

Investigation of Antiallergic and Antipruritic Activity Studies of *Shorea robusta* Oleoresin and *Wrightia tinctoria* Bark Extracts by Animal Models

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Abstract: Plants have formed the basis of sophisticated traditional medicinal systems that have been in existence for thousands of years and continue to provide mankind with new remedies. The study of total ethanol extract of *Shorea robusta* oleoresin and *Wrightia tinctoria* bark investigated the antiallergic activity study by sheep (*Capra hircus*) serum and compound 48/80 induced mast cell degranulation method using ketotifen fumarate and antipruritic activity study by compound 48/80 method using chlorpheniramine maleate as standard. The results showed both plants are excellent candidates.

Keywords: Traditional, investigated, antiallergic, antipruritic, compound 48/80.

INTRODUCTION

Shorea robusta and *Wrightia tinctoria* are widely distributed in Asia, Africa and Australia [1]. Both the plants are traditionally used for skin ailments [2, 3]. *Shorea robusta* oleoresin contains Nor-triterpenes 3- β -acetoxy-28-nor-urs-12-ene [4]. The stem bark of *W. tinctoria* is reported to contain β -amyrin, lupeol, β -sitosterol, stigmasterol, campesterol, a triterpenoid, flavonoids, steroids, alkaloids and phenolics [1].

Allergy is the fifth leading group of chronic diseases, affecting as much as 40% of the first-world population. Its pathophysiology has a genetic component and is driven by the immune system's sensitized response to antigens and environmental factors [5]. The incidence of allergic diseases is rising considerably worldwide in both developed and developing countries. These diseases include asthma, rhinitis, anaphylaxis drug, food, and insect allergy, eczema, urticaria and angioedema [6].

Pruritus is an important feature of atopic dermatitis (AD) with a high impact on the quality of life. Although the pathophysiology of atopic dermatitis itch is not fully understood the current studies have demonstrated a variety of mechanisms contribute to the induction and maintenance of the symptom [7]. Chronic pruritus is characteristic of several dermatologic diseases but also occurs in a variety of noncutaneous disorders [8].

Mast cells are best known for their role in IgE-associated allergic disorders and enhance the sensitization of certain cutaneous contact hypersensitivity responses, and increase inflammation and mortality during some severe bacterial infections [9]. Mast cells are found in the skin and in all mucosal tissues at homeostasis and their numbers are elevated in asthmatics lungs and gastrointestinal tract of inflammatory bowel disease [10]. Mast cells have cytoplasmic membrane-bound granules that contain a variety of biologically active mediators. The granules

also contain acidic proteoglycans that bind basic dyes such as toluidine blue. The cytoplasmic granules take purple to red colour, while nucleus is stained blue. Mast cells are activated by the cross-linking of high-affinity IgE Fc receptors [11].

EXPERIMENTAL SECTION

Plant materials

Wrightia tinctoria (*W.tinctoria*) bark and *Shorea robusta* (*S.robusta*) oleoresin were collected from Kulamavu Idukki district Kerala, India (Voucher No: 1180 and 1178). The Sample drug has been identified and authenticated by the Botanist Mr. Rogimon P. Thomas, Assistant Professor, Department of Botany, C.M.S. College Kottayam, Kerala, India.

Preparation of the plant extract

Shade dried and powdered drug of all the plant materials exudates of *S.robusta* (SR) and *W.tinctoria* (WT) bark were soaked in 95% ethanol in a round bottom flask. After soaking it for one day, it was

refluxed for 3 h and the clear solution was decanted off. The extraction was repeated thrice. The combined extracts were concentrated to a semisolid consistency. Thus total ethanolic extract (TEE) was obtained [12].

Animals (For antiallergic activity)

Sheep serum method [13]

Male *Wistar albino* rats of weighing between 150 - 200 g were sensitized by injecting subcutaneously 0.5 ml sheep (*Capra hircus*) serum along with 0.5 ml of triple antigen containing toxoids of Diphtheria, Tetanus and Bordetella pertusis organisms 20,000 million.

Compound 48/80 induced mast cell degranulation [14]

Healthy adult male *Wistar albino* rats of weighing between 150-200 g were selected for the study. Test extracts were given orally to overnight fasted animals.

Animals (For antipruritic activity) [15]

The study was performed on male *Wistar albino* rats (90 – 120 days old) weighing 150 – 200 g. They were maintained on standard conditions viz., (controlled, humidity and temperature), diet (Hindustan Lever Ltd) and water *ad libitum*. The study was conducted after obtaining institutional animal ethical committee (ref; id-MGU/DPS/IAEC/2016 PhD-2) clearance certificate.

Chemicals

Compound 48/80 (p-methoxy-N-methyl phenyl amine) was purchased from Sigma Aldrich, USA.

Chlorpheniramine maleate and ketotifen fumarate were obtained from Pfizer India Ltd. Mumbai. All other reagents used were analytical grade.

Acute toxicity study

Acute toxicity study was performed as per the Organisation for Economic Co-operation and Development as per the guidelines (OECD 423). A single dose acute toxicity study was conducted to determine the safety of the total ethanolic extracts and to determine the acute toxic category ("OECD guidelines for testing of chemicals –Acute Oral Toxicity – Acute Toxic Class Method" 2001).

Groups and treatment

Antiallergic activity

Sheep serum method

1. The sensitized rats were divided into six groups of six animals

Group 1: Control, received only vehicle (0.5% sodium carboxymethyl cellulose, 2 ml / Kg p.o)

Group II: Treated with ketotifen fumarate (1 mg/Kg p.o)

Group III and Group IV: Treated with ethanol extract of *S.robusta* oleoresin (200 and 400 mg/Kg p.o).

Group V and Group VI: Treated with ethanol extract of *W. tinctoria* bark (200 and 400 mg/Kg p.o).

2. Treatment was continued for 14 days. During the course of treatment the animals were maintained under controlled condition of temperature and were fed with standard diet.

3. On day 14th, 2 hrs after the assigned treatment, the rats were sacrificed and the intestinal mesentery was taken for studies on mast cells.

4. The mesenteries of sacrificed rats along with pieces of intestine were kept in Ringer-Locke solution at 37°C (NaCl- 9.0 g, KCl- 0.42 g, NaHCO₃- 0.15 g, glucose 1.0 g, CaCl₂ – 0.250 g/ litre of distilled water) at 37° C.

5. The mesenteric pieces were challenged with 5% sheep serum for 10 min and then transferred to a wide mouthed bottle containing 10% formalin for 24 hrs.

6. The mesenteric fans were fixed dried and stained with toluidine blue (0.1%) on a clean slide.

The excess of stain was washed with distilled water followed by dehydration in absolute alcohol. Finally the slides were cleared in xylene and mounted in diphenyl phthalein-xylene and examined microscopically for the number of intact and degranulated mast cells in at least 10 randomly selected high power fields.

Compound 48/80 induced mast cell degranulation

The animals were divided randomly into six groups and were given different doses of the extracts of *S.robusta* oleoresin and *W.tinctoria* bark by oral route.

Group I: Control, received only vehicle (0.5% sodium carboxymethyl cellulose, 2 ml / Kg p.o)

Group II: Treated with ketotifen fumarate (1 mg/Kg p.o)

Group III and Group IV: Treated with ethanol extract of *S.robusta* oleoresin (200 and 400 mg/Kg p.o).

Group V and Group VI: Treated with ethanol extract of *W.tinctoria* bark (200 and 400 mg / Kg p.o).

10 ml of normal saline was injected into the peritoneal cavity of normal male rats (150-200g) after a gentle massage. The peritoneal fluid was collected and transferred into the siliconised test tubes containing 7-10 ml RPMI-1640 medium (pH 7.2-7.4). Mast cells were washed thrice by centrifugation at low speed (400-500 rpm) followed by discarding the supernatant and

taking the pellets of mast cells into the medium. These cells were purified and incubated with compound 48/80 (5µg/ml) at 37°C for 10 min. After the incubation, these cells were spinned and stained with 0.1% toluidine blue solution and observed under a microscope. Test compounds were given to rats orally prior to collection of mast cells in doses of 100mg/kg for 14 days in two sets of experiments. Control animals received an equal volume of 2% w/v gum acacia solution 2 ml.

Antipruritic activity

Male *wistar albino* rats of weighing between 120-150 g were divided into six groups.

Group I: Control, received only vehicle (0.5% sodium carboxymethyl cellulose, 2 ml / Kg p.o)

Group II: Treated with Chlorpheniramine maleate (1 mg/Kg p.o)

Group III and Group IV: Treated with ethanol extract of *S.robusta* oleoresin (200 and 400 mg/Kg p.o).

Group V and Group VI: Treated with ethanol extract of *W. tinctoria* bark (200 and 400 mg/Kg p.o).

Assay of antipruritic activity

The antipruritic activity was evaluated by examining the incidence of scratching. Scratching behaviour was induced by subcutaneous injection of

0.1% solution of compound 48/80 in saline at 100 µl/site into the base of the neck on the back side of the rat. Scratching on the injected site by the hind paws were counted for 30 mins disregarding those at other site such as ears. Test compounds such as ethanol extract of *S.robusta* oleoresin and *W.tinctoria* bark (200 mg/kg and 400 mg/Kg) were given orally 1 hr before the injection of the compound 48/80. As a control, rats were administered 0.5 % sodium carboxy methyl cellulose (2 ml) orally. Chlorpheniramine maleate was used as the reference standard.

RESULTS AND DISCUSSION

Acute toxicity studies showed the nontoxic nature of the ethanol extracts of *S. robusta* oleoresin and *W.tinctoria* bark. There was no lethality or any toxic reactions found at any of the doses selected until the end of the study period.

Antiallergic activity

The inhibition of degranulation obtained with the TEE of *S. robusta* at a dose of 400 mg/kg is in good match with that obtained by the standard ketotifen fumarate (1mg/kg) as can be seen from table-1.

Even at a lower dose of 200 mg/kg fairly good inhibition of degranulation (75.39 ± 2.28) could be achieved. Only slightly less was the inhibition of degranulation achieved with the higher dose 400 mg/kg dose of TEE of *W. tinctoria*.

Table-1: Effect of TEE of the bark of *S.robusta* and *W.tinctoria* on mast cell degranulation induced by antigen sheep serum

Group	Treatment	Dose (mg/kg body weight)	n	Mast cells*	
				Intact (%)	Disrupted (%)
1	Control	0.5% sodium carboxy methyl cellulose	6	17.79 ±1.84	82.20 ± 1.84
2	Ketotifen fumarate	1mg/kg	6	81.91±1.09***	18.08 ±1.09
3	TEE of <i>S. robusta</i>	200 mg /kg	6	75.39±2.28***	24.60 ±2.28
4	TEE of <i>S. robusta</i>	400 mg /kg	6	80.44±1.93***	19.55 ±1.93
5	TEE of <i>W.tinctoria</i>	200 mg /kg	6	61.78±2.59***	38.22 ± 2.59
6	TEE of <i>W.tinctoria</i>	400 mg /kg	6	70.82± 1.23***	29.18 ±1.23

All values are expressed as Mean ± SEM. n = 6 n = number of animals per treatment

Significantly different from control *** p<0.001

Only slightly less was the inhibition of degranulation achieved with 400 mg/kg dose of TEE of *W. tinctoria*

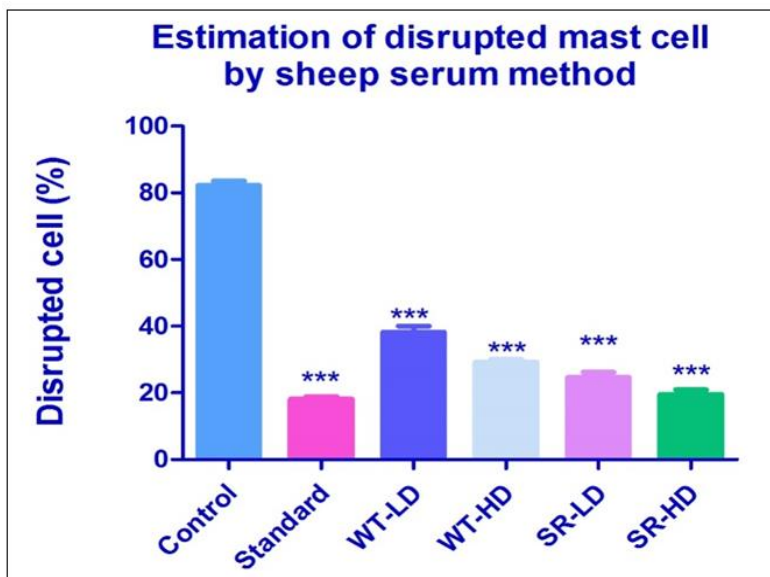


Fig-1: Estimation of intact mast cell by sheep serum method

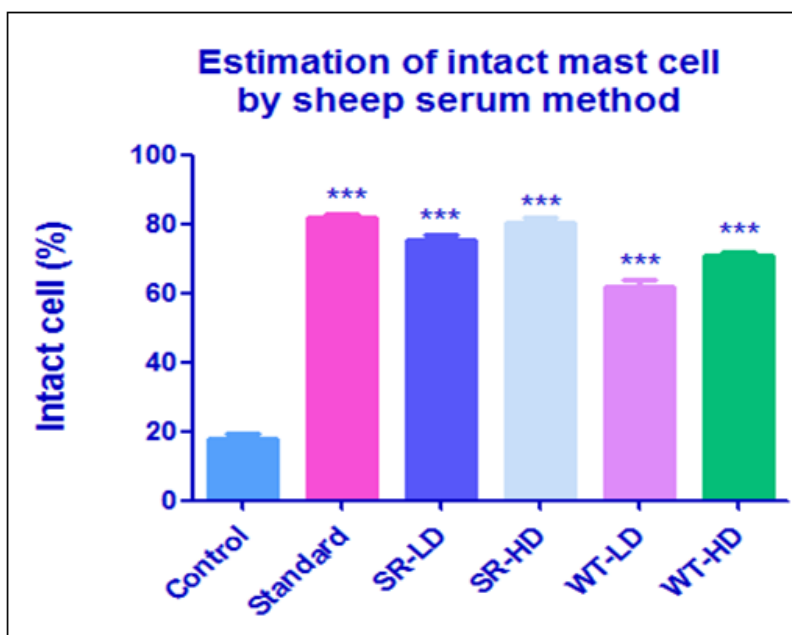


Fig-2: Estimation of disrupted mast cell by sheep serum method

The figure 1 & 2 showed the estimation of intact and disrupted mast cells by sheep serum in the TEE of *W. tinctoria* and *S. robusta*. WT-LD –

W.tinctoria 200mg/kg, WT-HD – *W.tinctoria* 400mg/kg, SR-LD – *S. robusta* 200mg/kg, SR-HD – *S.robusta* 400mg/kg dose.

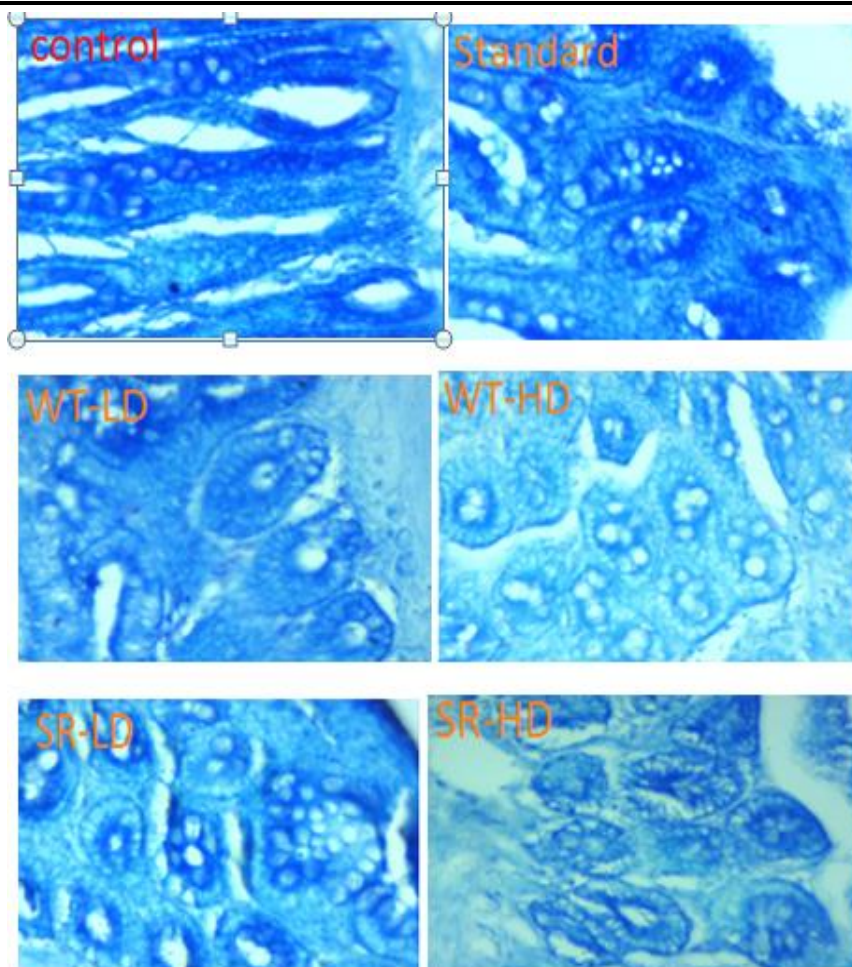


Fig-3: Intacts and disrupted mast cells present in the mesentery and intestine of rats SR-LD –*S. robusta*– 200mg/kg, SR-HD –*S. robusta* 400mg/kg, WT-LD – *W. tinctoria* -200 mg/kg, WT-HD – *W. tinctoria*400 mg/kg.

The above Figure-3 shows microscopic section of the intact and disrupted mast cells present in the mesentery and intestine of rat in the antiallergic study by sheep serum method. The treated group is compared

with control and standard group. The photographs show a clear picture of the disrupted mast cells in the higher dose when compared with standard.

Table-2: Effect of TEE of *S. robusta* and *W. tinctoria* on mast cell degranulation induced by Compound 48/80

Groups	Treatment	Dose (mg/kg body weight)	n	Mast Cells*	
				Intact (%)	Disrupted (%)
1	Control	0.5% sodium carboxymethylcellulose	6	16.65 ± 1.02	83.35 ± 1.02
2	Ketotifen fumarate	1mg/kg	6	77.88 ± 1.69***	22.12 ± 1.69
3	TEE <i>Shorea robusta</i>	200 mg /kg	6	63.71 ± 0.627***	36.29 ± 1.63
4	TEE <i>Shorea robusta</i>	400 mg /kg	6	71.89 ± 1.62***	28.19 ± 1.62
5	TEE <i>Wrightia tinctoria</i>	200 mg /kg	6	70.04 ± 1.88***	29.96 ± 1.88
6	TEE <i>Wrightia tinctoria</i>	400 mg /kg	6	74.39 ± 0.81***	25.61 ± 0.81

*All values are expressed as Mean ± SEM. n = 6, n = number of animals per treatment
Significantly different from control ***p<0.001

Intact mast cell assessment after compound 48/80 treatment of peritoneal fluid isolated mast cells showed better results with TEE of *W. tinctoria*

400mg/kg which was almost comparable with that of the standard. Fairly good was the result with that of *S.robusta* at the same dose.

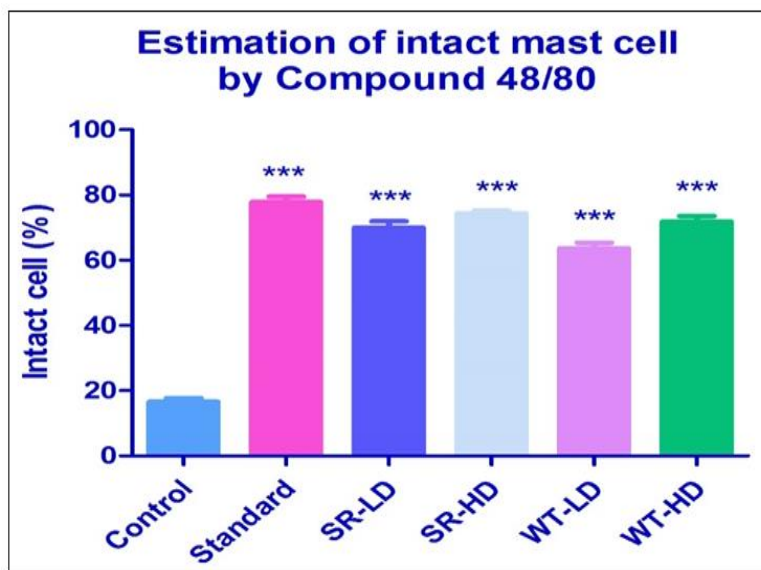


Fig-4: Estimation of intact mast cell by Compound 48/80. WT-LD – *W. tinctoria* 200 mg/kg, WT-HD – *W. tinctoria* 400 mg/kg, SR-LD – *S.robusta*200 mg/kg, SR-HD – *S. robusta* 400 mg/kg

The figure 4 & 5 showed the estimation of intact and disrupted mast cells by compound 48/80 for

antiallergic activity in the TEE of *W. tinctoria* and *S. robusta* at 200 and 400 mg/ kg body weight.

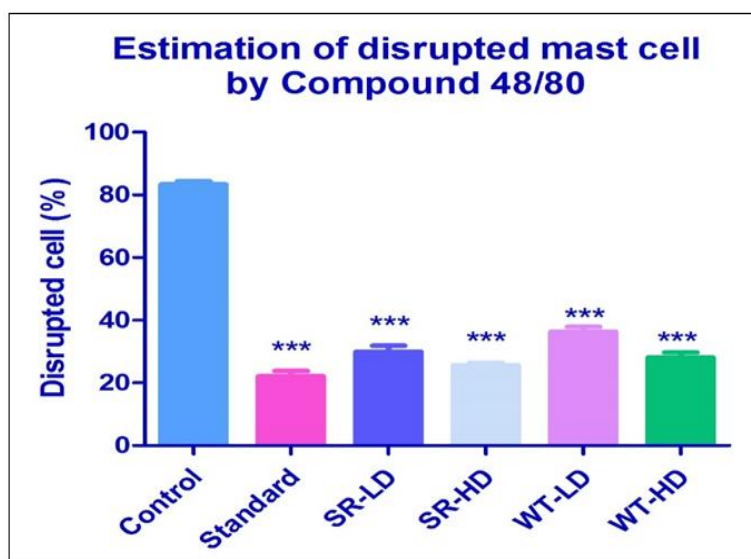


Fig-5: Estimation of disrupted mast cell by compound 48/80- WT-LD – *Wrightia tinctoria* 200 mg/kg, WT HD – *Wrightia tinctoria* 400 mg/kg, SR-LD - *Shorea robusta*200 mg/kg, SR-HD – *Shorea robusta* 400 mg/kg.

Ample support for the intact cells had been obtained from the visible proof of photograph taken under a light microscope for the antiallergic activity using compound 48/80. The photograph clearly shows the intact and degranulated cells present in the peritoneal fluid of the rats. More intact cells are present in the standard and the treated groups where as more degranulated cells present in the control.

Antipruritic activity

Compound 48/80 induced pruritis in rats

Antipruritic activity was studied using compound 48/80 induced scratch behaviour model [16].

Subcutaneous injection of compound 48/80 elicited a significant scratching response in rat. The average scratching frequency in the 10 min after the injection of compound 48/80 was 14.3 ± 0.8433 for TEE of *S.robusta* and 31.67 ± 1.745 *W. tinctoria* at a dose of 400 mg/kg. At a lower dose of 200 mg/kg was also in good match with the standard.

This marked reduction in scratching response was comparable to that obtained with the standard drug of chlorpheniramine maleate (Table-3).

Table-3: Antipruritic activity: Effect of TEE of *W.tinctoria* and *S.robusta* on mean incidence of scratching induced by Compound 48/80

Group	Treatment	Dose (mg/kg body weight)	n	Incidence of scratching*
1	Control	0.5% sodium carboxymethyl cellulose	6	47.33 ± 1.764
2	Chlorpheniramine maleate	1mg/kg	6	25.67 ± 0.8433***
3	Ethanolic Extract of <i>Shorea robusta</i>	200 mg /kg	6	27 ± 1.211***
4	Ethanolic Extract of <i>Shorea robusta</i>	400 mg /kg	6	14.3 ± 0.8433***
5	Ethanolic Extract of <i>Wrightia tinctoria</i>	200 mg /kg	6	41.50 ± 1.478
6	Ethanolic Extract of <i>Wrightia tinctoria</i>	400 mg /kg	6	31.67 ± 1.745***

*All values are expressed as Mean ± SEM. n = 6 n = number of animals per treatment

Significantly different from control ***p<0.001

The experimental results were expressed as mean ± SEM of 6 animals in each group. The data were statistically evaluated by one-way ANOVA, followed

by Dunnet's t-test for comparison of test groups with control. Values were considered statistically significant.

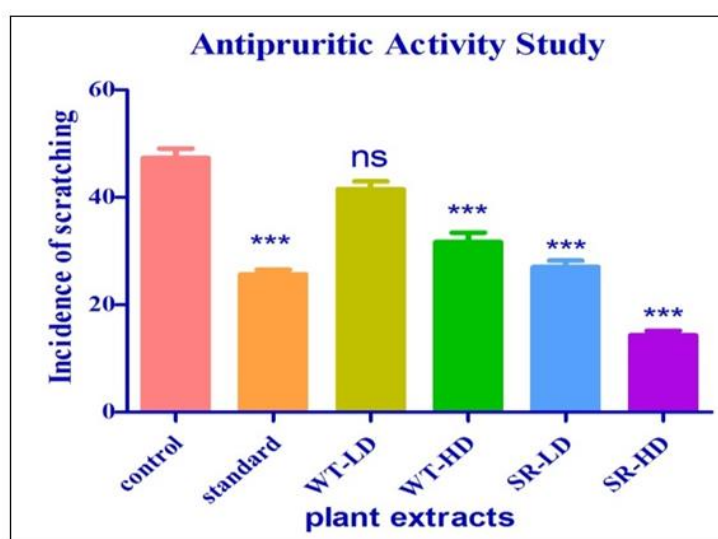


Fig-6: Scratching behaviour *S.robusta* and *W.tinctoria* induced by compound 48/80

On the basis of *in vivo* studies, it can be proved that TEE of *S.robusta* is effective against mast cell degranulation even at lower doses (sheep serum method). Whereas TEE of *W.tinctoria* is also proved to be effective at the dose of 400 mg/kg (compound 48/80). The scratching assay too showed the activity of *S.robusta*. Based on the above said events it can be proved that both the extracts especially *S.robusta* has good antiallergic and antipruritic activity.

The antiallergic and antipruritic properties of the ethanolic extracts of the plant *Shorea robusta* and *Wrightia tinctoria* taken up in our studies were evaluated using different experimental models. Analysis of results obtained revealed that significant antiallergic activities has been obtained with all the extracts comparable with that of standard drug ketotifen fumarate viz., 75% for sheep serum model and 74 % for the compound 48/80 model. Secondary metabolites which are normally present in the plant have been reported to possess antiallergic potential. A variety of *in vitro* and *in vivo* experiments have shown that

flavonoids possess antiallergic activities [17, 18]. Stimulation of mast cells with compound 48/80 or antiserum initiates the activation of signal transduction pathway which leads to histamine release. Some recent studies showed that compound 48/80 and other polybasic compounds are able to activate G proteins [19]. Antiallergic and anti-pruritic activities of *Shorea robusta* and *Wrightia tinctoria* can be attributed to the presence of the secondary metabolites like flavonoid, phenolics as well as triterpenes.

CONCLUSION

The total ethanol extract of *W.tinctoria* bark and *S.robusta* oleoresin was subjected to antiallergic and antipruritic activity. The promising activities recorded for the ethanolic extract *S.robusta* and *W.tinctoria* has much relevance to the main activity. Our results provide ample justification for the claims made in the indigenous system of medicine. This will encourage us in future for the isolation of flavonoids and triterpenes, may be the compound responsible for the activities.

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