ejpmr, 2021,8(10), 439-446

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

MAST CELL STABILIZATION ACTIVITY OF ROOTS OF BHARANGI (CLERODENDRU SERRATUM L MOON) AND LEAVES OF AGERATUM CONYZOIDES L. AN INVITRO STUDY

[*1]Dr. Kavana K. S., [2]Dr. Manasa R. and [3]Dr. Veena M. S.

[1,2] Final Year Pg Scholar, [3]Asistant Professor,
Department of Pg Studies Dravyaguna, Government Ayurveda Medical College Bangaluru -560009.

***Corresponding Author: Dr. Kavana K. S.**
Final Year Pg Scholar, Department of Pg Studies Dravyaguna, Government Ayurveda Medical College Bangaluru -560009.

Article Received on 14/09/2021 **Article Revised on 03/09/2021** **Article Accepted on 24/09/2021**

ABSTRACT

A mast cell is a type white blood cell. Specifically it is a type of granulocyte derived from the myeloid stem cell which is a part of the immune system and neuro immune systems and contains many granules rich in histamine and Heparin. Mast cells are involved in the manifestation of various disorders such as parasitic infections, mast cell activation disorders, allergic disorders, anaphylaxis, autoimmunity, Mastocytosis, clonal disorders etc.[1] Mast cell stabilizers are common medications used to prevent or control certain allergic disorders. They block a calcium channel essential for mast cell degranulation, stabilizing the cell and thereby Preventing the release of histamine and related mediators. Rat mesenteric bits are stored at laboratory in aseptic precautions, stock solutions to be prepared test samples to be prepared at different concentrations. Of aqueous and alcoholic extracts of Bharangi moola and leaves of *Agreatum conyzoids*. In this study number of intact mast cells, number of degranulated cells, percentage of stabilization is observed.[2]

KEYWORDS: *Bharangi moola*, *Ageratum conyzoides*, leaves, mast cell, invitro, mast cells.

INTRODUCTION

One of the common conditions that affect mankind is allergy in its diverse manifestations. Intensive research during the last several decades has highlighted the role of lymphocytes, immunoglobins, mast cells in the aetiopathogenesis of allergic conditions Mast cells play an important role in anaphylaxis and inflammation and have been used to test for newer agents for anti-allergic and anti-inflammatory activity. Mast cells are basophilic cells found in abundance in sub-epithelial layer of trachea-bronchial tree in the humans. They are also found in large numbers in mesentery of rats and contain numerous membrane bounded granules, which contain strong pharmacologically active mediators such as histamine, slow reacting substance for anaphylaxis (SRS-A), Serotonins, bradykinins etc Hypersensitivity reactions can be elicited by various factors: either immunologically induced, that is allergic reactions to natural (or) Synthetic compounds mediated by Ige, or Non-immunologically induced, direct contact, without the induction of or the mediation through immune response.[3,4]

METHODOLOGY

The effect of drugs on rat mesenteric mast cell degranulation is studied by incubating the drug at different concentrations and challenging the same with a degranulating agent.

The mast cells are examined microscopically after staining with 0.1% Toluidine blue, and the number of intact and disrupted cells is counted.[5]

Materials Required

1. Mesentry of albino rat

Mesentries are stored in asceptic precautions in laboratory environment.

Reagents required

Compound 48 / 80.
Disodium cromoglycate (DSCG).
Toluidine blue.

Hank's Balanced Buffer Solution (HBBS) - g/L

It is a mixture of,
Calcium chloride-0.14,
Potassium chloride-0.4,
Potassium dihydrogen phosphate -0.06
Magnesium chloride - 0.1
Magnesium sulphate-0.1
Sodium bicarbonate -0.35
Sodium chloride-8.0
Disodium hydrogen phosphate-0.09
D-Glucose 1.0.
All above chemicals were of analytical grade.

Preparation of stock solution
Reference standard: Disodium Cromoglycate – 0.2 mg/mL in HBBS.
Degranulating agent: Compound 48/80 - 0.2 mg/mL in HBBS.

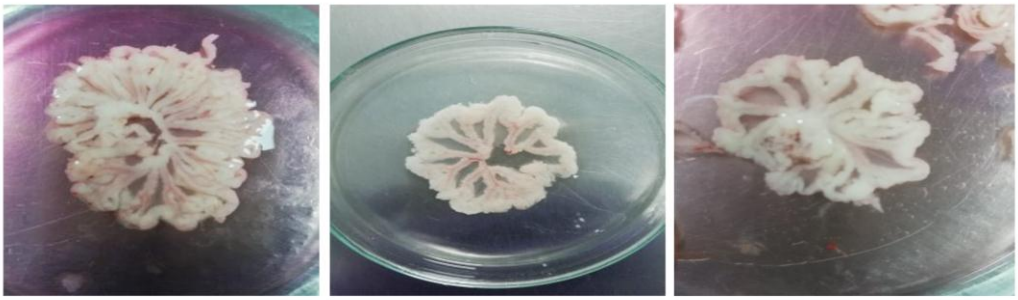
Preparation of stock solution
Reference standard: Disodium Cromoglycate – 2 mg/mL in HBBS.
Degranulating agent: Compound 48/80 - 1 mg/mL in HBBS.

Test samples
Test sample 1: Aqueous extract of root of Bharangi
Test sample 2: Alcoholic extract of root of Bharangi
Test sample 3 Aqueous extract of leaves Ageratum conyzoides
Test sample 4: Alcoholic extract of leaves of Ageratum conyzoides

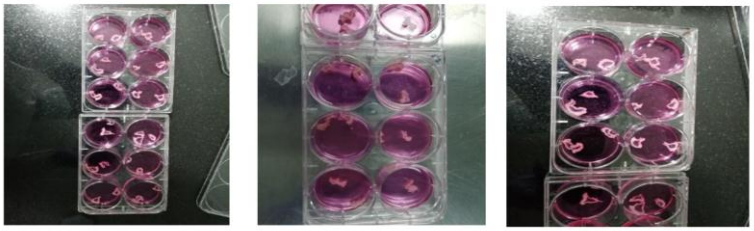
Preparation of test samples
All samples were prepared to 32mg/ml stock solution in water for test sample 1 and 3, and in DMSO for test sample 2 and 4. From this 3.2 mg/ml stock were prepared for all the samples.
Finally 200µg/ml stock was prepared.

Preparation of mesentry for assay
Pieces of mesenteries were harvested in Petri dishes containing about 10mL of HBBS.

Mesenteric bits



Distribution of mesenteric bits to HBBS wells



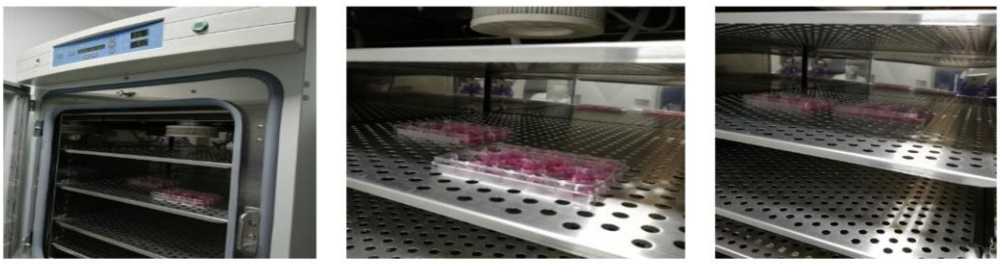
Hank's Balanced Buffer Solution (HBBS) in 6 well plate

Pre-incubation treatment mixture

Test solutions at different concentrations or DSCG (200µg/ml) were added to six well plate containing HBBS (2mL) and incubated at 37°C for 30 min in CO2 incubator (O2 and CO2 ratio was 95:5 with humidified atmosphere).

Test substances were replaced by buffer solution for a normal control.

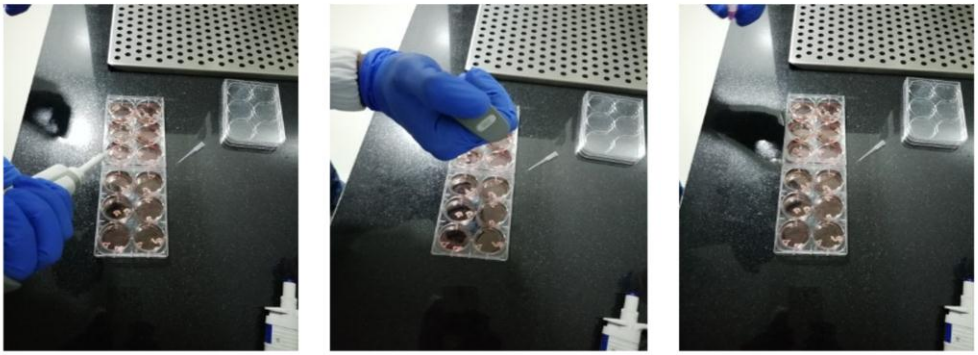
Incubation for 30 min



Degranulating agent

Compound 48/80 was added to the well plates to give the concentration of 50µg/ml (except normal control). The reaction mixture was incubated for 5 minutes in the CO2 incubator at 37°C for compound 48/80. The reaction was stopped by adding cold HBBS.

Compound 48/80 addition for 5 min incubation



Staining Procedure

Mesenteric bits were carefully taken and first fixed in 2 % formalin for 2 min and then washed with HBBS. Mesenteric bits again immersed in 0.1% Toluidine blue for 5 minutes and washed with HBBS.

Tissues were placed on a microscope slide and carefully stretched with needles. The unwanted tissues were trimmed off from the edges of the mesentery.

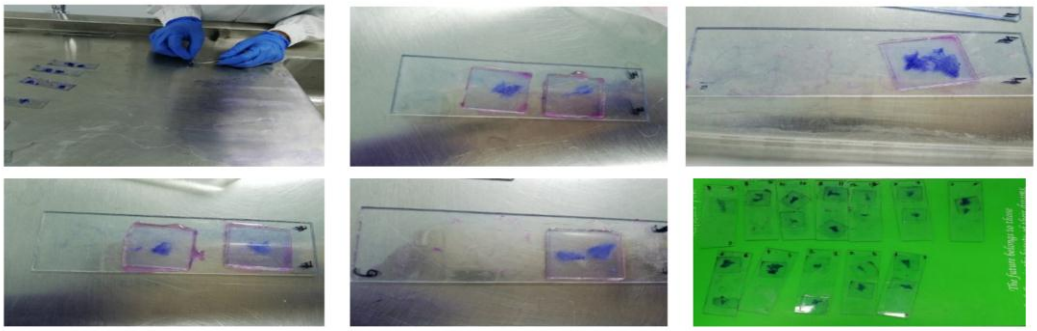
The number of intact and disrupted mast cells per field (40X & 100X) was counted.

Mast cell stabilization was assessed by scoring approx. 10 fields for 100 cells in total and tabulated.

Fixing the mesenteric bits with formalin

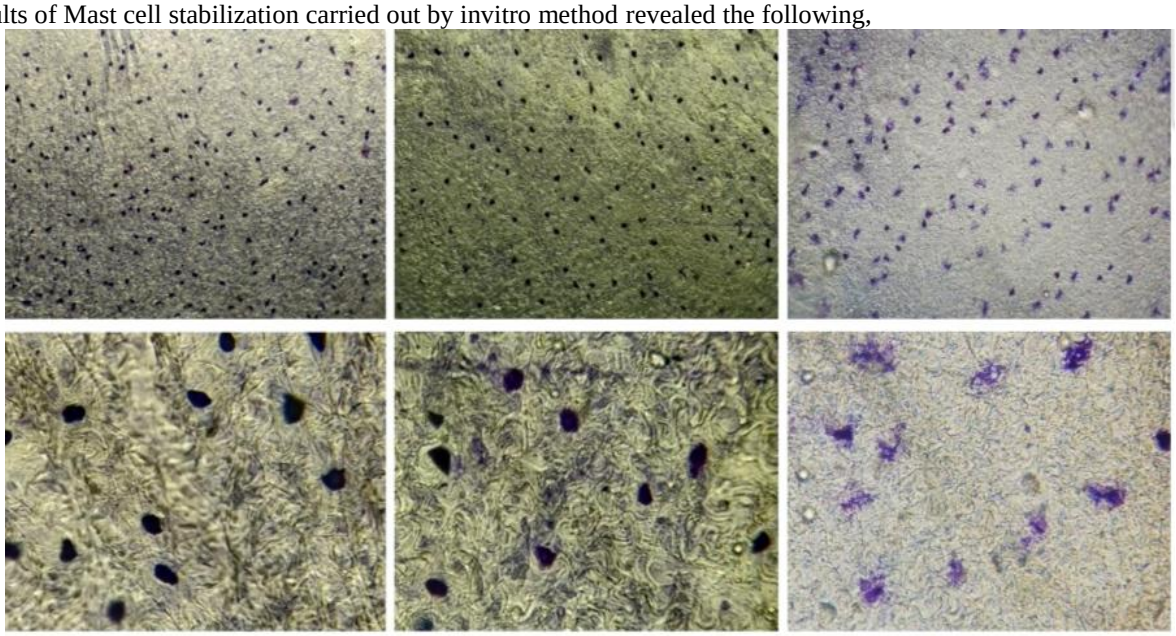
Staining with toluidine blue

Slide processing



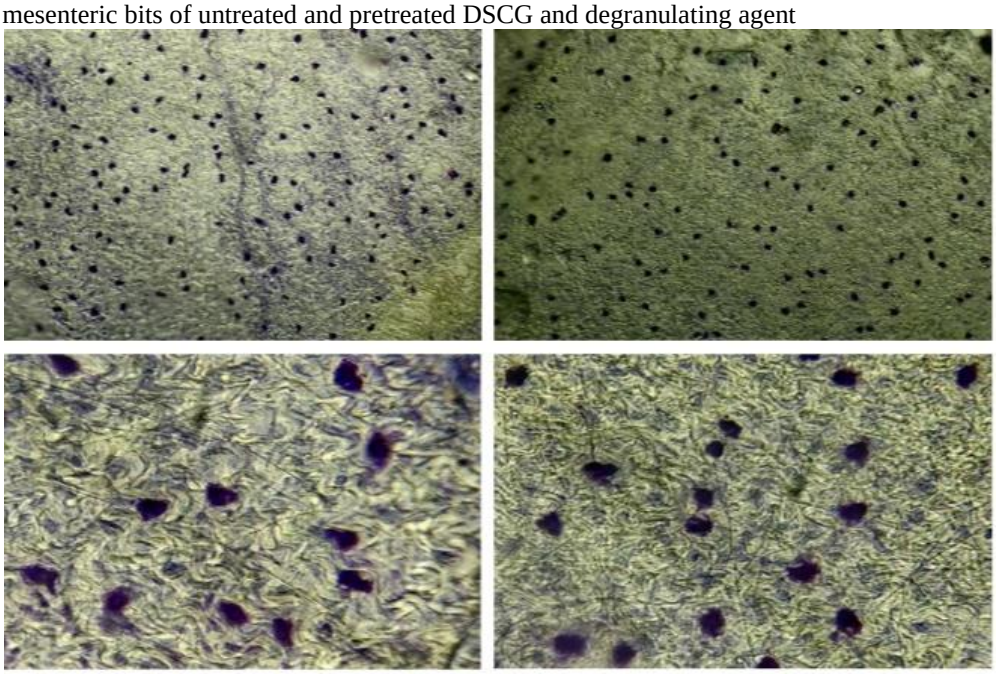
RESULT

Results of Mast cell stabilization carried out by invitro method revealed the following,



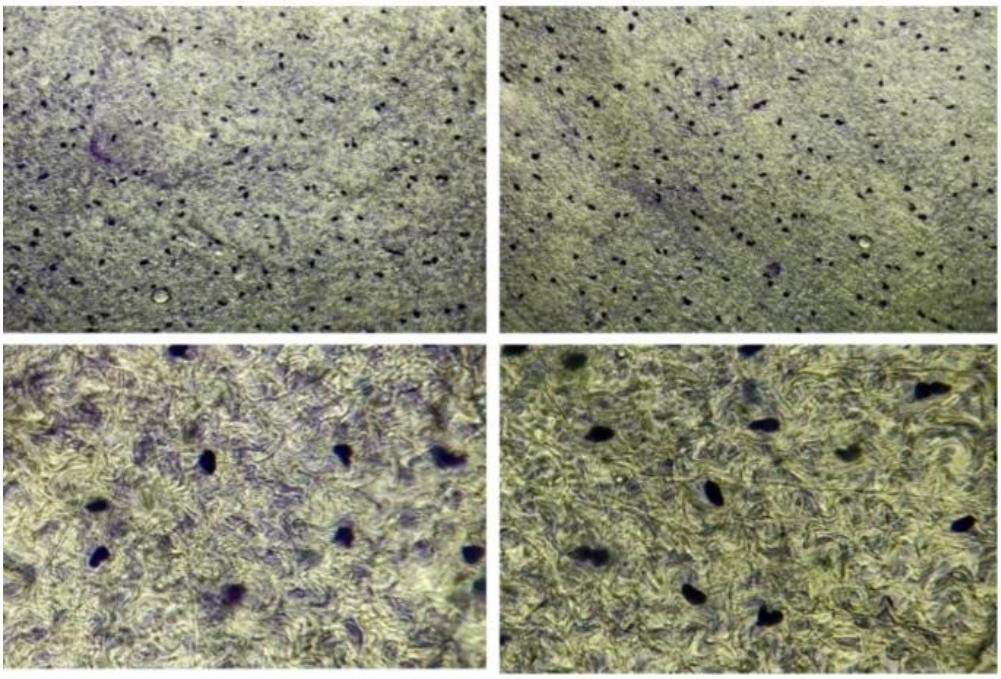
Control DSCG Compound 48/80

Mast cells in mesenteric bits of untreated and pretreated DSCG and degranulating agent



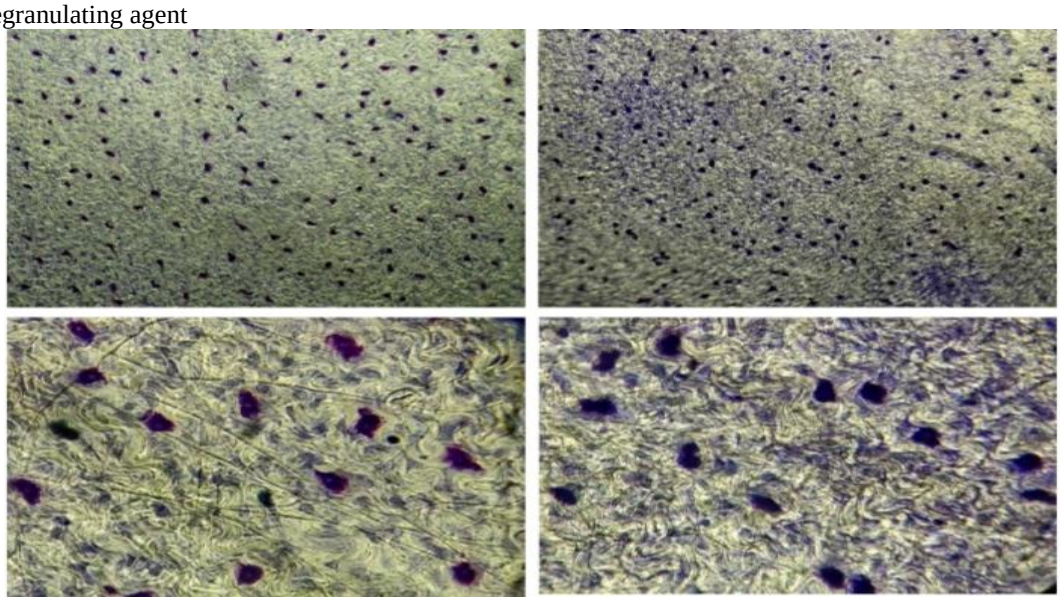
25µg/ml 50µg/ml

Mast cells in mesenteric bits pretreated with test sample of aqueous extract of Bharangi roots at different concentrations and post treated degranulating agent



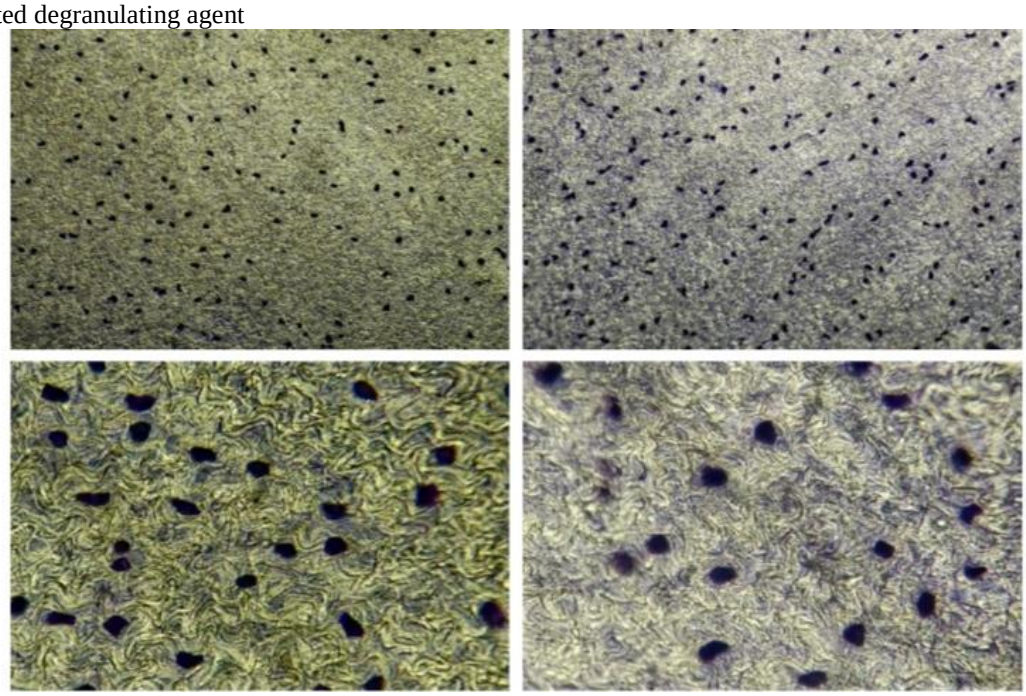
25µg/ml 50µg/ml

Mast cells in mesenteric bits pretreated with alcoholic extract of Bharangi moola at different concentration of and post treated degranulating agent



25µg/ml 50µg/ml

Mast cells in mesenteric bits pretreated with Aqueous extract of Ageratum conyzoides at different concentration and post treated degranulating agent



25µg/ml 50µg/ml

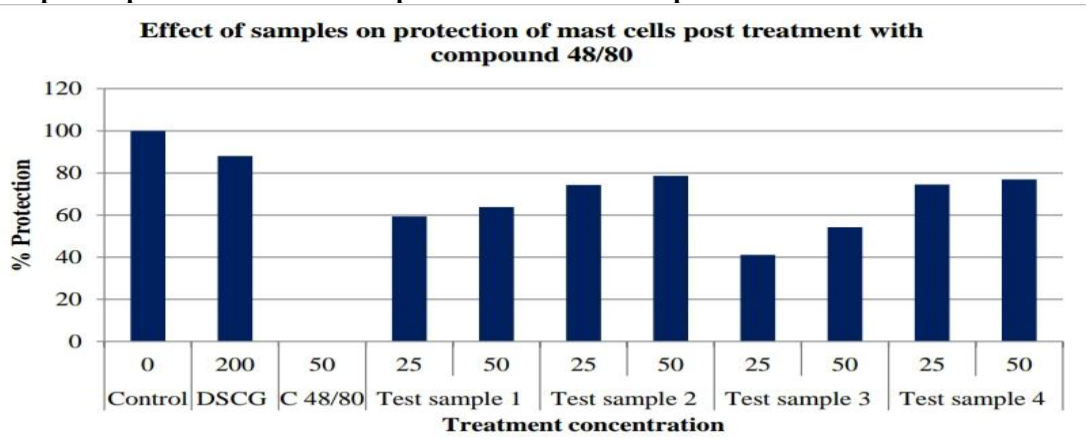
Plate no 18

Mast cells in mesenteric bits pretreated with alcoholic extract of leaves of Ageratum conyzoides at different concentration and post treated degranulating agent.

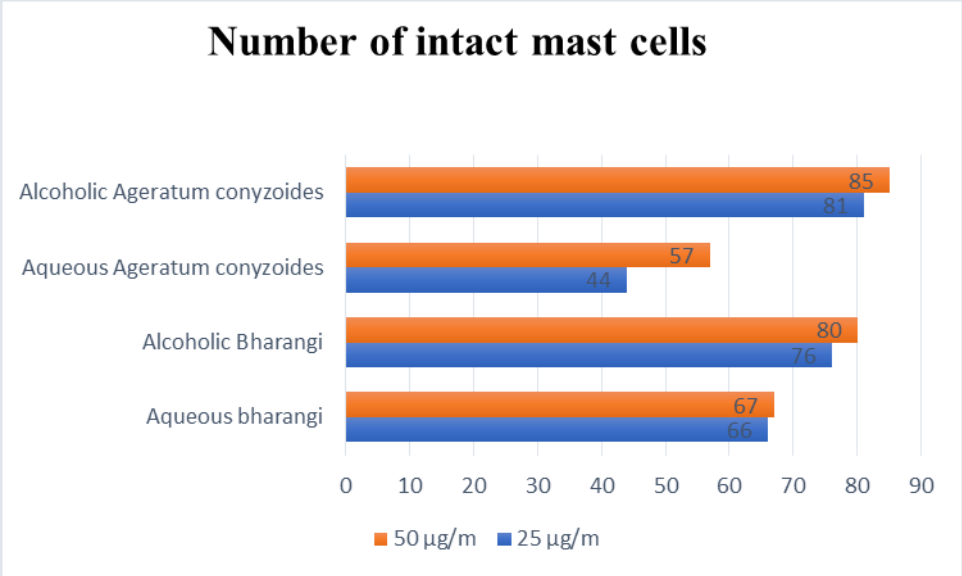
Table No 53: Effect of test samples on mast cell stabilization and their protective activity

Sample	Conc µg/ml	Total number of cells	No. of intact cells	No. of degranulated cells	% Protection	% degranulation
Control	0	100	100	0	100	0
DSCG (Disodium cromoglycate)	200	100	88	12	88	12
Compound 48/80	50	105	0	105	0	00
Aqueous extract of Bharangi roots	25 50	111 105	66 67	45 38	59.4 63.8	40.6 36.2
Alcoholic extract of Bharangi roots	25 50	109 108	81 85	28 23	74.3 78.7	25.7 21.3
Aqueous extract of leaves of Ageratum conyzoides	25 50	107 105	44 57	63 48	41.1 54.2	58.9 45.8
Alcoholic extract of leaves of Ageratum conyzoides	25 50	102 104	76 80	26 24	74.5 76.9	25.5 23.1

Effect of samples on protection of mast cells post treatment with compound 48/80



DSCG: Disodium cromoglycate
Test sample 1: Aqueous extract of Bharangi moola
Test sample 2: Alcoholic extract of Bharangi moola
Test sample 3: Aqueous extract of leaves of Ageratum conyzoides
Test sample 4: Alcoholic extract of leaves of Ageratum conyzoides



DISCUSSION

A mast cell (also known as a mastocyte or labrocyte) is a type white blood cell. Specifically, it is a type of granulocyte derived from the myeloid stem cell which is a part of the immune system and neuro immune systems. Mast cells play a key role in inflammatory process. When activated, a mast cell can either selectively release (piece meal degranulation) or rapidly release (anaphylactic degranulation) histamines. In allergic reactions, mast cells remain inactive until an allergen binds to antibody IgE already coated upon the cell. Other membrane activation events can either prime mast cells for subsequent degranulation or act in synergy with Fc and RI transduction. Mast cells are involved in the manifestation of various disorders such as mast cell activation disorders, allergic disorders, anaphylaxis, etc. Mast cell stabilizers are common medications used to prevent or control certain allergic disorders. They block a calcium channel essential for mast cell degranulation, stabilizing the cell and thereby Preventing the release of histamine and related mediators. Standard drug Disodium chromoglycate showed 88% of mast cell stabilization. There was significant response in all trial groups in stabilizing mast cells. Bharangi moola at the concentration of 50µg/ml showed 78.7% of mast cell stabilization, Ageratum leaves at 50µg /ml concentrations showed 76.9% of mast cell stabilization On comparing with trial drugs roots of Bharangi(Clerodendrum serratum) and leaves of Ageratum conyzoides, alcoholic extracts showed more mast cell stabilization activity when compared with aqueous extracts. Among alcoholic extracts of roots of Bharangi (Clerodendrum serratum) and leaves of Ageratum conyzoides, alcoholic extract of roots of Bharangi showed highest result.

CONCLUSION

Among the trial drugs alcoholic extract of *Bharangi moola* and leaves of Ageratum conyzoides exhibited better mast cell stabilization, among them alcoholic extract of Bharangi moola (Clerodendrum serratum) shown better result.

REFFERENCES

1. Susan, Berttan, G Katzung. Basic and clinical pharmacology. 12th ed. Tata Mc Graw Hill education pt Ltd, 2012; 1229.
2. K.D Tripathi, essentials of medical pharmacology, 7th edition, 2013, New Delhi, Jaypee brothers medical publishers, P1002.
3. Malick A, Andrew G. Antihistamines in the treatment of asthma, allergy, 1997.
4. Padmalatha K., Venkatraman B.V., Roopa R. (2000) *Indian J. Pharmacol*, 32: 1-10.
5. Das S.N., Sharma N., Chatterjee S. (1995) *Indian J. Ind. Med*, 17(1): 79-82.
6. Vogel H.G., Vogel W.H. (1997) Drug Discovery and evaluation, *Springer-Verlag: Berlin Heidel barg*, 433.
7. Rajasekaran M., Sudhakaran C., Pradhan S.C., Baopna S.S., Nair A.G.R. (1989) *J.Drug Dev*, 2(3): 179–182.
8. Mukherjee K.C. (1989) *Medical Laboratory Technology, Tata McGraw-Hill Publising Company Ltd: New Delhi*, (1): 263.
9. Lee Y.M., Leim D.K., Kim S.H., Shin T.Y., Kim H.M. (1996) *J.Ethnopharmacol*, 54: 77-84.
10. Fransesca Levi – Schaffer and Ron Eliashar, mast cell stabilizing properties of antihistaminic journal of investigative dermatology, 2009; 2549-2551.
11. Swapana SBS W Radhapiyari Devi1, Marina A1. Ethnobotany, phytochemistry and pharmacology of Ageratum conyzoides Linn (Asteraceae). 31) Research gate 31)[Internet]. Available from: https://www.researchgate.net/publication/236123857 .
12. Vinay J.A comparative pharmaceutico pharmaco clinical study of effect of different formulations of Tulasi in Shwasa 2006 Jamnagar.