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### Mast cell stabilizing activity of *Inula racemosa* linn.

#### ABSTRACT

The influence of the ethanolic extract of roots of *Inula racemosa* linn on degranulation of rat peritoneal mast cell induced by compound 48/80 and Egg albumin was studied. The inhibitory effect of the extract was significant in immunologically induced degranulation of mast cells.

**KEYWORDS:** Flavonoids, Kitotifen, Mast cell degranulation, *Inula racemosa*.



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## INTRODUCTION

Mast cells and basophils play a central role in inflammatory and immediate allergic reactions. On stimulation, they are able to release potent inflammatory mediators, such as histamine, proteases, chemotactic factors, cytokines and metabolites of arachidonic acid that act on the vasculature, smooth muscle, connective tissue, mucous glands and inflammatory cells. Mast cells settle in connective tissues and usually do not circulate in the blood stream. Basophils are the smallest circulating granulocytes with relatively the least known function. They arise in the bone marrow, and following maturation and differentiation, are released into the blood circulation. Adequately stimulated basophils may settle in the tissues. There are two categories of inflammatory (anaphylactic) mediators in mast cells and basophils. Preformed mediators, stored in secretory granules and secreted upon cell activation, include a biogenic amine, typically histamine, proteoglycans, either heparin, over-sulphate chondroitin sulphates or both, and a spectrum of neutral proteases. Released histamine acts at H1, H2 and H3 receptors on cells and tissues, and is rapidly metabolized extracellularly. The proteoglycan imparts the metachromatic staining characteristic of mast cells when exposed to certain basic dyes such as toluidine blue. It has two functions, (1) may package histamine and basic proteins into secretory granules, and in mast cells and (2) appears to regulate the stability of the protease called tryptase. Neutral proteases, which account for the vast majority of the granule protein, serve as markers of mast cells found in serosal, mucosal and brain region. Newly generated mediators, often absent in the resting mast cells, are typically produced during IgE-mediated activation, and consist of arachidonic acid metabolites, principally leukotriene C4 (LTC4) and prostaglandin D2 (PGD2) and cytokines. Of particular interest in humans is the production of tumour necrosis factor , IL-4, IL-5 and IL-6<sup>1</sup>. *Inula racemosa* (IR) is an ornamental plant of the asteraceae family. It grows in the temperate and alpine western Himalayas, and it is common in Kashmir., commonly known as puskarmula, it is well known herb in India for its medicinal properties. The roots of puskarmula are used for the medicinal purpose. Externally, the paste of its roots is used effectively, in dressing the wounds and ulcers as the herb possesses antiseptic property. It alleviates the pain along with the oedema. The essential oil of the roots of puskaramula show antibacterial and antifungal activity and is moderately effective against *S. aureus*, *Ps aeruginosa*, *B. subtilis* and mildly against *E. coli* and *B. anthracis*. The paste of its roots is specially recommended, to be applied on the chest in pleurisy and inflammatory conditions of pleura, to mitigate the pain. Internally, puskaramula is useful to boost the appetite and to digest undigested toxic metabolites. Hence it is beneficial in anorexia (loss of appetite) and dyspepsia (indigestion). It also alleviates the flatulence and abdominal pain. Puskaramula is the highly praised panacea for cough, hiccup and bronchial asthma .In pulmonary functions by abolishing the bronchospasm, relieving the mucous and hence, the obstruction in bronchial asthma. It also effectively curbs the frequency of paroxysms of bronchospasms, when the root powder is licked with honey. Puskaramula works well in pleurisy, even tubercular, by ameliorating the infection, fever, pain and th cough. It imparts a stimulant action on the heart and reduces the breathlessness due to cardiac asthma. It possesses a mild diuretic property, hence is used with benefit in dysuria. In ayurveda it is widely used for various disorders, it is mostly used in heart and respiratory disorders. The rhizome act as antiseptic, anti-bacterial, anti-fungal, anti-inflammatory, analgesic and mild diuretic. It is used in the treatment of contagious fevers, angina pectoris, heart disease and ischemic heart disease. It is also used in cough, hiccup, bronchial asthma, indigestion, flatulence, inanorexia and in fever<sup>2-3</sup>.

**Principle constituent:** At least four sesquiterpene lactones have been isolated from *Inula*. These compounds obtained are dihydroisoalantolactone, isoalantolactone and alantolactone. From the roots, sitosterol, octadecanoic acid and D-mannitol have been isolated. Two biologically active new sesquiterpene lactones, inunal and isoalalantolactone are isolated. Alantolactone, isoalantolactone and dihydroisoalantolactone isolated from roots.

A germacranolide – inunolide – from root oil. Also alloalantolactone isolated from roots and characterized. Two new sesquiterpene lactones inunal and isolloalantolactone isolated and characterized<sup>2-3</sup>.

## 2. Materials and methods

### 2.1 Plant material

The dried root powder of *Inula racemosa* was purchased from Dravid Herbs World, Pondicherry, India. Egg albumin was purchased from Hi-Media Lab., Mumbai and compound 48/80 was purchased from sigma chemicals company, USA. Kitotifen (Ketovent) was purchased from Intas Pharm. Ltd., India.

### 2.2 Extraction

*Inula racemosa* root powder extracted with 90% ethanol in a soxhlet extractor. The extract was concentrated under reduced pressure at a temperature below 50°C to yield a syrupy mass (Yield -7.10%), which was used for the present investigation

### 2.3 Preliminary phytochemical investigation

Preliminary phytochemical analysis shows the presence of glycosides, terpenoids and flavonoids<sup>4</sup>.

### 2.4 Animals

Animals-Male Wistar rats (200-250g) were obtained from the experimental animal house, School of life science, Devi Ahilya University, Indore. They were maintained under standard housing condition (Room temperature 25±2°C and 45-55% RH with 10:14h, L:D cycles). The animals were given standard laboratory feed and water ad libitum. The study was cleared by Animal ethics committee (School of life science, Devi Ahilya University, Indore). All the animals received humane care according to criteria outlined in the guide for the care and use of laboratory animals prepared by the national academy of the sciences and published by national institute of health.

### 2.5 Degranulation studies

Male Wistar rats (200-250gms) were exsanguinated and injected intraperitoneally with 10 ml of physiological solution of the following composition in mM NaCl 137, NaHCO<sub>3</sub> 12, NaH<sub>2</sub>PO<sub>4</sub> 0.3, KCl 2.7, MgCl<sub>2</sub> 1.0, CaCl<sub>2</sub> 1.8 dextrose 5.6. The abdomen was gently massaged for about 1 minute and the peritoneal exudates was collected over ice and centrifuged at 2000 rpm for 5 minutes. The cells were washed twice with physiological solution and was resuspended in 1 ml of the salt solution. Sensitized mast cell were obtained from animals sensitized with egg albumin (350mcg). The doses being given on the 1<sup>st</sup>, 3<sup>rd</sup> and 5<sup>th</sup> day. The sensitized mast cells were degranulated using egg albumin (1mg/ml) on the 10<sup>th</sup> day of sensitization. The normal mast cell were degranulated using compound 48/80 (100mcg/ml). The cell suspension of mast cells was treated as follows. To 0.1 ml of the peritoneal mast cell suspension, 0.1ml of the test agent in the saline was added and incubated in a constant temperature water bath (37°C) for 15 minutes. Then 0.1 ml of degranulating agent (Egg albumin 1 mg/ml or compound 48/80 100mcg/ml) was added and further incubated for a period of 10 minutes. The cell were then stained with 0.1% toluidine blue for 5-10 min. The stained cells were viewed through a digital light microscope at 100x magnification and 100 mast cells were counted. The number of intact and fragmented or disrupted mast cells was noted. A mast cell was considered disrupted if four or five granules were found around the mast cells. The number of fragmented or disrupted mast cells as well as of the intact mast cells were counted<sup>5-8</sup>.

## 2.6 Analysis

Values were expressed as mean  $\pm$  SE. The values were statistically analyzed using one-way Analysis of Variance (ANOVA) followed by Tukey's multiple comparison test. The analysis was carried out using Graph Pad Prism software V.4.

S.No.	Treatment	Dose mcg/ml	Number of mast cell	Percent Inhibition
1.	Control	-	7 $\pm$ 2	-
2.	Ketotifen	10	82 $\pm$ 4	78.22*
3.	IR extract	05	30 $\pm$ 3	18.85*
4.	IR extract	10	46 $\pm$ 5	39.96*
5.	IR extract	20	61 $\pm$ 6	58.97*
6.	IR extract	40	74 $\pm$ 4	71.65*

Values are mean $\pm$ S.E.,

\*P<0.001 when compared with control.

Table: Effect of *Inula racemosa* extract on egg albumin induced mast cell degranulation in rats.

S.No.	Treatment	Dose mcg/ml	Number of mast cell	Percent Inhibition
1.	Control	-	8±2	-
2.	Ketotifen	10	80±4	77.52*
3.	IR extract	05	33±4	20.36*
4.	IR extract	10	46±3	37.08*
5.	IR extract	20	61±4	59.52*
6.	IR extract	40	43±3	41.28*

Values are mean±S.E.,

\*P<0.001 when compared with control.

TableII: Effect of *Inula racemosa* extract on compound 48/80 induced mast cell degranulation in rats.

### 3. Results

*Egg albumin induced degranul* Kitotifen as a reference standard was found to inhibit degranulation to an extent of 78.22. *Inula racemosa* extract at concentration 5,10,20 and 40 mcg/ml produced dose related inhibition of 18.85,39.96,58.97 and 71.65 respectively (Table-I). *Compound 48/80 induced degranulation studies* Ketitifen at a concentration of 10mcg/ml was found to inhibit degranulation to an extent of 77.52. *Inula racemosa* extract at concentration of 5,10,20 and 40 mcg/ml showed reduction in degranulation of mast cell to 20.36,37.08,59.52 and 41.28 respectively (Table-II).

#### 4. DISCUSSION

The mast cells have a crucial role in the development of many physiological changes during anaphylactic and allergic responses. Immunoglobulin- E antibodies bind to receptors on the surface of mast cell. Allergen-IgE interaction on mast cell leads to the release of histamine, heparin, proteases and other mediators and the synthesis and secretion of leukotrienes and prostaglandins. These products result in bronchoconstriction, changes in blood vessel tone, increased vascular permeability and myriad other proinflammatory effects<sup>9</sup>. The functions of mast cells can be manipulated for therapeutic ends by regulating their function with appropriate drugs. Plant origin constituents may influence differentiation into mast cells, chemical composition and or architecture of mast cell surface membrane. It may influence the synthesis of IgE molecules or binding of IgE on mast cell surface. It is also possible, that the plant drug may reduce the life span of mast cells.

Extract of *Inula racemosa* markedly protected the sensitized mast cells. However, the effect was less than that observed with the standard drug (Kitotifen) used. The pathological mechanism involved in Type-I allergy has been explained as the degranulation of mast cells and basophils, followed by the release of mediators such as histamine, leukotrienes and prostaglandins from these cells<sup>10</sup>. The degranulation of mast cells occurs in response to the immunological stimuli in which the antigen-antibody interaction on the cell surface predominates.

Mast cells after degranulation shows demonstrated that transgranulation occurs between mast cells and fibroblasts with mast cells apparently transferring their granules to the cytoplasm of fibroblasts or to the mesothelium. It has been reported that mast cell granules are internalized in fibroblasts 1-3 h after C48/80 injection<sup>11</sup>. In the mast cell the extruded granules might be degraded by the extracellular, as the initial compact morphological appearance of the discharged granules is gradually lost, and the granule contents are discharged<sup>12</sup>. Mast cells are well known for their close appositions to the nervous system, such as to the enteric nerves of the intestine, vagus nerves of the mesentery in the rat, and trigeminal sensory fibers in the rat dura mater. Electrical trigeminal stimulation promotes mast cell secretion and degranulation in the dura mater and tongue, and this activation of mast cells by neurogenic mechanisms appears to be important in the development of neurogenic inflammation<sup>13</sup>.

The present investigation indicates the ethanolic extract of *Inula racemosa* is active in the Type-I allergic conditions because of their ability to inhibit the release of mediators from mast cells and thus influence the course of the disease by preventing the harmful effects of the released mediators. The preliminary phytochemical tests showed the presence of flavonoids in the ethanolic extract. Plant flavonoids are known to inhibit basophil histamine release and neutrophilbetagluconidase release, and thereby possess in-vivo antiallergic activity<sup>14</sup>. The flavonoids also inhibited the histamine release induced by 48/80. Plants containing flavonoids have been reported to possess antihistaminic, antiallergic and mast cell degranulation properties<sup>15-16</sup>.

Based on these results, it could be suggested that *Inula racemosa* stabilizes mast cells in the rat. As mast cells play a major role in Type I hypersensitivity-mediated diseases like allergic asthma and rhinitis, studies are under way to evaluate the efficacy of *Inula racemosa* due to its mast stabilization property in these animal allergic models.

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