

**EFFECT OF *INULA RACEMOSA* ROOT EXTRACT  
ON VARIOUS ASPECTS OF ASTHMA**

Gautam P.Vadnere\*<sup>1</sup>, Ram S.Gaud<sup>2</sup>, Abhay Kumar Singhai<sup>3</sup>, R.S.Somani<sup>4</sup>

<sup>1</sup>Smt.S.S.Patil College of Pharmacy, Chopda-425 127, (MS), India

<sup>2</sup>School of Pharmacy and Technology Management, NMIMS, Mumbai-400 056,  
(MS), India

<sup>3</sup>Department of Pharmaceutical Sciences, Dr. Hari Singh Gour Vishwavidyalaya,  
Sagar-470003, (MP), India

<sup>4</sup>Sinhgad College of Pharmacy, Pune-411 041(MS), India

**Summary**

*Inula racemosa* Hook. F. commonly known as 'Pushkaramula' in indigenous system of medicine in India. The roots are bitter, acrid, thermogenic, aromatic, stimulant, antiseptic, alexipharmic, deodorant, anti-inflammatory, digestive, carminative, expectorant, bronchodilator, diuretic, uterine stimulant, aphrodisiac, febrifuge, tonic. They are useful in vitiated conditions of Kapha and vata, foul ulcers and wounds. Use of this herb in treatment of asthma like conditions by ayurvedic practioners in India prompted us to evaluate the actions of test extracts on various aspects of asthma like bronchoconstriction, eosinophilia, stress, mast cell degranulation and allergy associated with change in vascular permeability using various *in vitro* and *in vivo* animal models. Petroleum ether (60-80°), ethanol (95%), water extract of air dried roots of *Inula racemosa* obtained by successive extraction. Petroleum ether extract (PEEIR) at a dose of 4 mg/ml (55.41±3.04) and 10 mg/ml (48.87±1.36) exert significant antagonistic effect (p<0.05) on histamine induced (1.6µg/ml) contraction as compared to its ethanol and water extract. A dose dependent contraction was observed in goat tracheal chain preparation. Significant control of milk-induced eosinophilia in mice was seen at a dose of 50 & 100mg/kg i.p. by petroleum ether extract (44.77 % & 54.36 % respectively) as compared control group (43.1±2.41). Same dose dependent inhibition of milk induced leukocytosis 59.53 % and 77.47% by petroleum ether extract supports the adaptogenic potential of drug. Challenge with clonidine induces mast cell degranulation in mice and clonidine- induced mast cell degranulation was inhibited by standard mast cell stabilizer disodium cromoglycate (DSCG 200µg/kg, i.p.) as 14±1.22 (83.57%) when compared with control group. Pretreatment with petroleum ether extract at a dose of 100 mg/kg i.p significantly (p<0.05) offered 74.68% of protection against mast cell degranulation when compared with control group. Altering significantly (p<0.05) the capillary permeability as evident again from the optical density value by treatment group of petroleum ether extract at a highest dose of 100 mg/kg i.p (212±18.9) as compared to control group (602±27.8). Results thus obtained substantiate the potential role of herb in immunologically, physiologically and biochemically heterogeneous disorder, asthma and related conditions.

**Key Words:** antihistaminic, capillary permeability, eosinophilia, *Inula racemosa*.

\*Address of corresponding author: Smt.S.S.Patil College of Pharmacy, Chopda-425 127, (MS), India. E-mail ID: [gautamvadnere31@rediffmail.com](mailto:gautamvadnere31@rediffmail.com)  
Phone No.: +91 9890022892, Fax: +91 2572210894

## Introduction

The complex nature of asthma has allowed the development of a number of diverse approaches to its treatment and as a result the disease is controlled to some extent by existing drug regimens with certain improvement in treatment. According to the Global Initiative for Asthma (GINA) program initiated with the U.S. National Heart, Lung, and Blood Institute, NIH and the World Health Organization (WHO) estimates that as many as 300 million people of all ages, and all ethnic backgrounds, suffer from asthma. It is estimated that asthma accounts for about 1 in every 250 deaths worldwide. The limited availability of asthma medications is a major problem in the countries like Southern Asia (1). The strongest risk factors for developing asthma are exposure to indoor allergens and same remains a major risk factor for respiratory disease, including asthma, in Southern Asia. It has been estimated that over a half million premature deaths can be attributed annually to the use of biomass fuels in India (2). Indeed, large parts of the Indian urban populations are exposed to some of the highest air pollutant levels in the world (3). In its various forms asthma comprises a syndrome of bronchial inflammation, hyper responsiveness and airflow obstruction. Pathogenesis of this disease is believed to be atopic in more than 50% adults and at least 80% of affected children (4). According to numerous epidemiological studies from different parts of the world, the prevalence of the atopic diseases has increased over the past 30–40 years (5). Ayurveda is the most ancient health care system of India, Srilanka and other countries (6). The World Health Organization has also recognized the importance of traditional medicine and has created strategies, guidelines and standards for botanical medicines. Every now and then we come across patients of asthma seeking ayurvedic treatment and advice. This is because of the versatile approach of ayurveda to the root cause of the problem and its belief in preventing the disease rather than treating it. *Inula racemosa* H.F. is highly valued in “Indian System of medicine” possessing potential role in allergic asthma and related respiratory affliction as reported.

Much of the investigations support the therapeutic efficacy of the plant as anti-hyperglycemic (7),  $\beta$  adrenergic blocking activity (8), cardiac activity (9), repellent, insecticidal and phytotoxic (10), antidermatophytic (11, 12), anti-inflammatory and hepatoprotective (13), antifungal and antibacterial effect (14) as well plant is a major ingredients in many ayurvedic formulations. In this context, systematic investigation thought to be much more mandatory and aimed to validate its traditional claims by screening the possible antiasthmatic properties of plant in various aspects of asthma like bronchoconstriction, leukocytosis, eosinophilia, allergy and mast cell degranulation.

## Methods

### Plant material

*Inula racemosa* H.F. identified at satpuda hills near to Chopda, Dist. Jalgaon, MS, India,, as it usually grows at higher altitude in hilly areas with the help of Botanist and plant identity was confirmed by comparing the samples with the description mentioned in different floras and text. Further to authenticate plant species herbarium sheet was prepared and forwarded to BSI (Botanical Survey of India, Pune). A voucher specimen (No. 285176) deposited at BSI. Roots of fully established plant (nearly two years old) were collected in the month of September by uprooting method, washed thoroughly with water then air dried roots was stored at 25 °C in air tight container.

### Extraction of plant material

Air dried roots of *Inula racemosa* H.F. coarsely powdered with the use of hand grinder removed excessive fine then weighed 3 kg of powder and subjected for continuous hot extraction (Soxhletion) in batch primarily to defatt the plant material with petroleum ether [60-80°C] for 72 hrs After complete deffating, extracted material was filtered and the solvent was evaporated under reduced pressure up to dryness. Further the marc was subjected extraction with ethanol [95%] for 72 hrs then extract was filtered and concentrated under reduced pressure. Finally ethanol exhausted marc was macerated with purified water for 7 days. Aqueous extract was then filtered and concentrated under reduced pressure. All the concentrated extracts were finally dried in a water bath at temperature of 30-35°C and stored in cool and dried place.

### Animals

Healthy adult female Wistar rats weighing 200-250 g and adult albino Swiss mice weighing 25-30 g were housed in groups of five under standard laboratory conditions of temperature ( $25 \pm 2^\circ\text{C}$ ) and 12/12 hr light/dark cycle. Animals had free access to standard pellet diet and water *ad libitum*. All animal experimentation was carried out after approval of the protocol by the Institutional Ethical Committee of Dr. H.S. Gour University with strict adherence to the guidelines of CPCSEA, India.

### Chemicals and reagents

All standard drugs required for the study was purchased and solvents and chemicals used for physiological salt solution were of laboratory grade. Histamine from Sigma, USA; Clonidine of Unichem, India; Disodium cromoglycate from Cipla, RPMI Buffer medium 1640 of Hi Media, India has been purchased. Reagents like Eosin solution, WBC diluting fluid purchased from Qualigens, India. Toluidine blue and Evans blue were purchased from Research Lab, India. Bovine serum albumin and Freund's adjuvant obtained as gift sample from Serum Institute of Pune, India.

### Preparation of samples

Extract solutions were prepared in 5 % Polyethylene glycol (PEG-400) for *in vitro* and *in vivo* studies.

### Goat tracheal chain preparation

Goat trachea brought from slaughter house were cut into individual ring and tied together in series to form a chain (15). Then suspended in a bath containing Kreb's solution (Concentration in mMoles/ L as NaCl, 118; KCl, 4.7; CaCl<sub>2</sub>, 2.5; MgSO<sub>4</sub>, 1.2; NaHCO<sub>3</sub>, 25.0; KH<sub>2</sub>PO<sub>4</sub>, 1.2; Glucose, 11.1) maintained at  $37 \pm 1^\circ\text{C}$ . One end of the tracheal chain was attached to an S-shaped aerator tube and other attached to an isotonic frontal writing lever to smoked drum (magnification 10-12 folds), a stream of 5% CO<sub>2</sub> in oxygen should be bubbled through organ tube. Tissue was allowed to equilibrate for 45 min. under to load of 400 mg (15). A dose response curve for histamine in variant molar concentrations, by maintaining 45 min time cycle was taken as,

**Group-I**– Control group, **Group-II**–Vehicle treated (PEG-400, 5 % /ml), **Group-III, IV, V,**–Received test extract added to reservoir at a dose of (2mg/ml, 4mg/ml, 10mg/ml respectively). Graph of percentage of maximum contractile response on ordinate and negative logarithm of molar concentration of histamine on abscissa was plotted to record dose response curve of histamine, in absence and in presence test extract.

### **Acute toxicity studies for dose selection**

The acute oral toxicity study of different extracts was carried out as per the OECD guideline no.423. Healthy adult female Wistar rats weighing between 200 and 250 g were randomly selected, and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The test extracts were administered in a single dose by gavage using a stomach tube. Animals were fasted prior to dosing, following fasting period, the animals were weighed and test substance was administered. After the dose was administered, food was withheld for a further 3-4 h in rats. The literature survey showed that the plant material and their various extracts were used for several studies. Hence, on the basis of available information limit test for crude extracts was conducted at the highest starting dose level 2000 mg/kg of body weight. Animals were observed initially after dosing at least once during the first 30 minutes, periodically during the first 24 hours. The animals were alive, healthy and active during the observation period. There were no changes in skin and fur, eyes and mucous membranes, and respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern were normal. Highest dose at which no toxic signs are seen, one fifth or less of that can be taken as effective dose.

### ***In vivo* studies**

#### **Milk- induced eosinophilia**

A blood eosinophilia is hallmark of both allergic and non allergic asthma. Mice were divided into five groups, five animals each group.

**Group-I** serves as control, receives vehicle 1ml /kg, i.p. and boiled and cooled milk in a dose of 4 ml /kg, s.c.

**Group-II** received only vehicle (5 % PEG-400, 1ml /kg, i.p.).

**Group-III, IV and V** were treated with test extracts at a dose of (25, 50,100 mg/kg, i.p.) respectively, 1 hr before milk injection.

Blood samples were collected from retro-orbital plexus under light ether anesthesia, the eosinophil count is done in each group before drug administration and 24 h after the milk injection (boiled and cooled, 4 ml/kg, s.c.). Difference in the eosinophil count before and 24 h after milk administration was noted using modified method described by authors (16, 17).

#### **Milk- induced leukocytosis**

Mice were divided into five groups, five animals each group.

**Group-I** serve as control and treated with vehicle 1ml/kg, i.p. and boiled and cooled milk in a dose of 4 ml/kg, s.c.

**Group-II** received only vehicle (5 % PEG-400, 1ml/kg, i.p.).

**Group-III, IV and V** were treated with test extracts at a dose of (25, 50,100 mg/kg, i.p.) respectively, 1 hr before milk injection.

Blood samples were collected from retro- orbital plexus under light ether anesthesia, the leukocyte count is done in each group before drug administration and 24 h after the milk injection (boiled and cooled, 4 ml/kg, s.c.). Difference in the leukocyte count before and 24 h after milk administration was noted using modified method described by authors (17).

**Mast cell degranulation**

Mice divided in five groups, five animals each. The three days drug treatment schedules were followed.

**Group-I** serve as control and treated with vehicle (10 ml/kg, i.p.).

**Group-II** treated with standard drug disodium cromoglycate (DSCG, 200 $\mu$ g/ml, i.p.).

**Group-III, IV and V** were treated with test extracts at a dose of 25, 50 and 100 mg/kg, i.p. respectively.

On day fourth each animal were injected with 4 ml/kg, 0.9% NaCl solution into peritoneal cavity. By gentle massage, peritoneal fluid collected after 5 min. and transferred into siliconised test tube containing 7-10 RPMI- 1640 buffer medium (pH 7.2- 7.4). This solution then centrifuged at 400- 500 rpm. Pellets of mast cell were washed with same buffer medium twice by centrifugation, discarding supernatant. The cells were challenged with clonidine (50  $\mu$ g) incubated at 37 °C in a water bath for 10 min. Followed by staining with 1 % toluidine blue and observed under microscope. Total 100 cells were counted from different visual area. Percent protections against degranulation were calculated using method described by (18, 19).

**Capillary permeability**

Mice were divided into four groups of five animals each.

**Group-I** serve as control, mice in this group were sensitized with bovine albumin and Freund's adjuvant 0.05 ml given i.p. Three weeks later the animals were challenged with the same dose of bovine albumin. At the same time Evan's blue injected at a dose of 200mg/kg, i.v. The mice were sacrificed. 30 min. later 5 ml of saline was injected i.p. and the abdominal wall was gently massaged for a minute. The abdomen was then incised and peritoneal fluid was collected and filtered after passage through glass wool. It was centrifuged at 3000 rpm for 15 min.

**Group-II, III and IV** were treated with test extracts at a dose of 25, 50 and 100 mg/kg, i.p. 24 h and 2 h before the challenge. The transmittance of the dye depends on capillary permeability was determined by measuring the optical density on a spectrophotometer by modification of the method used by (20).

**Statistical analysis**

The data presented as mean $\pm$ SEM. The statistical significance between the groups has been tested by ANOVA followed by Dunnett's test. A probability value less than 0.05 were considered as significant.

**Results****Acute toxicity Studies**

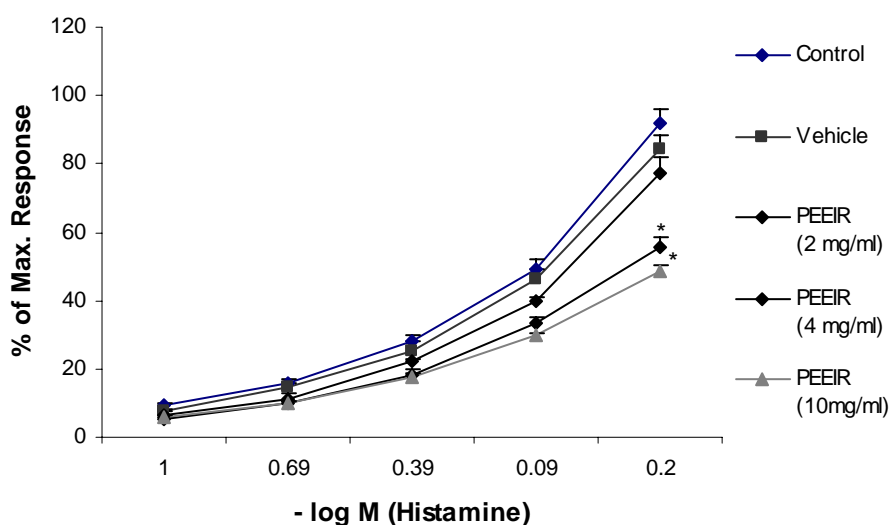
Studies done to assess the safety and therapeutic profile of the plant extract, Administration of single dose of petroleum ether (60-80 °C), alcohol (95 %) and water extracts of flowers of roots of *Inula racemosa* at the limit dose of 2000 mg/kg, p.o did not have any toxic effects. The animals were alive, healthy and active during the observation period. Thus, different extracts emerged safe for administration for further studies.

**Effect of crude extract on isolated goat tracheal chain preparation**

A notable contraction produced by histamine at a dose of 1.6 $\mu$ g/ml on isolated goat tracheal chain preparation (82 mm taken as 100 %). Petroleum ether extract of roots *Inula racemosa* (PEEIR) at a dose of 4 mg/ml (55.41 $\pm$ 3.04) and 10 mg/ml (48.87 $\pm$ 1.36) as shown in Figure 1 and Table 1, exert significant antagonistic effect

( $p < 0.05$ ) on histamine induced (1.6 $\mu$ g/ml) contraction as compared to its ethanol and water extract.

**Figure 1. Effect of PEEIR on goat tracheal chain preparation**



n= 4, values are expressed in mean $\pm$ SEM.

\* $p < 0.05$  compared with histamine induced contraction (82 mm taken as 100 %).

**Table 1. Effect of crude extracts on goat tracheal chain preparation**

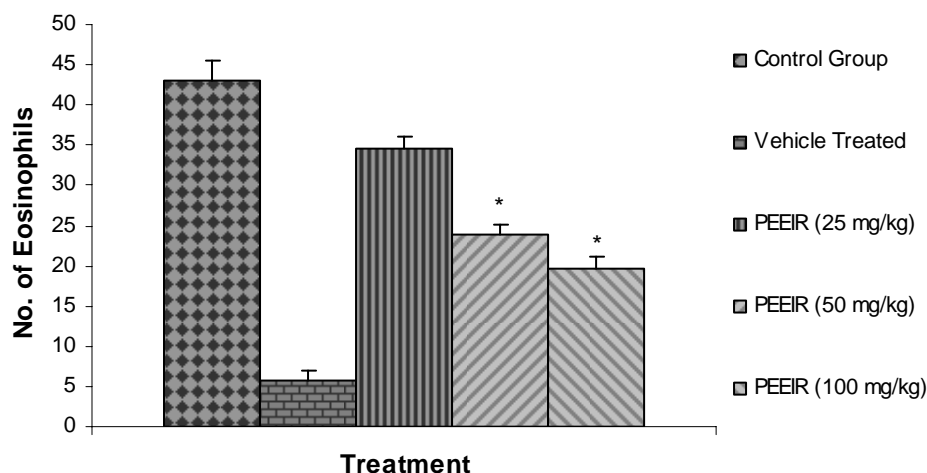
| Sr. No | Extract   | Dose           | Effect on the tissue | Effect on histamine-induced contraction (%) |
|--------|-----------|----------------|----------------------|---|
| 1.     | Histamine | 1.6 $\mu$ g/ml | Contraction          | 92.07 $\pm$ 3.8                             |
| 2.     | PEEIR     | 2 mg/ml        | Contraction          | 77.42 $\pm$ 4.49                            |
| 3.     | PEEIR     | 4 mg/ml        | <b>Relaxation</b>    | <b>55.41<math>\pm</math>3.04*</b>           |
| 4.     | PEEIR     | 10mg/ml        | <b>Relaxation</b>    | <b>48.87<math>\pm</math>1.36*</b>           |
| 5.     | EEIR      | 2 mg/ml        | Contraction          | 93.9 $\pm$ 0.996                            |
| 6.     | EEIR      | 4 mg/ml        | Contraction          | 87.2 $\pm$ 0.584                            |
| 7.     | EEIR      | 10mg/ml        | Contraction          | 89.6 $\pm$ 1.041                            |
| 8.     | WEIR      | 2 mg/ml        | Contraction          | 89.32 $\pm$ 3.69                            |
| 9.     | WEIR      | 4 mg/ml        | Contraction          | 78.56 $\pm$ 2.98                            |
| 10     | WEIR      | 10mg/ml        | Contraction          | 85.37 $\pm$ 3.65                            |

n =4, values are expressed in Mean $\pm$ SEM

\* $P < 0.05$  compared with histamine- induced contraction (82mm taken as 100%).

### Effect of crude extract on milk-induced eosinophilia in mice

Pretreatment with petroleum ether extract of roots of *Inula racemosa* (PEEIR) at a dose of 50 & 100mg/kg i.p. significantly reduced milk-induced eosinophilia in mice (23.8 $\pm$ 1.265, 19.67 $\pm$ 1.408 respectively) as compared control group (43.1 $\pm$ 2.41). Ethanol and water extract of *Inula racemosa* (EEIR, WEIR) unable to control the eosinophilia in all the doses administered. Figure 2 shows the control of eosinophilia (44.77 % & 54.36 %) in mice by PEEIR in dose dependent manner might substantiate the use of this herb in allergic asthma.

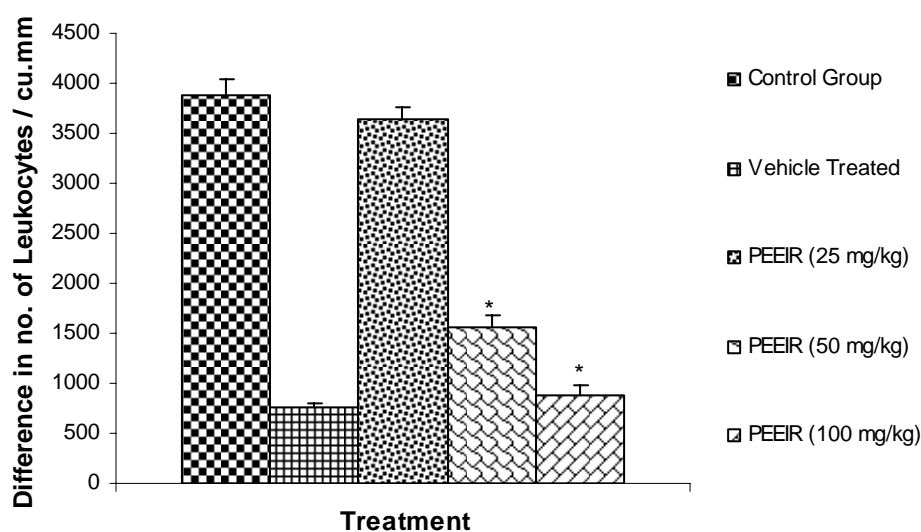
**Figure 2. Effect of PEEIR on Milk induced eosinophilia in mice**

n=5, values are expressed in mean±SEM

\*p< 0.05 compared with control group (ANOVA followed by Dunnett's test)

#### Effect of crude extract on milk-induced leukocytosis in mice

In continuation of these study the effect of PEEIR, EEIR, WEIR at same dose assessed for control of leukocytosis. A perusal of result in figure 3, reveals that PEEIR at a dose of 50 & 100 mg/ kg i.p controls the milk induced leukocytosis  $1570 \pm 102.64$ ,  $874 \pm 102.56$  respectively as compared with control group. The results clearly indicate that dose dependent inhibition of leukocytosis 59.53 % and 77.47% by *Inula racemosa* petroleum ether extract and no sign of this potential seen in ethanol and water extract of drug.

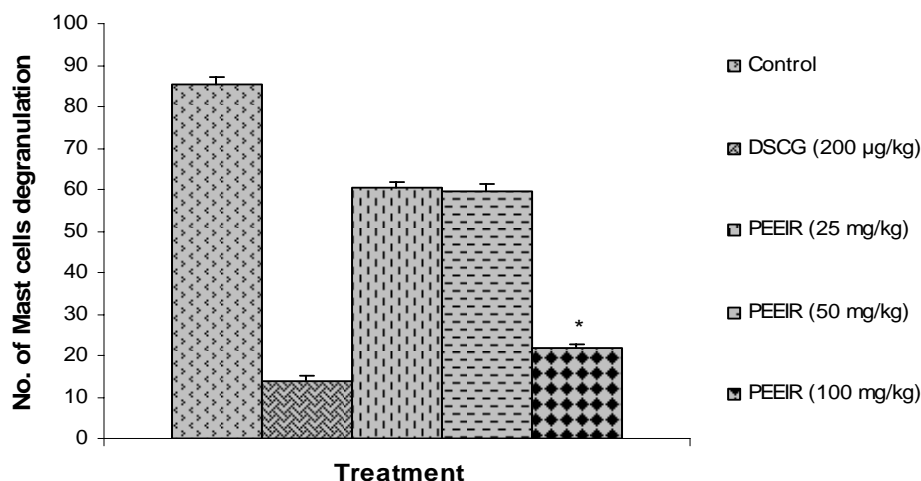
**Figure 3. Effect of PEEIR on Milk induced leukocytosis in mice**

n=5, values are expressed in mean±SEM

\*p< 0.05 compared with control group (ANOVA followed by Dunnett's test)

**Effect of crude extracts on clonidine-induced mast cell degranulation in mice**

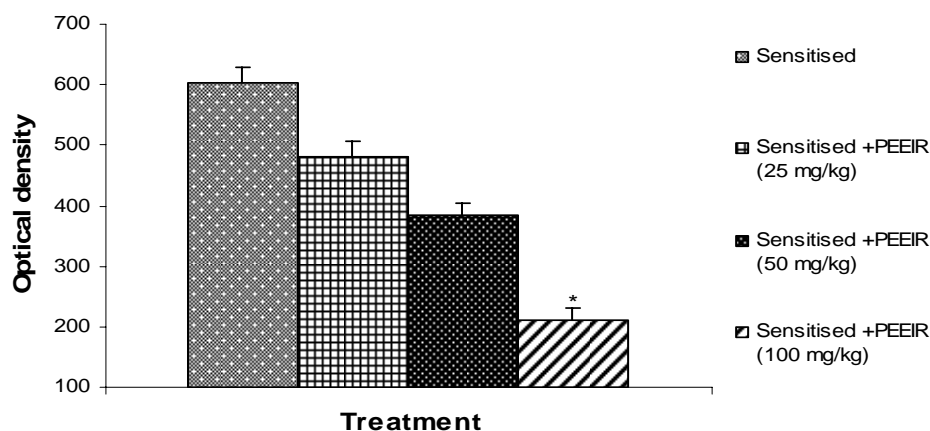
Treatment group with the dose of 100 mg/kg i.p of petroleum ether extract of roots of *Inula racemosa* (PEEIR) significantly ( $p < 0.05$ ) offered 74.68% of protection against mast cell degranulation when compared with control group (Figure 4). Ethanol and water extract unable to show that substantial protection against degranulation.

**Figure 4. Effect of PEEIR on mast cells degranulation in mice**

n=5, values are expressed in mean±SEM; Control = Vehicle (5 % PEG-400, 1ml / kg, i.p); \* $p < 0.05$  compared with control group (ANOVA followed by Dunnett's test)

**Effect of crude extracts on capillary permeability in mice**

Significantly ( $p < 0.05$ ) altering the capillary permeability as evident again from the optical density value by treatment group of petroleum ether extract of roots of *Inula racemosa* (PEEIR) at a highest dose of 100 mg/ kg i.p ( $212 \pm 18.9$ ) as compared to control group ( $602 \pm 27.8$ ) as senn in figure 5. Ethanol and water extract of roots was unable to show that alteration in vascular permeability.

**Figure 5. Effect of PEEIR on capillary permeability in mice**

n=5, values are expressed in mean±SEM; \* $p < 0.05$  compared with control group (ANOVA followed by Dunnett's test)



### Discussion

Crude extracts tested for direct antihistaminic activity *in vitro* using isolated goat tracheal chain preparation, dose relative (0.1-1.6 µg/ml) contractile response of histamine using goat tracheal chain preparation demonstrates the sensitivity of histamine H<sub>1</sub> receptors present and potentiates its utility for screening the direct antihistaminic action of different extracts under investigation for their claim in asthma treatment. It is reported that isolated goat trachea contracts in response to acetylcholine (0.1-12.8 µg), histamine (0.1-102.4 µg), and barium chloride (0.1-51.2 µg) in a dose dependent manner and to 5-HT in a narrow dose range. Pheniramine maleate (H<sub>1</sub>-receptor antagonist) blocks contractions to histamine while cimetidine (H<sub>2</sub>-receptor antagonist) potentiates the contraction. These observations suggest the presence of both H<sub>1</sub>-excitatory and H<sub>2</sub>-inhibitory receptors for histamine on the isolated goat trachea (21).

A notable contraction produced by histamine at a dose 1.6µg/ml, as 82 mm taken as 100 % for *Inula racemosa* study. Significant inhibition of histamine induced contractions produced by petroleum ether extract of roots of *Inula racemosa* (PEEIR) in dose dependent manner (4-10 mg/ml) on isolated goat tracheal chain preparation may be by blockade of H<sub>1</sub> receptors leading to inability of smooth muscle to respond to histamine induced spasm leading to inhibition of bronchoconstriction.

Further assessment of antiallergic activity *in-vivo* by studying the effect of extracts on milk-induced eosinophilia in mice where (PEEIR) at a dose of 50 & 100mg/kg i.p. significantly reduced milk-induced eosinophilia in mice (23.8±1.265, 19.67±1.408 respectively) as compared control group (43.1±2.41). Drug shows the control of eosinophilia (44.77 % & 54.36 %) in mice by PEEIR in dose dependent manner might substantiate the use of this herb in allergic asthma.

Increases in TLC (Total leukocyte count) or nervous debility may aggravate symptoms of asthma. After parental administration of milk there was increase in TLC, and this stress full condition can be made normalized by administration of an antistress or adaptogenic drugs. PEEIR at a dose of 50 & 100 mg/ kg i.p controls the milk induced leukocytosis was indicative of the antistress effect of PEEIR when compared with control group.

Clonidine, a α<sub>2</sub> adrenoceptor agonist causes disruption of mast cell wall similar to compound 48/80 act through the dynamic expulsion of granules without causing any change to the cell wall (19). Released mediators from mast cells, each having more than one potent effect on airway inflammation (22). Challenge with clonidine induces mast cell degranulation in mice and clonidine- induced mast cell degranulation was inhibited by standard mast cell stabilizer disodium cromoglycate (DSCG 200µg/ml, i.p.) as 14±1.22 (83.57%) when compared with control group. Treatment group with the dose of 100 mg/kg i.p of petroleum ether extract of roots of *Inula racemosa* (PEEIR) significantly (p<0.05) offered 74.68% of protection against mast cell degranulation when compared with control group thereby justifies its mast cells stabilizing potential.

Change in vascular permeability is predominant in atopic conditions. Allergy and anaphylaxis in the rat is associated with a marked increase in intestinal capillary permeability (23). An increased capillary permeability in the early days after the sensitization was considered to be caused by the irritability of Freund's adjuvant. But increasing leakage of dye was seen in animal sensitized passively. Thus the increasing leakage of dye may be convincingly accepted as one of criteria of these immunological experiments in redefining the role of these test extracts in acute

allergic conditions. In this study the result of increasing leakage of dye was observed in the form of optical density as transmittance of the dye depends on change in capillary permeability. Significantly ( $p < 0.05$ ) altering the capillary permeability as evident again from the optical density value by treatment group of petroleum ether extract of roots of *Inula racemosa* (PEEIR) at a highest dose of 100 mg/ kg i.p ( $212 \pm 18.9$ ) as compared to control group ( $602 \pm 27.8$ ). The promising result of these present investigations confirms the utility of *Inula racemosa* in various aspects of asthma and promotes the further study for specific mechanistic approach and phytoconstituents responsible for the significant results.

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