MICHELIA CHAMPACA: A USEFUL REMEDY FOR MAST CELL STABILIZATION, ANTIALLERGIC AND ANAPHYLACTIC ACTIVITY IN MANAGEMENT OF ASTHMA

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ABSTRACT

The anti-allergic, anti-anaphylactic and mast cell stabilizing activity of ethanolic extract of Michelia champaca (MC) was evaluated pharmacologically by using milk induced leukocytosis and eosinophilia in mice, compound 48/80 induced mast cell degranulation in rats and egg albumin induced passive paw anaphylaxis in rats. In the milk induced eosinophilia and leukocytosis, MC showed significant inhibition of the total number of eosinophils and leukocyte count in mice; in the compound 48/80 induced mast cell degranulation MC showed significant protection of mast cells in rats; and in passive paw anaphylaxis, MC showed significant reduction in the paw edema volume in rats. These results suggest that MC may prove to be a potential therapeutic agent in management of asthma which may be due to anti-stress, mast cell stabilizing and anti-inflammatory activity.

KEYWORDS: Michelia champaca, milk, compound 48/80, anaphylaxis.

1. INTRODUCTION

Asthma is a clinical syndrome characterized by paroxysmal dyspnea and wheezing due to increase resistance to flow of air through narrow bronchi. The spasm of bronchial smooth muscles narrows the lumen and edema occurs. This produces blockage of the lumen. Asthma occurs often due to response to various triggers such as environmental stimulants, allergens, cold air, exercise and emotional stress. When treatment is inadequate, however, airway remodeling can occur and reversibility of obstruction becomes incomplete.[1, 2]
Michelia champaca (Family: Magnoliaceae), locally known as Swarna Champa, is a tree with golden-yellow fragrant flowers and aggregate fruits, grows wild in Chittagong Hill Tracks and planted in gardens in other areas of Bangladesh.\[^3\] Flowers of Michelia Champaca (Linn.) were used traditionally in treatment of inflammation, expectorant, cough and antispasmodic.\[^4\] Flower of Michelia champaca Linn are the potent source of novel bioactive compounds with wide range of medicinal properties like antimicrobial activity,\[^5\] anti-inflammatory activity, antipyretic activity,\[^6\] antioxidant activity,\[^7\] antiulcer activity,\[^8\] burn wound healing activity.\[^9\] Preliminary phytochemical studies reported that Michelia champaca Linn, shown presence of flavonoids, p-coumaric acid, caffeic acid and catechin which are also reported for antioxidant activity.\[^10,11,12\]

Taking into consideration the traditional claims, reported chemical constitution and reported pharmacological activities the present study was planned to evaluate the anti-asthmatic activity of flowers of Michelia champaca.

2. MATERIALS AND METHODS

Experimental animals

The rats and mice of either sex were purchased from National Toxicology Center, Pune. They were housed in group of five under standard laboratory conditions of temperature (25±2°C) and 12/12 hr. light/dark cycle. Animals had free access to standard pellet diet (Amrut laboratory animal feed, Sangli-Maharashtra.) and water ad libitum. The distribution of animals in the groups, the sequence of trials and the treatment allotted to each group were randomized, throughout the experiment. Laboratory animal handling and experimental procedures were performed in accordance with the guidelines of CPCSEA (CPCSEA NO.198/99) with IAEC clearance proposal number DYPIPSR/IAEC/11-12/P-04.

Plant material Collection and authentification of plant material

The flowers of Michelia champaca were collected from the local market of Pune and the sample was authenticated from Botanical survey of India, Pune. [BSI/WRC/Tech/2012)]

Preparation of Extract

The extraction of flowers of Michelia champaca were carried by soxhlation method using Soxhlet apparatus. The 1000 gm. of dried flowers were coarsely powdered and extracted using 95% ethanol in a Soxhlet extractor. The last traces of solvents were evaporated in
rotary evaporator under reduced pressure to produce ethanolic extract of *Michelia champaca* flowers (MC). The percentage yield of flower ethanolic 23.50 % w/w.[12]

**Phytochemical Screening**

Phytochemical screening of MC was performed to determine the presence of various phytochemicals such as steroid, saponin, alkaloid, flavonoid, tannin, phenolic compound and glycosides.[13]

**Acute toxicity study and Dose selection**

Albino rats of either sex weighing 200-250 gm. were used in the study. Acute oral toxicity study was performed as per Organization for Economic Co-operation and Development (OECD)-423 guideline.[14] The animals were divided in 4 groups (n=3) and were fasted overnight prior to drug administration. Following the period of fasting, the animals were weighed and the test substance was administered. The animals were administered with test extract at the dose of 5, 50, 300 and 2000 mg/kg body weight orally. The animals were observed for 5 min every 30 min till 2 h and then at 4, 8 and 24 h after treatment for any behavioral changes/mortality. They were further observed daily for 7 days for mortality. No mortality up to 7 days after treatment was observed with the ethanolic extract of *Michelia champaca* flowers at the dose of 2000 mg/kg, p.o. and therefore they were found safe up to dose of 2000 mg/kg. Thus the animal doses were selected on the basis of acute oral toxicity study. Hence, 1/10th (200 mg /kg) of this dose was selected for this study. For the doses of mouse, the dose of rat was extrapolated by using conversion factor i.e., 140,280,560 (mg/kg, p.o.).[15]

**Milk- induced leukocytosis and eosinophilia in mice**[16]

Mice were divided into five groups (n=5). Animals belonging to group I served as positive control and was administered with only boiled and cooled milk (4 ml/kg, s.c.). Animals belonging to group II served as standard and were administered with Dexamethasone (50 mg/kg i.p.) while animals belonging to group III to V served as test group and were received respective doses of MC (140, 280 or 560 mg/kg p.o.) and 1 hr later boiled and cooled milk (4 ml/kg, s.c.) was administered to the same animals. After 24 h of milk administration, the blood samples were collected from retro-orbital plexus under light ether anesthesia. Total leukocytes and eosinophils counts were recorded in each group.
Compound 48/80 Mast cell degranulation in rats

Rats were divided into five groups (n = 5). On the 1st day of sensitization, all the animals from each group were injected with Compound 48/80 (1 mg/kg, s.c.). Rats of the vehicle control group received distilled water (10 ml/kg, p.o.). Rats of the standard group received ketotifen fumarate (1 mg/kg, p.o.), while rats of the test groups received 100, 200 or 400 mg/kg p.o. of MC for 15 days. On day 15th, 2 h after the assigned treatment, 10 ml of normal saline solution was injected into the peritoneal cavity and the abdomen was gently massaged for 90 s. The peritoneal cavity was carefully opened and the fluid containing mast cells were aspirated and collected in siliconised test tube containing 7-10 ml of RPMI-1640 medium (pH 7.2-7.4). The mast cells were then washed thrice by centrifugation at low speed (400-500 rev/min) and the pellets of the mast cells were taken in the RPMI-1640 medium. The mast cell suspension (approximately 1 × 106 cells/ml) was challenged with 5 μg/ml of compound 48/80 solution, stained with 0.1% toluidine blue and observed under high power microscope (45X). A total of 100 cells were counted from different visual areas. The numbers of intact and degranulated cells was counted and the percent protection was calculated using the formula:

\[
\text{% Protection} = \left[1-\left(\frac{T}{C}\right)\right] \times 100
\]

Where, T- No. of degranulated cells in test.

C- No. of degranulated cells in control.

Passive paw anaphylaxis in rats

Wistar rats were divided into five groups (n=5). Antiserum to egg albumin was raised in rats by using aluminum hydroxide gel as an adjuvant. Animals were given three doses of 250 μg of egg albumin (s.c.) adsorbed on 12 mg of aluminum hydroxide gel prepared in 0.5 ml of saline on 1st, 3rd and 5th day. On 10th day of sensitization, the blood was collected from the retro orbital plexus under light ether anesthesia. The collected blood was allowed to clot and serum is separated by centrifugation at 1500 rpm. The animals were passively sensitized with 0.1 ml of the undiluted serum into the left hind paw. The right hind paw was received an equal volume of saline. Animals belonging to group I served as control and was administered with only distilled water (10 ml/kg, p.o.). Animals belonging to group II served as standard and were administered with Dexamethasone (0.5 mg/kg, i.p.), whereas animals belonging to Group III to Group V received MC at different concentrations respectively 24 hrs. after sensitization. One hr. after test drug administration, animals was challenged by giving 10 μg
of egg albumin in 0.1 ml of saline in the left hind paw and the paw inflammation was measured by using a Plethysmometer (UGO Basile, 7140). The difference in the reading prior to and after antigen challenge represents the edema volume and the percent inhibition of edema was calculated by using the formula,

\[
\text{% Inhibition} = \left[1 - \left(\frac{T}{C}\right)\right] \times 100
\]

Where, T-Mean relative change in paw volume in test group.
C- Mean relative change in paw volume in control group.

Statistical analysis
The results were expressed as Mean ± SEM and statistically analyzed by one-way analysis of variance (ANOVA) followed by Dennett’s test and p <0.05 was considered significant.

3. RESULT
Phytochemical Screening
The phytochemical investigation of MC showed the presence of alkaloids, glycosides, triterpenoids, proteins, carbohydrates and flavonoids.

Acute Toxicity Study
Dose was selected based on basis of acute oral toxicity study done on ethanolic extract of *Michelia champaca* flowers. Extract was found to be safe up to the dose level 2000 mg/kg. There was no behavioral abnormality and zero mortality was recorded till 48 h post treatment with no signs of acute toxicity. Therefore 1/10\(^{th}\) of the dose 2000 mg/kg of ethanolic extract of *Michelia champaca* flowers was selected i.e., 200 mg/kg as middle dose in rats. In rats, three doses were used, i.e., 100, 200, and 400 mg/kg (1/20th; 1/10th; and 1/5th of 2000 mg/kg, the highest dose used in acute toxicity study). Whereas, in mice, the doses were 140, 280 and 560 (i.e., dose used in rat X 1.4, as suggested by Ghosh)\(^{[15]}\)

Effect of MC on Milk-Induced leukocytosis and eosinophilia in Mice
Subcutaneous injection of milk at dose of 4 ml/kg produced an increase in the leucocytes count after 24 hr of its administration. Animals treated with Dexamethasone has shown significant inhibition of milk-induced leukocytosis as compared to positive control (p<0.01). In the groups of mice pretreated with ethanolic extract of *Michelia champaca* (MC) at the dose of 140, 280 and 560 mg/kg, there was significant inhibition (p<0.01) of milk-induced leukocytosis and Eosinophilia. (Graph 1 & 2)
3.2. Effect of MC on Compound 48/80-induced mast cell degranulation in rats

Compound 48/80 induced mast cell degranulation was significantly \((p<0.01)\) inhibited by Ketotifen (1 mg/kg, i.p.) and percent protection was found to be 65.9\%. In the groups pretreated with ethanolic extract of *Michelia champaca* at the dose of 100 mg/kg, p.o. has not shown any significant protection of mast cells and the percent protection was found to be 4.5\%. In the groups pretreated with ethanolic extract of *Michelia champaca* at the dose of 200 & 400 mg/kg, p.o., has shown significant \((p< 0.05)\) protection of mast cells and the percent protection was found to be 34.7\% and 56.38\% respectively. (Graph 3)
3.3. Effect of MC on passive paw anaphylaxis in rats

Antiserum to egg albumin was injected 24 hr before administration test drugs. In the vehicle or distilled water treated group, egg albumin increased the paw volume in the sensitized animals, which was measurable up to the time period of 4 hrs. Dexamethasone significantly reduced (p<0.01) the paw volume at 0.5, 1, 2, 3 and 4 hrs. time intervals and the percentage inhibition was 41.93%, 54.54%, 60.37%, 41.66% and 52.38% respectively. Pretreatment with MC at the dose of 100 mg/kg, p.o. has not shown any significant reduction in the paw edema volume at 0.5 and 1st hr. but showed significant reduction in the paw edema volume at 2nd, 3rd (p<0.05) and 4th hr (p<0.01). The percentage inhibition was found to be 8.06%, 10.90%, 9.43%, 8.33% and 26.19% respectively. MC at the dose of 200 & 400 mg/kg, p.o. has shown significantly reduced (p<0.01) the paw edema volume at 0.5, 1st, 2nd 3rd and 4th hr time interval. The percentage inhibition shown by MC at the dose of 200 was found to be 25.80%, 18.54%, 15.09 %, 31.25% and 28.57% respectively. The percentage inhibition shown by MC at the dose of 400 was found to be 38.70%, 40.40%, 41.50%, 39.58% and 40.47 % respectively. (Graph 4)

![Graph 3 Effect of MC on Passive Paw Anaphylaxis in Rats](image)

4. DISCUSSION

Airway inflammation is one of the important factors in pathologic progress of asthma and is current target for suppressing asthma.[19] Herbal medicines are being increasingly utilized to treat a wide variety of diseases, though the knowledge about their mode of action is relatively scanty. So there is a growing interest regarding the pharmacological evaluation of various plants used in traditional system of medicine.[20]
‘Adaptogen’, a naturally occurring compound allows body to fight off with the effects of stress and strengthen body’s own innate healing powers. Adaptogen put an individual into a state of non-specific heightened resistance in order to resist the stress and adaptation to external challenges of a physical, chemical and biological nature.\textsuperscript{[21, 22]} Many times asthma attacks are induced by stress and thus adaptogens can be useful significantly in the asthma attacks as an anti-stress agent. After parenteral administration of milk in rats, there is increase in total number of leukocyte and erythrocyte count. This stress full condition can be normalized by administration of an anti-stress or adaptogenic drug.\textsuperscript{[23]} White blood cells or leukocytes are the cells of the immune system involved in defending the body against both infectious disease and foreign materials. An increase in the number of leukocytes i.e., leukocytosis and an increase in the number of eosinophils i.e., eosinophilia in the blood is often an indicator of allergies such as asthma, hay fever and hives and also parasitic infections. In the present study, the ethanolic extract of \textit{Michelia champaca} flowers has significantly inhibited milk induced leukocytosis and eosinophilia indicating that \textit{Michelia champaca} may possesses adaptogenic and antistress activity which may contribute to its antiasthmatic activity. The same findings were observed by Suralkar & Kasture (2013).

Compound 48/80 which is a mixed polymer of phenethylamine cross-linked by formaldehyde causes release of histamine from mast cells and thus can be useful as a direct and convenient reagent to study the mechanism of allergy and anaphylaxis.\textsuperscript{[20]} Compound 48/80 initiates the generation of superoxide anion by A-kinase inactivation through decreasing the intracellular cAMP concentration in mast cells.\textsuperscript{[24]} Generated superoxide anion results in the inositol 1, 4, 5- triphosphate or GTP induced calcium release from endoplasmic reticulum which increases intracellular calcium content which leads to histamine release from mast cells which is known as degranulation of mast cells.\textsuperscript{[25]} Stimulation of mast cells with compound 48/80 by degranulation leads to histamine release which exerts many effects related to the immediate phase of allergic inflammation including vasodilatation, increased vascular permeability, tissue edema, contraction of bronchial and intestinal smooth muscles and increased mucus production. Thus, compound 48/80 has been used as a direct and convenient reagent to study the mechanism of allergy and anaphylaxis.\textsuperscript{[26,27]} In present study, the ethanolic extract of \textit{Michelia champaca} flowers significantly reduced the mast cell degranulation and probably the subsequent release of histamine and further array of inflammatory cytokines. Thus, the ethanolic of \textit{Michelia champaca} may possess the mast cell stabilizing activity which may
further contributes to its antiasthmatic activity. The same findings were observed by Srivastava et al., 1999.

Administration of egg albumin (s.c.) to rat raises the antibodies to albumin in the plasma. The sub plantar injection of plasma containing these antibodies then challenged with egg albumin leads to passive paw anaphylaxis in rats. Passive paw anaphylaxis is a chronic inflammatory process occurring due to exposure of allergen resulting in the activation of T-lymphocytes with subsequent release of inflammatory mediators. Immunomodulating agents are useful in treatment of asthma by virtue of inhibiting the antigen-antibody (AG:AB) reaction thereby inhibiting release of inflammatory mediators. The present study revealed that, there was a significant reduction in the paw volume by the ethanolic extract of *Michelia champaca* flowers which could be due to inhibition of AG:AB reaction or by mast cell stabilization and thus may contribute in antiasthmatic activity. The same findings were suggested by Kumar et al., (2010).

During Preliminary photochemical evaluation *Michelia champaca* flowers have shown presence of flavonoids, alkaloids, glycosides, proteins, carbohydrates, and saponins. Flavonoids are known to possess anti-inflammatory and anti-oxidant activities. Thus, the presence of these phytoconstituents in the ethanolic extract of *Michelia champaca* may further contribute in anti-allergic and anti-anaphylactic activities in the management of asthma.

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