

ANTIASTHMATIC ACTIVITY OF AQUEOUS EXTRACT OF *PISTACIA INTEGERRIMA* GALLSSURENDRA ADUSUMALLI^{1*}, PUSAPATI MADAN RANJIT², M.SANKARANARAYAN HARISH¹¹Nargund College of Pharmacy, Dattatreyanagar, Bangalore, Karnataka, India, ²NRI College of Pharmacy, Pothavarappadu (V), Agiripalli (M), Krishna Dist., Andhra Pradesh, India. *Email: surendracology@gmail.com

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ABSTRACT

Objective: Antiasthmatic activity of aqueous extract of *Pistacia integerrima* galls in various experimental models.

Methods: The mast cell stabilization, antihistaminic and spasmolytic activity of aqueous extract of galls was evaluated using albino Wistar rats and guinea pigs. The effect on mast cell stabilization was performed by *ex vivo* challenge of antigen in sensitized rat intestinal mesenteries. Antihistaminic activity was studied in guinea pigs using histamine-induced bronchospasm where preconvulsive dyspnea (PCD), was used as an end point following exposure to histamine aerosol and spasmolytic activity was studied on isolated guinea pig tracheal chain preparation where percentage inhibition in histamine induced contraction was measured.

Results: Treatment with aqueous extract of galls showed a dose dependent effect on disruption rate of actively sensitized mesenteric mast cells of albino rats when challenged with antigen (horse serum along with triple antigen vaccine). Aqueous extract of galls treatment for ten days resulted in significant protection against histamine aerosol-induced bronchospasm in guinea pigs and showed the spasmolytic activity against histamine induced contractions in isolated guinea pig tracheal chain preparation.

Conclusion: Antiasthmatic activity extract galls may be due to the membrane stabilizing potential, suppression of antibody production and inhibition of antigen induced histamine release.

Keywords: Sensitization, Mast cell disruption, Preconvulsive dyspnea (PCD), Histamine aerosol, *Pistacia integerrima*, Tracheal chain preparation.

INTRODUCTION

Asthma has been known since antiquity, yet it is a disease that still defies precise definition. The word asthma is of Greek origin and means "panting"[1]. Asthma is chronic characterized by acute exacerbation of coughing, dyspnoea, wheezing and chest tightness[2]. Airway inflammation causes various symptoms of asthma which are often associated with widespread airflow obstruction and also cause an associated increase in airway responsiveness to a variety of stimuli. Asthma causes an attack accompanied by wheezing, shortness of breath, chest tightness and coughing[3]. Though many effective drugs to treat acute symptoms of asthma are available, asthma medications are mostly taken with an inhaler which allows the medicine to reach the lungs effectively. The modern asthma therapy is the regular use of inhaled corticosteroids[4]. There are no satisfactory and completely safe drugs in market. Hence the research has been on to look back to traditional medicinal plants to treat asthma. Many plants have been alleged to have curative properties for asthma. Ayurveda, An Indian system of medicine has described several drugs from indigenous plant sources for use in the treatment of bronchial asthma and allergic disorders.

Herbal medicine is currently in the lime light and is given more popularity than ever before as sales figures in some countries, for example the USA, have risen beyond the expectations of some producers. The reasons for this change are complex but clearly are connected with what could be described as the 'greening of medicine'[6]. Plants and their extracts continue to provide effective treatment for diseases of all kinds including asthma. In Ayurveda and Chinese traditional medicine *Pistacia integerrima* galls and stem bark were used as a good remedy for the patients having asthma, cough and other allergic conditions.

Pistacia integerrima (Family. Anacardiaceae) is called as Karkatakashringi in Ayurveda and Indian traditional medicine. It consists of gall-like excrescences formed by insects on the leaves, petioles and branches of the plant. Karkatshringi is used in indigenous system of medicine (Ayurveda, Unani and Siddha) as a remedy for cough, asthma, fever, respiratory and in liver disorders[7,8]. Karkatshringi also finds usage in the treatment of children's ear infections, suppress haemorrhage from gums and used to suppress bleeding from nose [9,10]. Hakims consider galls useful in pulmonary infections, diarrhoea and vomiting [11].

In the present study the effect of aqueous extract of *Pistacia integerrima* galls was studied on the mast cell stabilization in rats, antihistaminic and spasmolytic activity in guinea pigs.

MATERIALS AND METHODS

Plant galls extract

A sample of aqueous extract of *Pistacia integerrima* galls dry powder purchased from Liala Chemiloids, Vijayawada, A.P, India, on 2/11/2009 under the trade name Karkatakashringi. The product code is C/SHP/PIIN-01; Batch number is L9101152, it was approved by QC manager.

SCREENING THE ANTI-ASTHMATIC ACTIVITY

Antiasthmatic activity of aqueous extract of *pistacia integerrima* galls was screened in animal models (Albino Wistar rats and guinea pigs) by using different methods. All experimental procedures were followed in strict accordance with the guidelines prescribed by the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA), proposal No. IAEC/NCP/09/09. This proposal was approved by Intuitional animal ethics committee (IAEC), Nargund College of Pharmacy, Bangalore, Karnataka, India, on 23/5/2009.

Mast cell stabilizing activity in rats[12-14]

All the animals (120-160 g weight animal were used for the study) were fed with standard diet and water ad-libitum. Of the five groups, six animals were taken in each group and maintained under standard laboratory conditions. The rats were sensitized by 0.5 ml of subcutaneous injection of horse serum along with 0.5 ml of triple antigen vaccine (20 thousand million organisms of *Bordetella pertussis*). Then sensitized rats were divided into 4 groups and treatment was started on the 7th day of the sensitization for 14 days according to following dose schedule. One group kept as saline control without sensitization. Group I: 0.5 ml horse serum + 0.5 ml triple antigen vaccine per subcutaneously (Sensitized control), Group II: 0.5 ml of 0.9% (w/v) saline per orally (Normal control without sensitization), Group III: Aqueous extract of *Pistacia integerrima* galls 27 mg/kg b.w.p.o., Group IV: Aqueous extract of *Pistacia integerrima* galls 54 mg/kg b.w. p.o. and Group V: Prednisolone (Used as standard for mast cell stabilizing activity) 10 mg/kg b.w.p.o.

On 14th day 2 hours after the last dose treatment rats were sacrificed and intestinal mesenteries were isolated for the study of mast cells. Mesenteries of sacrificed rats along with intestinal pieces were kept in a Ringer-Locke's solution at 37°C. Then mesenteric pieces were challenged with 5% horse serum for 10 minutes. Pieces of mesentery were stained supravitaly with toluidine blue by the following method. Tissue was first immersed in 0.1% toluidine blue in 4% aqueous formal saline for 10 minutes. The tissue was then transferred to xylene for 5-10 minutes. Finally it was rinsed with acetone twice, placed on microscope slide and stretched with the help of needles. The intestinal pieces were cut and removed. The tissue was examined under a microscope. The numbers of intact, disrupted and partially disrupted mast cells per high power field, 10X, 40X, 100X were counted. The cells in at least more than 10 such randomly selected fields from each tissue were counted.

Histamine- induced bronchospasm in guinea pigs [14-18]

All the animals were fed with standard diet and water ad-libitum. Of the four groups, six animals were taken in each group and maintained under standard laboratory conditions. Bronchospasm was induced in guinea pigs by exposing them to 1% histamine aerosol under constant pressure (160 mm Hg) in an aerosol chamber (28 x 28 x 14) made up of perplez glass. Of the four groups of six animals each, group I served as control and group II received aqueous extract of *P.integerrima* galls 23.25 mg/kg b.w. p.o. (low dose), group III received aqueous extract of *P.integerrima* galls 46.50 mg/kg b.w.p.o. (high dose) and group IV received Ketotifen (Used as standard for this method) 1 mg/kg b.w.p.o. used as standard drug once a day for 10 days.

The animals were exposed to 1% histamine aerosol under constant pressure (160 mm Hg) in an aerosol chamber on day 0 without any treatment. The end point, preconvulsive dyspnea (PCD) was determined from the time of aerosol exposure to onset of dyspnea leading to the appearance of convulsions. As soon as PCD commenced the animals were removed from the chamber and exposed to fresh air. This PCD was taken as day 0 value. On day1, day5 and day10, 2 h after administration of aqueous extract of *P.integerrima* galls, the time for the onset of PCD was recorded as on

day 0. The protection offered by the treatment was calculated by the following formula. Percentage protection = $[1 - T_1 / T_2] \times 100$. Where: T_1 is time for PCD onset on day 0 and T_2 is time for PCD onset on day 10.

Spasmolytic activity in isolated guinea pig tracheal chain preparation [19-20]

All the animals were fed with standard diet and water ad-libitum and maintained under standard laboratory conditions. Guinea pigs of either sex, weighing 250-300 g were sacrificed by cervical dislocation and carotid bleeding. The trachea was dissected out and transferred to a dish containing kerb's solution (composition (g/l): NaCl (6.8), KCl (0.35), CaCl₂ (0.28), MgSo₄7H₂O (0.25), NaHCO₃ (2.1), KH₂PO₄ (0.16) and glucose (2.0)) and cut transversely between the segments of the cartilage so as to give a number of rings of the trachea. About 5-6 rings these were tied to form a chain of approximately 4-5 cm length, which was in kerb's solution, contained in an organ bath maintained at 37°C and continuously aerated with carbogen (95% O₂+5% CO₂). One end of the tracheal chain was attached to a tissue holder at the base of organ bath and the other end to a frontal lever; the responses were recorded on a slow moving kymograph.

The suspended tracheal was allowed to stabilize for at least 30 minutes. During stabilization, the bath was supplied with fresh kerb's solution ones per every 15 minutes. Then cumulative concentration response to histamine in the absence and presence of aqueous extract of *P.integerrima* galls were recorded with a slow moving (0.25 mm/sec) kymograph.

Statistical Analysis

The analysis was performed using Graph pad Prism software version 5. The results of various studies were expressed as mean \pm SEM. Data analyzed using one-way ANOVA, followed by Dunnet's Multiple Comparison Test in mast cell stabilizing activity, students paired-T-test in histamine induced bronchospasm in guinea pigs to find out the level of significance. $P < 0.05$ was considered statistically significant.

Table 1: Showed the mast cell stabilizing activity in rats.

Group	Treatment	Mast cells (%)		
		Intact	Disrupted	Partially Disrupted
I	Sensitized control (0.5ml horse serum+ 0.5ml triple antigen vaccine)	2.048 \pm 0.582	90.75 \pm 1.812	7.202 \pm 1.356
II	Control (0.5ml of 0.9% NaCl)	79.89 \pm 0.725 ***	8.88 \pm 0.678 ***	11.23 \pm 0.746 ns
III.	<i>P.integerrima</i> treated (27 mg/kg b.w.p.o.)	26.11 \pm 4.840 ***	30.73 \pm 1.420 ***	43.16 \pm 4.044 ***
IV	<i>P.integerrima</i> treated (54 mg/kg b.w.p.o.)	56.16 \pm 2.073 ***	22.46 \pm 2.640 ***	21.38 \pm 1.664 ***
V	Prednisolone.Treated (10mg/kg b.w.p.o.)	68.01 \pm 2.008 ***	17.65 \pm 1.127 ***	14.34 \pm 1.946 ns

Values represents mean \pm SEM, n = 6 in each group, (Data analysed by One-way ANOVA Followed by Dunnet's Multiple Comparison Test), significantly different from Sensitized Control group *** $P < 0.001$, ns- non significant.

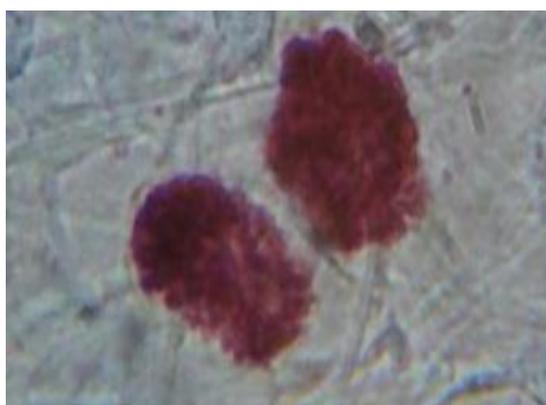


Fig. 1a: Aqueous extract of *Pistacia integerrima* galls treated group and prednisolone treated group shows more no of intact mast cells when compared to sensitized control group and other groups.

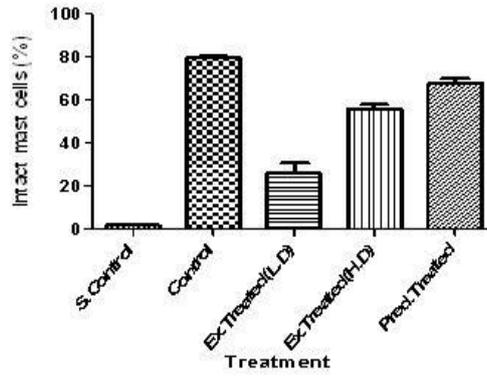


Fig. 1b: Percentage of intact mast cells in different groups of rats in mast cell stabilizing activity model.

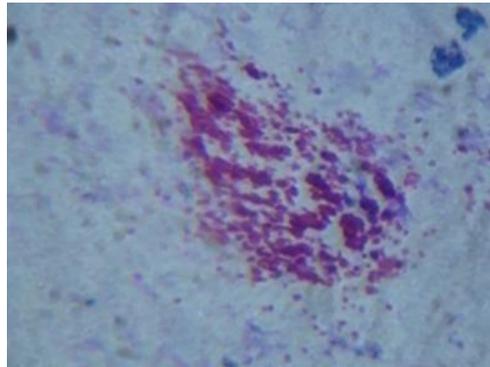


Fig. 2a: Sensitized control group shows more no of disrupted mast cells when compared to the other groups.

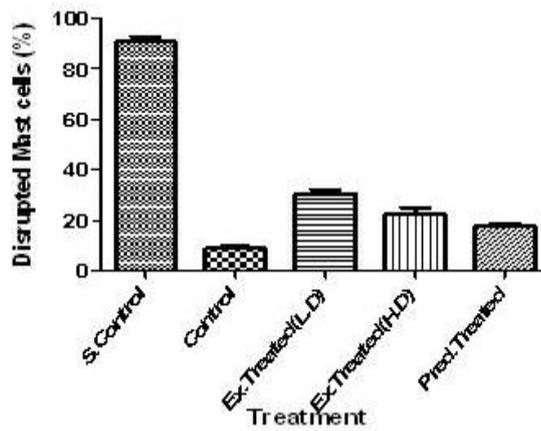


Fig. 2b: Percentage of disrupted mast cells in different groups of rats in mast cell stabilizing activity model.

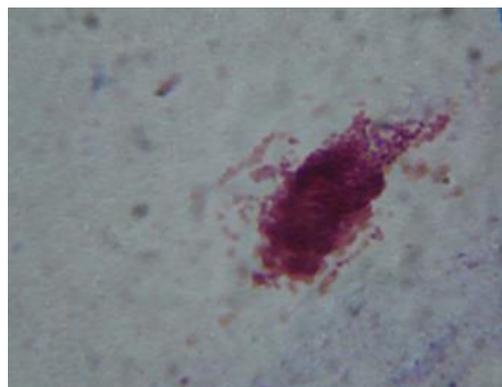


Fig. 3a: Aqueous extract of *Pistacia integerrima* galls (low dose) treated group shows the more no of partially disrupted mast cells when compared to Sensitized control group and other groups.

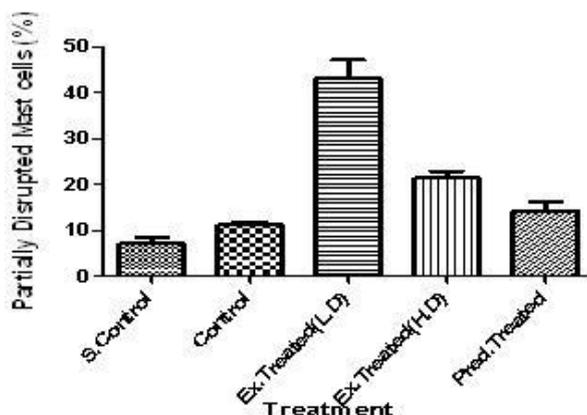


Fig. 3b: Percentage of partially disrupted mast cells in different groups of rats in mast cell stabilizing activity model.

RESULTS

Mast cell stabilizing activity in rats

One week after sensitization, the antigen challenge disrupted about 90% of the mast cells. When sensitized animals treated with aqueous extract of *Pistacia integerrima* galls (27 and 54 mg/kg p.o.) for two weeks and then challenged with an antigen there was a significant reduction in the number of disrupted mast cells. Table 1 showed the effect of aqueous extract of *Pistacia integerrima* galls at 54mg/kg was well comparable with that of prednisolone. Fig 1a and Fig 1b showed aqueous extract of *Pistacia integerrima* galls treated group and prednisolone treated group shows more no of intact mast cells when compared to sensitized control group and other groups, percentage of intact mast cells in different groups of rats in mast cell stabilizing activity model respectively. In another Fig 2a and Fig 2b showed sensitized control group shows more no of disrupted mast cells when compared to the other groups, percentage of disrupted mast cells in different groups of rats in mast cell stabilizing activity

model respectively. Like this Fig 3a and Fig 3b aqueous extract of *Pistacia integerrima* galls (low dose) treated group shows more no of partially disrupted mast cells when compared to sensitized control group and other groups, percentage of partially disrupted mast cells in different groups of rats in mast cell stabilizing activity model respectively.

Histamine- induced bronchospasm in guinea pigs

Table 2 showed aqueous extract of *Pistacia integerrima* galls significantly prolonged the latent period of convulsion as compared to control following exposure to histamine aerosols. Fig 4a, Fig 4b and Fig 4c showed the increase in the PCD time in aqueous extract of *Pistacia integerrima* galls (low dose) treated group when compared to controls, the increase in the PCD time in aqueous extract of *Pistacia integerrima* galls (high dose) treated group when compared to controls, the increase in the PCD time in ketotifen (used as a standard drug) treated group when compared to controls respectively.

Table 2: Showed histamine induced bronchospasm in guinea pigs.

Group	Treatment	PCD time (Sec) before treatment		Protection (%)
		On day 0	On day 10	
I	<i>P.integerrima</i> Treated (23.25 mg/kg b.w.p.o.)	70.50 ± 3.274	178.7 ± 11.31***	60.55
II	<i>P.integerrima</i> Treated (46.50 mg/kg b.w. p.o.)	75.83 ± 2.822	211.7 ± 14.93***	64.19
III	Ketotifen Treated (1mg/kgb.w.p.o)	96.17 ± 7.560	279.3 ± 13.93***	65.57

Values represents mean ± SEM, n = 6 in each group, significantly different from Control group. (Data analysed by Students paired -T-test), ***P < 0.001. Preconvulsive dyspnea (PCD).

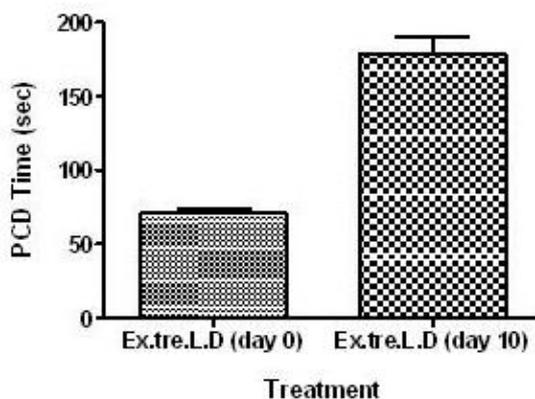


Fig. 4a: The increase in the PCD time in aqueous extract of *Pistacia integerrima* galls (low dose) treated group when compared to controls.

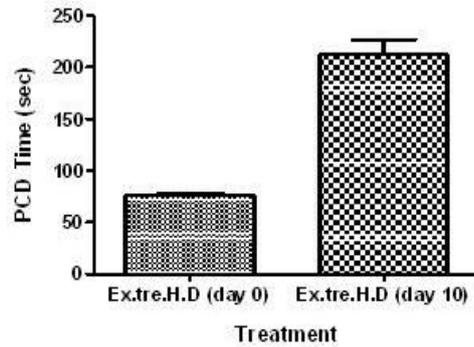


Fig. 4b: The increase in the PCD time in aqueous extract of *Pistacia integerrima*galls (high dose) treated group when compared to controls.

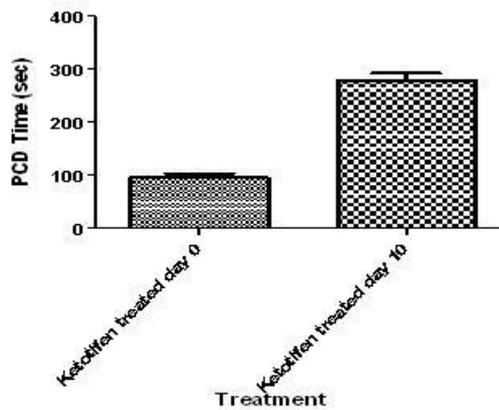


Fig. 4c: The increase in the PCD time in Ketotifen (used as a standard drug) treated group when compared to controls.

Spasmolytic activity in isolated guinea pig tracheal chain preparation

Table 3 showed the aqueous extract of *P.integerrima* galls showed complete antagonism against histamine induced

contractions in guinea pig tracheal chain preparation. Fig 5 showed the effect of aqueous extract of *Pistacia integerrima* galls on histamine induced contractions in isolated guinea pig tracheal chain preparation.

Table 3: Showed spasmolytic activity on isolated guinea pig tracheal chain preparation.

Treatment	0.2ml Hist.	0.1ml Ex. +	0.2ml Hist.	0.2ml Ex. +	0.2ml Hist.	0.4ml Ex. +	0.2ml Hist.	0.2ml Hist.
Mean contraction response in mm	8	6.0		4.5		0		7.5
Percentage (%) inhibition in contraction response	-	25		43.75		100		

Hist. = Histamine hydrochloride, Ex. = Aqueous extract of *Pistacia integerrima* galls.

Data showing the effect of aqueous extract of *Pistacia integerrima* galls on histamine induced contractions in isolated guinea pig tracheal chain preparation.

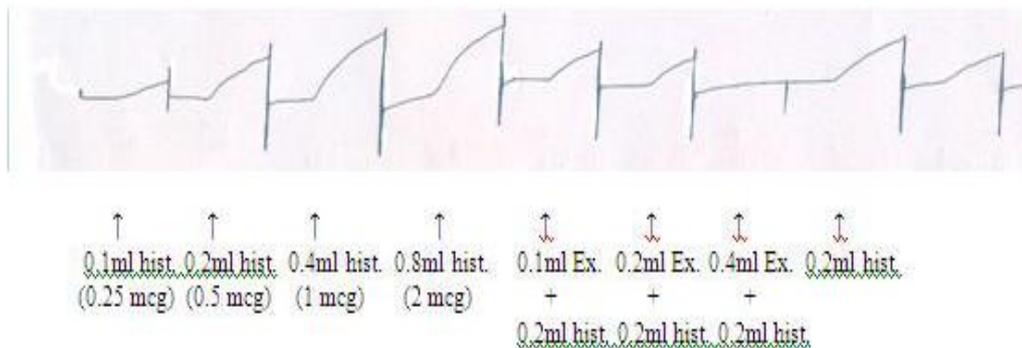


Fig. 5: Effect of aqueous extract of *Pistacia integerrima* galls on histamine induced contractions in isolated guinea pig tracheal chain preparation.

CONCLUSION

All these findings revealed the antiasthmatic activity of aqueous extract of *P.integerrima* galls. Previous studies are reported that, presence of glycosides, flavonoids, tannins and phenolic constituents in aqueous extract of *P.integerrima* galls[21]. Some other studies are also reported are methanol extract (ME) and ethyl acetate fraction of methanol extract (EAFME) of *P. integerrima* was rich in phenolic and flavonoid content, it showed *in vitro* antioxidant activity of galls[22]. Our conclusion is antiasthmatic activity extract galls may be due to the presence of rich content of phenolic chemical constituents, these chemical constituents are may responsible of these membrane stabilizing potential, suppression of antibody production inhibition of antigen induced histamine release and showed spasmolytic activity on isolated guinea pig tracheal chain. Further studies are required to find out mechanism at molecular level and whose chemical constituents are responsible for this antiasthmatic activity.

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