

Mast cell stabilising activity of various subfractions of leaves of *Vitex Negundo*

Jignesh I. Patel, Shrikalp S. Deshpande¹

Department of Pharmacology, B.K. Mody Government Pharmacy College, Polytechnic Campus, Rajkot, ¹K.B. Institute of Pharmaceutical Education and Research, Gandhinagar, Gujarat, India

To evaluate anti-asthmatic, anti-inflammatory, and anti-allergic activity of various subfractions of ethyl acetate fraction of leaves of *Vitex negundo*. Ethyl acetate fraction (EAF) of leaves of *V. negundo* was further subfractionated into NaOH fraction, acetone fraction, and chloroform fraction to separate chemical constituents present in EAF using thin layer chromatography technique and different solvent systems. The stabilisation potential of the various subfractions of EAF of leaves of *V. negundo* against mast cell degranulation was studied on isolated mesentery of rat by exposure to compound 48/80. Dexamethasone (5 mg/kg) was used as a reference standard. AF of EAF of leaves of *V. negundo* (500 µg/ml) showed significant protection of rat mesenteric mast cells from disruption caused by compound 48/80. This study concluded that AF of EAF of leaves of *V. negundo* shows mast cell stabilising activity. It may be useful as anti-asthmatic, anti-inflammatory, and anti-allergic agent.

Key words: Asthma, compound 48/80, mast cell, *Vitex negundo*

INTRODUCTION

The mechanism of the inflammatory response resulting in asthma is complex and involves numerous cell types, including mast cells. The mast cell has long been associated with asthma, because it releases a variety of preformed (histamine, leukotrienes, prostaglandins, and platelet activating factor) and newly synthesised mediators [eosinophils and neutrophils chemotactic factors, tumor necrosis factor- α , and cytokines (IL-4, IL-8, and IL-13)] that could account for several features of asthma-like bronchoconstriction and airway inflammation, respectively. Among the mediators released from mast cells, histamine is a well-characterised and the most potent vasoactive mediator in acute bronchoconstriction.^[1] In this study, compound 48/80 is used as a mast cell degranulating agent. It was described as a histamine liberator.^[2]

Vitex negundo is a species reported under the genus *Vitex*. Family: Verbenaceae. It is distributed throughout India, Ceylon-Afghanistan, tropical Africa, Madagascar,

China, and Philippines.^[3] Ethyl acetate fraction (EAF) of leaves of *V. negundo* shows anti-asthmatic activity.^[4] Ethanolic extract of *V. negundo* leaves showed inhibitory effect of degranulation of rat peritoneal mast cells induced by compound 48/80 and egg albumin.^[5] Aqueous extract of mature leaves of *V. negundo* shows anti-inflammatory analgesic and anti-histaminic activity.^[6] In this study, the various subfractions of EAF of leaves of *V. negundo* were screened for mast cell degranulation properties.

MATERIALS AND METHODS

Plant Collection

The leaves of plant *V. negundo* were collected from Agriculture Research Centre, Jagudan, Mehsana, North Gujarat, India. Its botanical identification was confirmed by the department of Pharmacognosy, KBIPER.

Preparation of Various Subfractions

- Leaves of plant were dried under shade for a week. Then it is powdered and successively fractionated with petroleum ether, ethyl acetate, and distilled water as solvent. Among these, EAF has anti-asthmatic activity.^[2]
- EAF was further subfractionated into NaOH fraction (AQF), acetone fraction (AF), and chloroform fraction (CF) to separate chemical constituents present in EAF using thin layer chromatography technique and different solvent systems.

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Address for correspondence: Jignesh I. Patel, Department of Pharmacology, B.K. Mody Government Pharmacy College, Polytechnic Campus, Near Aji-dam Chokdi, Rajkot - 360 003, Gujarat, India. E-mail: jigneshmpharma@yahoo.com

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Animals

Protocol for this project was approved by IAEC, K.B. Institute of Pharmaceutical Education and Research, Gandhinagar. Protocol approval no. is KBIPER 08/102. Albino rats of Wistar strain were used.

Method

The method used in this study was described by Norton S.^[7]

The pieces of mesentery were collected in following Petri dishes containing Ringer Locke solution (NaCl 0.9%, KCl 0.042%, CaCl₂ 0.024%, NaHCO₃ 0.015%, and dextrose 0.1%) in after the animals were killed.

- Petri dish no. 1 Ringer Locke solution (vehicle control)
- Petri dish no. 2 Ringer Locke solution (positive control)
- Petri dish no. 3 Dexamethasone (10 µg/ml)
- Petri dish no. 4 AQF of EAF of *V. negundo* leaf (500 µg/ml)
- Petri dish no. 5 AF of EAF of *V. negundo* leaf (500 µg/ml)
- Petri dish no. 6 CF of EAF of *V. negundo* leaf (500 µg/ml)

Each Petri dish was incubated for 10 min at 37°C.

Later 0.2 ml of compound 48/80 having concentration 10 µg/ml was added and again incubated for 10 min at 37°C. After that all pieces were transferred to 4% formaldehyde solution containing 0.1% toudine blue and kept a side for 20-25 min. After staining and fixation of mast cells, mesentery pieces were transferred through acetone and xylene two times and mounted on slides.

All the pieces were examined under light microscope with ×450 magnification. A minimum of 100 cells were counted for intact and disrupted mast cells and from that % protection from degranulation was calculated.

Statistical Analysis

Results were analyzed statistically using one-way analysis of variance followed by Turkey's test. Data were considered statistically significant at $P < 0.001$.

RESULTS

Compound 48/80 produced significant disruption of mast cells. AQF of EAF of *V. negundo* leaves produced lesser degranulation of mast cell [Figure 1]. The effect was comparable to dexamethasone-induced protection.

AQF of EAF of *V. negundo* leaves (500 µg/ml) showed significant protection (80.99 ± 0.7231 ; $P < 0.001$) when compared with positive control group (50.76 ± 2.1597 ; $P < 0.001$). Dexamethasone produced significant protection (83.50 ± 0.4355 ; $P < 0.001$).

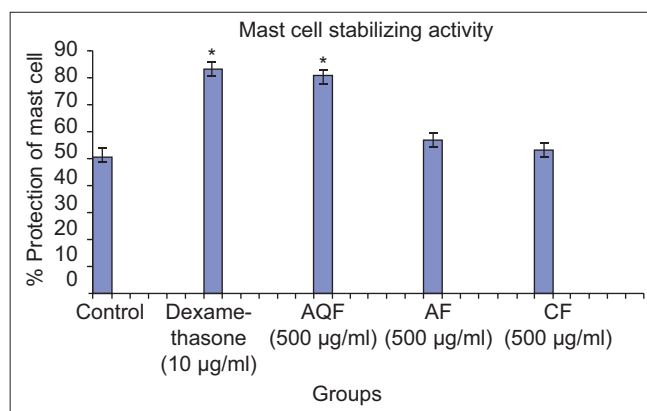


Figure 1: Effect of various subfractions of *Vitex negundo* leaves on mast cell degranulation induced by compound 48/80. *Significantly different from control at $P < 0.001$. Each bar in the graph represents mean ± SEM of six observations

DISCUSSION

It is now well known that mast cells are extensively involved in the pathophysiology of bronchial asthma.^[8] To evaluate the effectiveness of drug on mast cell stability, rat mesentric mast cells have long been considered useful tool. In this study, this model is used to evaluate the effectiveness of the test drug on mast cell stability.

When a piece of mesentery is placed in a solution of 48/80, the cell swells and becomes balloon-like, sometimes twisting and turning as it swells, possibly in response to the mechanical forces of the surrounding tissue.

Because living cells swell before they fragment, and because stained intact cells appear swollen, it was assumed that at least one of the effects of 48/80 was to increase the osmotic pressure within the mast cell and hence cause an inflow of water.

This increase in osmotic pressure might come about in several ways:

- (1) The mast cells might preferentially concentrate 48/80
- (2) A substance that was previously not available for osmotic effect might be liberated or formed in the cell
- (3) The cell might take up or become more permeable to ions present in the extracellular fluid.

But the evidence is in favour of the possibility that 48/80 may make the cell more permeable to extracellular ions and thereby cause disruption of the cell.^[7]

Here in our study a significant protection of rat mesentric mast cells from disruption caused by compound 48/80 was observed with AQF of EAF of *V. negundo* leaves. Therefore, AQF of EAF of *V. negundo* leaves may make the mast cell very less permeable to extracellular ions as compared with control group and give protection to mast cell degranulation.

CONCLUSION

From the results of this study, it can be concluded that AQF of EAF of *V. negundo* leaves shows mast cell stabilising activity. It may be useful as anti-asthmatic, anti-inflammatory, and anti-allergic agent.

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REFERENCES

1. Church MK, Bradding P, Walls AF. Human mast cells and basophils. Vol. 1. In: Kay AB, editor. Allergy and allergic diseases. Oxford, UK: Blackwell Science Ltd.; 1997. p. 149-70.
2. Feldberg W, Paton WD. Release of histamine from skin and muscle in the cat by opium alkaloids and other histamine liberators. *J Physiol* 1951;114:490-509.
3. Kirtikar KR, Basu BD. Indian Medicinal Plants. Vol. 3. Dehradun: International book Distributors, Book Sellers and Publishers; 1996. p. 1935-44.
4. Patel JI, Shah SK, Deshpande SS, Shah GB. Evaluation of the anti-asthmatic activity of leaves of *Vitex negundo*. *Asian J Pharm Clin Res* 2009;2:81-6.
5. Nair AM, Tamhankar CP, Saraf MN. Studies on the mast cell stabilizing activity of *Vitex negundo* Linn. *Indian Drug* 1995;32:277-82.
6. Dharmasiri MG, Jayakody JR, Galhena G, Liyanage SS, Ratnasooriya WD. Anti-inflammatory and analgesic activities of mature fresh leaves of *Vitex negundo*. *J Ethnopharmacol* 2003;87:199-206.
7. Norton S. Quantitative determination of mast cell fragmentation by compound 48/80. *Br J Pharmacol Chemother* 1954;9:494-7.
8. Black J. The role of mast cells in the pathophysiology of asthma. *N Engl J Med* 2002;346:1742-3.

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