

Antihistaminic Effect of *Moringa Oleifera* Seed Extract

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Abstract

To evaluate the antihistaminic activity of *Moringa oleifera* (Moringaceae) to validate its traditional use, The antihistaminic activity of ethanolic seed extract of *Moringa oleifera* was evaluated on horse serum and triple antigen induced active anaphylaxis Wistar albino rats. Ethanolic seed extract (500 mg/kg body weight) significantly decreases histamine secretion and de-granulation of mast cells in rats (09.35±2.32). Phytochemical study revealed the presence of steroid saponins, alkaloids, flavonoid and glycoside. The glycoside saponin was reported to possess mast cell degranulation inhibiting and antihistaminic activity.

Key words: *Moringa oleifera*, Anti-histamines, mast cell, active anaphylaxis.

1. Introduction

Asthma is a disease of upper respiratory tract which is associated with change in the levels of eosinophils, mast cells, lymphocytes, cytokines and other inflammatory cell products. Histamine is an organic nitrogen compound involved in local immune responses as well as regulating physiological function in the gut and acting as a neurotransmitter.^[1] Histamine triggers the inflammatory response. As part of an immune response to foreign pathogens, histamine is produced by basophils and by mast cells found in nearby connective tissues. Histamine increases the permeability of the capillaries to white blood cells and some proteins, to allow them to engage pathogens in the infected tissues.^[2] Antihistamines are a type of medicine that is often used to treat a number of allergic disorders viz. hay fever, rhinitis, skin eczema or urticaria, conjunctivitis. Due to several side effects of antihistaminic allopathic medicines, certain antihistaminic phytoconstituents have been investigated in ethnomedicinal plants. Hence, in the present study, antihistaminic constituent glycoside saponin was reported from the plant *Moringa oleifera*. People in the Indian sub-continent have

long used *Moringa* pods for food. However, In India, it is traditionally used for asthma, bronchitis, catarrh, chest congestion, cough, glandular swelling and other respiratory disorders, like sore throat and tuberculosis^[3].

2. Materials and methods

2.1. Plant material

Seeds of *Moringa oleifera* were collected in June, July and August 2010, from Vidisha localities (M.P., India), and authenticated by Prof. S. K. Jain, Department of Botany, S.S.L. Jain P.G. College Vidisha (M.P.) India where voucher specimen was deposited in herbarium.

2.2. Preparation of extract

Dried and coarsely powdered seeds of *M. oleifera* (500 g) was extracted successively with ethanol using Soxhlet extractor. The extract was concentrated to dryness in rotary evaporator under reduced pressure to yield ethanol extract of *M. oleifera* seeds (12.28% yields).

2.3. Animals

Wistar albino rats (150-170 g) of either sex were housed under standard laboratory conditions.

The animals had free access to food and water. The animal ethical committee of the institute approved all the protocols of the study (Registration No.804/03/CPCSEA).

2.4. Preliminary phytochemical screening

To determine the chemical constituents, qualitative phytochemical screening of ethanol extract of *M. oleifera* seed was carried out for alkaloids, flavonoids, saponins, steroids and glycoside following standard procedure^[4, 5].

2.5. Experimental bioassay

Albino rats of either sex were divided into six groups each containing two animals. Rats were fasted for 18 h with water *ad-libitum*. Control group did not received herbal extract solution orally but test groups received ethanolic extract of *M. oleifera* at four different doses of 50, 100, 250 and 500 mg/kg. All the animals were sacrificed after 2 hr of the treatment and the mesenteric pieces were taken out and kept in Ringer Locke solution then histological sections were cut with the help of microtome [6]. For the observation of mast cell de-granulation activity, the mast cells was stained with 1% toluidine blue and observed under microscope. De-granulated mast cells were observed under microscope was like burst instead of intact. Total 100 cells were counted from different visual areas and protection against de-granulations was calculated [7, 8].

2.6. Statistical analysis

The results were reported as mean $\pm$ SEM and analyzed for statistical significance using One way ANOVA followed by Student 't' test. $P < 0.05$ was considered significant.

3. Results

3.1. Preliminary phytochemical screening

Preliminary phytochemical study of seeds extract of *M. oleifera* revealed the presence of steroids, saponins, alkaloids, flavonoid and glycosides. But saponins, glycosides and flavonoids were found to be more positive in ethanolic extract (Table 1).

3.2 Bioassay for anti-histaminic activity.

For the evaluation of anti-histaminic activity, group I of albino rats was served as controlled and was sensitized by 0.5 ml horse and 0.5 ml triple antigen i. p. injection on day 0, 7 and 14 and sacrificed 2 hrs after the challenge for the observation of mast cells which showed

88.20 $\pm$ 2.56 mast cells de-granulation. For the observation of prophylaxis activities, group II treated with 50 mg/kg body weight ethanolic extract of *Moringa oleifera* and it was observed that this group of herbal extract showed significant protection that is 20.50 $\pm$ 2.20 against mast cell de-granulation. In the III group which was treated with 100 mg/kg body weight of active fraction of *Moringa oleifera* extract, it was noticed that mast cell de-granulation was found to be continuously decreased with 15.60 $\pm$ 2.44 reduction. In another two doses of 250 and 500 mg/kg body weight, the mast cell de-granulation was found to be significantly decreased that is 11.55 $\pm$ 2.47 and 09.35 $\pm$ 2.32, respectively. Besides this, when these doses were compared with the dose of standard/reference drug prednisolone 10 mg/kg/b. w., it was observed that the de-granulation of mast cells was a very lowest level that is 8.90 $\pm$ 2.20. It appears that the de-granulation of mast cells is quite dosed dependent. It is inversely proportional to the doses, as the doses increases, the de-granulation of mast cells decreases. However, the anti-histaminic activity is directly proportional to the doses because the number of mast cells was found to be stabilizing simultaneously with increasing the doses. The results when compared to the control seem to be quite significant at $p < 0.05\%$ when student "t" test was applied (Table 2 and Graph 1).

4. Discussion

The de-granulation of mast cell occurs in response to the immunological stimuli in which antigen antibody reactions are predominant. Ethanolic seeds extract of *M. oleifera* at doses of 50, 100, 250 and 500 mg/kg significantly protect egg albumin induced de-granulation of mast cell in dose dependent. Ethanolic extract of *M. oleifera* at 500 mg/kg protect mast cell more (09.35 $\pm$ 2.32) which compared to Prednisolone standard drug (10 mg/kg. body weight) and found to be very near to the result of standard drug (8.90 $\pm$ 2.20). This indicates that ethanolic extract of *M. oleifera* are effective in type I hypersensitivity reactions and also effective in stabilizing mast cell. The anaphylactic allergic reaction is a life-threatening reaction inducing release of mediators such as histamine and pro-inflammatory cytokines and can be elicited by various stimuli when compared to control group. Control group showed 88.20 $\pm$ 2.56 increased number of de-granulated mast cells. Hence *M. Oleifera* shows antihistaminic and anti-inflammatory mechanism by inhibiting horse

serum and triple antigen induced anaphylaxis in rats. Phytochemical screening of ethanolic extract of *M. oleifera* revealed the presence of steroids, saponin, alkaloids, flavonoids and glycosides. Glycoside saponin was reported to possess mast cell stabilizing property. Flavonoid has also been shown to possess smooth muscle relaxant and bronchodilator activity [9]; the flavonoids including apigenin and luteolin were known to inhibit basophil histamine release and neutrophil beta glucuronidase release, and thereby possess in-vivo anti-allergic activity. These flavonoids also inhibited the histamine release induced by 48/80. Hence anti-asthmatic

activity of ethanolic extract of *M. oleifera* may be due to presence of flavonoid or glycoside saponins. Present study revealed that ethanolic extract of *M. oleifera* stabilize horse serum and triple antigen induced mast cell and inhibit release of histamine in anaphylactic reaction and possesses anti-allergic action. In conclusion, ethanolic extract of *M. oleifera* are effective in treatment of asthma as it shows anti-allergic and mast cell stabilizing potential. Very recently, Nivedithadevi et al. [10] have evaluated anti-histaminic activity 50% alcoholic extract of *Tephrosia purpurea* in guinea pig isolated ileum.

Table 1. Preliminary phytochemical screening of *M. oleifera* extract.

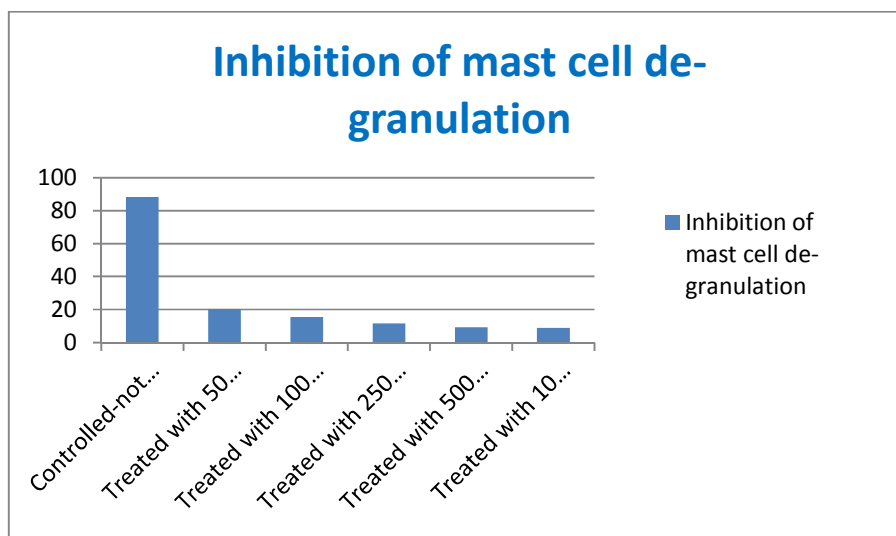
Se. No.	Phytochemical constituents	Benzene extract	chloroform extract	Acetone extract	Ethanol extract	water extract
1	Saponins	—	+	+	++	+
2	Tannins	—	—	—	+	+
3	Phenols	—	—	—	+	+
4	Glycosides	+	—	+	++	+
5	Alkaloids	—	—	—	+	+
6	Volatile oils	—	—	+	—	—
7	Flavonoids	+	+	+	++	+

(+) indicates for the presence, (—) indicates for absence, (++) indicates for more positive.

Table 2. Effect of active fraction (Mo-4) of *Moringa oleifera* extracts on albino rats.

Group	Treatment	Dose (mg/kg b. w.)	Route of administration	Inhibition of mast cells de-granulation
I	Controlled (Anaphylactic)	Not treated	-----	88.20±*2.56 (Increased number of de-granulated mast cells)
II	Treated (Prophylactic)	50	Oral with Acacia gum	20.50±2.20
III	Treated (Prophylactic)	100	Oral with Acacia gum	15.60±2.44
IV	Treated (Prophylactic)	250	Oral with Acacia gum	11.55±2.47
V	Treated (Prophylactic)	500	Oral with Acacia gum	09.35±2.32 (Reduced number of de-granulated mast cells).
VI	Prednisolone	10	Intra muscular	8.90±2.20

P value < 0.05, *SEM



Graph 1. Showing anti-histaminic effect of *Moringa oleifera* active fraction (Mo-4) against the mast cell de-granulation

Cite this article"

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