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A preliminary study on the effect of *Azadirachta indica* on bronchial smooth muscles and mast cells

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Abstract

Objective: The present study was designed to assess the protective effect of *Azadirachta indica* on bronchial smooth muscles and mast cells. Methods: *Azadirachta indica* (200 mg/kg p.o.) leaf juice was administered to animals and its protective effect was determined on histamine aerosol induced bronchospasm in guinea pigs, comp 48/80 induced mast cell degranulation and active anaphylaxis in rats. Results: A significant protection against histamine induced bronchospasm in guinea pigs was observed. There was 63% delay in onset of dyspnoea in *A. indica* treated animals against that of 16% in control animals. *A. indica* neither could prevent mast cell degranulation nor could protect against active anaphylaxis. Conclusion: The results of the present preliminary study reveal that *A. indica* has bronchoprotective role. Moreover, it also suggests that mechanisms other than that associated with mast cell degranulation or active anaphylaxis are possibly involved in bronchoprotective role of *A.indica.*

Key words: *Azadirachta indica,* bronchospasm, mast cell degranulation, active anaphylaxis.

1. Introduction

Azadirachta indica (Family: Meliaceae, Hindi: Neem) is an indigenous plant widely available in India. Different parts of this plant have been reported to possess hypoglycemic, antiseptic, antiulcerogenic [1] properties. Recently, it has been shown to possess anti-inflammatory [2] antioxidant [3] immunomodulatory [4] and adaptogenic [5] actions. Consideration of these properties leads to the speculation of possible beneficial effect of *A.indica* in bronchial asthma. Earlier report from our laboratory indicated anxiolytic activity of *A. indica* leaf juice, which was comparable to diazepam [6]. Careful perusal of literature revealed no study in this regard. The present study with *A.indica* leaf juice on different parameters of allergy was undertaken keeping in view its reported immunomodulatory, anti-inflammatory and adaptogenic activities.

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2. Materials and methods

2.1 Plant material

Fresh green leaves of *A.indica* were collected from the campus of Institute of Medical Sciences, BHU, Varanasi. Fresh leaves were separated from stem and crushed with the help of pestle and mortar to make a paste and then squeezed to get the juice. The juice was directly used for the experimentation. Fresh leaf juice was used in the study because Ayurvedic literature suggests use of *A.indica* in this form. The dose was calculated in terms of weight of green leaves of *A. indica* and was given orally with the help of an orogastric tube. A fixed dose of 200 mg/kg of *A.indica* was used in all the experiments on the basis of our earlier report [6].

2.2 Animals

After Institutional Ethics Committee's approval, albino rats (CF strain) and guinea pigs of either sex weighing between 150-200g and 250-300g respectively were either obtained from the Institute Animal House or Zoological Emporium, Varanasi. They were housed in colony cages and fed standard Hind Lever pellet diet at room temperature 25 ± 2°C and 45-55% RH with 10:14 h, L: D cycles. The animals were kept fasting for 24 h prior to actual experimentation but water was given *ad libitum*.

2.3 Aerosol induced bronchospasm in guinea pigs

Armitage *et al.* [7] described that when 1% histamine acid phosphate was used as aerosol, guinea pigs showed progressive signs of difficulty in breathing leading to convulsions and death. The time until signs of convulsion appeared is called pre-convulsion time. The criterion used in the present experiments was onset of dyspnoea, and percent protection was calculated. *A. indica* fresh leaf extract was administered (200mg/kg, p.o.) once a day for 3 days. Actual experiment was done on day 3, 1 h after *A. indica* administration.

2.4 Mast cell degranulation by Comp. 48/80 in rats

This was assessed by adopting the method of Norton [8]. Albino rats (CF strain) of either sex were divided in to four groups of 6 animals each. Rats in group I and II received 1 ml/kg, p.o. of double distilled water and served as control. Group III and IV rats were administered with *A.indica* (200 mg/kg, p.o.) once a day for 4 days.

On day 4, 1 h after treatment, the rats were sacrificed by cervical dislocation. The abdomen was opened and mesentery of the jejunum and ileum was carefully exposed. The mesentery along with small pieces of jejunum and ileum (excepting the distal most 2cm which showed maximum variation in the degree of spontaneous degranulation) were taken out and placed in a petridish containing oxygenated Ringer Locke's solution NaCl 9.0, KCl 0.42, $CaCl_2$ 0.24, $NaHC0_3$ 0.5 and glucose 1.0 g/L double distilled water, pH 7.4 at 37 ± 0.5°C.

Tissues of groups II and IV were challenged with *comp. 48/80* (2.5µg/ml) *in vitro* for 10 min. The tissues were then stained supravitally. The tissues were immersed in 0.1% toludine blue in 4% formaldehyde in saline for 15-20 min. The tissues were next transferred and kept in acetone (two changes) for 10 min and then kept in xylene for 2 min (two changes) and subsequently mounted on slides. Before mounting, excess pieces of fat were trimmed off from the edges of the mesentery. The tissue mesentery was stretched with the help of a needle.

Usually 5-6 pieces of mesentery of each animal were used for each group. From these pieces, five microscopic fields were selected at random under 100 X magnification at widely separated areas of the mesentery. In each field using 430 X magnification the first 10 mast cells were examined starting from the left-hand side of the

field and proceeding clockwise. Each cell was considered either disrupted or not disrupted. The term disrupted was selected instead of fragmented, since granules were found around many cells that did not appear to be in fragments. The sole criterion for calling a cell disrupted was the presence of granules which appeared swollen at lower concentration of *comp. 48/80.* For each group 100 to 150 mast cells were examined and average % disruption was calculated.

2.5 Active anaphylaxis

This was done by following the method of Gupta and Tripathi [9]. Rats were sensitised by injecting subcutaneously 0.5 ml of horse serum with 0.5 ml of triple antigen containing 20,000 million *Bordetella pertussis* organism (CRI, Kasauli, India). The sensitised rats were divided into 2 groups of 6 animal each. Rats of group I received double distilled water and served as control. Rats of group II were administered *A.indica* (200mg/kg, p.o.) once a day for 14 days.

On day 14, rats were sacrificed 1h after treatment and the intestinal mesentery was taken for the study of mast cells. *In vitro* mesenteric pieces were challenged with 5% horse serum for 10 min after which the mast cells were stained and examined microscopically.

2.6 Statistical Analysis

Statistical analysis was done by using student's *t* - test for unpaired data.

3. Results

3.1 Aerosol induced bronchospasm in guinea pigs

The results are summarised in Table 1. *A.indica* (200 mg/kg, p.o.) once a day for 3 days protected guinea pigs significantly against bronchospasm induced by histamine aerosol. A significant delay in the onset of dyspnoea was observed in *A.indica* treated animals.

3.2 Mast cell degranulation by comp.48/80 in rats.

Comp 48/80 induced significant mast cell degranulation. *A.indica* also produced mast cell degranulation *per se* and it did not show any protective role against *comp. 48/80* challenge. The results are summarised in Table 1.

3.3 Active anaphylaxis

A.indica did not have any protective role on mast cell degranulation in actively sensitized rats (Table 1).

4. Discussion

A.indica, apart from its diverse uses in Ayurveda, has recently been shown to possess anti-inflammatory [2], antioxidant [3] immunomodulatory [4] and adaptogenic [5] properties. The present study on histamine aerosol induced bronchospasm, *in vitro* mast cell degranulation, and active anaphylaxis was undertaken to find out possible anti allergic effect of *A.indica.*

The data of the present study indicate that *A.indica* protects guinea pigs against histamine induced bronchospasm. Bronchial hyperreactivity is a characteristic feature of bronchial asthma and immune responses as well as inflammatory reactions play an important role in causation of bronchial hyperreactivity [10].

Therefore, the finding suggests a protective role of *A.indica* in bronchial asthma. This finding is also in accordance with the earlier reports of anti-inflammatory, immunomodulatory and adaptogenic activities especially in the light of the role of immune response and inflammatory processes in bronchial asthma.

Effect of *A. indica* on *comp 48/80* induced mast cell degranulation and active anaphylaxis was evaluated to ascertain the possible mode of action. *A. indica* did not reveal any effect on

Table 1
Effect of *Azadirachta indica* leaf juice on bronchial smooth muscles and mast cells.

Effect on 1% histamine acid phosphate induced bronchospasm in guinea pigs. (*A.indica* 200 mg/kg/day p.o. x 3 days, number of animals- 20 per group)

Treatment	% delay in onset of dyspnoea (sec)
Control	16.10 ± 1.15
A. indica	63.18 ± 7.94[a]

Effect on *in vitro* comp. 48/80 (2.5 µg/ml) induced rat mesenteric mast cell degranulation. (*A.indica* 200 mg/kg/day p.o.x 4 days, n = 6 in each group)

Treatment	% degranulation
Control	15.83 ± 1.32
Comp. 48/80	90.07 ± 1.66[a]
A.indica	84.81 ± 2.99[a]
A.indica + comp. 48/80	91.89 ± 1.02[NS]

Effect on mast cell degranulation in actively sensitized rats. (*A.indica* 200 mg/kg/day x 14 days, n = 6 in each group)

Treatment	% degranulation
Control	94.21 ± 0.53
A. indica	95.91 ± 0.38*

[a] $p < 0.001$ in respect of control; [NS]Not significant in respect of comp. 48/80
*Not significant in respect of control.
Values are mean ± SEM (Statistical analysis by Student's *t* - test).

active anaphylaxis and it failed to prevent *comp 48/80* induced mast cell degranulation. Additionally, it resulted in mast cell degranulation *per se.* These findings are not in concurrence to the observed bronchoprotective effect of *A.indica*. However, advances in understanding of pathophysiology of bronchial asthma dictate that, though mast cell degranulation with subsequent release of histamine is an important factor in bronchial hyperreactivity, it is not the only or even main spasmogen. Other mediators like leukotrienes, inflammatory cytokines, chemotaxins, chemokines and leukocytes play an equally important role [11].

Van der Nat and coworkers [12] reported that *A. indica* causes a dose dependant decrease in the chemiluminscence of leukocytes and a dose dependant increase in the production of migration inhibition factor by the lymphocytes. Additionally, in an attempt to determine the possible mode of anti-inflammatory action of *A. indica,* Chattopadhay [2] showed that the action is through the stabilization of lysosomal membranes, antiproliferative and antioxidant like mechanisms. Chemical investigation on leaves of *A.indica* resulted in isolation of flavonol-o-glycosides, quercetin and beta-sitosterol [2,13]. Quercetin and other plant flavonoids have been reported to possess potent antioxidant,

anti-inflammatory and immunomodulatory activities [14]. Beta-sitosterol is a plant sterol having anti-inflammatory and immuno-modulatory activities [15]. The presently observed bronchoprotective effect of *A. indica* might be explained in the light of these reports.

Taken together, the present preliminary investigation suggests the protective role of *A. indica* in bronchial asthma. Though the exact mode of action of *A. indica* can not be pinpointed on the basis of present findings, it helps to exclude mast cell stabilization as a mode of action and suggests that other mechanisms like anti-inflammatory and immunomodulatory activities may be responsible for the bronchoprotective effect of *A. indica.*

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