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Inhibition of histamine mediated responses by *Mirabilis jalapa*: confirming traditional claims made about antiallergic and antiasthmatic activity

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The roots of *Mirabilis jalapa* are used traditionally in allergic skin disorders and asthma. The effect of an ethanol:acetone (1:1) extract of the roots of *M. jalapa* was studied for antihistaminic activity using a guinea pig tracheal chain preparation and clonidine-induced mast cell granulation in mice. Its antiallergic activity was evaluated using milk-induced eosinophilia and albumin-induced paw oedema in mice. The extract (0.5 mL of 100 mg mL⁻¹) inhibited histamine-induced guinea pig tracheal chain contractions non-competitively. The extract (100 or 200 mg kg⁻¹ i.p.) inhibited milk-induced eosinophilia, albumin-induced paw oedema and protected mast cells against clonidine-induced granulation. The study justified the folkloric use of *M. jalapa* in the treatment of allergic diseases and asthma.

Keywords: albumin; mast cells; paw oedema

1. Introduction

Allergic inflammatory responses are the cause of the symptoms of many types of diseases, including atopic dermatitis and asthma. It is well documented that the eosinophils are of prime importance in allergic responses (Gleich, 1990). Eosinophils are thought to contribute to the inflammation associated with several diseases, including bronchial asthma, allergic rhinitis and hypereosinophilic syndrome. Blood and tissue eosinophilia are also characteristic of atopic dermatitis, drug-induced skin eruptions, eosinophilic cellulitis and eosinophilic pustular folliculitis. *Mirabilis jalapa* Linn (Family: Nyctaginaceae) is distributed as an ornamental plant throughout the tropics of the world. Mainly it is found in Mexico, Chile, India, South America, France, Brazil and Italy. Traditionally, in India, *M. jalapa* is used as a diuretic, carminative, stomachic, vermifuge, cathartic, uterine stimulant and antispasmodic (Nadkarni, 1954). The roots of *M. jalapa* also possess antiviral activity (Vivanco, Savary, & Flores, 1999). In folklore medicine it is used in the treatment of allergic skin disorders and asthma. Therefore, we investigated the antiallergic activity of an ethanol:acetone (1:1) extract of the roots of *M. jalapa* using

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a histamine-induced contraction of guinea pig ileum, milk-induced eosinophilia, clonidine-induced mast cell degranulation and egg albumin-induced active paw anaphylaxis in mice.

2. Results

2.1. Evaluation of antihistaminic activity using isolated guinea pig tracheal preparation

Histamine produced a dose-dependent contraction of the isolated guinea pig tracheal chain preparation. *Mirabilis jalapa* extract (MJE) significantly ($p < 0.05$) inhibited the histamine-induced contractions of the tracheal chain preparation. In the presence of the extract, the agonist could not produce a maximal effect and indicated non-competitive antagonism. MJE *per se* did not produce any effect on the tracheal chain. The observations are given in Figure 1.

2.2. Milk-induced eosinophilia

In mice treated with vehicle, the difference in eosinophil counts before and 24 h after subcutaneous injection of milk was 8.33 ± 4.81 . Subcutaneous injection of milk (4 mL kg^{-1}) increased the number of eosinophils by 120.00 ± 10.20 . In the animals pretreated with MJE (100 and 200 mg kg^{-1}), the difference in the eosinophil count before and after milk was 75.0 ± 8.35 , 47.5 ± 5.6 , respectively ($p < 0.05$), whereas the difference in the eosinophil count in the diphenhydramine-treated group was 14.2 ± 2.0 ($p < 0.001$). The observations are given in Table 1.

2.3. Clonidine-induced mast cell degranulation

The clonidine-induced mast cell degranulation was inhibited by mast cell stabiliser DSCG. The percentage protection offered by DSCG was $85.22 \pm 6.89\%$. Pretreatment with MJE

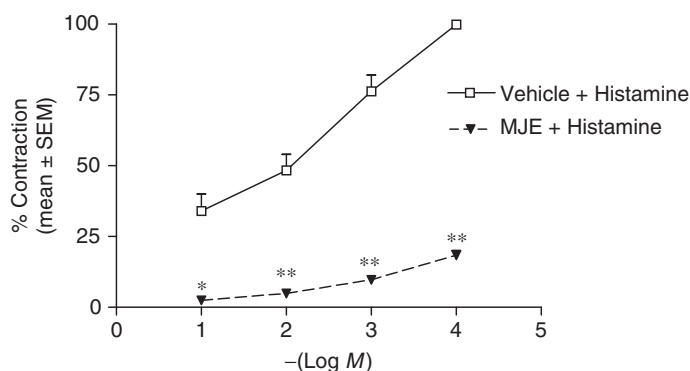


Figure 1. Effect of MJE on histamine-induced contraction of isolated guinea pig tracheal chain preparation. A dose response curve (DRC) of histamine in variant molar concentrations in the absence and presence of aqueous solutions of MJE (100 mg mL^{-1} , 0.5 mL) were recorded. 1 = 7.26, 2 = 6.96, 3 = 6.66, 4 = 6.36; $n = 5$, ** $p < 0.0001$, * $p < 0.001$, compared to vehicle + histamine-treated group by Student's *t*-test (unpaired). The values are expressed as mean $\pm$ SEM.

Table 1. Effect of *M. jalapa* root extract on milk-induced eosinophilia, clonidine-induced mast cell degranulation and egg albumin-induced paw oedema.

Treatment (mg kg ⁻¹)	Eosinophil count	% Protection from mast cell degranulation	Volume of paw oedema (mL)
Vehicle	8.33 ± 4.81	—	0.82 ± 0.02
Milk	120.0 ± 10.2		
MJE (100)	75.0 ± 8.35*	48.5 ± 7.68**	0.40 ± 0.03*
MJE (200)	47.5 ± 5.6*	66.75 ± 10.45**	0.42 ± 0.02*
Diphen (1)	14.2 ± 2.0**		
DSCG (1 µg)		85.22 ± 6.89**	
Dexa (0.5)			0.28 ± 0.03**

Notes: Values are mean ± SEM of five observations. * $p < 0.01$, ** $p < 0.001$ compared to respective control. Diphen = Diphenhydramine; DSCG = disodium cromoglycate, Dexa = dexamethasone.

(100 and 200 mg kg⁻¹) offered significant mast cell protection to 48.75 ± 7.68 and 66.75 ± 10.45%.

2.4. Active paw anaphylaxis

Dexamethasone (0.5 mg kg⁻¹) and MJE (100 and 200 mg kg⁻¹) significantly ($p < 0.01$) decreased egg albumin-induced paw thickness from 0.82 ± 0.02 (vehicle-treated group) to 0.28 ± 0.03, 0.40 ± 0.03, and 0.42 ± 0.03 mm, respectively. Observations are given in Table 1.

3. Discussion

The histamine released from degranulated mast cells regulates airway smooth muscle (ASM) function in asthma (Bradding, Walls, & Holgate, 2006). In asthma patients there is an exaggerated bronchoconstrictor response of the ASM to direct and indirect stimuli, such as histamine and exercise. Thus targeting histamine, either by prevention of its release from mast cells or the use of histamine receptor antagonists, becomes part of antihistaminic therapy in asthma. The inhibition of histamine's contractile response by MJE indicated its ability to block the postsynaptic histamine H₁ receptors. The presence of peripheral blood eosinophilia and activated eosinophils in the chronic inflammatory infiltrate of the airways is a characteristic of both allergic and non-allergic asthma. Mast cell-derived cytokines are known to regulate both IgE synthesis and the development of eosinophilic inflammation (Bradding & Holgate, 1999). Eosinophilic inflammation of the airways is correlated with the severity of asthma. These cells are likely to play a part in the epithelial damage seen in this disease (Bousquet, Jeffery, Busse, Johnson, & Vignola, 2000). The inhibition of milk-induced eosinophilia suggests the usefulness of MJE in treatment of eosinophilia associated with different clinical conditions.

Mast cells play a major role in the pathophysiology of asthma due to their ability to release a host of pleiotropic autacoid mediators, proteases and cytokines in response to activation by both immunoglobulin IgE-dependent and diverse non-immunological stimuli. (Bradding et al., 2006; Cruse et al., 2005). Secretion of autacoid mediators such as histamine, prostaglandin D₂ and leukotriene C₄ induces bronchoconstriction, mucus secretion and mucosal oedema, thereby contributing to acute symptoms. Gupta et al. (1994)

showed that clonidine releases histamine from mast cells in a similar manner to selective liberators like compound 48/80. The MJE stabilised the mast cells in a dose-dependent way, revealing its mast cell stabilising activity. Wu, Chun, Ebizuka and Sankawa (1991) have reported inhibition of ovalbumin-induced paw anaphylaxis by *Centipeda minima*, which is suggestive of antiallergic activity. *M. jalapa* also exhibited egg albumin-induced paw oedema. Thus, the study concludes that *M. jalapa* has potential antihistaminic activity, which justifies its folkloric use in the treatment of asthma and allergic skin diseases.

4. Experimental

4.1. Plant and preparation of extract

Fresh roots of *M. jalapa* were harvested from the Botanical Garden, Cagliari, Sardinia, Italy in August. The plant material was authenticated at the Herbarium of the Department of Botanical Sciences, University of Cagliari by comparison with a voucher specimen (1423). The fresh roots (500 g) were macerated for eight days with ethanol:acetone (1:1). The extract was concentrated under reduced pressure (yield: 24 g).

4.2. Animals

Albino male Swiss mice (18–25 g) and guinea pigs (275–300 g) were housed under standard laboratory conditions (light period of 12 h per day, temperature $25 \pm 2^\circ\text{C}$ and humidity $55 \pm 5\%$) with free access to food (standard pellet chow, Lipton, India) and water *ad libitum*. Food but not water was withdrawn overnight and during the experiment. The experimental procedures were carried out in strict compliance with Institutional Animal Ethics Committee regulations, constituted under the provisions of the Prevention of Cruelty to Animals Act.

4.3. Drugs

Clonidine (Unichem Laboratories Pvt. Ltd, India), dexamethasone (Zydus Laboratories, India), diazepam (Ranbaxy Laboratories, India), diphenylhydramine (Glenmark Pharmaceuticals, India), disodium chromoglycate (DSCG) (Cipla Pharmaceuticals, India), and histamine (Sigma, USA), were used in this study.

4.4. Reagents and chemicals

Eosin solution and white blood cell dilution fluid (Qualigens, India), toluidine blue (Research Lab, India), RPMI buffer medium 1640 (Hi-Media, India) and other chemicals of analytical grade were purchased from Modern Scientifics, Nashik, India.

4.5. Evaluation of antihistaminic activity using isolated guinea pig tracheal preparation

Guinea pig trachea was cut into individual rings and tied together in a series to form a chain, as described by Goyal (1998). This was suspended in an organ bath containing Krebs's solution (concentration in mM L^{-1} : NaCl, 118; KCl, 4.7; CaCl_2 , 2.5; MgSO_4 , 1.2; NaHCO_3 , 25.0; KH_2PO_4 , 1.2; Glucose, 11.1), maintained at $37 \pm 1^\circ\text{C}$ while a stream of 5% CO_2 in oxygen was bubbled through the organ tube. One end was tied to aerator tube and the other attached to an isotonic frontal writing lever to a smoked drum. The tissue was

allowed to equilibrate for 45 min under a load of 400 mg. A DRC for histamine (0.5963×10^{-9} M) was taken in variant molar concentrations. A time cycle of 45 min was followed. DRCs of histamine in the absence and presence of MJE dissolved in distilled water were recorded. Five animals were used for the experiment.

4.6. Milk-induced eosinophilia

Mice were divided into four groups of five animals each. Blood samples were collected from retro-orbital plexus under light ether anaesthesia. The eosinophile count was done in each group before drug administration and 24 h after buffalo milk injection (4 mL kg^{-1} , s.c.), as described by Brekhman and Dardymov (1969). One group received distilled water (0.5 mL kg^{-1} i.p.) and milk. The other groups received MJE (100 and 200 mg kg^{-1} , i.p.) and diphenhydramine (1 mg kg^{-1} , i.p.). After 30 min of drug treatment each animal was injected with milk. The difference in eosinophile count before and 24 h after drug administration was calculated.

4.7. Clonidine-induced mast cell degranulation

Mice were divided in five groups of five animals each. A three-day drug treatment schedule was followed. Group I received vehicle (0.1 mL , i.p.). Group II was treated with a standard drug, i.e. DSCG ($10 \mu\text{g kg}^{-1}$ i.p.). Groups III–V received MJE (100 and 200 mg kg^{-1} , i.p. each). On the fourth day each mouse received 4 mL kg^{-1} of normal saline into the peritoneal cavity. Peritoneal fluid was collected after 5 min of gentle massage and transferred into siliconised test tubes containing 10 mL RPMI-1640 buffer medium (pH 7.2–7.4). This solution was then centrifuged at $400\text{--}500 \text{ rev min}^{-1}$ for 15 min. Pellets of mast cells were washed with the same buffer medium twice by centrifugation, discarding the supernatant. These cells were challenged with clonidine ($50 \mu\text{g}$) and incubated at 37°C in a water bath for 10 min. This was followed by staining with 1% toluidine blue and observed under microscope ($10\times$). A total of 100 cells were counted from different visual areas. Percent protection against degranulation was calculated (Gupta, Srimal, Verma, & Tandon, 1994).

4.8. Active paw anaphylaxis

Mice of either sex were sensitised by injecting $0.25 \mu\text{g}$ of egg albumin adsorbed on 6 mg of aluminium hydroxide gel subcutaneously. On day 12 of sensitisation, the animals were challenged with $10 \mu\text{g}$ of egg albumin in saline in the sub plantar region of the hind paw. The contralateral paw received an equal volume of saline. The paw thickness was measured using a micrometer 15 min after challenge. The difference in paw thickness reflected the oedema due to antigen antibody reaction (Nair, Tamhankar, & Saraf, 1994). Mice received vehicle, MJE (100 and 200 mg kg^{-1} , i.p.) and dexamethasone (0.5 mg kg^{-1} , s.c.) 30 min prior to antigen challenge.

4.9. Statistical analysis

All observations are presented as mean $\pm$ SEM. The data was analysed by Student's *t*-test or one-way ANOVA followed by Dunnett's test. $p < 0.05$ was considered significant.

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