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**INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES**<http://doi.org/10.5281/zenodo.1299896>Available online at: <http://www.iajps.com>**Research Article****ROLE OF BIOENHANCER IN THE TREATMENT OF ASTHMA****M. Ashoka Shenoy¹, A.R.Shabaraya¹, Karunakar Hegde¹**¹Srinivas College of Pharmacy Valachil, Farangipete Post, Mangalore – 574143**Abstract:**

The alcoholic extract of Moringa oleifera at 200 and 400 mg/kg doses were tested for anti-asthma activity against passive paw anaphylaxis by measuring paw volume using plethysmograph, mast cell stabilization by ex-vivo challenge of antigen in sensitized rat intestinal mesenteries and vascular permeability induced by acetic acid in mice. Dexamethasone, Prednisolone and Indomethacin were used as standard reference drugs. Moringa oleifera exhibited significant anti-asthmatic activity in all above three models and activity was comparable with standard drug. The findings from various studies reveal that the anti-asthmatic activity of Moringa oleifera may be due to the mast cell stabilizing potential, suppression of IgE, and inhibition of release of inflammatory mediators. Moringa oleifera, antiallergic, anaphylaxis, mast cell stabilization, vascular permeability

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INTRODUCTION:

Asthma remains the most common chronic respiratory disease in India affecting a major portion of the population. Although asthma is often believed to be a disorder localized to the lungs, current evidence indicates that it may represent a component of systemic airway disease involving the entire respiratory tract, and this is supported by the fact that asthma frequently coexists with other atopic disorders, particularly allergic rhinitis [1].

Despite significant improvements in the diagnosis and management of asthma over the past decade, as well as the availability of comprehensive and widely-accepted national and international clinical practice guidelines for the disease, asthma control remains suboptimal. Poor asthma control contributes to unnecessary morbidity, limitations to daily activities and impairments in overall quality of life [2].

New drugs are needed because those that are currently available cannot control symptoms and exacerbations in all patients and can cause adverse reactions. In the past 10 years there have been substantial advances in the understanding of asthma genetics, airway biology and immune cell signalling. These advances have led to the development of small molecule therapeutics and biogenic agents that may improve asthma care in the future [1].

Herbs have been the highly esteemed source of medicine throughout human history. They are widely used today, is not a throwback to the dark ages but an indication that herbs are a growing part of modern, high-tech medicine. About 25-30 percent of today's prescription drugs contain chemicals derived from plants. Unlike synthetic substances the natural drugs do not give symptomatic relief rather provide complete cure of many diseases. Due to these salient features the importance of herbal drugs has been realized seriously and they are becoming a preferred way of therapy throughout the globe [3].

Moringa oleifera is commonly referred as "miracle tree" or a "wonder tree" due to its socioeconomic importance, nutritional values, industrial applications, and its wide use in folk medicine. Its leaves contain important trace elements, proteins, vitamins, beta-carotene, amino acids, various phenolics, and other phytoconstituents and these are used in Siddha medicine. Different extracts of its roots, bark, leaves, flowers, immature pods, and mature fruits have been reported to possess cardiac and circulatory stimulant, antifertility, antitumor, antipyretic, antispasmodic, antiinflammatory, antiulcer, hypotensive, hypolipidemic, hypoglycemic, hepatoprotective,

antioxidant, antifungal and antibacterial activities, and thus promising therapeutic potential. Aqueous extract of its leaves has been reported to regulate thyroid hormone and can be used to treat hyperthyroidism [4].

In the present study anti asthmatic activity of alcoholic extract of *Moringa Oleifera* leaves was studied using passive paw anaphylaxis, mast stabilization activity and vascular permeability methods.

MATERIALS AND METHODS:

Animals

In bred Wistar rats (175-200 g) and Swiss albino mice (17-25 g) of either sex were used for the study. They were maintained under standard conditions (temperature $22\pm 2^\circ\text{C}$, relative humidity $50\pm 5\%$ and 12 h light/dark cycle). The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They have free access to standard pellet diet and water ad libitum. The Institutional Animal Ethics Committee approved the experimental protocol. All the animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the "National Academy of Sciences" and published by the "National Institute of Health".

Drugs and Chemicals

All the drugs and chemicals like Dexamethasone, Indomethacin, Prednisolone procured from yarrow chemicals, Mumbai and Toluidine blue, Evans blue, Egg albumin, Horse serum procured from Hi-media suppliers. Bordetella pertussis organisms obtained from Serum Institute of India Ltd., Pune.

Preparation of the alcoholic extract of *Moringa oleifera* leaves:

Fresh leaves were collected and shade dried for few weeks. Dried leaves were ground in an electric grinder to obtain coarse powder. The powder was stored in an air tight container. The powdered material (150 g) was packed in Soxhlet extractor and extracted using ethanol as solvent. The temperature was maintained on an electric heating mantle with thermostat control. Appearance of colourless solvent in the siphon tube was taken as the termination of extraction. The extract was concentrated to syrupy consistency by using rotary flash evaporator. The concentrated extract was then air dried at room temperature, weighed and percentage yield was calculated and stored in air tight container in $2-8^\circ\text{C}$ until used.

Passive Paw Anaphylaxis Using Rat by Measuring the Paw Volume by Plethysmometer

1. Preparation of Antiserum from Rats

The rats of either sex were injected intraperitoneally with 0.2 ml, 10% egg albumin and 0.2 ml of Bordetella pertussis vaccine on day 1, 3, and 5. After 21 days of the first immunization, blood was collected from orbital plexus under light ether anesthesia. The collected blood was allowed to clot and serum was separated by centrifugation at 1500 rpm. The separated serum was stored at -20°C until it was used for the experiment ⁵.

2. Passive Paw Anaphylaxis

The rats of either sex randomly divided into four groups each containing six animals. The drugs were administered orally in distilled water. The animals were dosed once daily for seven days. Group I served as a Control, Group II received Dexamethasone (5 mg/kg) as a Standard. Group III and IV received *Moringa oleifera* extract at 200 and 400 mg/kg respectively. Two hours after the last dose of drug administration (on 7th day), rats were passively sensitized into the left hind paw with 0.1 ml of the undiluted serum. The contra lateral paw received an equal volume of saline. 24 hours after sensitization, the rats were challenged in the left hind paw with 10 mg of Egg albumin in 0.1 ml saline. The hind paw volume was measured after 30 minutes by volume displacement method using mercury column plethysmometer ⁶.

3. Vascular Permeability Induced by Acetic Acid

The mice of either sex randomly divided into four groups of six each. All the drug preparations have been done in distilled water and administered orally. Group I animals served as a Control and received distilled water. Group II animals received Indomethacin (20 mg/kg) as a Standard reference drug, Group III and IV animals received *Moringa oleifera* extract at 200 and 400 mg/kg. Thirty minutes after the drug treatment, mice received an

intravenous injection of 0.5% Evan's blue saline solution (0.1 ml/10 g body weight) and an intraperitoneal injection of 0.6% acetic acid (10 ml/Kg body weight). After 20 min, the dye that leaked into the peritoneal cavity collected by lavaging with 10 ml of distilled water and transferred to 10 ml volumetric flask through glass wool. To each flask, 0.1 ml of 0.1 N sodium hydroxide solution added and volume made up to the mark with distilled water followed by measurement of absorbance at 610 nm ⁶.

4. Mast Cell Stabilizing Activity

The rats of either sex randomly divided into four groups of six each. The drugs were administered orally in distilled water. The animals were dosed once daily for fourteen days. Group I animals Served as a control. Group II, III IV and V were sensitized by injecting 0.5 ml of horse serum subcutaneously along with 0.5 ml of triple antigen containing 20,000 million Bordetella pertussis organisms. Group II were sensitized control received vehicle. Group III received Prednisolone (10 mg/kg) and group IV & V received *Moringa oleifera* extract at 200 and 400 mg/kg respectively. On day 14, the rats sacrificed 2 h after the treatment and the intestinal mesenteries taken out for the study on mast cells. Mesenteries along with intestinal pieces excised and kept in Ringer Locke solution (NaCl 154, KCl 5.6, CaCl₂ 2.2, NaHCO₃ 6.0, glucose 5.55 mM/L of distilled water) at 37°C. The mesenteric pieces challenged with 5% horse serum for 10 min after which the mast cells stained with 1.0% toluidine blue and examined microscopically for the number of intact and degranulated mast cells ^{7,8}.

5. STATISTICAL ANALYSIS

The data were expressed as mean \pm SEM. The total variation present in the data was analyzed by one way analysis of variance (ANOVA) followed by Post hoc Dunnett's test by the software SPSS Version 15.

RESULTS:

Table 1: Effect of alcoholic extract of *Moringa oleifera* on passive paw anaphylaxis

S. No.	Treatment	Paw Volume
1	Control	0.48 \pm 0.031
2	Toxic Control	1.29 \pm 0.39
3	Standard (Dexamethasone 5mg/Kg)	0.69 \pm 0.050*
4	<i>Moringa oleifera</i> (200mg/Kg)	1.01 \pm 0.090
5	<i>Moringa oleifera</i> (400mg/Kg)	0.78 \pm 0.039*

*P<0.05 as compared to toxic control (One way ANOVA followed by Dunnett's test).

Table 2: Effect of Alcoholic extract of *Moringa oleifera* on Vascular Permeability induced by Acetic Acid

S. No.	Treatment	Evans Blue Concentration (µg/ml)	% Inhibition
1	Control	1.2851±0.2260	-
2	Indomethacin (20 mg/kg)	0.5415±0.0940**	57.89
3	<i>Moringa oleifera</i> (200mg/Kg)	0.8425±0.0973ns	34.43
4	<i>Moringa oleifera</i> (400mg/Kg)	0.0615±0.0615*	46.84

Values are expressed as mean±SEM. *P<0.05, **P<0.01 as compared to control. (One way ANOVA followed by Dunnett's test).

Table 3: Effect of Alcoholic Extract of *Moringa oleifera* on Mast Cell Stabilizing Activity

S. No.	Treatment	Granulated Mast cells (%)	Degranulated Mast Cells (%)
1	Control ^a	77.49±3.096	22.51±3.096
2	Sensitized control	14.17±2.040	85.83±2.040
3	Prednisolone (10mg/Kg)	66.34±3.081**	33.66±3.081**
4	<i>Moringa oleifera</i> (200mg/Kg)	27.64±3.809 ^{ns}	72.36±3.809 ^{ns}
5	<i>Moringa oleifera</i> (400mg/Kg)	43.85±9.042*	56.15±9.042*

^a Not treated with horse serum and triple antigen. Values are expressed as mean±SEM. *P<0.01, **P<0.001 as compared to sensitized control. (One way ANOVA followed by Dunnett's test).

DISCUSSION:

The effect of alcoholic extract of *Moringa oleifera* on the passive paw anaphylaxis was studied in rats by passively sensitized by the administration of rat anti serum and then challenged by administration of injection of egg albumin to hind paw. There was significant increase in the paw volume in sensitised animals compared to normal animals. The treatment with *Moringa oleifera* significantly reduced the paw volume which is comparable to the standard reference drug dexamethasone.

Treatment with *Moringa oleifera* extract on Vascular Permeability induced by Acetic Acid, significantly reduced the leakage of Evans blue dye. The results were comparable to standard reference drug indomethacin.

The effect of alcoholic extract of *Moringa oleifera* on the mast cell stabilising activity was studied following active anaphylaxis. *Moringa oleifera* resulted in marked protection against the mast cell degranulation following antigen challenge in sensitised animals.

Basophils, mast cells, and their preformed de novo synthesized mediators, play a pivotal role in the pathogenesis of allergic disorders. These molecules are potent vasoactive and bronchoconstrictor agents and they modulate local immune responses and inflammatory cell infiltration^{9, 10}. Immunoglobulin E (IgE)-mediated mast cell stimulation is an important initial event in the development of type I allergic reactions such as asthma and atopic disorders.

Clinical studies have found a close association between asthma and serum IgE levels, as well as IgE-dependent skin test reactivity to allergens¹¹. Antigen challenge, in sensitized animals, results in the degranulation of mast cells, which is an important feature of anaphylaxis. In the present study, *Moringa oleifera* showed marked protection against the mast cell degranulation following antigen challenge in sensitized animals. Mast cell stabilizing activity of *Moringa oleifera* may be attributed to the presence of herbal extracts, which are known for their mast cell stabilizing potential against antigen-antibody reaction and/or due to the suppression of IgE antibody production, which is responsible for degranulation of mast cells. This antianaphylactic effect may be caused by the stabilization of the mast cell membrane, suppression of IgE, and inhibition of pathological effects induced by the release of inflammatory mediators in *Moringa oleifera* treated animals. Bronchial asthma is one such allergic process characterized by inflammation of the airways usually accompanied by increased vascular permeability, resulting in plasma exudation. Plasma protein leakage has been implicated to play a role in the induction of a thickened, engorged, and edematous airway wall, resulting in the airway luminal narrowing correlating with bronchial hyper-reactivity and airway inflammation^{12, 13}. All the above findings lend credence to the beneficial use of *Moringa oleifera* in the treatment of allergy and related conditions. However, further studies with other experimental models, especially to explore the

role of cytokines are warranted to substantiate the antiasthmatic activity of *Moringa oleifera*.

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