ANTI-HISTAMINIC AND ANTI-ANAPHYLACTIC ACTIVITY OF *RANDIA DUMETORUM*

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Summary

In the present study ethanolic extract of *Randia dumetorum* (Retz.) Poir. (Rubiaceae) fruits (126, 252 and 504 mg/kg p.o.) were studied for its anti-histaminic and anti-anaphylactic activity. The extract has significant anti-anaphylactic activity. The extract protected the guinea pigs against histamine induced bronchospasm and also inhibited the disruption of mast cells induced by clonidine in rats.

Key words: *Randia dumetorum*; Anti-histaminic; Anti-anaphylactic; Passive paw anaphylaxis

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Introduction

Randia dumetorum (Retz.) Poir. (Rubiaceae) is a small thorny tree found all over India upto an altitude of 1350 meters in the hills. *Randia dumetorum* has been recommended in Ayurvedic system of medicine for variety of diseases. The fruit of the drug is said to be anti-asthmatic, emetic, expectorant, diaphoretic, nauseant, anthelmintic, abortifacient and antispasmodic and used in bronchitis. Bark is a sedative and nervine calmative. It is administered internally and applied externally in the form of a paste in rheumatism and to relieve pain of bruises and bone-aches during fevers and to disperse abscesses. It also acts as an astringent and is useful in diarrhea and dysentery [1].

Antigen-induced allergic responses are closely implicated in the pathogenesis of allergic inflammatory diseases such as bronchial asthma, allergic rhinitis and dermatitis. In bronchial asthma, various chemical mediators released from antigen-stimulated mast cells or basophils elicit immediate hypersensitivity reactions such as bronchial contraction and airway plasma extravasation [2]. Following the immediately occurring events, late-phase inflammatory responses such as airway edema, infiltration of inflammatory leukocytes and airway hyperresponsiveness are observed [3,4,5].

The present study was undertaken to evaluate the anti-histaminic and anti-anaphylactic activity of ethanolic extract of *Randia dumetorum* (Retz.) Poir. fruits against allergen (egg albumin) induced passive paw anaphylaxis, clonidine induced degranulation of mast cells and histamine induced bronchoconstriction.

Materials and methods

Plant material

Fruits of *Randia dumetorum* (Retz.) Poir. (Rubiaceae) were collected from local market of Pune and authenticated by Botanical Survey of India, Pune, where a sample specimen (Voucher number: PBN 01) has been deposited.

Extraction

Dried and coarsely powdered fruits of *Randia dumetorum* (Retz.) Poir. were subjected to solvent extraction in soxhlet extractor using ethanol as solvent (Hot method). Ethanol extract was dried and mixed with equal parts of gum acacia. The yield obtained was 6 % w/w.

Animals

Wistar rats (150-250 g) and Dunkin-Hartley Guinea pigs (350-400) were housed under standard laboratory conditions of temperature ($22^o \pm 2^o$ C), relative humidity (60 ± 5 %) and light and dark cycle (12:12), in groups of five each. The animal had free access to food and water. The ethical committee of the institute approved the protocol of the study.

Drugs and chemicals

The following drugs and chemicals were used for this study:

Drugs*:* Clonidine – Unichem, India.
Sodium cromoglycate – Cipla Ltd, India.
Dexamethasone - Cadila Healthcare Ltd, India.
Chlorpheniramine maleate - Research Lab Fine Chem. Industries, India.

Chemicals*:* Histamine diphosphate – Sigma Aldrich, USA.
Egg albumin - Burgoyne Burdidges Company, India.
RPMI buffer medium 1640 – Himedia, Mumbai, India.
Ethanol AR grade

Effect on passive paw anaphylaxis [6]

Anti serum to egg albumin was raised in rats using aluminium hydroxide gel as an adjuvant. Animals were given three doses of 100 mcg of egg albumin (s.c.) adsorbed on 12 mg of aluminium hydroxide gel prepared in 0.5 ml of saline on 1st, 3rd and 5th day. On 10th day of sensitization, the animals were bled from the orbital plexus. The collected blood was allowed to clot and the serum was separated by centrifugation at 1500 rpm.

Animals were divided into five groups each containing 5 animals. Animals belonging to group I served as control and were administered only the vehicle (10 ml/kg, p.o.). Animals belonging to group II were administered Dexamethasone (0.5 mg/kg, i.p.). Whereas animals belonging to groups III, IV and V received ethanolic extract of *Randia dumetorum* (Retz.) Poir. (126, 252 and 504 mg/kg, p.o.) respectively. The animals were passively sensitized with 0.1 ml of the undiluted serum into the left hind paw. The contralateral paw received an equal volume of saline. The *Randia dumetorum* (Retz.) Poir. drug extract were administered 24 hour after sensitization. 1 hr. after *Randia dumetorum* (Retz.) Poir. extract administration, the animals were challenged in the left hind paw with 10 mcg of egg albumin in 0.1 ml of saline and paw inflammation was measured by using a Plethysmometer (UGO Basile, 7140). The difference in the reading prior to and after antigen challenge represented the edema volume and the percent inhibition of edema was calculated by using the formula.

$$\% \text{ Inhibition} = 1-(T / C) \times 100$$

T - Mean relative change in paw volume in test group.
C - Mean relative change in paw volume in control group.

Effect on mast cell degranulation [7]

Rats were divided into six groups, five animals in each group. Animals belonging to group-I received vehicles 5 ml/kg, (p.o). Animals belonging to group-II received Sodium cromoglycate 50 mg/kg, (i.p.). Animals belonging to group-III, IV, V received *Randia dumetorum* (Retz.) Poir. (126, 252 and 504 mg/kg, p.o.) respectively. The treatment was continued for 7 days. On day 7 th, 2 hour after the assigned treatment, mast cells were collected from the peritoneal cavity. The rats were anesthetized with ether and were injected with 10 ml of normal saline solution into peritoneal cavity and abdomen was gently massaged for 90 second. The peritoneal

cavity was carefully opened and the fluid containing mast cells was aspirated and collected in siliconised test tube containing 7 to 10 ml of RPMI-1640 Medium (pH 7.2- 7.4). The mast cells were then washed thrice by centrifugation at low speed (400-500 rpm) and the pallet of mast cells was taken in the medium. The mast cells suspension approximately (1 x 10^6 cells/ml) was challenged with 0.5 µg/ml of clonidine solution and stained with 1 % toluidine blue and observed under high power microscope field (400 X). Total 100 cells were counted from different visual areas and the number of intact and degranulated cells was counted. The percent protection was calculated.

Effect on histamine-induced bronchoconstriction in guinea pigs [7]

Fasted guinea pigs were randomly divided into 5 groups, containing 5 animals each. Group-I-received Chlorpheniramine maleate (2 mg/kg, p.o.).Group-II, III & IV received three doses (110.25, 220.50, 441.00 mg/kg p.o) of ethanolic extract of *Randia dumetorum* (Retz.) Poir. Prior to drug treatment each animal was placed in the histamine chamber and exposed to 0.2 % histamine aerosol. The time for preconvulsion dyspnoea (PCD) (The time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsion) was noted. As soon as PCD commenced, animals were removed from the chamber and placed in fresh air to recover. This time for PCD value was taken for basal value. Guinea pigs were then allowed to recover from dyspnoea for 4 hours. After 4 hr. the animals of group II, III and IV were administered with the test drug extract and group I received Chlorpheniramine maleate. These animals were again subjected to histamine aerosol later at interval of 1 hr, 4 hr and 24 hr of drug administration and time for PCD was determined. The protection offered by treatment was calculated by using the following formula (Mitra et al, 1999).

$$\% \text{ Protection} = (T_2 - T_1)/ T_2 \times 100$$

Where,
T_1 = The mean time for PCD before administration of test drug.
T_2 = The mean time for PCD after administration of test drug at 1hr, 4 hr and 24 hr.

Statistical analysis

The results of various studies were expressed as mean ± SEM and analysed statistically using one-way ANOVA followed by Dunnett's test or unpaired student 't' test to find out the level of significance. $p < 0.05$ was considered statistically significant.

Results

Passive paw anaphylaxis

In the vehicle treated group, egg albumin increased the paw volume in the sensitized animals, which was measurable up to the time period of 4 hrs. Pretreatment with *Randia dumetorum* (Retz.) Poir. extract (126 mg/kg, p.o) significantly reduced ($p<0.01$) the paw volume at 0.5, 1, 2, 3, and 4 hr time interval and the percentage inhibition was 29.83%, 35.12%, 37.81%, 40.10% and 34.69 % respectively.

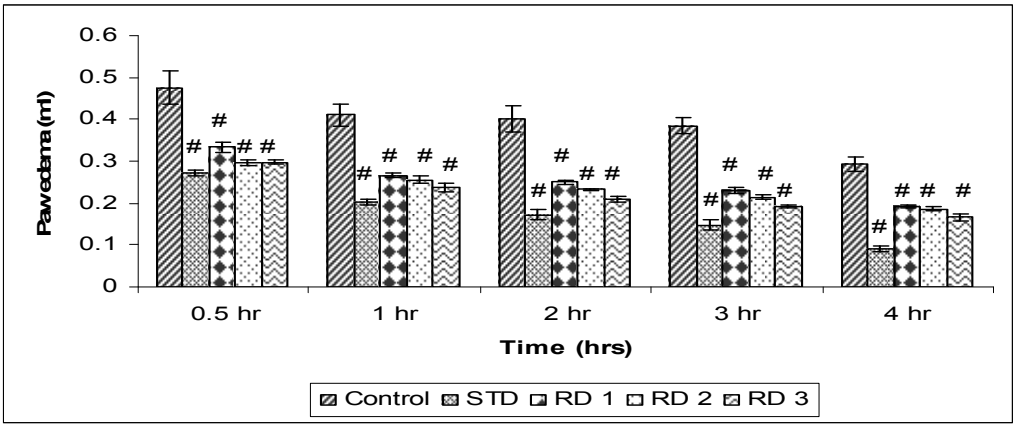
Randia dumetorum (Retz.) Poir. extract (252 mg/kg, p.o) significantly reduced ($p<0.01$) the paw volume at 0.5, 1, 2, 3 and 4hr time interval and the percentage inhibition was 37.81%, 37.56%, 42.28%, 44.27 and 36.73% respectively. *Randia dumetorum* (Retz.) Poir. (504 mg/kg, p.o) significantly reduced ($p<0.01$) the paw volume at 0.5, 1, 2, 3 and 4 hrs time interval and the percentage inhibition was 37.39%, 42.43%, 47.78%, 50.00% and 43.53 % respectively. Dexamethasone (0.5 mg/kg, p.o) significantly reduced ($p<0.01$) the paw volume at 0.5, 1, 2, 3 and 4 hrs time intervals and the percentage inhibition was 42.82%,50.73%,57.21%, 61.45% and 69.38 % respectively.

Table 1: Effect of *Randia dumetorum* on Passive paw anaphylaxis in rats

Groups (n=5)	Paw Edema Volume (ml) (Mean ± SEM)				
	0.5 hr	1 hr	2 hr	3 hr	4hr
Control	0.476 ± 0.039	0.410 ± 0.025	0.402 ± 0.031	0.384 ± 0.019	0.294 ± 0.017
STD	0.272 ± 0.008**	0.202 ± 0.006**	0.172 ± 0.013**	0.148 ± 0.012**	0.090 ± 0.007**
RD 1	0.334 ± 0.013**	0.266 ± 0.006**	0.250 ± 0.005**	0.230 ± 0.007**	0.192 ± 0.005**
RD 2	0.296 ± 0.007**	0.256 ± 0.009**	0.232 ± 0.003**	0.214 ± 0.005**	0.186 ± 0.005**
RD 3	0.298 ± 0.005**	0.236 ± 0.010**	0.210 ± 0.007**	0.192 ± 0.005**	0.166 ± 0.008**

Values are mean ± SEM; n = 5 in each group, ** $p<0.01$, compared to control group (One way ANOVA followed by Dunnett's test).

Figure 1: Effect of *Randia dumetorum* on Passive paw anaphylaxis in rats.



# $p<0.01$, compared to control group (One way ANOVA followed by Dunnett's test).

Clonidine induced mast cell degranulation in rats

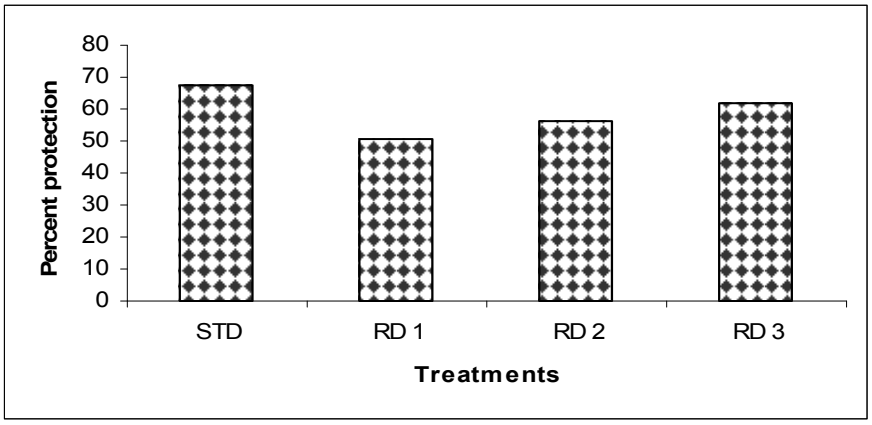
Clonidine induced mast cell degranulation was significantly ($p<0.01$) inhibited by sodium cromoglycate (50 mg/kg, i.p.). In the groups pretreated with ethanolic extract of *Randia dumetorum* (Retz.) Poir. (126, 252 and 504 mg/kg, p.o) there also significant protection ($p< 0.01$) of mast cells was observed.

Table 2: Effect of *Randia dumetorum* (Retz.) Poir. on clonidine induced mast cell degranulation in rats

Groups (n=5)	Dose	Mast cells %		Percent Protection
		Intact	Disrupted	
Control	5 ml/kg, p.o	21.4 ± 0.509	78.6 ± 0.509	-------
STD	50 mg/kg, i.p.	74.6 ± 0.509**	25.4 ± 0.509**	67.68
RD 1	126 mg/kg, p.o	61.4 ± 1.503**	38.6 ± 1.503**	50.89
RD 2	252 mg/kg, p.o	65.4 ± 0.509**	34.6 ± 0.509**	55.97
RD 3	504 mg/kg, p.o	70.2 ± 0.663**	29.8 ± 0.663**	62.08

Values are mean ± SEM; n = 5 in each group, ** $p<0.01$, compared to control group (One way ANOVA followed by Dunnett's test).

Figure 2: Effect of *Randia dumetorum* (Retz.) Poir. on clonidine induced mast cell degranulation in rats.



Histamine Induced Bronchoconstriction in Guinea Pig

The ethanolic extract of *Randia dumetorum* (Retz.) Poir. (110.25, 220.50 and 441 mg/kg, p.o) significantly prolonged ($p<0.01$) the latent period of convulsions as compared to control following exposure to histamine aerosol at 1st and 4th hour. *Randia dumetorum* (Retz.) Poir. extract (441 mg/kg, p.o) showed significant ($p<0.05$) action at 24th hour also.

Table 3: Percent protection against Histamine Induced Bronchoconstriction in Guinea Pig

Groups	% Protection		
	1 hr.	4 hr.	24 hr.
STD	61.76	71.73	28.90
RD 1	38.51	55.39	23.52
RD 2	49.18	56.88	14.54
RD 3	52.21	60.08	21.13

Figure 3: Effect of *Randia dumetorum* (Retz.) Poir. extract against histamine induced bronchoconstriction in guinea pigs.

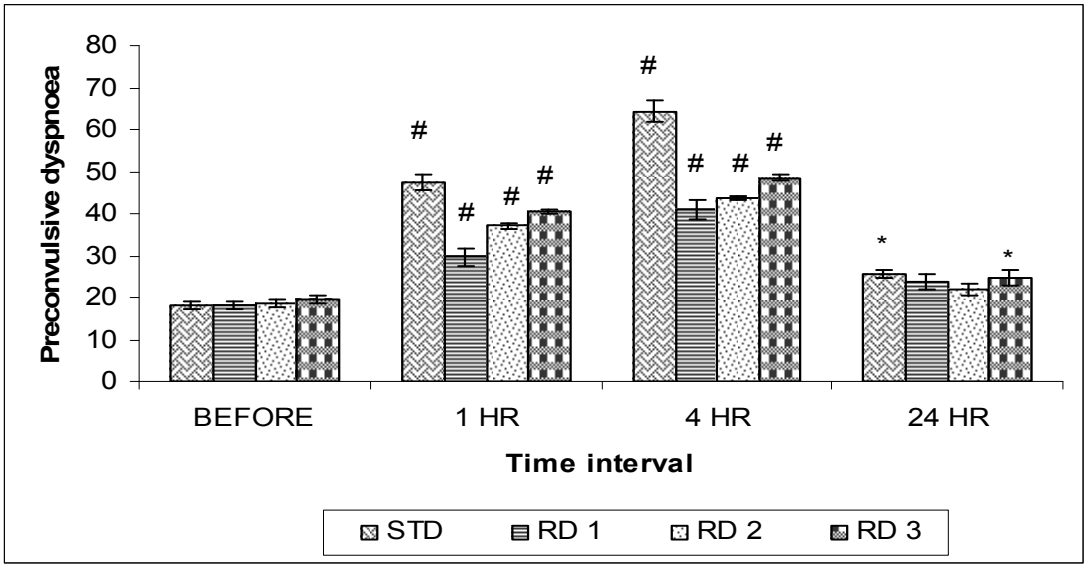
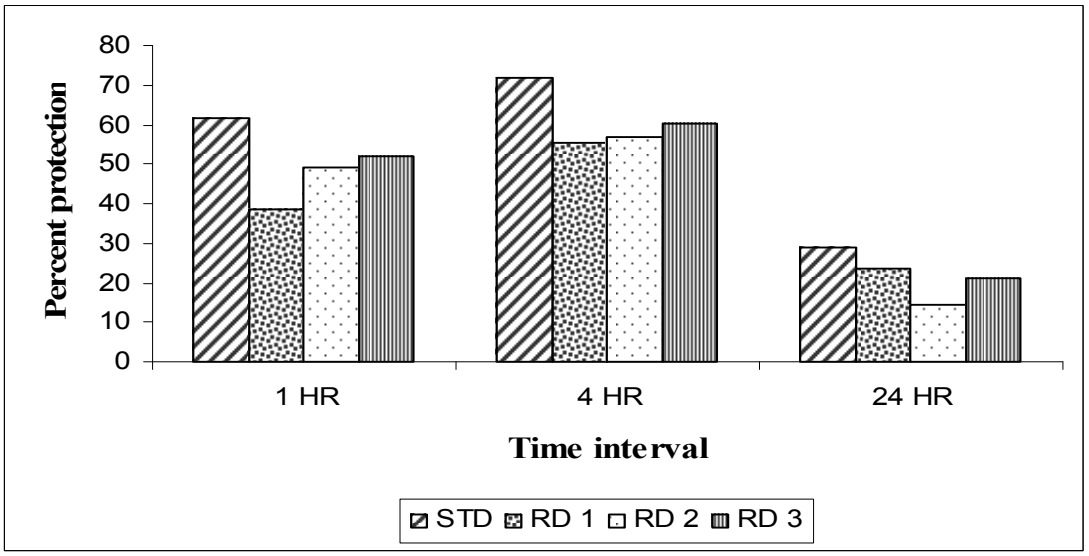


Figure 4: Percent protection against Histamine Induced Bronchoconstriction in Guinea Pig.



Discussion

Basophils, mast cells and their preformed de novo synthesized mediators, play a pivotal role in the pathogenesis of allergic disorders. These molecules are potent vasoactive and bronchoconstrictor agents and they modulate local immune responses and inflammatory cell infiltration [8, 9].

Immunoglobulin E (IgE) mediated mast cell stimulation is an important initial event in the development of type I allergic reactions like asthma and atopic disorders. Clinical studies have found a close association between asthma and serum IgE levels as well as IgE dependent skin test reactivity to allergens [10]. Antigen challenge, inn sensitized animals, results in degranulation of mast cells, which is an important feature of anaphylaxis. In the present study *Randia dumetorum* (Retz.) Poir. showed marked protection against the mast cell degranulation following antigen challenge in sensitized animals [11].

The present findings reveal that the anti-histaminic and antianaphylactic activity of *Randia dumetorum* (Retz.) Poir. may be due to the mast cell stabilizing potential, suppression of antibody production and inhibition of histamine-induced bronchoconstriction. Thus, the antigen and antibody reaction taking place on the surface of mast cells leading to release of mediators [12] seem to have modulated. Degranulated mast cells release a number of mediators like leukotrienes, platelet-activating factor, eosinophilic chemotactic factor and eosinophil-derived neurotoxin [13]. The prevention of degranulation process by the extract indicates a possible stabilizing effect on the biomembrane of mast cells [14]. Its ability to afford protection against histamine induced bronchospasm in guinea pigs shows antihistamine like action [15]. Histamine is a central mediator in the pathogenesis of allergic and inflammatory disorders. In the present study *Randia dumetorum* (Retz.) Poir. prolonged the latent period of PCD in guinea pigs following histamine aerosol. This may be suggestive of an antihistaminic activity following treatment with *Randia dumetorum* (Retz.) Poir. It also offered protection against anaphylactic shock-induced bronchospasm in rats [11].

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