

## Full Length Research Paper

# Mast cell stabilizing activity of *Myrica nagi* bark

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Accepted 9 May, 2011

***Myrica nagi* Hook. (Myricaceae), commonly known as box berry (English), possesses diverse pharmacological activities in animals, little is known about its mast cell stabilizing activity. The present study evaluated the mast cell stabilizing activity of ethyl acetate and aqueous extracts of bark of *M. nagi* using compound 48/80 and egg albumin induced allergy tests. Adult Wistar albino rats were subjected to compound 48/80 and egg albumin induced allergy tests. The effects of ethyl acetate and aqueous extract at 100 and 200 mg/kg showed slightly better protection of mast cell degranulation 45 and 62% respectively, than the standard drug prednisolone (65%) in egg albumin induced degranulation of mast cells in rats. These extracts also showed better mast cell stabilizing activity 70 and 78% respectively, than the standard drug (65%) when peritoneal mast cells are treated with compound 48/80. The phytochemical screening revealed the presence of flavonoids and steroids. The results of the study for the first time show that the plant possesses mast cell stabilizing activity, confirming the traditional claims. Future research should focus on the identification and the mast cell stabilizing activity of the constituents from this plant.**

**Key words:** Mast cell stabilization, *Myrica nagi*, compound 48/80, egg albumin.

## INTRODUCTION

Mast cells are constituents of virtually all organs and tissues and are important mediators of inflammatory responses such as allergy and anaphylaxis (Church and Levi-Schaffer, 1997) in which histamine remains the best-characterized and most potent vasoactive mediator implicated in the acute phase of immediate hypersensitivity upon release (Petersen et al., 1996). Mast cell degranulation can also be evoked by the compound 48/80, which is a mast cell degranulator and has been used as a direct and convenient reagent to study the mechanism of anaphylaxis (Ennis et al., 1980). The influence of natural products derived from plants is broadly recognized for their great structural diversity as well as their wide range of pharmaceutical activities (Mukherjee, 2001). *Myrica nagi* Hook (syn. *Myrica esculenta* Buch. and Ham) (Myricaceae) is a subtropical shrub commonly known as box berry. The medicinal uses and chemical constituents of *M. nagi* have been widely studied (Malterud et al., 1996). The constituents of *M. nagi* have been shown to inhibit toxicity in a number of animal model systems (Rastogi and Mehrotra, 1995; Chopra et al., 1996). A number of the chemical constituents of *M. nagi* have been identified as strong

antioxidants (Malterud et al., 1996), and a number of pharmacological effects of *M. nagi* have been reported (Rastogi and Mehrotra, 1995; Chopra et al., 1996; Mathiesen et al., 1997). It has been traditionally used for the treatment of various disorders such as liver diseases, fever, asthma, anemia, chronic dysentery, ulcer and inflammation (Nadkarni et al., 1954; Rastogi and Mehrotra, 1995). *M. nagi* bark contains gallic acid, myricanol, myricanone, epigallocatechin 3-*O*-gallate, two prodelphinidin dimers [epigallocatechin-(4 $\beta$ →8)-epigallocatechin 3-*O*-gallate and 3-*O*-galloyl epigallocatechin-(4 $\beta$ →8)-epigallocatechin 3-*O*-gallate], and the hydrolyzable tannin castalagin (Sum et al., 1988). Considering the available information and folklore use of the plant, the present study was designed to evaluate the mast cell stabilizing activity of the ethyl acetate and aqueous extracts of bark of *M. nagi* using experimental models.

## METHODS

### Collection of plant materials

Barks of *M. nagi* (MN) were purchased from a local market. The plant was identified and authenticated by S. Kitchlu, Indian institute of integrative medicine (CSIR), Jammu, India.

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A voucher specimen (SU/DPS/Herb/32) of the same has been deposited in the Department of Pharmaceutical Sciences, Saurashtra University, Rajkot for future reference.

### Preparation of plant extract

Barks were dried in shade, moderately ground by electric grinder and subjected to soxhlet extraction using ethyl acetate and later solvent was evaporated at reduced pressure to afford ethyl acetate extract (yield-5.2% w/w). *M. nagi* aqueous extract was obtained by boiling fresh powder in distilled water (100°C) and later by evaporating water from the decanted portion under reduced pressure (yield-29.4% w/w). The extracts were stored in refrigerator and prepared freshly in sodium carboxy methyl cellulose (SCMC) solution just before the experiments.

### Experimental animals

Male Wistar albino rats (250 to 300 g) were subjected to compound 48/80 and egg albumin induced allergy tests (n=5, in each group). All the animals were housed in groups in polypropylene cages and placed in climate controlled central animal house having temperature  $22 \pm 2^\circ\text{C}$ , relative humidity  $60 \pm 5\%$ , and a 12 h light/dark cycle (lights on at 08:00 h and off at 20:00 h). The animals were fed with standard pellet diet (Amrut, Pranav Agro Industries Ltd, India) and water *ad libitum*. All the protocols were approved (approval no-SU/DPS/IAEC/1005) by Institutional Animal Ethics Committee (IAEC) of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India.

### Administration of drugs

Disodium cromoglycate and prednisolone were dissolved in distilled water. While ethyl acetate and aqueous extract were prepared as suspension in distilled water using 0.5% SCMC as the suspending agent. Animals were assigned to different treatment groups (n=5, in each group). The control group received 0.5% SCMC, (1 ml/kg, p.o.), whereas test groups received ethyl acetate and aqueous extract. All drugs, ethyl acetate and aqueous extract were prepared before 1.00 h of experimentation. All the doses of extracts were administered orally.

### Acute toxicity study

The acute toxicity study was performed as per the method described by Litchfield and Wilcoxon (1949) and  $LD_{50}$  was calculated accordingly. Briefly, the Ethyl acetate and aqueous extract in the dose range of 10-1600 mg/kg were administered orally to different groups of mice (n = 10). The animals were examined every 30 min up to a period of 3 h and then, occasionally for additional period of 4 h; finally, overnight mortality was recorded. All tests on rats were performed at three dose levels 100 and 200 mg/kg, p.o. body weight corresponding to 10 and 20% of  $LD_{50}$  value (1000 mg/kg, i.p.), respectively.

### Compound 48/80 induced allergy test

Compound 48/80 induced allergy test (Lee et al., 1990; Loeffler et al., 1971) was performed on healthy adult albino rats. The animals were sensitized by subcutaneous injection of compound 48/80, 1 mg/kg body weight. This sensitized animals were divided into 6 groups (n=5 in each group). Control group received 0.5% SCMC

solution (1 ml/kg, p.o.). Positive control group received standard drug disodium cromoglycate (50 mg/kg, p.o.). The test groups received different doses (100 and 200 mg/kg) of ethyl acetate extract and aqueous extract respectively for 14 days. On the 14th day, 2 h after the assigned treatment, 10 ml of normal saline was injected into the peritoneal cavity of rats, after a gentle massage, the peritoneal fluid was collected and transferred into the siliconised test tubes containing 7 to 10 ml of RPMI-1640 medium (Roswell Park Memorial Institute medium-1640) (pH 7.2 to 7.4). Mast cells were purified by Percoll method (Yurt et al., 1977). Mast cells were washed thrice by centrifugation at low speed (400 to 500 rpm) followed by discarding the supernatant and taking the pellet of mast cells into the medium. These cells were purified and incubated with compound 48/80 (5  $\mu\text{g/ml}$ ) at  $37^\circ\text{C}$  for 10 min. After incubation, these cells were spinned and stained with 0.1% toluidine blue and observed under a microscope.

### Egg albumin induced allergy test

A method described by Norton (1954) was used. The rats were sensitized by 0.1 ml of 1% w/v egg albumin solution intraperitoneally as well as subcutaneously on the first, third, fifth and twelfth day of first egg albumin administration. The animals were administered drug as per the following schedule from sixth day to twelfth day.

Group I Control (0.5 % sodium CMC),  
Group II Prednisolone (2 mg/kg. p.o.),  
Group III Ethyl acetate extract of MN (100 mg/kg),  
Group IV Ethyl acetate extract of MN (200 mg/kg),  
Group V Aqueous extract of MN (100 mg/kg),  
Group VI Aqueous extract of MN (200 mg/kg).

On Day 12, animals were again exposed to 0.05 ml of 1% egg albumin. The animals were sacrificed with ether on the twelfth day after one hour of oral administration. Pieces of mesentery with connecting lobes of fat and blood vessels were rapidly dissected out and placed in Ringer-Locke solution (NaCl 0.9%, KCl 0.042%,  $\text{CaCl}_2$  0.024%,  $\text{NaHCO}_3$  0.015% and dextrose 0.1%) in different Petri dishes with labels and then treated with 0.1 ml of 1% w/v solution of egg albumin for 20 min. All these processes were done at room temperature. Then all these mesenteries were transferred in separately labeled Petri dishes containing 10% formaldehyde solution for 24 h to stop further physiological changes in mast cell structure. Then these mesenteries were stained with 0.1% toluidine blue for 10 min. Then these mesenteries were wash with 95% alcohol to remove traces of water and then with xylene. Usually five pieces of mesentery were used for each concentration of drug. Each piece was observed under microscope with 45 $\times$  and 10 $\times$  magnification and 100 cells were counted and from it, percentage protection from degranulation was calculated.

### Statistical analysis

All the data were expressed as mean  $\pm$  SEM from five animals. The data obtained was analyzed using the one-way ANOVA followed by Dunnett Multiple Comparisons Test for determining the level of significance and  $p < 0.05$  was considered statistically significant.

## RESULTS

### Acute toxicity studies

The acute toxicity studies showed that the  $LD_{50}$  of the

**Table 1.** Effect of different extracts of MN on compound 48/80 induced mast cell degranulation<sup>a</sup>.

Treatment	Dose	Mast cells	
	(mg/kg, p.o.)	Intact cell (%)	Disrupted cell (%)
Control	---	19.20 ± 0.80	80.80 ± 0.80
Disodium cromoglycate	50 <sup>b</sup>	65.80 ± 0.58**	34.20 ± 0.58**
Ethyl acetate extract	100	70.00 ± 0.70**	30.00 ± 0.70**
Ethyl acetate extract	200	75.60 ± 1.50**	24.40 ± 1.50**
Water extract	100	78.60 ± 0.50**	21.40 ± 0.50**
Water extract	200	79.80 ± 0.37**	20.20 ± 0.37**

<sup>a</sup>Values are expressed as mean ± SEM (n = 5). <sup>b</sup>intraperitoneal route \*\*p < 0.01; compared with control (one-way ANOVA followed by Dunnett Multiple Comparisons test)

**Table 2.** Effect of different extracts of MN on egg albumin induce mast cell degranulation<sup>a</sup>.

Treatment	Dose	Mast cells	
	(mg/kg, p.o.)	Intact cell (%)	Disrupted cell (%)
Control	---	25.33 ± 1.17	74.66 ± 1.17
Prednisolone	2 <sup>b</sup>	65.33 ± 1.52**	34.66 ± 1.52**
Ethyl acetate extract	100	45.66 ± 1.64**	54.33 ± 1.64**
Ethyl acetate extract	200	62.33 ± 1.22**	37.67 ± 1.22**
Water extract	100	44.33 ± 1.14**	55.66 ± 1.14**
Water extract	200	58.33 ± 1.56**	41.66 ± 1.56**

<sup>a</sup>Values are expressed as mean ± SEM (n = 5). <sup>b</sup>intraperitoneal route, \*\*p < 0.01, compared with control (one-way ANOVA followed by Dunnett Multiple Comparisons test).

ethyl acetate and aqueous extracts in mice was 1000 mg/kg by i.p. route. Preliminary phytochemical tests indicated the presence of flavonoids and steroids in the plant.

### Compound 48/80 induced allergy test

As compared to the control group, the pretreatment with ethyl acetate and aqueous extract of *M. nagi* (100 and 200 mg/kg, p.o.) showed significant better protections against the compound 48/80 induced degranulation of mast cells in a dose dependent manner as shown in Table 1. The *M. nagi* at 200 mg/kg shows protection similar to those of the standard group.

### Egg albumin induce allergy test

As compared to the control group, the ethyl acetate and aqueous extract of *M. nagi* bark showed (100 and 200 mg/kg, p.o.) a dose-dependent significantly better mast cells protection (p < 0.01) as shown in Table 2. In the same experimental conditions, the egg albumin induced allergy test of the reference drug prednisolone (2 mg/kg, i.p.) was clearly evident (p < 0.01).

## DISCUSSION

The mast cell has a prominent role in the pathogenesis of asthma. However, current asthma therapies inadequately target the mast cell. Prospective approaches, highlighted by the emergence of anti-IgE therapies, target the mast cell more effectively, especially in asthma that has an allergic basis. As such, future strategies aimed at impeding the activity and expression of mast cells could be a valuable approach to treat asthma.

*M. nagi* is traditionally used for the treatment of liver diseases, fever, asthma, anemia, chronic dysentery, ulcer, and inflammation (Nadkarni et al., 1954; Rastogi and Mehrotra, 1995). Scientific data on these properties of the plant are not available. Therefore, we investigated the effects of different doses of ethyl acetate and aqueous extract using compound 48/80 and egg albumin induces allergy test models. Mast cells play a key role in the immediate type of allergic reactions through the release of numerous mediators and cytokines. Mast cell degranulation also can be elicited by the synthetic compound 48/80 and it has been used as a direct and convenient reagent to study the mechanism of anaphylaxis (Ennis et al., 1980). Numerous reports established that stimulation with compound 48/80 or IgE initiates the activation of signal transduction pathway

which leads to histamine release. Several recent studies shown that compound 48/80 and other polybasic compounds directly activate G-proteins (Mousil et al., 1990). Compound 48/80 increases the permeability of the lipid bilayer membrane by causing the perturbation in the membrane. The intracellular calcium pathways are crucial to the degranulation of mast cells. Agents that stimulate an intracellular calcium level have been shown to induce mast cell degranulation (Tasaka et al., 1986). Calcium movements in mast cells represent a major target for effective anti-allergic drugs, as this is an essential event linking stimulation to secretion. Most of the studies of plant extracts and flavonoids as an anti-allergic agents showed that attenuation of compound 48/80 induced intracellular calcium in mast cells was strongly speculated that decreased intracellular calcium involved in the inhibitory effect of histamine release and might have membrane stabilizing activity through inhibition of G-protein activation (Shina et al., 2005). Here, the reference standard used was disodium cromoglycate in case of compound 48/80 induce mast cell degranulation; it is a well known mast cell stabilizer. It reduces synthesis of Prostaglandin E<sub>2</sub>, Thromboxane A<sub>2</sub>, Leukotriene C<sub>4</sub> and B<sub>4</sub> etc. Also it inhibits release of histamine, serotonin and other inflammatory mediators. Simultaneously it blocks H<sub>1</sub> receptors. Prednisolone was used as reference standard in case of egg albumin induce allergy test, which increase the production of anti-inflammatory mediators such as Lipocortin 1, endopeptidases and endonucleases. It also reduces the synthesis and release of several proinflammatory cytokines such as IL-1, GM-CSF, IL-3, IL-4, IL-5, IL-6 and IL-8; reducing inflammatory cell activation, recruitment and infiltration and decreasing vascular permeability (Lee et al., 1990; O'Bryne., 1997).

## Conclusions

The present study for the first time provides evidence for the mast cell stabilization activity of ethyl acetate and aqueous extract in experimental animals. The presence of flavonoids and steroids in ethyl acetate and aqueous extract could be responsible for these activities. The need of the hour is to identify and isolate the phytoconstituents responsible for the observed central effects in animals and to understand their molecular mechanisms.

## ACKNOWLEDGEMENTS

The authors are grateful to the Head of the Department of Pharmaceutical Sciences, Saurashtra University, Rajkot, Gujarat, India, for providing the facilities used during the course of this study. Special thanks to Prof. P. Parmar, Botanical Survey of India for identification and authentication of the plant.

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