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IN VITRO MAST CELL STABILIZATION ACTIVITY OF *ONOSMA BRACTEATUM* WALL.

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ABSTRACT

The influence of the ethanolic extract of the aerial parts of *Onosma bracteatum* wall 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, *Onosma bracteatum*.

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 andinflammatory 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

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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. In the cytoplasm of both mastocytes and macrophages are special lipid bodies, where metabolism of arachidonic acid occur and their products, including leukotrienes, may be stored[1].

Onosma bracteatum(OB), Wall (Family Boraginaceae, commonly known as Gaozaban, Gojihva) which has been reported to be used in the treatment of asthma and bronchitis . The drug is used as tonic, alterative, demulcent, diuretic and is considered cooling. It is useful as a spasmolytic. A decoction is used in the treatment of rheumatism, syphilis and leprosy. The plant is considered to be useful in relieving excessive thirst and restlessness in febrile excitement, and also to be useful in relieving functional palpitation of the heart, irritation of the bladder and stomach, and strangury. Aerial parts of *Onosma bracteatum* are prescribed by many Ayurvedic practitioners in bronchial asthmatic patients. However, no scientific studies are so far carried out to investigate the mast cell stabilizing activity of *Onosma bracteatum* [2,3].

MATERIALS AND METHODS

Plant material

The dried aerial parts of *Onosma bracteatum* 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.

Extraction

Onosma bracteatum aerial parts extracted with 90% ethanol in a soxhlet extractor. The extract was concentrated under reduced pressure at a temperature below 50^0c to yield a syrupy mass (Yield -7.45%), which was used for the present investigation

Preliminary phytochemical investigation

Preliminary phytochemical analysis shows the presence of glycosides, phenolic compounds and flavonoids[4].

Animals

Animals-Male Wister 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. The animals were given standard laboratory feed and water ad libitum. The study was cleared by Animal ethics committee. 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.

Degranulation studies

Sensitized mast cell were obtained from animals sensitized with egg albumin. The doses being given on the 1st, 3rd and 5th day. The sensitized mast cells were degranulated using egg albumin (1mg/ml) on

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the 10th 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 degranulaing 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 and the tissue was then washed in acetone and then xylene (2 changes each) for 5 min each wash.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[58].

Analysis

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

Table 1.

Effect of Onosma bracteatum extract on egg albumin induced mast cell degranulation in rats.

S.No.	Treatment	Dose mcg/ml	Number of mast cell	Percent Inhibition
1.	Control	-	7±2	-
2.	Ketotifen	10	82±4	78.22*
3.	OB extract	05	30±2	18.12*
4.	OB extract	10	45±4	37.9*
5.	OB extract	20	59±4	58.78*
6.	OB extract	40	74±4	71.18*

Values are mean±S.E.,

*P<0.001 when compared with control.

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Table 2.
Effect of Onosma bracteatum extract on compound 48/80 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.	OB extract	05	33±2	20.44*
4.	OB extract	10	46±3	36.68*
5.	OB extract	20	61±3	58.98*
6.	OB extract	40	43±3	40.18*

Values are mean±S.E.,
*P<0.001 when compared with control.

RESULTS

Egg albumin induced degranulation studies.Kitotifen as a reference standard was found to inhibit degranulation to an extent of 78.22. Onosma bracteatum aerial parts extract at concentration 5,10,20 and 40 mcg/ml produced dose related inhibition of 18.12,37.9,58.78 and 71.18 respectively (Table 1.). Compound 48/80 induced degranulation studies. Ketitifen at a concentration of 10mcg/ml was found to inhibit degranulation to an extent of 77.52. Onosma bracteatum extract at concentration of 5,10,20 and 40 mcg/ml showed reduction in degranulation of mast cell to 20.44,36.68,58.98 and 40.18 respectively (Table 2.).

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[9]. 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.

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Extract of *Onosma bracteatum* 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[10]. The degranulation of mast cells occurs in response to the immunological stimuli in which the antigen–antibody interaction on the cell surface predominates. The present investigation indicates that the extract of *Onosma bracteatum* is active against Type-I allergic condition because of their ability to inhibit the release of mediators from mast cells and basophils and thus influences the course of the disease. The preliminary phytochemical tests showed the presence of flavonoids and phenolic compounds in the *Onosma bracteatum* ethanolic extract.

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[11]. 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[12]. 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 [13].

The present investigation indicates the ethanolic extract of *Onosma bracteatum* 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 neutrophilbetaglucuronidase release, and thereby possess in-vivo antiallergic activity[14]. 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[15-16].

Thus it can be concluded that flavonoids and phenolic compounds present contribute to the effect of *Onosma bracteatum* on mast cell stabilization in the animal experiments in the present study.

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