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RESEARCH ARTICLE

Mast Cell Stabilizing Activity of *Carica Papaya* Leaves

Chaudhari SG, Chaudhari HR*, Mishra PA, Shendkar AK, Desai JS

Dr. L. H. Hiranandani College of Pharmacy, Ulhasnagar, 421003, Maharashtra, India.

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ABSTRACT

Carica papaya (Caricaceae), commonly known as papaya possesses diverse pharmacological activities. The present study evaluated the mast cell stabilizing activity of hydroalcoholic extract of leaves of *carica papaya* using albumin as an inducer. The present study aimed to evaluate the mast cell stabilization activity as a mechanism for antiasthmatic activity of leaves of *Carica papaya* using experimental paradigm. Wistar rats are used for experimental work on mast cell degranulation inhibition. Present study demonstrates the mast cell degranulation inhibition with the extract of leaves of *Carica papaya*. Albumin is used as an inducer for degranulation. Dose 200mg/kg ($p<0.05$) and 400mg/kg ($p<0.01$) showed significant inhibition in degranulation. Mast cell stabilization activity was shown by the leaves of *Carica* papaya in our research study. It also decreases mast cell degranulation and thus depletes the release of histamine which is one of the reasons for spasmogenic response.

KEYWORDS

Anti-asthmatics, *Carica Papaya*, Mast Cell Stabilization, Degranulation, Albumin

INTRODUCTION

Asthma is chronic pulmonary disorder accompanied by recurrent episodes of wheezing, chest tightness, cough, breathlessness. These episodes are associated with variable airflow obstruction which can be reversible if treated. The inflammation also associated with airway hyper responsiveness to variety of stimuli.

It is characterized by airway inflammation, i.e. redness and swollen airway, similarly airway obstruction i.e. tightening of the airway muscles which makes difficulty in air movement, and airway hyperresponsiveness (AHR) i.e. muscles of airways respond more quickly to minute quantity of allergen or stimuli.[1]

In airway inflammatory cells, nearly 20% of the cells are mast cells. These cells are found in airways at many sites like beneath the basement membrane of airways, near blood vessels in submucosa, spread in muscle bundles and also in bronchial luminal space.[2] These are multifunctional cells and perform many inflammatory activities. A huge array of mediators is released after activation of mast cells.[3,4] These cells are traditionally recognized for their role in hypersensitivity reactions. In 1878, Paul Ehrlich firstly describes mast cell. Mast cell houses numerous granules which contain many preformed mediators like leukotrienes, histamine, tryptase and many cytokines. These cell have high affinity FCεRI IgE receptors on their surface, which on cross-linking, and on binding with IgE antibodies, activate mast cells consequently initiating it's degranulation.[5] The release of cytokines and mediators occurs at this step. This is a sufficient trigger for bronchoconstriction, airway inflammation.[6] These are long lived cells and have capacity to get activated more than one time. Mast cell activation results into release as

***Address for Correspondence:**
H. R. Chaudhari
Dr. L. H. Hiranandani College of Pharmacy,
Ulhasnagar, 421003, Maharashtra, India
E-Mail Id: chaudharisg@gmail.com

well as de novo synthesis of mediators such as IL-4, IL-5, thus it contributes to a continued inflammatory response.[7]

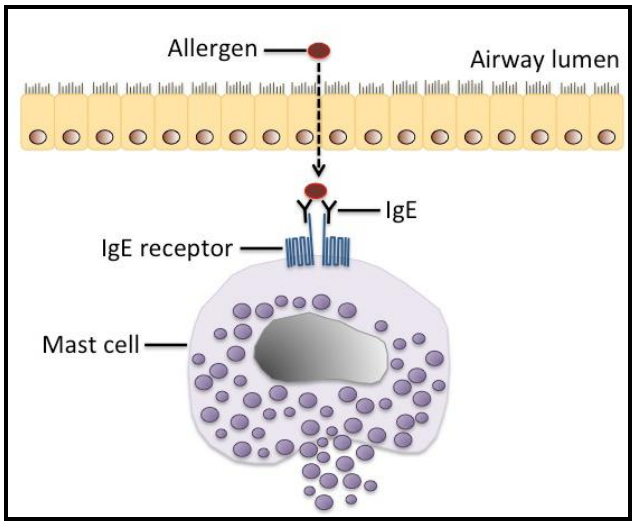


Figure 1: Mast cell degranulation due to Ag:Ab complex and IgE receptor on mast cell

MATERIALS AND METHOD

Collection of Plant Material

The leaves of plant *Carica papaya* (Family-*Caricaceae*) were collected from the region thane, Maharashtra, India in the month of August and September 2012. The leaves were authenticated at the Blatter Herbarium, St. Xavier's College, Mumbai, matched with specimen no. PD-3755

Extraction of Plant Material

Around 20 % ethanol was taken in a 250 ml R.B.F (Round Bottom Flask) and was attached to Soxhlet apparatus. 50-60 grams of the leaf powder was packed in thimble, Solvent in RBF got evaporated and then condensed. Condensed solvent entered to the thimble part, and through siphon arm, the content was dropped in the RBF. Cycles were allowed to repeat for two days. The content in RBF then subjected to rotary evaporator. The condensed extract was macerated with little quantity of water and slightly warmed. Then it was filtered through Buchner Funnel, the liquid extract then allowed to get condensed.

Phytochemical evaluation of extract showed that it contains alkaloids, glycosides, flavonoids. The extract was found to contain flavonoids, alkaloids, glycosides and tannins.

Experimental Animals

Animal Specifications

Species- Wistar rats

Age / weight / size – 250-300 gm

Gender – Either

Wistar rats weighing between 250- 300gm procured from Animal house of Dr. L. H. Hiranandani College Of Pharmacy, Ulhasngar , Animals were housed at ambient temperature 22±1º, relative humidity of 45 – 55%, 12 h light/dark cycle, in an animal house approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Protocol No. IAEC/PCOL-06/2013. The animals had free access of standard diet and water and housed in a poly propylene cages.

Studies on mesenteric mast cell degranulation induced by albumin.

In Vivo

Procedure

Wistar rats were sensitized with 0.1 ml of 1%w/v solution of albumin intraperitoneally on first, third, fifth and twelfth day. The extract was administered from sixth to twelfth day orally.

Group I – Control

Group II- Disease control

Group III – standard - Disodium cromoglycate 50mg/kg

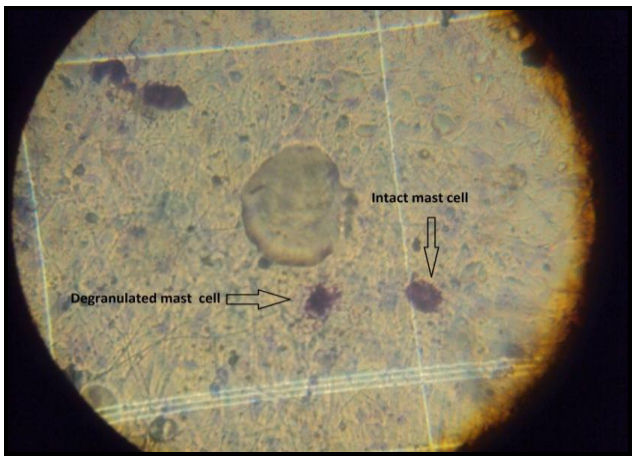
Group IV –Test 1- Extract of leaves of *Carica papaya* 100 mg/kg

Group V –Test 2- Extract of leaves of *Carica papaya* 200 mg/kg

Group VI – Test 3- Extract of leaves of *Carica papaya* 400 mg/kg

On twelfth day, after sensitization, rats were sacrificed and the mesentery was collected onto the slide, followed by staining with 0.1% toludine blue solution for 10 minutes. After

staining of mast cells mesenteric pieces were then observed under light microscope (power 450X).Percent of degranulation of the mast cells in the control group and the treated groups were calculated by counting the number of intact mast cells.[8,9]



Statistical Analysis

The results were expressed as mean ± standard error of mean. One way ANOVA was applied. $p<0.05$ as considered statistically significant.

In Vitro

Procedure

Saline solution was injected intra-peritoneally into the peritoneal cavity of the lightly anaesthetized Wistar rats. After giving abdominal massage for optimum distribution of the injected fluid, the peritoneal fluid was collected in the centrifuge tubes placed over ice. Peritoneal fluid obtained from 4-5 rats was pooled together and was subjected to centrifuge at the speed of 2000 rpm for 5 minutes. Supernatant solution was discarded while the underlying cells (pellets) obtained were resuspended in 1 ml of saline. 0.1 ml of this cell suspension was divided into six test tubes as shown below.

Test tube 1- 0.1 ml of the cell suspension obtained intra-peritoneally

Test tube 2- 0.1 ml of the cell suspension obtained intra-peritoneally

Test tube 3 - 0.1 ml of the cell suspension obtained intra-peritoneally + Disodium Cromoglycate 20 µ/ml[8]

Test tube 4 - 0.1 ml of the cell suspension obtained intra-peritoneally + Hydro-alcoholic Extract of leaves of *C. papaya* 50 µg/ ml

Test tube 5 - 0.1 ml of the cell suspension obtained intra-peritoneally + Hydro-alcoholic Extract of leaves of *C. papaya* 100 µg/ ml

Test tube 6 – 0.1 ml of the cell suspension obtained intra-peritoneally + Hydro-alcoholic Extract of leaves of *C. papaya* 200 µg/ ml

Each test tube was incubated for 15 min at 37°C. Then, 0.1 ml of 1% w/v egg albumin was added into each test tube, except test tube no. 1 and all the test tubes were further incubated under same conditions for 10 min. The cells were stained with 0.1% toluidine blue for 10 minutes and were observed under microscope for stained mast cell.

Statistical Analysis

All values were expressed as mean ± SEM. One way ANOVA was applied. The results were considered to be statistically significant when $p< 0.05$.

RESULTS DISCUSSION

The results of this study reveal that hydroalcoholic extract of *Carica papaya* possesses significant anti-asthmatic activity by inhibiting mast cell degranulation. In this, mast cell degranulation was induced by albumin.

***In Vivo* Model**

Mast cell degranulation in the mesentery of the rats induced by albumin was found to be 44.97% in positive control group. Addition of disodium cromoglycate inhibited degranulation significantly ($p<0.0001$) when compared with disease control, and it was reduced to 12.11% according to Table No. 1. With the doses of hydroalcoholic extract of *Carica papaya*, (100, 200, 400 mg/kg) the degranulation lowers to 43.28, 26.47 and 21.30% respectively. Inhibition of degranulation of mast cells was seen significantly ($p<0.05$) in CPLE dose 200 mg/kg. It also shows significant effect ($p<0.01$) at 400 mg/kg.

Table 1: Observation table for Inhibition of Mast cell degranulation *(in vivo)*

Treatment	**Dose [mg/kg, p.o.]**	**No. of Intact mast cells seen in the Neubeur's chamber**	**% of Intact mast cells**	**% of Degranulated mast cells**
Control	Saline	359.3 ± 28.27***	100	-
Disease Control	Saline	198.7 ± 10.61	55.03	44.97
Standard	Disodium cromoglycate 50 mg/kg	315.8 ± 14.28***	87.89	12.11
Test 1	100 mg/kg	203.8 ± 12.65^{ns}	56.72	43.28
Test 2	200 mg/kg	264.2 ± 8.514*	73.53	26.47
Test 3	400 mg/kg	282.8 ± 18.27**	78.70	21.30

Values are expressed as mean ± SEM (n = 6 animals), n.s- non significant,
***p < 0.0001, **p<0.01, * p<0.05, compared with disease Control Group (oneway ANOVA followed by Dunnett's Multiple Comparisons test).

Table 2: Observation table for Inhibition of Mast cell degranulation *(in vitro)*

Treatment	**Dose [µg/kg, p.o.]**	**No. of Intact Mast Cells Seen in the Neubeur's Chamber**	**% of Intact Mast Cells**	**% of Degranulated Mast Cells**
Negative Control	Saline solution	229.8 ± 5.294***	100	-
Disease Control	Saline solution	119.0 ± 6.763	51.78	48.22
Standard	Sodium dicromoglycate 20 µg/ml	208.3 ± 7.210***	90.64	9.36
Test 1	50 µg/ml	150.3 ± 10.26^{ns}	65.40	34.60
Test 2	100 µg/ml	171.7 ± 16.06**	74.71	25.29
Test 3	200 µg/ml	173.7 ± 8.894**	75.58	24.42

Values are expressed as mean ± SEM (n = 6), n.s- non significant,
***p < 0.0001, **p < 0.01, compared with Positive Control Group (oneway ANOVA followed by Dunnett's Multiple Comparisons test).

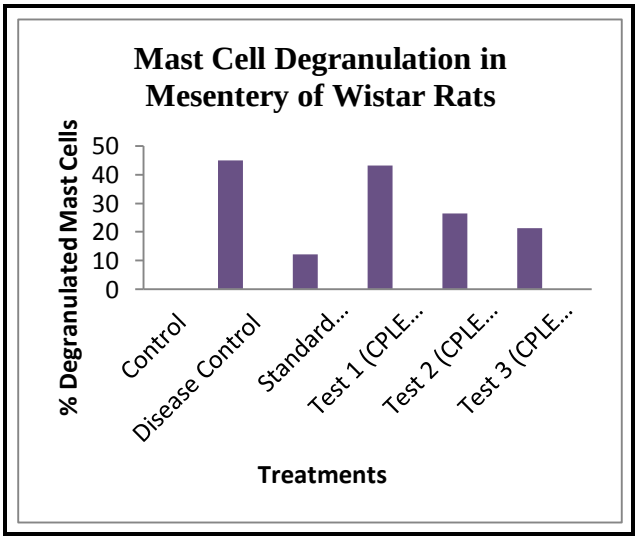


Figure 2: Graphical representation of inhibition of mast cell degranulation due to CPLE (in vivo)

It shows dose dependent effect as we can see from Figure No. 2. The protection given by the doses of extract was comparable with that of disodium cromoglycate which is potent mast cell degranulation inhibitor.

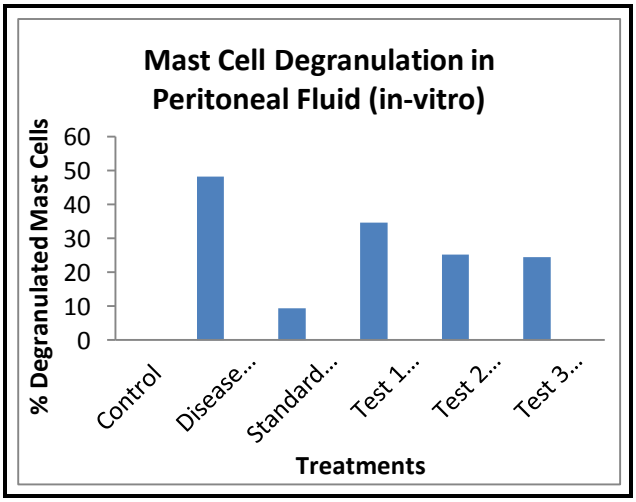


Figure 3: Graphical representation of inhibition of mast cell degranulation due to CPLE (in vitro)

Mast cell degranulation induced by albumin was found to be 48.22 % in positive control group. Addition of disodium cromoglycate inhibited degranulation, and it was reduced to 9.36 %. With the doses of hydroalcoholic extract of *Carica papaya*, (100 µg/ml, 200 µg/ml) lower the degranulation significantly ($p<0.01$) to 25.29 and 24.42% respectively as explained in Table No. 2. The protection given by the doses of extract was comparable with that of disodium cromoglycate which is potent mast cell degranulation inhibitor. The decrease in mast cell degranulation can be observed in Figure No.3.

Mast cell plays a critical role in immediate hypersensitivity. After activation of mast cells, they show their biological effects and release the preformed mediators and de novo synthesized mediators like histamine, leukotrienes, and other cytokines. Mast cells are found adjacent to blood vessels in the lamina propria in normal human airways, but in asthma they migrate into three key structures: the airway epithelium; the airway mucous glands; and the ASM (airway smooth muscle). It was hypothesized that the distribution and activation of mast cells across the airway wall may reflect their function in asthma. Following a laboratory allergen challenge, secretion of the autacoid mediators like histamine, PGD_2 and leukotriene (LTC_4) induces bronchoconstriction, mucus secretion and mucosal oedema, thus contributing to acute symptoms.

CONCLUSION

It can be concluded that, CPLE possess significant mast cell stabilizing activity against albumin induced mast cell degranulation in mesentery of rats. Inhibition of degranulation of mast cells was seen significantly ($p<0.05$) in CPLE dose 200 mg/kg. It also shows significant effect ($p<0.01$) at 400 mg/kg. So, de novo synthesized mediators like histamine also inhibited from its release, which results into decreased inflammation of airways and release of mediators.

From mast cell degranulation inhibition model, it can be concluded that, CPLE possess significant mast cell stabilizing activity against albumin induced mast cell degranulation peritoneal fluid collected from rats. So, the release of histamine and other mediators are also inhibited.

In pathogenesis of asthma, mast cells have a very prominent role. Degranulation of these

mast cells releases many mediators like histamine, leukotrienes, platelet activating factor for eosinophils, neutrophils, chemotactic factors, etc[10]. Through this release of numerous mediators and cytokines, mast cell plays a key role in immediate type of allergic reactions, airway hyperresponsiveness[11]. Extent of hyperresponsiveness is closely related to the degree of inflammation[12]. Herbs which produce anti-inflammatory effect by inhibiting the release of such mediators, may exhibit anti-asthmatic property.

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REFERENCES

1. Ismail, M. Y. M. (2010). Antiasthmatic Herbal Drugs A Review. *International Journal of Pharmacy & Pharmaceutical Sciences*, *2*(3), 28-29.
2. Kaliner, M. (1987). Mast cell mediators and asthma. *Chest Journal*, *91*(6), 171S-176S.
3. Kambayashi, T., & Koretzky, G. A. (2007). Proximal signaling events in FcεRI-mediated mast cell activation. *Journal of allergy and clinical immunology*, *119(3)*, 544-552.
4. Church, M. K., & Levi-Schaffer, F. (1997). The human mast cell. *Journal of Allergy and Clinical Immunology*, *99*(2), 155-160.
5. Okayama, Y., Ra, C., & Saito, H. (2007). Role of mast cells in airway remodeling. *Current Opinion in Immunology*, *19*(6), 687-693.
6. Brightling, C. E., Ammit, A. J., Kaur, D., Black, J. L., Wardlaw, A. J., Hughes, J. M., & Bradding, P. (2005). The CXCL10/CXCR3 axis mediates human lung mast cell migration to asthmatic airway smooth muscle. *American Journal of Respiratory and Critical Care Medicine*, *171*(10), 1103-1108.
7. Plante, S., Semlali, A., Joubert, P., Bissonnette, É., Laviolette, M., Hamid, Q., & Chakir, J. (2006). Mast cells regulate procollagen I (α 1) production by bronchial fibroblasts derived from subjects with asthma through IL-4/IL-4δ2 ratio. *Journal of Allergy and Clinical Immunology*, *117*(6), 1321-1327.
8. Mehta, A., & Agrawal, B. (2008). Investigation into the mechanism of action of Moringa oleifera for its anti-asthmatic activity. *Oriental Pharmacy and Experimental Medicine*, *8*(1), 24-31.
9. Patel, T., Rajshekar, C., & Parmar, R. (2011). Mast cell stabilizing activity of Myrica nagi bark. *Journal of Pharmacognosy and Phytotherapy*, *3*(8), 114-117.
10. Cushing, J. E., & Campbell, D. H. (1957). Manifestations of Antigen-Antibody reactions in Principles of Immunology. *McGrew-Hill Book Co. Inc. NY*, 278.
11. Bellanti, J. A. (1971). Mechanism of Tissue Injury produced by Immunologic Reactions *In Immunology, Asian Edn*: p. 184.
12. Bousquet, J., Jeffery, P. K., Busse, W. W., Johnson, M., & Vignola, A. M. (2000). Asthma: from bronchoconstriction to airways inflammation and remodeling. *American Journal of Respiratory and Critical Care Medicine*, *161*(5), 1720-1745.

