$ mM), 5HT ($5.678\ X\ 10^{-8}$ mM) and $BaCl_2$ ($1.38\ X\ 10^{-3}$ mM) nor did the drug cause any inhibition of 5HT ($5.678\ X\ 10^{-8}$ mM) induced contraction of guinea pig tracheal chain preparations.

Mast cell stabilizing activity of A. indicum on rat peritoneal mast cell degranulation

(1) Compound 48/80 induced mast cell degranulation (n = 6): Compound 48/80 (10 µg/ml) was found to induce mast cell degranulation to the extent of 78.46%. Ketotifen (10 µg/ml) as a reference standard produced an inhibition of 85.42%.*A. indicum* extract (11.11-33.33 mg/ml) produced dose dependent inhibition of mast cell degranulation (Fig. 2).

(2) Egg albumin induced mast cell degranulation (n = 6): Egg albumin (1 mg/ml) was found to induce mast cell degranulation to the extent of 78.50%. Ketotifen (10 µg/ml) as a reference standard produced an inhibition of 70.39%. *A. indicum* extract (10 – 30 mg/ml) produced dose dependent inhibition of mast cell degranulation (Fig. 3).

Mast cell stabilizing activity of A. indicum on compd. 48/80 induced paw edema: A. indicum extract at the dose of 250 and 500 mg/kg, p.o. significantly decreased rat paw edema as compared to control. Ketotifen (1 mg/kg p.o.) used as a positive reference standard also showed significant reduction of rat paw edema as compared to control (Fig. 4).

Anti-inflammatory activity of A. indicum on carageenan induced rat paw edema (n = 6): A. indicum extract at the dose of 250 and 500 mg/kg, p.o. significantly decreased rat paw edema as compared to control. Diclofenac sodium (20 mg/kg p.o.) used as a positive reference standard also showed significant reduction of rat paw edema as compared to control (Fig. 5).

Inhibition of rat neutrophil adhesion to nylon fibers by A. indicum (n = 6): A. indicum extract at 100 and 200 mg/kg, p.o. did not cause statistically significant inhibition of the adhesion of neutrophils to nylon fibers (Fig. 6).

DISCUSSION

A. indicum is growing as a weed in the waste places throughout the tropical parts of India. It is widely used as an anti-inflammatory drug in various inflammatory conditions by Ayurvedic and Unani practitioners [5]. It is now known that airway inflammation is a persistent feature of asthma, even between acute flare-ups and plays a critical role in the alteration in lung function. In susceptible individuals, this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness and coughing, particularly at night or in the early morning. The inflammation also causes an associated increase in the existing bronchial hyperresponsiveness to a variety of stimuli [22].

A. indicum is used as an anti-asthmatic drug in several southern parts of India [6]. In our previous clinical studies done on Indian asthmaticpatients, powder of dried aerial parts of *A. indicum* was found to be effective in decreasing the severity of commonly observed symptoms of bronchial asthma viz. dyspnoea, cough, chest tightness and wheezing. The drug was

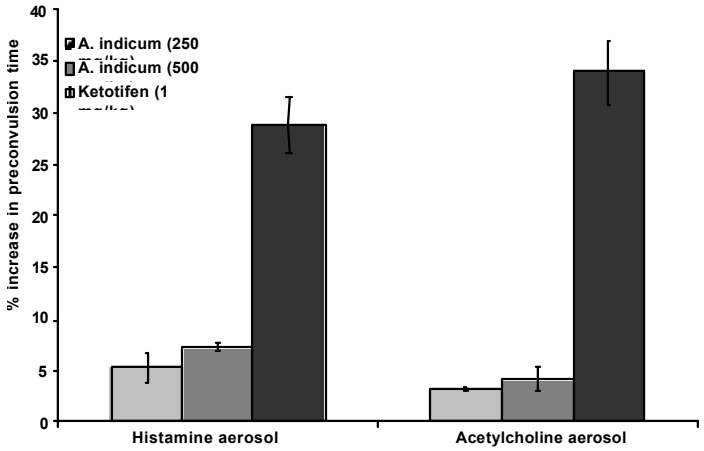

Fig. 1: Bronchodilating activity of *A. indicum* on histamine and Ach induced bronchospasm in guinea pigs

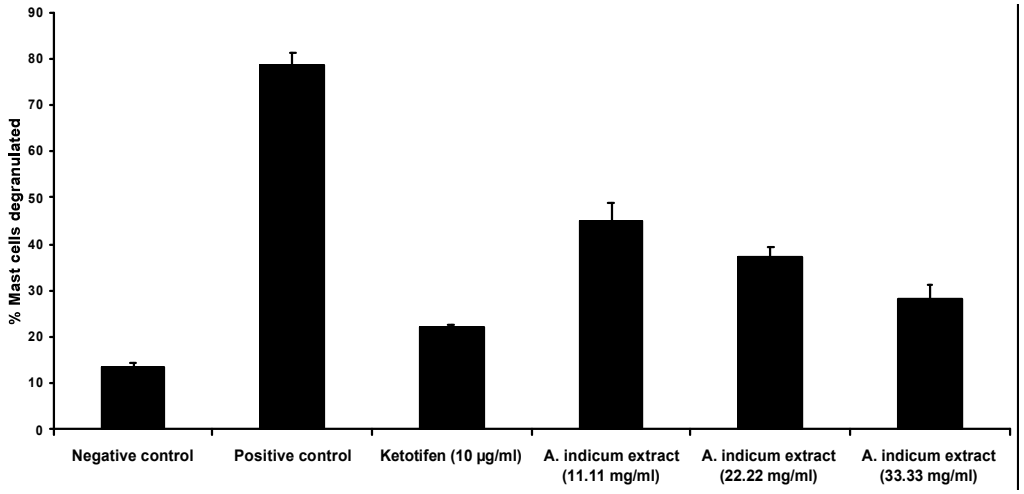

Fig. 2: Mast cell stabilizing activity of *A. indicum* on Compound 48/80 induced rat mast cell degranulation (n = 6)
* Significantly different from control, $p<0.05$

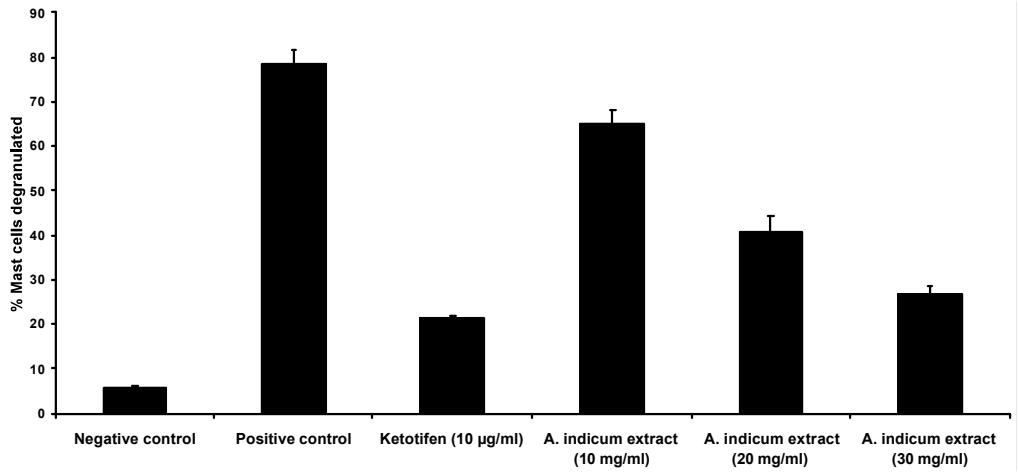

Fig. 3: Mast cell stabilizing activity of *A. indicum* on egg albumin induced rat mast cell degranulation (n = 6)
* Significantly different from control, $p<0.05$

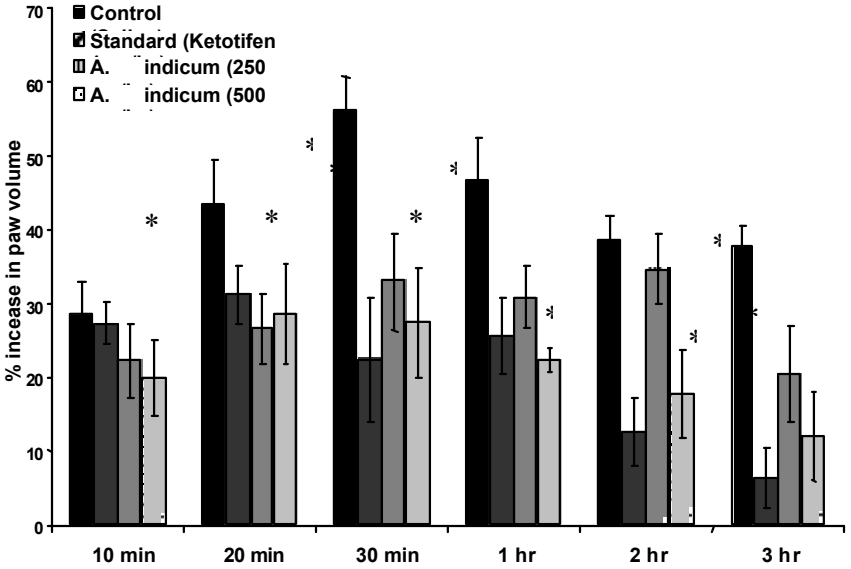

Fig. 4: Mast cell stabilising activity of *A. indicum* on Compd. 48/80 induced rat Paw edema (n = 6)
* Significantly different from control, $p < 0.05$

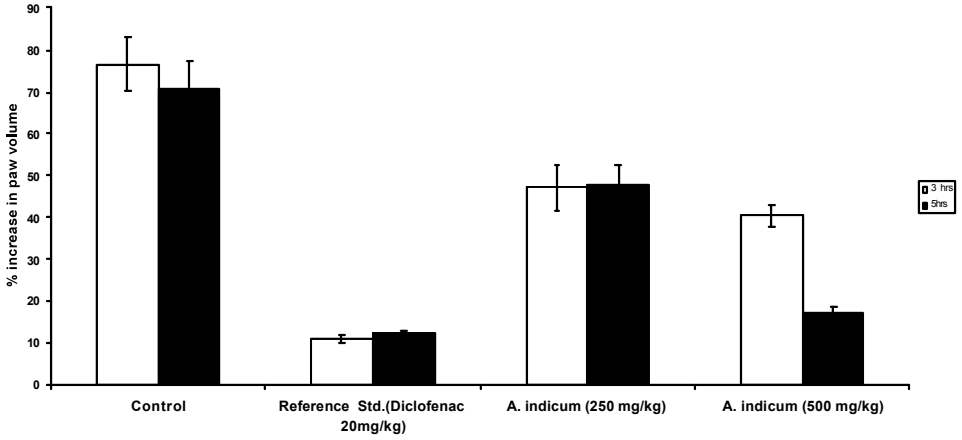

Fig. 5: Anti-inflammatory activity of *A. indicum* on Carageenan induced rat Paw edema (n = 6)
* Significantly different from control, $p < 0.05$

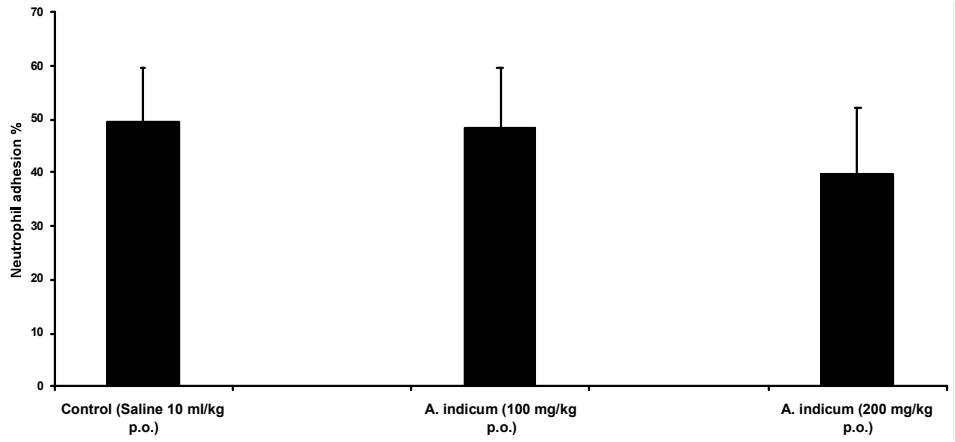

Fig. 6: Inhibition of *in vitro* rat neutrophil adhesion to nylon fibers by *A. indicum* (n = 6)

also found to significantly increase the lung function parameters like Forced Vital Capacity (FVC), forced expiratory volume in 1 second (FEV_1) and peak expiratory flow rate (PEFR) in patients having mild to moderate bronchial asthma [17]. Thus, the present investigation was performed to determine the mechanism of action of *A. indicum* in bronchial asthma.

Since bronchodilators, mediator release inhibitors and anti-inflammatory drugs are the different classes of drugs used conventionally in the treatment of bronchial asthma, various animal models were used to determine which of these mechanisms is responsible for antiasthmatic activity of *A. indicum*.

In the present study, bronchodilating effect of *A. indicum* was evaluated by observing the effect of methanolic extract of its aerial parts on histamine and Ach aerosol induced bronchoconstriction in guinea pigs. *A. indicum* was not found to increase the preconvulsion time against histamine and Ach aerosol as compared to control animals. Also, *A. indicum* was not found to inhibit agonist induced contractions of isolated smooth muscle preparations like guinea pig ileum and tracheal chain. This shows that *A. indicum* does not possess any antispasmodic activity. So some other mechanism must be responsible for its anti-asthmatic effect observed in our clinical study.

In addition to bronchodilators, a significant number of therapeutic approaches for bronchial asthma have been designed based on antagonizing specific mediators released from mast cells and on selectively inhibiting the activation of these cells. Mast cells play a key role in the induction of allergic disorders, such as bronchial asthma through the release of mediators including histamine, arachidonate products, proteases and several cytokines, which are found in relatively high quantities in these cells [23]. Several medicinal plants used in asthma like *Albizzia lebbeck*, *Ocimum sanctum, Eleocarpus sphaericus* etc. have been reported to have mast cell stabilizing activity [24-26]. In the present study, methanolic extract of *A. indicum* was found to significantly inhibit *in vitro* rat peritoneal mast cell degranulation induced by compound 48/80 and egg albumin. *A. indicum* was also found to significantly inhibit the edema formation induced by compound 48/80. Compound 48/80 is one of the more potent releasers of histamine and other mediators of inflammation from mast cell granules, which on release lead to immediate inflammatory response [27]. The inhibition of compound 48/80 induced edema supports the finding with *in vitro* mast cell degranulation studies and confirms the mast cell stabilizing activity of *A. indicum*.

Increased appreciation of the role of inflammation in the pathophysiology of asthma has led to new treatment strategies. The current emphasis is on long-term anti-inflammatory therapy in patients with persistent asthma. Various herbal drugs used in asthma have been reported to have anti-inflammatory activity e.g. *Picorrhiza kurroa, Boswelia serrata, Ginkgo biloba* etc [28-30]. The time course of edema development in carageenan induced paw edema model in rats is generally represented by a biphasic curve [31]. The first phase occurs within an hour of injection and is partly due to trauma of injection and also to serotonin component [32]. Prostaglandins play a major role in the development of second phase of reaction which is measured around 3 hour time [33]. The presence of PGE_2 in the inflammatory exudates from the injected foot can be demonstrated at 3 hr and period thereafter [34]. In the present study, *A. indicum* extract at the dose of 250 and 500 mg/kg, p.o. significantly decreased carageenan induced rat paw edema as compared to control which was comparable with that of standard drug. However, *A. indicum* did not cause statistically significant inhibition of the adhesion of neutrophils to the nylon fibers.

Thus significant anti-inflammatory activity and mast cell stabilizing activity seen of *A. indicum* in the present study suggests the possible mechanism responsible for anti-asthmatic activity of this medicinal plant.

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