International Journal of Pharma and Bio Sciences V1(2)2010

MAST CELL STABILIZING ACTIVITY OF *OCIMUM SANCTUM* LEAVES.

GAJENDRA P. CHOUDHARY

School of Pharmacy, (Pharmacognosy Lab), Devi Ahilya University
Indore (M.P.)-452018, India.

Corresponding Author choudhary.gp@gmail.com

ABSTRACT

The mast cell has long been associated with asthma, since it releases a variety of preformed and newly synthesized mediators that account for several features of asthma. Among the mediators, histamine is a well-characterized and the most potent vasoactive mediator in acute bronchoconstriction.
In the present study, albino rats were sensitised by horse serum along with triple antigen containing *Bordetella pertussis*.Treatment was given with *Ocimum sanctum* ethanolic extract and flavonoid fraction and standard for 14 days. On the 14th day 3 hours after the last dose treatment, rats were sacrificed and intestinal mesentery taken out for study of mast cell. In the unsensitized rats, 12.55% of the mast cells were in the process of degranulation. In the sensitized untreated rats, 80.90% of the mast cells degranulated when challenged with the antigen. Prednisolone used as reference standard was found to inhibit degranulation of mast cells to an extent of 72.25%. The ethanolic extract at 100 and 200 mg/kg body weight inhibited degranulation of mast cells to an extent of 62.44 and 67.24%, respectively. Preliminary phytochemical analysis showed positive response for flavonoids.The isolated flavonoidal fraction of *Ocimum sanctum* at 75 and 150 mg/kg body weight inhibited degranulation of mast cell to an extent of 54.62 and 60.48% respectively.

KEY WORDS

Asthma ,Flavonoids,*Ocimum sanctum,* ,Mast cell, Prednisolone

MAST CELL STABILIZING ACTIVITY OF *OCIMUM SANCTUM* LEAVES.

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 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].

Ocimum sanctum Linn (OS) (Labiatae) commonly known as 'Tulsi' has been extensively used in Ayurvedic system of medicine for various ailments. The whole plant of as has medicinal value, few of them are aromatic, stomachic, demulcent, diaphoretic, digestive, diuretic, expectorant, febrifuge, vermifuge and alexiteric properties[2]. Pharmacologyically important active principles of Ocimum sanctum are a large group of polyphenolic flavonoids like apigenin, vicenin-1 and vicenin-2, caffeic and urosolic acid[3] . Flavonoids isolated from *Ocimum* Sanctum scavenged free radicals in vitro and showed antilipoperoxidant activity *in vivo* at very low concentration[4]. Ocimum Sanctum may act at various levels in the immune mechanism, such as antibody production, release of mediators of hypersensitivity reactions and tissue responses to these mediators in the target organs in modulating the humoral immune responses[5].Chemical constituents *Ocimum sanctum* leaves contain 0.7% volatile oil comprising about 71 % eugenol and 20% methyl eugenol. The oil also contains carvacrol and sesquiterpine hydrocarbon caryophyllene[6]. Ursolic acid has been isolated from the OS leaves[7]. Apart from ursolic acid, Nair et al. [8] also isolated apigenin, luteolin, apigenin7 -O-glucuronide, luteolin-7 -O-glucuronide, orientin, molludistin. Isolation of two flavonoids, orientin and vicenin from the aqueous leaf extract of OS is also reported[9]. KeIrn et al[10] have extracted, and purified the following phenolic compounds from the fresh leaves and stems of OS: cirsilineol, cirsimaritin, isothymusin, isothymonin, apigenin, rosmarinic acid and appreciable quantities

MAST CELL STABILIZING ACTIVITY OF *OCIMUM SANCTUM* LEAVES.

of eugenol. The structures of these compounds have also been elucidated. Experimental studies in albino rats showed the efficacy of basil leaves in decreasing blood glucose in hyperglycemic rats and rabbits[11].

Ocimum sanctum leaf powder supplementation at 1 % dose level showed significant hypoglycemic and hypolipidaemic effects in diabetic rats which could be associated with the essential oil, eugenol present in OS leaf powders[12].

The ethanolic leaf extract of this plant at 100 mg/kg daily dose when fed orally for 7 days was found to normalize the noise induced changes in total and differential leucocytes in rats[13]. The immunostimulant capacity of OS may be responsible for the adaptogenic action of the plant[14]. Antifertility activity of OS leaves has been reported in rats, mice and rabbits. The cold water extract at 3g/100g body weight dose when fed orally for 6 days was found to be effective against carbon tetrachloride (0.2 ml/100 g subcutaneously) induced liver injury (necrosis, fatty degeneration and hydropic degeneration) in albino rats[15].Steam distilled extract from the fresh leaves showed modification humoral immune response in albino rats which could be attributed to such mechanisms as antibody production, release of mediators of hypersensitivity reactions and tissue responses to these mediators in the target organs[16].

Protective role of aqueous leaf extract against radiation induced lipid peroxidation, glutathione and allied antioxidant enzymes in liver of mice was ob served[17]. OS leaf extract exhibited significant antioxidant activity against several paradigms of oxidative stress induced by a variety of techniques in different rat tissues, which was comparable to that of vitamin E[18].Recently cycloxygenase inhibitory properties of OS have also been reported along with its antioxidant properties[19]. OS significantly decreased the incidence of benzo(a)pyrene induced neoplasia of stomach and 3'-methyl-4-dimethylaminoazobenzene induced hepatomas in rats[20]. Ethanolic leaf extract also had significant modulatory influence on carcinogen metabolizing enzymes (cytochrome P450, cytochrome. B_5 and aryl hydrocarbon hydroxylase, glutathione-S-transferase) and glutathione levels in mouse [21] . An acute (carrageenan induced paw edema) and chronic inflammation (croton oil induced granuloma and exudate formation) models, the response observed with 500 mg/kg methanolic extract and aqueous suspension of OS was comparable to 300 mg/kg of sodium salicylate. Both the formulations showed analgesic activity, reduction in typhoid paratyphoid ME vaccine induced pyrexia, but the antipyretic action was weaker and of shorter duration than 300 mg/kg sodium salicylate. They also delayed castor oil induced diarrhoea in rats when fed orally on prophylactic basis[22]. Ethanolic extract (50%) of fresh leaves and fixed oil from the seeds exerted significant antiasthmatic (against histamine and acetylcholine induced preconvulsive dyspnoea in guinea pigs) and anti-inflammatory activity (against carrageenan, serotonin, histamine and PGE_2 induced inflammation in rats). The antiasthmatic activity of the ethanolic extract of the fresh leaves is suggested to be due to the presence of volatile oil consisting of several components[23]. The aqueous extract exerted protective effect against radiation induced chromosome damage in mouse bone marrow and modified bone marrow radio sensitivity which could be attributed to its free radical scavenging activities[24]. The effect of aqueous extract of leaves of this plant on wound healing was assessed using excision and incision wound models in Wistar rats[25]. Scientific knowledge concerning the use of medicinal plants in asthma is very limited. It is likely that some of the plants being used have no significant effect on respiratory disorders. In the present study, we investigated mast cell stabilizing activity of *Ocimum sanctum* to inhibit mediators release.

MAST CELL STABILIZING ACTIVITY OF *OCIMUM SANCTUM* LEAVES.

MATERIALS AND METHODS

Collection of plant material

The leaves of *Ocimum sanctum* was collected from the plantation in the Medicinal Garden, School of pharmacy, Devi Ahilya Vishwavidhyalaya, Indore. The plant material was identified by a botanist , Dr.A.B.Sheerwani (Retd. Prof. and Head), Department of botany, Holkar Science College, Indore, and their voucher specimens were deposited in the Department of Pharmacognosy, School of Pharmacy, Devi Ahilya Vishwavidhyalaya, Indore .

Preparation of plant extract

The air dried plant material was reduced to coarse powder and subjected to extraction with ethanol(90%) in soxhlet extractor. After the complete extraction, the solvent was distilled off and concentrated on a water bath to a dry residue. The extracts were concentrated by distilling of the solvent and then evaporated to dryness on water bath.

Animals

Swiss albino rats (150-200g) of either sex were obtained from the experimental animal house ,School of Life Science, Devi Ahilya Vishwa Vidhyalaya, Indore. They were maintained under standard housing condition. The animals were given standard laboratory feed and water *ad libitum*. 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, USA. The study was approved by Animal Ethics Committee.

Acute toxicity study

Acute toxicity study was carried out according to Miller and Tainter methods in albino mice of either sex (wt.20-25gm.) were used [26]. The LD^{50} of *Ocimum sanctum* for the ethanolic extract when given orally and tested in albino mice were found to be non toxic up to the dose of 3.0 gm/kg body weight. Based on result of LD^{50}, $1/10^{th}$ of the LD^{50} were taken as therapeutic doses[27]. After identification of constituent in ethanolic extract of plant drug, mast cell stabilizing activity was performed.

Histamine and horse serum were procured from Hi-media laboratories, Mumbai, DPT antigen which contain *Bordetella pertussis* from Serum Institute of India, Pune, toluidine blue and tween 80 from Lobachemie, Mumbai and prednisolone 10mg (I.P.), Wyeth
limited, Goa. Other chemicals and reagents were procured from Merck, Mumbai.

Evaluation of mast cell stabilizing activity

Rats were divided into five groups of six animals in each group. Group I served as control and received vehicle (Tween 80, 1%). Group II (sensitized control group, received only Tween 80, 1%), Group III (prednisolone), Group IV (*Ocimum sanctum* 100mg), Group V (*Ocimum sanctum* 200mg), were sensitized by injecting 0.5 ml of horse serum subcutaneously along with 0.5 ml of triple antigen containing *Bordetella pertussis* organisms (Serum Institute of India Ltd., Pune). Reference drug (Prednisolone) and extracts were administered orally once a day for 14 days. On day 14, the rats were sacrificed 3 h after the treatment and the intestinal mesentry was taken out for the study on mast cells. Mesentries along with intestinal pieces were excised and kept in Ringer Locke solution (NaCl 154, KCl 5.6, CaCl2, 2.2 NaHCO3 , 6.0, glucose 5.55 mM/L of distilled water) pH-7.4 for 30 min at 37°C. The mesenteric pieces were challenged with 5% horse serum for 10 minutes after which the mast cells were stained with 1.0%

MAST CELL STABILIZING ACTIVITY OF *OCIMUM SANCTUM* LEAVES.

toludine blue and examined microscopically for the number of intact and degranulated mast cells[28].

Mast cell staining

Pieces of intestinal mesentery were mounted on slides. All slides were air dried, then stained with 1.0% toludine blue, at room temperature for 5min. Mast cells were readily identified by their metachromatic cytoplasmic granules under a light microscope.

Statistical analysis

The results were expressed as mean ±SEM and analyzed statistically using *Students t-test* to find out the level of significance.

Table1

Mast Cell Stabilizing Activity of Ethanolic Extracts of Ocimum sanctum.

Group	Treatment	Dose (mg/kg body weight)	Route of administration	Granulated mast cells(%) (mean ±S.E.)	Degranulated mast cells(%) (mean ±S.E.)
I	Control(Tween 80,1%)[a]	_	Oral	87.44±4062	12.55±1.80
II	Control(Tween 80,1%)sensitize	_	Oral	21.32±1.78	80.90±4.65
III	Prednisolone	10	Oral	72.25±3.91*	28.14±1.34*
IV	Ethanolic extract	100	Oral	62.44±3.80*	37.61±1.65*
V	Ethanolic extract	200	Oral	67.24±2.94*	45.62±2.40*

[a]*Not treated with horse serum and triple antigen.*
values are mean±S.E., n=6,
**P<0.001 when compared with control.*

Mast cell stabilizing activity of Flavonoid Fraction (Isolated from ethanolic extract)

The ethanolic extract of, *Ocimum sanctum* exhibited significant mast cell stabilizing activity so it was subjected to detailed chemical investigation. The preliminary qualitative tests revealed the presence of phenolic compound and flavonids.Therefore ethanolic extract of *Ocimum sanctum* was subjected to seperation of flavonoids

.

MAST CELL STABILIZING ACTIVITY OF *OCIMUM SANCTUM* LEAVES.

Scheme of Fractionation of Flavonoids from Ethanolic Extracts

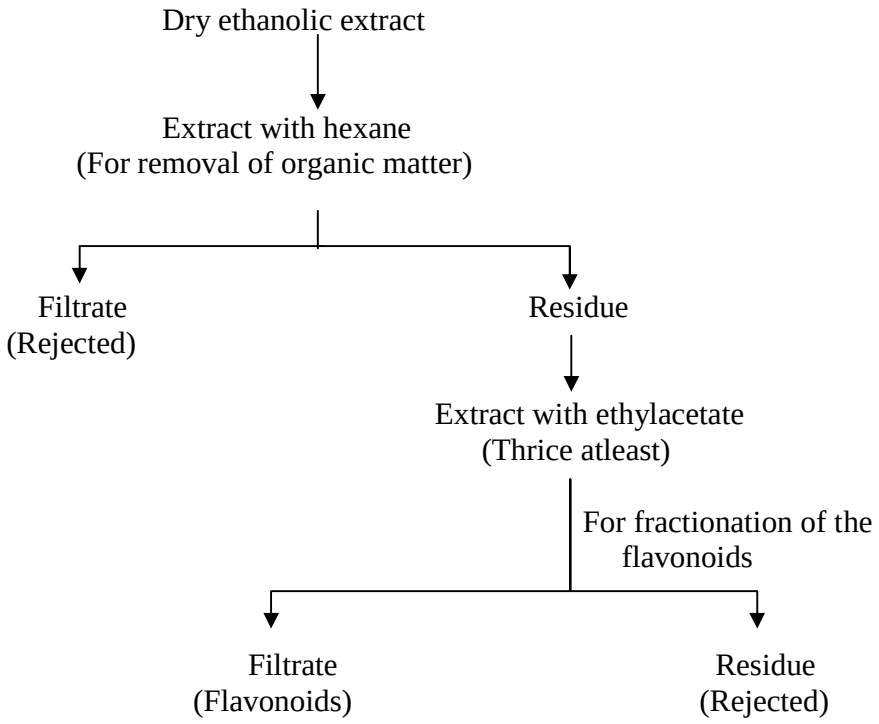

Table 2

Influence of Flavonoid Fraction of ocimum sanctum on Mast Cell Degranulation

Group	Treatment	Dose (mg/kg body weight)	Route of administration	Granulated mast cells (%) (Mean ±S.E.)	Degranulated mast cells (%) (mean ± S.E.)
1.	Control (Tween 80,1%)[a]	_	Oral	87.44±4.62	12.55±1.80
2.	Control(Tween 80,1%),Sensitize	_	Oral	21.32±1.78	80.90±4.65
3.	Prednisolone	10	Oral	72.25±3.91*	28.14±1.34*
4. (a)	*Ocimum sanctum* (Fla.Frac.)	150	Oral	60.48±2.72*	39.14±1.23*
(b)	*Ocimum sanctum*(Fla.Frac.)	75	Oral	54.62±1.76*	46.12±3.22*

[a] *Not treated with horse serum and triple antigen. Values are mean ±S.E., n=6,P < 0.001 when compared with control.*

MAST CELL STABILIZING ACTIVITY OF *OCIMUM SANCTUM* LEAVES.

RESULTS

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[29]. 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.The effect of ethanolic extract of *Ocimum sanctum* was studied on the degranulation of sensitized peritoneal cells of albino rats using DPT antigen containing *B. pertussis*. Extract of *Ocimum sanctum* markedly protected the rats against antigen-induced challenge of mast cells. However, the effect was less than that observed with the standard drug (Prednisolone) 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[30]. 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 *Ocimum sanctum* is active against Type-I allergic conditions 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 flavone glycosides in the *Ocimum sanctum* ethnolic extract.In the present study the (control) unsensitized rats, 12.55±1.8% of the mast cells were degranulated. In the sensitized untreated rats, 80.90±4.65% of the mast cells were degranulated when challenged with the antigen. At the doses used, prednisolone as reference standard were found to inhibit degranulation of mast cells to an extent of 72.25±3.91%. The ethanolic extract of *Ocimum sanctum* at 100 and 200 mg/kg body weight was found to inhibit degranulation of mast cells to an extent of 62.44±3.92 and 67.24±3.56%, respectively The percentage yield of isolated flavonoids of *Ocimum sanctum* was obtained 0.6. The isolated flavonoids of the Ocimum sanctum was inhibit degranulation of mast cell 60.48 and 54.62 at the doses 150 and 75 mg/kg body weight.

DISCUSSION

The influence of flavonoidal fraction treatment on mast cell stabilization shown from figure (1-4). The number of mast cell per unit area of the mesentery were also decreased significantly by the flavonoidal fraction treatment. Mast cells can be induced to release their secretory granules by exocytosis after various stimuli, such as certain chemical compounds, hypotonic solutions[31] and incubation with anti-IgE antibody [32].

It has been reported that mast cells can releasegranule matrix materials slowly (days) or morerapidly. The "slow-release reaction" or "piecemealdegranulation" is morphologically expressed in the progressive loss of granular materials which are transported by vesicles to the cell's surface. In contrast, stimulated mast cells can rapidly extrudegranular matrix materials through multiple membrane openings to the cell's exterior, and this is regarded as a "rapid-release reaction"[33].

MAST CELL STABILIZING ACTIVITY OF *OCIMUM SANCTUM* LEAVES.

Mast cells were evenly distributed in the mesentery. They displayed abundant granules and clear cell boundaries by toluidine blue staining to be loaded with abundant purple metachromatic cytoplasmic granules and to have clear cell boundaries .

Mesenteric mast cells were evenly distributed. They displayed abundant granules and clear cell boundaries in the control group (Fig. 1). Mast cell degranulation was seen with numerous extruded granules both near and at a distance from the cell body in sensitize group. The majority of granules were seen at adistance from the cell body, and toluidine blue staining intensity of the granules was significantly reduced after different extract treatment.

Microscopic examination of mast cell

In control and treated animals, granulation was seen in mast cell granules (Fig. 1-4).In the control group, the surface folds extended straight from the cell surface. Numerous granules with Immunoreaction were present at the cell periphery. Few cells immunoreactive granules appeared to be extruded from the cell bodies and were seen near the cell surfaces (Fig.1). After extract treatment, there were more immunoreactive granules located outside the cell body. In addition, profiles of long and curved surface folds were observed .In the mesentery, mast cells were located in the connective tissue and were surrounded by abundant collagen fibrils. The degranulated cells contained fewer perinuclear granules, most of which were not immunolabeled. Very few cell membrane folds were seen in the degranulated cell. At this time, some of the extruded granules were enclosed by the processes of connective tissue cells, around which numerous collagenous fibrils were identified. 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[34]. 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[35]. 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 [36].

The present investigation indicates the ethanolic extract of *ocimum sanctum* 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. From the ethanolic extract of *ocimum sanctum*, two known flavonoids, vicenin and orientin have been reported. The preliminary phytochemical tests showed the presence of flavonoids in the ethanolic extract of the plant. Plant flavonoids are known to inhibit basophil histamine release and neutrophilbetaglucuronidase release, and thereby possess *in-vivo* antiallergic activity [37]. 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 [38-39].

HISTOPATHOLOGICAL STUDIES

Histological study of the intestinal muscle tissue provides further evidences on the mast cell stabilizing efficacy of the flavonoid fraction of

MAST CELL STABILIZING ACTIVITY OF *OCIMUM SANCTUM* LEAVES.

Ocimum sanctum . The section of the intestinal muscle tissue of the control animal intestinal muscle layer was slightly thicker than the treated with exract. In control, cytoplasm of fibroblast was highly granulated which is the indication of high secretory activity compared to treated rats. Observations indicate that there might be release of histamine in control rats. Mast cells after degranulation show transgranulation, which occur between mast cells and fibroblast with mast cell apparently transferring their granules to the cytoplasm of fibroblast. The section of the intestinal muscle tissue of the animals treated with flavonoidal fraction of the, *Ocimum sanctum* showed very less granulation of fibroblast tissue and transgranulation was also less between mast cell and fibroblast (Figure5-8).

In conclusion, ethanolic extract of *Ocimum sanctum* shows significant mast cell stabilizing activity. On the basis of above evidence, it is possible that flavonoids present could be responsible for this activity. However this claim demands further research to isolate mast cell stabilizer principle.

REFERENCES

1. Foreman JC. Non-immunological stimuli of mast cells and basophil leucocytes. Immunopharmacology of Mast cells and Basophils. London: Academic Press. 1993; p.57-69.
2. Varier N V K, Ocimum tenuiflorum Linn., in Indian Medicinal Plants: A Compendium of 500 Species (Orient Longman Ltd., Madras, India), vol 4, 1997, 168.
3. Nair A G R , Gunasegaran R , Joshi B S , chemical investigations of some South Indian Plants, *Indian J chem*, 21B,1982 979.
4. Uma Devi P, Ganasoundari A, Vrinda B, Srinivasan K K and UnniKrishanan M K, Radiation protection by the Ocimum falvonoids orientin and vicenin- mechanism of action, *Radiat Res*, 2000,154, 455.
5. Mediratta P K, Sharma K K and Singh S, Evaluation of immunomodulatory potential of Ocimum sanctum seed oil and its possible mechanism of action, *J Ethanopharamacol*, 2000,80,15.
6 Shah C S & Qadry J S, Volatile oils, in A Text Book of Pharmacognosy, (B S Shah Prakashan, Ahmedabad, India), 1988,216.
7. Balaneliru S & Nagarajan B, Protective effect of oleanolic acid and ursolic acid against lipid peroxidation, *Biochem Int*, 24 ,1991, 981.
8 Nair A G R, Gunasegaran R & Joshi B S, Chemical investigation of certain south Indian plants, *Indian J Chem*, 21B ,1982, 979.
9. Uma Devi P, Bisht K S & Vinitha M, A comparative study of radioprotection by Ocimum flavonoids. and synthetic aminothiol protectors in the mouse, Br J Radiol, 71 1998,782.
10. KeIrn M A, Nair M G, Stratsburg G M & DeWitt D L, Antioxidant and cyclo-oxygenase inhibitory phenolic compounds from Ocimum sanctum Linn., *Phytomedicine*, 7,2000, 7.
11. Dhar M L, Dhar M M, Dhawan B N, Mehrotra B N & Ray C, Screening of Indian plants for biological activity: Part I, *Indian J Exp BioI*, 6,1968, 232.
12. Sarkar A, Lavania S C, Pandey D N & Pant M C, Changes in the blood lipid profile after administration of Ocimum sanctum (Tulsi) leaves in the normal albino rabbits, *Indian J Physiol Pharmacol*, 38 ,1994,311.
13. Brekhman I I & Dardymov I V, New substances of plant origin which increase non-specific response, *Ann Rev Pharmacol*, 9, 1969, 419.

MAST CELL STABILIZING ACTIVITY OF *OCIMUM SANCTUM* LEAVES.

14. Godhwani S, Godhwani J L & Vyas D S, Ocimum sanctum: A preliminary study evaluating its iimnunoregulatory profile in albino rats, *J Ethnopharmacol*, 24, 1988 ,193.
15. Kantak N M & Gogate M G, Effect of short term administration ofTulsi (Ocimum sanctum Linn) on reproductive behaviour of adult male rats, *Indian J Physiol Pharmacol*, 36,1992,109.
16. Chattopadhyay R R, Sarkar S K, Ganguly S, Medda C & Basu T K, Hepatoprotective activity of Ocimum sanctum leaf extract against paracetamol induced hepatic damage in rats. *Indian J Pharmacol*, 24, 1992, 163.
17. Balanehru S & Nagarajan B, Intervention of adriamycin induced free radical damage, *Biochem Int*, 28 ,1992, 735.
18. Vma Devi P & Ganasoundari A, Modulation of glutathione " and antioxidant enzymes by Ocimum sanctum and its role in. ; the protection against radiation injury, *Indian J Exp Bioi*, 37, 1999, 262.
19. Bhattacharya S K, Bhattacharya A, Das K, Muruganandam A. V & Sairam K, Further investigations on the antioxidant activity of Ocimum sanctum using different paradigms of oxidative stress in rats, *J Nat Rem*, 1, 2001, 6
20. Aruna K & Sivaramakrishnan V M, Anticarcinogenic effects of some Indian plant products, *Food Chern Toxicol*, 30 J,1992, 953.
21. Banerjee S, Prashar R, Kumar A & Rao A R. Modulatory in- . fluence of thanolic extract of Ocimum leaves on carcinogen metabolizing enzyme activities and reduced glutathione levels in mouse, *Nutr Cancer*, 25, 1996, 205.
22. Godhwani S, Godhwani J L & Vyas D S, Ocimum sanctum: An experimental study evaluating its anti-inflammatory, analgesic and antipyretic activity in animals, *J Ethnopharmacol*, 21 ,1987,153.
23. Singh S & Agrawal S S, Antiasthmatic and antiinflammatory activity of Ocimum sanctum, *lilt J Pharmacog*, 29, 1991, 306.
24. Ganasoundari A, Uma Devi P & Rao M N A, Protection against radiation fnduced chromoso~e damage in mouse. bone marrow by Ocimum sanctum, *Mutat Res*, 373 ,1997, 271.
25. Somashekar Shetty B, Udupa S L, Udupa A L, Vilas K & Bhaskar, Effect of Ocimum sanctum on wound healing, *Indian J Pharmacol*, 31 ,1999,78.
26. Miller and Tainter "Quantal Response Calculation of LD^{50}" in "Screening methods in Pharmacology" Ed;Robert A.Turner, Newyork Academic Press,1944.
27. Jalalpure S.S., Patil M.B., Prakash N.S., Hemalatha K. and manvi F.V., Hepatoprotective activity of fruits of piper longum L.*Indian Jour. of Pharm.Sci.*,,65,2003,363.
28. Sengupta K, Kolhapure SA. Evaluation of efficacy and safety of HK-07 tablets in upper and lower respiratory tract allergic disease in children. *Med Update* 12,2004, 37-46.
29. Subramoniam A, Evans DA, Valsaraj R, Rajasekharan S, Pushpangadan P. Inhibition of antigen-induced degranulation of sensitized mast cells by *Trichopus zeylanicus* in mice and rats. *J. Ethnopharmacol.* 68,1999,137–143.
30. Crossland J. Pharmacological Aspects of Allergy. Lewis Pharmacology. Churchill Livingstone, New York, 1980, p. 393.
31. Bloom GD, O Haegermark. 1965. A study on morphological changes and histamine release induced by C48/80 in rat peritoneal mast cells. *Exp. Cell Res*. 40: 637-654.
32. Chen XJ, L Enerback. 1995. Influence of genetic and environmental factors on surface expression and occupancy of IgE receptors and on histamine releasability of mast cells. *Int. Arch. Allergy Imm*. 106: 241-249.

33. Norrby K. 1981. On the 48/80-induced secretion of tissue mast cells and its mitogenic effect on nearby cells in the intact rat. Virchows Arch. B Cell Pathol.Incl. Mol. Pathol. 38: 57-65.
34. Norrby K, S Enestrom. 1984. Cellular and extracellular changes following mast-cell secretion in avascular rat mesentery. *Cell Tissue Res*. 235: 339-345.
35. Takayama I, Y Fujii, S Ohno, MA Fujino. 1994. Ultrastructural study of mast cells stimulated with C48/80 as revealed by quick-freezing method. *Virchows Arch*. 424: 287-294.
36. Dimitriadou V, MG Buzzi, MA Moskowitz, TC Theoharides. 1991. Trigeminal sensory fiber stimulation induces morphological changes reflecting secretion in rat dura mater mast cells. *Neuroscience* 44: 97-112.
37. Pathak, D., Pathak, K., Singla, A.K., 1991. Flavonoids as medicinal agents — recent advances. *Fitoterapia* 62, 371–389.
38. Tripathi RM, Sen PC, Das PK. Studies on the mechanism of action of *Albizia lebbeck*, an Indian indigenous drug used in the treatment of atopic allergy. *J. Ethnopharmacol.* 1979; 1: 385–396.
39. Havsteen B. Flavonoids: a class of natural products of high pharmacological potency. *Biochem. Pharmacol.* 1983; 32: 1141–1148.