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ANTI ASTHMATIC ACTIVITY OF AQUEOUS EXTRACT OF MYRICA NAGI BARK

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ABSTRACT

Antiasthmatic activity of aqueous extract of *Myrica nagi* barks in various experimental models. The mast cell stabilization, antihistaminic and spasmolytic activity of aqueous extract of bark 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 dyspnoea (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. Treatment with aqueous extract of bark showed a dose dependent (at 27 and 54 mg/kg p.o.) 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 bark 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. Antiasthmatic activity of bark extract may be possibly 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, *Myrica nagi*, 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 a chronic inflammatory disorder of the airway [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 [3,4]. 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 cornerstone of modern asthma therapy is the regular use of inhaled corticosteroids [5]. There are no satisfactory and completely safe drugs in market. Hence the research has been on to look back to traditional medicinal plant to treat asthma. Many plants have been alleged to have curative properties for asthma.

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 *Myrica nagi* bark were used as a good remedy for the patients having asthma, cough and other allergic conditions.

Myrica nagi (Fam. Myricaceae) is a subtropical shrub commonly known as Box berry. The medicinal uses and chemical constitutes of *M. nagi* have been widely studied [7,8,9,10]. The constituents of *M. nagi* have been shown to inhibit toxicity in a number of animal model systems [11,12]. A number of the chemical constitutes of *M. nagi* have been identified as strong antioxidants [7], and a number of pharmacological effects of *M. nagi* have been reported [11,12,13]. It has been traditionally used for the treatment of various disorders such as liver diseases, fever, asthma anemia, chronic dysentery, ulcer and inflammation. In the present study the effect of aqueous

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extract of *Myrica nagi* bark was studied on the mast cell stabilization in rats, antihistaminic and spasmolytic activity in guinea pigs.

MATERIALS AND METHODS

Plant bark extract

A sample of aqueous extract of *Myrica nagi* bark dry powder purchased from Liala Chemiloids, Vijayawada, A.P, India, under the trade name Kaiphal. The product was approved by QC manager.

SCREENING THE ANTIASTHMATIC ACTIVITY

Antiasthmatic activity of aqueous extract of *Myrica nagi* bark 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. SU/DPS/IAEC/1005. This proposal was approved by Intuitional animal ethics committee (IAEC), Department of Pharmaceutical Sciences, Saurashtra University, Rajkot, Gujarat, India.

Mast cell stabilizing activity in rats [14, 15]

All the animals (Albino Wistar rats, either sex with weight of (120-160g) 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.

Bronchospasm was induced in guinea pigs by exposing them to 1% histamine (Sigma-Aldrich) aerosol under constant pressure (160 mm Hg) in an aerosol chamber (28 x 28 x 14) made up of perplex glass. The four groups of six animals each, group I served as control and group II received aqueous extract of *M. nagi* bark 23.25 mg/kg b.w.p.o. (low dose), group III received aqueous extract of *M. nagi* bark 46.50 mg/kg b.w.p.o. (high dose) and group IV received Ketotifen 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 day 1, day 5 and day 10, 2 h after administration of aqueous extract of *M. nagi* bark, 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 [16,17,18]

All the animals (Albino guinea pigs, either sex with Weight of 200-450g) were fed with standard diet and water ad-libitum and maintained under standard laboratory conditions.

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Spasmolytic activity in isolated guinea pig tracheal chain preparation [16, 17, 18]

All the animals (Albino guinea pigs, either sex with Weight of 200-450g) 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), $CaCl_2$ (0.28), $MgSo_47H_2O$ (0.25), $NaHCO_3$ (2.1), KH_2PO_4 (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% O_2+5% CO_2). 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 *M. nagi* bark 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 ± 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.

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 *Myrica nagi* bark (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 *Myrica nagi* bark at 54mg/kg was well comparable with that of prednisolone. Figure 1a and Figure 1b showed aqueous extract of *Myrica nagi* bark 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 Figure 2a and Figure 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 Figure 3a and Figure 3b aqueous extract of *Myrica nagi* bark (low dose) treated group shows the 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 *Myrica nagi* bark significantly prolonged the latent period of convulsion as compared to control following exposure to histamine aerosols. Figure 4a, Figure 4b and Figure 5 showed the increase in the PCD time in aqueous extract of *Myrica nagi* bark (low dose) treated group when compared to controls, the increase in the PCD time in aqueous extract of *Myrica nagi* bark (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.

Figure 1(a). Aqueous extract of Myrica nagi bark treated group and prednisolone treated group shows more no of intact mast cells when compared to sensitized control group and other groups

Figure 1(b). Percentage of intact mast cells in different groups of rats in mast cell stabilizing activity model

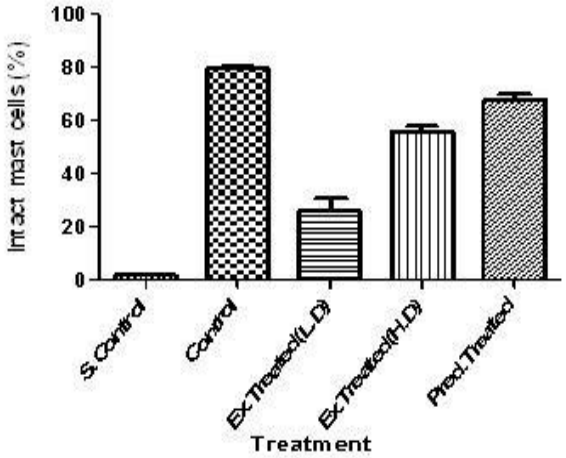

Figure 2(a). Sensitized control group shows more no of disrupted mast cells when compared to the other groups

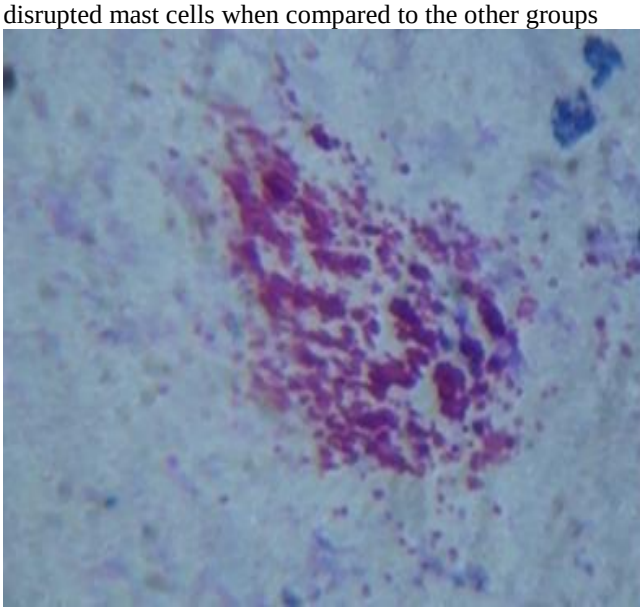

Figure 2(b). Percentage of disrupted mast cells in different groups of rats in mast cell stabilizing activity model

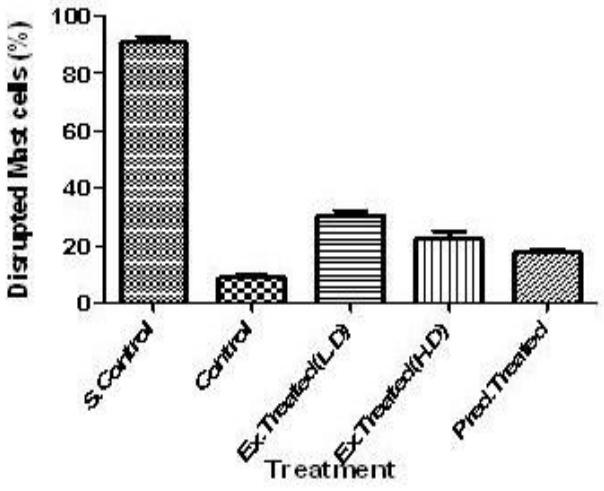

Figure 3(a). Aqueous extract of Myrica nagi bark (low dose) treated group shows the more no of partially disrupted mast cells when compared to Sensitized control group and other groups

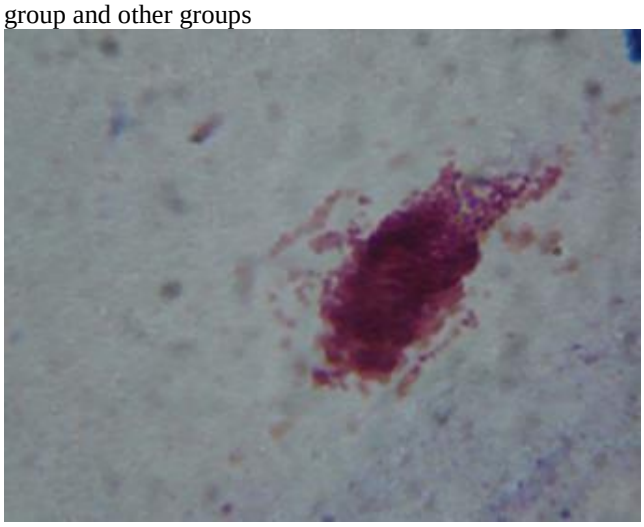

Figure 3(b). Percentage of partially disrupted mast cells in different groups of rats in mast cell stabilizing activity model

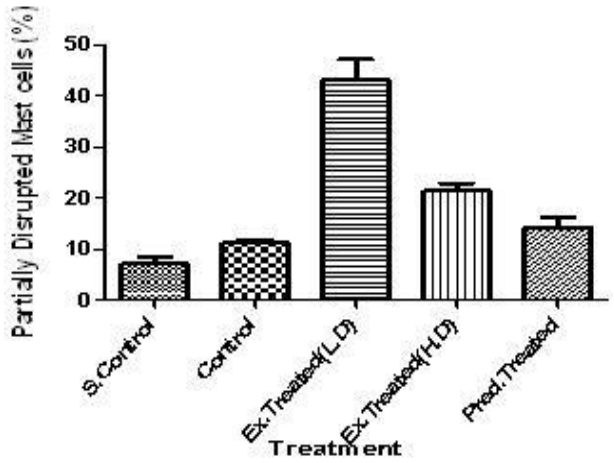

Figure 4(a). The increase in the PCD time in aqueous extract of Myrica nagi bark (low dose) treated group when compared to controls

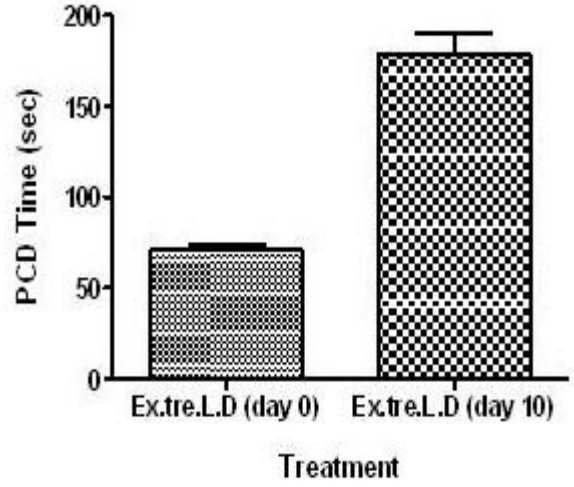

Figure 4(b). The increase in the PCD time in aqueous extract of Myrica nagi bark (high dose) treated group when compared to controls

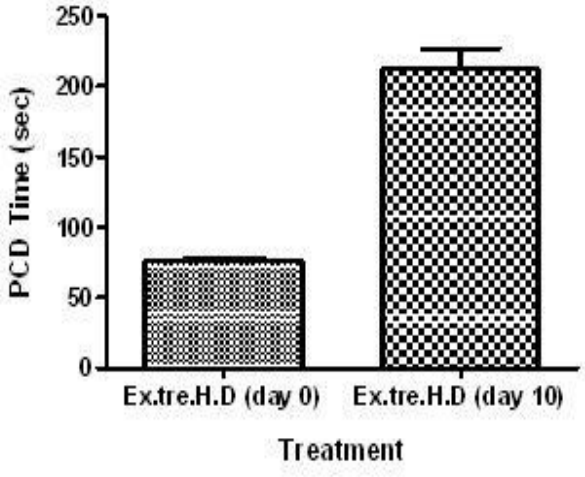

Figure 5. The increase in the PCD time in Ketotifen (used as a standard drug) treated group when compared to controls

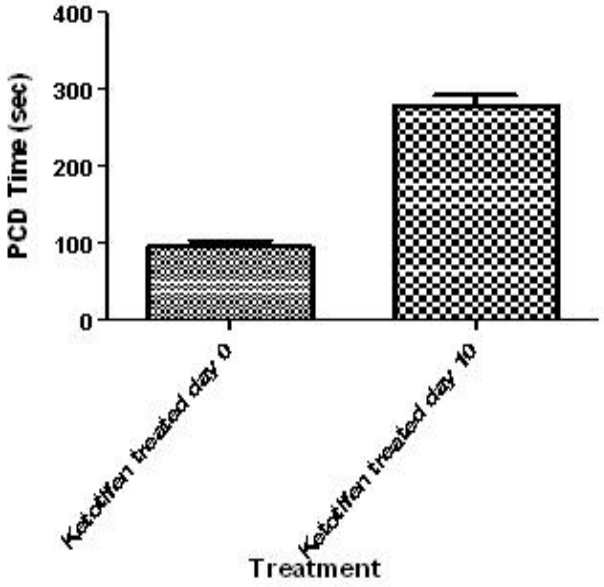

Figure 6. Effect of aqueous extract of Myrica nagi bark on histamine induced contractions in isolated guinea pig tracheal chain preparation

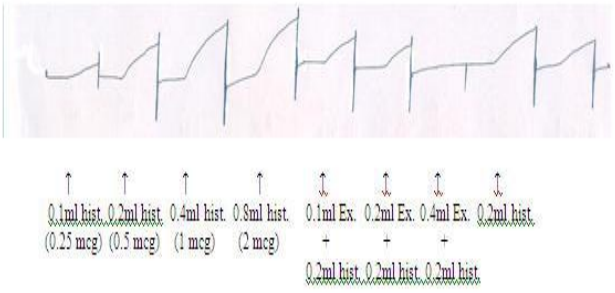

Table 1. Showed the Mast cell stabilizing activity in rats. Values represents mean ± SEM, n = 6 in each group, (Data analysed by One-way ANOVA Followed by Dunnet's Multiple Comparison Test), significantly different from Sensitized Control group ***P < 0.001, ns- non significant

Group	Treatment	Mast cells (%)		
		Intact	Disrupted	Partially Disrupted
I	Sensitized control (0.5ml horse serum + 0.5ml triple ntigen vaccine)	2 .048 ± 0.582	90.75 ± 1.812	7.202 ± 1.356
II	Control (0.5ml of 0.9% Nacl)	79.89 ± 0.725 ***	8.88 ± 0.678 ***	11.23 ± 0.746 ns
III	*M. nagi* treated (27 mg/kg b.w.p.o.)	26.11 ± 4.840***	30.73 ± 1.420***	43.16 ± 4.044***
IV	*M. nagi* treated (54 mg/kg b.w.p.o.)	56.16 ± 2.073***	22.46 ± 2.640***	21.38 ± 1.664***
V	Pred. Treated (10mg/kg b.w.p.o.)	68.01 ± 2.008***	17.65 ± 1.127***	14.34 ± 1.946 ns

Table 2. Showed histamine induced bronchospasm in guinea pigs. 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)

Group	Treatment	PCD time (Sec)	before treatment	Protection (%)
		On day 0	On day 10	
I	*M. nagi* Treated (23.25 mg/kg b.w.p.o.)	70.50 ± 3.274	178.7± 11.31***	60.55
II	*M. nagi* 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

Table 3. Showed spasmolytic activity on isolated guinea pig tracheal chain preparation. Data showing the effect of aqueous extract of *Myrica nagi* bark on histamine induced contractions in isolated guinea pig tracheal chain preparation

Treatment	0.2ml hist.	0.1ml Ex. +	0.2ml Ex. +	4ml Ex. +	0.2ml hist.
			0.2ml hist.	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	0

Hist. = Histamine hydrochloride, Ex. = Aqueous extract of *Myrica nagi* bark

Spasmolytic activity in isolated guinea pig tracheal chain preparation

Table 3 showed the aqueous extract of *M. nagi* bark showed complete antagonism against histamine induced contractions in guinea pig tracheal chain preparation. Figure 6 showed the effect of aqueous extract of *Myrica nagi* bark on histamine induced contractions in isolated guinea pig tracheal chain preparation.

DISCUSSION

All these findings reveal the antiasthmatic activity of aqueous extract of *M. nagi* bark may the presence of rich content flavonoids and phenolic constituents. Methanol extract (ME) and ethyl acetate fraction of methanol extract (EAFME) of *M. nagi* was rich in phenolic and flavonoid content, it showed in vitro antioxidant activity of bark [19]

CONCLUSION

Further studies are required to find out mast cell stabilizing activity, bronchial smooth muscle relaxation mechanism at molecular level and which chemical constituents is responsible of this antiasthmatic activity.

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