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EVALUATION OF HYDRO-ALCOHOLIC LEAF EXTRACT OF *FICUS BENGALENSIS* FOR THE ANTI-ASTHMATIC ACTIVITY USING VARIOUS ANIMAL MODELS.

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ABSTRACT

Objective- To evaluate the anti-asthmatic activity of *Ficus bengalensis* leaves. **Materials & Methods**- The hydroalcoholic (30% ethanolic) *Ficus bengalensis* leaf extract (HFBLE) was prepared. The antiasthmatic activity of HFBLE was evaluated using various experimental models like histamine induced bronchospasm in guinea pigs, *Invivo* and *invitro* inhibition of Mast cell degranulation studies on isolated guinea pig ileum and goat tracheal strip preparation. **Results**- 500 mg / kg of FBHLE significantly ($p<0.01$) inhibited the contraction induced by the histamine on guinea pig ileum and bronco-constriction. FBHLE also showed significant ($p<0.01$) and very significant ($p<0.0001$) inhibition of invivo and invitro mast cell degranulation, at 500mg/kg and 200µg/ml, respectively. Over 50% inhibition of contraction was exhibited at the highest test dose of 200µg/ml of *Ficus bengalensis* hydroalcoholic leaf extract of (FBHLE) in guinea pig ileum and goat tracheal tissue. **Conclusion**- Anti-athmatic activity of *Ficus bengalensis* (FBHLE) can either be due to spasmolytic activity, bronchodilation properties, or because of inhibition of mast cell degranulation.

Key Words: Ficus bengalensis, asthma, trachea, histamine, mast cells, rats, guinea pig, goat trachea.

INTRODUCTION

Asthma is known to be a commonly encountered complaint with increasing environmental hazards. It is a common complaint both in Pediatric and adult population, characterized by chronic airway–inflammation and increased airway responsiveness resulting in symptoms of wheeze, cough, chest tightness and dyspnea. Asthma has reached proportions and more than 200 million individuals affected worldwide in all groups related to increased indoor allergens, changes in immune responses to infectious diet, healthcare delivery systems and adverse effects. Airway inflammation characterizing the diseases occurs when genetically susceptible individual are exposed to environmental factors but, this may vary from person to person the timing, intensity and mode of exposure to aero allergens from environmental factors, which stimulate the immune system to produce IgE. [1] The inhalation of allergens in a sensitized atopic asthmatic patient results in two phase broncho-constrictor responses. The inhaled allergens rapidly interact with mucosal mast cells via an IgE dependent mechanism resulting in release of mediators such as histamine and Leukotrienes (LTs), which cause reversible bronchoconstriction. Asthma can also be exercise induced. Airway changes during asthma include wall thickening sub-epithelial fibrosis, increase mucus production and goblet cell mass, myofibroblast hyperplasia, myocyte-hyperplasia and hypertrophy and epithelial hypertrophy. In this condition mucus glands are also present in peripheral bronchioles resulting in mucus hypersecretion and airway obstruction due to mucus impaction. [2]

There are two kinds of therapy available in management of asthma. First by ‘Curative’ approach, in which medicines like Cromolyn sodium, Nedocronium sodium and steroid are used for long duration; [3] second by ‘Symptomatic’ approach, in which bronchodilators, including β2 agonist like Methyl-xanthines are used. [3, 4] But these drugs failed to overcome/cure asthma and remained uniformly an unacceptable and non satisfactory because of adverse effects and duration of dosage regimen. The WHO has also recognized the role of traditional system of medicine which depends largely upon the medicinal plants to achieve its gole “health for all by 2020”. [5]

Ficus bengalensis (*FB*) (Moraceae) is commonly known as Banyan tree or Vata or Vada tree in Ayurveda. There are more than 800 species and 2000 varieties of *Ficus* species, most of which are native to the old world tropics. [6] It is endemic to Bangladesh, India and Sri Lanka. It is also known as Bengal fig, Indian fig and East Indian fig, Indian Banyan or simply Banyan (English), also borh, nyagrodha (Sansikrat), Bat, Bargad and Bar (Hindi). The triad

Ganges, the Himalayas and the Banyan tree symbolize the images of India, for this reason it is considered as National Tree. Ficus means fig and bengalensis means belonging to or is of Bengal. [7]

According to Ayurveda, it is astringent to bowels; useful in treatment of biliousness, ulcers, erysipelas, vomiting, vaginal complains, fever, inflammations, leprosy. According to Unani system of medicine, its latex is aphrodisiac, tonic, vulnerary, maturant, lessens inflammations; useful in piles, nose-diseases, gonorrhea, etc. The aerial root is styptic, useful in syphilis, biliousness, dysentery, inflammation of liver, etc. [8] Milky juice is used for pains, rheumatism, lumbago and bruises. For the treatment of spermatorrhea, 2 drops of fresh latex in a lump of sugar are taken once daily on empty stomach early in the morning. Seeds are cooling and tonic in nature. [9] Its leaf buds are astringent, leaves infusion is given in diarrhea and dysentery, poultice of hot leaves is applied on abscesses. The bark is astringent and tonic and used in diabetes and leucorrhoea, lumbago, sores, ulcers pains and bruises. [10] Some important Ayurvedic marketed formulations are Nyagrodhaadi churnam (Bhaishajya Rutnavali), Saarivaadya Chandanaasava, Dineshavalyaadi Taila (Sahasrayoga). [11] In the present study, effect of hydroalcoholic (30% ethanolic) leaf extract of *Ficus bengalensis* was studied on various *in vivo* and *in vitro* methods for evaluation of anti-asthmatic activity.

MATERIALS AND METHODS

Plant material

The leaves of plant *Ficus bengalensis* (Family- Moraceae) were collected from the botanical garden of Dr. L. H. Hiranandani College of Pharmacy, Ulhasnagar, Maharashtra, India in the month of September and October 2012. The leaves were authenticated at the Blatter Herbarium, St. Xavier's College, Mumbai (specimen matches with Shah-6555 of G.L. Shah).

Preparation of plant extract

The leaves were dried, powdered mechanically, and extracted with 30% ethanol as the solvent. The filtrate obtained after extraction was subjected to acid hydrolysis, followed by the separation of flavonoids into ethyl-acetate. The dry extract obtained by evaporating ethyl-acetate was stored in an air-tight container. Preliminary phytochemical analysis of the hydroalcoholic extract of the leaves of *Ficus bengalensis* indicated the presence of quercetin, alkaloids, tannins, glycosides, saponins, terpenoids, sterols, and carbohydrates. [12]

Chemicals

All the necessary chemicals used in the study were provided by H(S)NCB's Dr. L. H. Hiranandani College of Pharmacy, Ulhasnagar.

Animals

Dunkin-Hartley strain guinea pigs (350-400 gm) of either sex, were procured from Haffkine Biopharmaceutical Corporation Limited, and were maintained under standard conditions husbandry with room temperature- 26 ± 2ºC, 12 h light/dark cycle. Wistar rats of either sex (250-300 gm) were procured from H(S)NCB's facility for breeding and experimentation, Dr. L. H. Hiranandani College of Pharmacy, Ulhasnagar. and were housed at ambient temperature 22±1º, 12 h light/dark cycle. Animals had free access to standard pallet diet and water *ad libitum*. The protocol of the experiment was approved by the Institutional Animal Ethical Committee of Dr. L.H. Hiranandani college of Pharmacy, Ulhasnagar, India (**IAEC/PCOL-05/2013**), as per the guidance of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Acute toxicity testing [13]

Under the procedures of Guideline, OECD 425 (Organization for Economic Cooperation and Development) 100 mg/kg, 500 mg/kg and 1000 mg/kg doses of extract were administered to confirm the absence of acute toxicity of the extract. Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter, for a total of 14 days. The extract was safe at all the tested doses for acute toxicity.

PROCEDURE

Histamine Induced Bronchospasm In Guinea Pigs (In Vivo) [14]

Twelve animals were used for the stabilization of the dose and method. Guinea pigs were examined for their sensitivity towards histamine spray by subjecting them to histamine aerosol (in ascending concentration) before conducting the actual model. Guinea pigs which were found to be sensitive to histamine at relatively higher doses were segregated from the remaining population and were not used for model. The sensitive guinea pigs were divided in 5 groups containing 5 animals in each. They were fasted for 24 h and then they were exposed to an atomized fine mist of 2% histamine dihydrochloride aerosol (dissolved in normal saline) using nebulizer at a pressure of 300 mm Hg in the histamine chamber (24 x 14 x 24

cm, made of perplex glass). Guinea pigs exposed to histamine aerosol showed progressive signs of difficulty in breathing leading to convulsions, and asphyxia. The time until signs of convulsion appeared was called pre-convulsion time (PCT). By observation experience was gained so that the pre-convulsion time can be judged accurately. As soon as PCT commenced, animals were removed from the chamber and placed in fresh air to recover. Ketotifen (1 mg/kg) and all fractions of hydro-alcoholic extract (150 mg/kg, 300 mg/kg and 500 mg/kg) were administered orally 90 min prior to the histamine exposure according to the groups.

Mast Cell Degranulation In Mesentery Of Wistar Rats [14, 15]

Wistar rats divided into six groups, each containing six animals, were sensitized with 0.1 ml of 1% w/v solution of albumin intra-peritoneally on first, third, fifth and twelfth day of first albumin administration. The extract was administered from sixth to twelfth day orally.

Group I – Control

Group II- Disease control

Group III – Standard drug (Disodium cromoglycate) 20µ/ml

Group IV –Test 1- Hydro-alcoholic Extract of leaves of *Ficus bengalensis* 150 mg/ml

Group V –Test 2- Hydro-alcoholic Extract of leaves of *Ficus bengalensis* 300 mg/ml

Group VI –Test 3- Hydro-alcoholic Extract of leaves of *Ficus bengalensis* 500 mg/ml

On twelfth day, after sensitization, rats were sacrificed and sections of mesenteries were collected onto the slide from each rat, followed by staining of the same with 0.1% toluidine blue solution made in water for 10 minutes. Toluidine blue stains mast cells. After staining the mesenteric pieces were then observed under light microscope (power 450X). Percent degranulation of the mast cells in the control group and the treated groups were calculated by counting the number of degranulated mast cells.

Mast Cell Degranulation In Peritoneal Fluid [14, 15]

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

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

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

Test tube 6 – 0.1 ml of the cell suspension obtained intra-peritoneally + Hydro-alcoholic Extract of leaves of *Ficus bengalensis* 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.

Spasmolytic Activity Of Isolated Guinea Pig Ileum [14]

Overnight fasted guinea pigs of either sex were sacrificed by cervical dislocation method. Ileum will be quickly dissected out and mounted in organ bath maintained at 37°C±1°C along with continuous aeration. Organ bath contained Tyrode's solution (NaCl 0.1, $NaHCO_3$ 1.0, NaH_2PO_4 0.05, and Glucose 1.0 gm/lit.). Responses of contractions were recorded with histamine 10 µg/ ml in absence and in presence of extract of concentrations 50 µg/ml (Test 1), 100 µg/ ml (Test 2) and 200 µg/ ml (Test 3).

Spasmolytic Activity Of Isolated Goat Trachea [16]

Goat trachea was obtained from nearby slaughterhouse. Trachea was cleaned off unwanted tissues and then it was cut into uniform rings. The rings were tied together in series to form a chain. This chain was suspended in organ bath containing Kreb's solution (NaCl 6.9., KCl 0.35, $CaCl_2$ 0.28, $MgSO_4$ 0.28, $NaHCO_3$ 2.1, KH_2PO_4 0.16, Glucose 2.0 gm/lit) maintained at 37±0.5ºC. Aerator was used to continuously bubble air into the organ tube. One end of the tissue was attached to the aerator tube by thread, while the other end was tied to the isotonic frontal lever. The dose response curve was taken on the kymograph paper present on the rotating drum. The dose response curve was obtained with histamine (10 µg/ ml) in absence and in presence of extract of concentrations 50 µg/ml (Test 1), 100 µg/ ml (Test 2) and 200 µg/ ml (Test 3).

Statistical Analysis

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

RESULT AND DISCUSSION

Table 1 Histamine Induced Bronchospasm In Guinea Pigs (In Vivo)

Sr. No.	Treatment	Pre-Convulsion Time (seconds)
1	Disease Control	145 ± 19.77
2	Standard (Ketotifen 1mg/kg)	415.8 ± 27.97***
3	Test 1 (FBHLE 150 mg / kg)	190.8± 37.47^{ns}
4	Test 2 (FBHLE 300 mg / kg)	255.4 ± 11.24*
5	Test 3 (FBHLE 500 mg / kg)	298.2 ± 13.19**

Values are expressed as mean ± SEM (n=5 animals) *p<0.05. **p<0.01, ***p<0.0001; ns = non significant, compared with Disease Control Group (one-way ANOVA followed by Dunnett's Multiple Comparisons test).

The controlled (Disease Control) animals showed convulsion during the first 3 min of spraying the inducer (histamine). Owing to its anti-histaminic property, the standard drug (ketotifen) showed maximum inhibition. Test drug showed significant inhibition of broncho-constriction at the highest dose (500 mg/kg), and non-significant inhibition at the lowest dose (150 mg/kg).

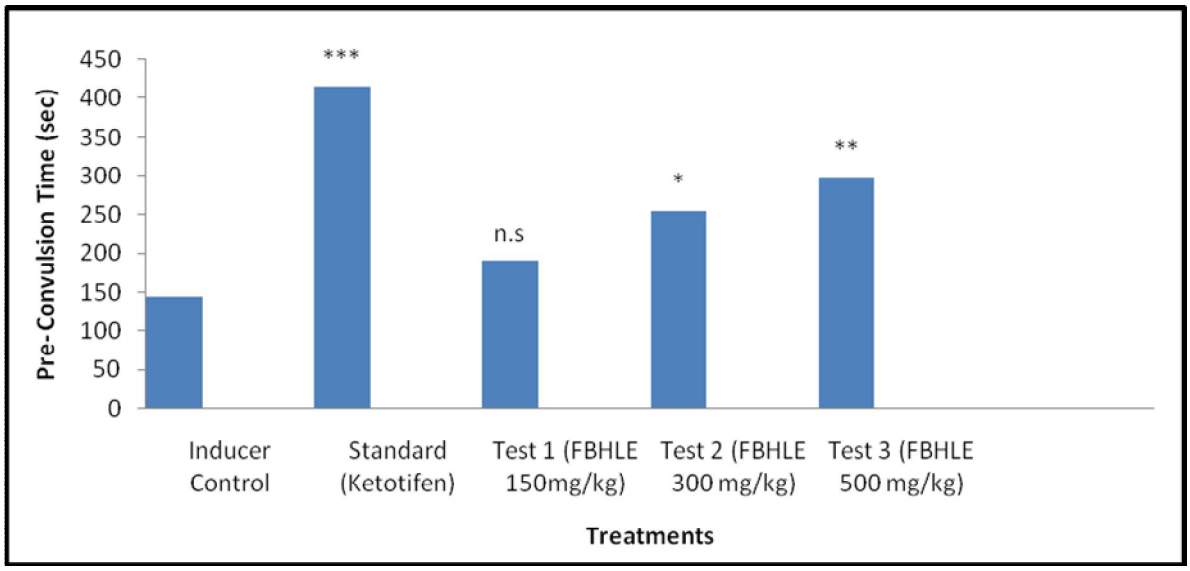

Fig. 1 Histamine Induced Bronchospasm In Guinea Pigs (In Vivo)

The acute response to allergen challenge in guinea pigs is nearly identical to that evoked in atopic human subjects because of the mast cells residing in the airways.[17] Histamine was used to induce bronchoconstriction in guinea pigs by action on H1 receptors present in the

airways. Hence, Ketotifen, a H1 receptor antagonist was used as a standard drug. Histamine binds to H1 receptor and activates the G-protein Gq, which inturn activates membrane bound phospholipase C (PLC) that hydrolyses phosphatidyl inositol 4, 5-biphosphate (PIP2), a membrane bound phospholipid. The products inositol 1, 4, 5-triphosphate (IP3) and diacylglycerol (DAG) act as second messengers. The primary action of IP3 is facilitation of Ca^{++} mobilization from intracellular organellar pools, while DAG in conjunction with Ca^{++} activates protein kinase C (PKC) which phosphorylates and alters the activity of a number of functional and structural proteins. Cytosolic Ca^{++} is a veritable messenger which combines with calmodulin (CAM) to activate myosin light chain kinase (MLCK) inducing contraction, and another important regulator calcium-calmodulin protein kinase (CCPK). [18]

Table 2 Mast Cell Degranulation In Mesentery Of Wistar Rats

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 solution	359.3 ± 28.27***	100	-
Disease Control	Saline solution	198.7 ± 10.61	55.03	44.97
Standard (Disodium cromoglycate)	50 mg/kg	315.8 ± 14.28***	87.89	12.11
Test 1 (FBHLE 150 mg / kg)	150	189.8 ± 11.09^{ns}	52.82	47.18
Test 2 (FBHLE 300 mg / kg)	300	260.5 ± 4.137*	72.50	27.50
Test 3 (FBHLE 500 mg / kg)	500	283.0 ± 11.98**	78.76	21.24

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

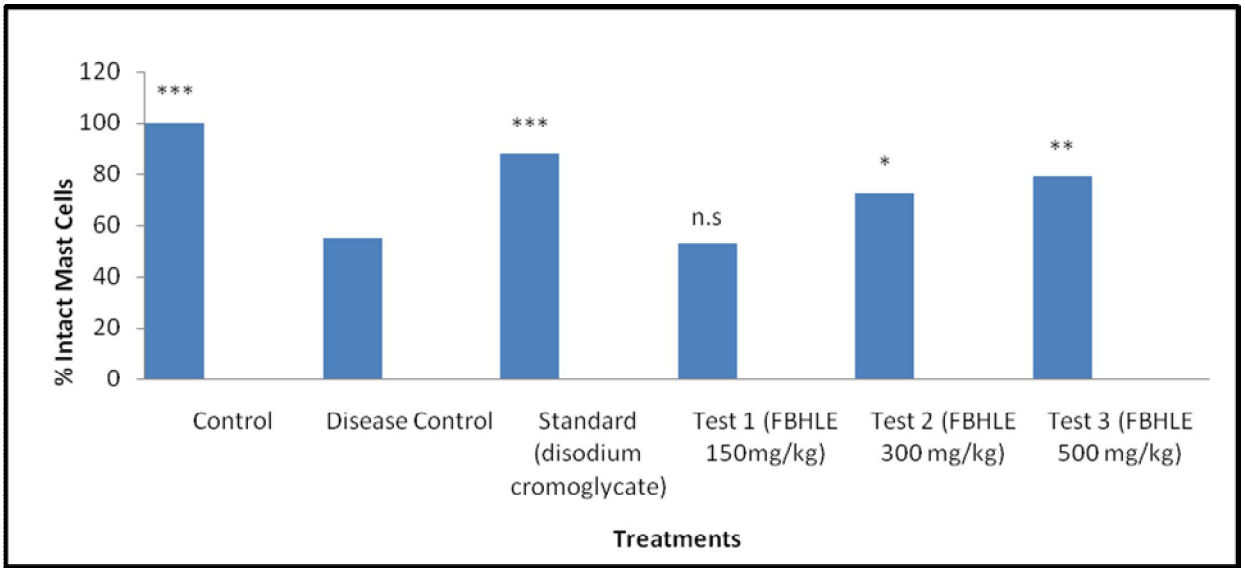

Fig. 2 Mast Cell Degranulation In Mesentery Of Wistar Rats (Intact mast cells)

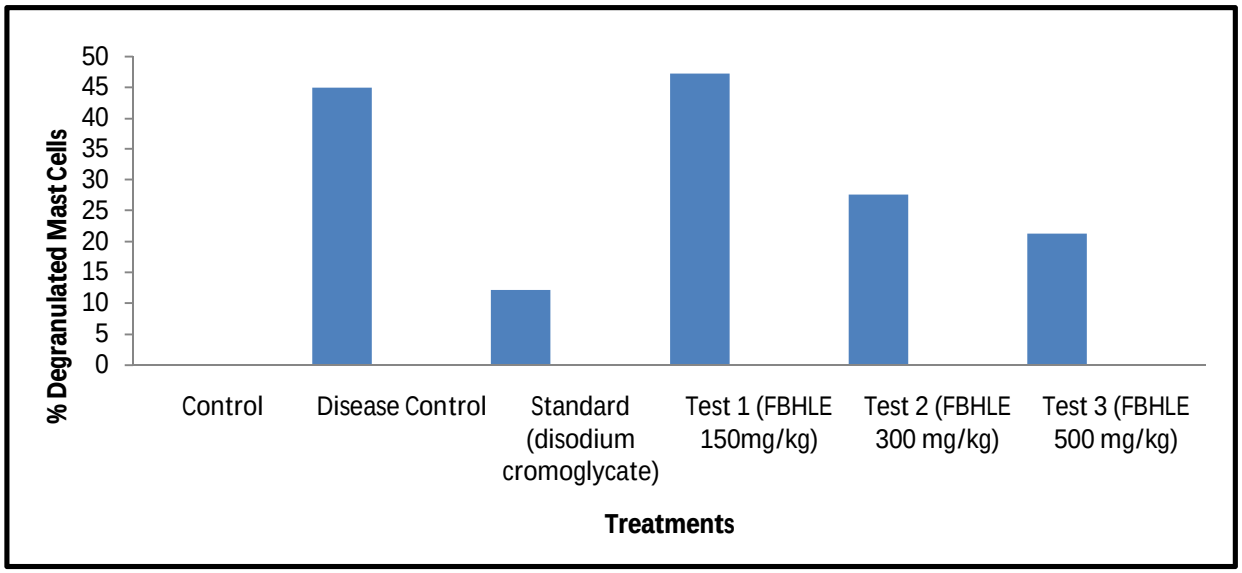

Fig. 3 Mast Cell Degranulation In Mesentery Of Wistar Rats (Degranulated mast cells)

In this study mast cell degranulation was studied in the mesentery of the Wistar rats. Mast cell degranulation in the mesentery of the rats induced by albumin was found to be 44.97% in the Disease Control group. Addition of Disodium cromoglycate inhibited degranulation significantly ($p<0.0001$) and hence amount of intact (non-degranulated) mast cells was 87.89 % when compared with 55.03% of Disease Control. Test doses, 150, 300, 500 mg/kg, of the hydro-alcoholic extract of *Ficus bengalensis*, showed 52.82, 72.50 and 78.76 % of intact mast cells (non-degranulated), respectively. Inhibition of degranulation of mast cells was dose dependent and significant ($p<0.01$) at the two higher doses. The protection given by the doses

of extract was comparable with that of disodium cromoglycate which is potent mast cell degranulation inhibitor.

Following are the images of mast cells (intact and degranulated), as seen under the microscope

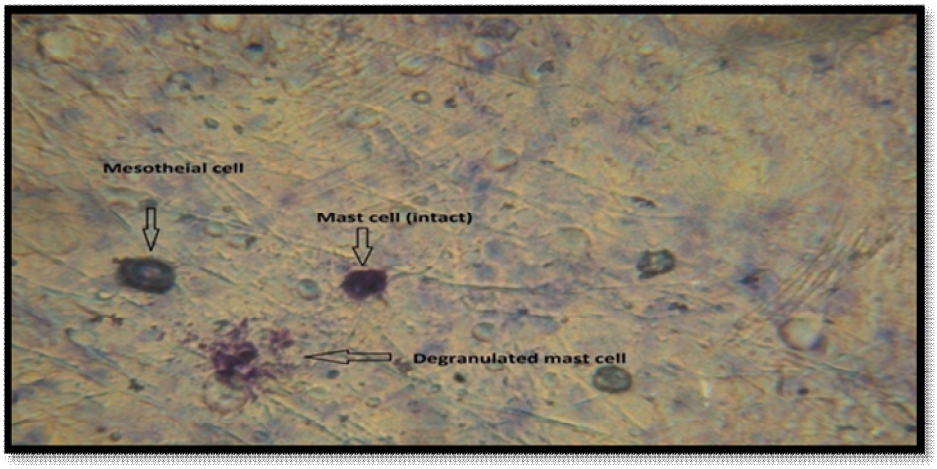

Fig. 4 Cells seen under microscope in the peritoneal fluid

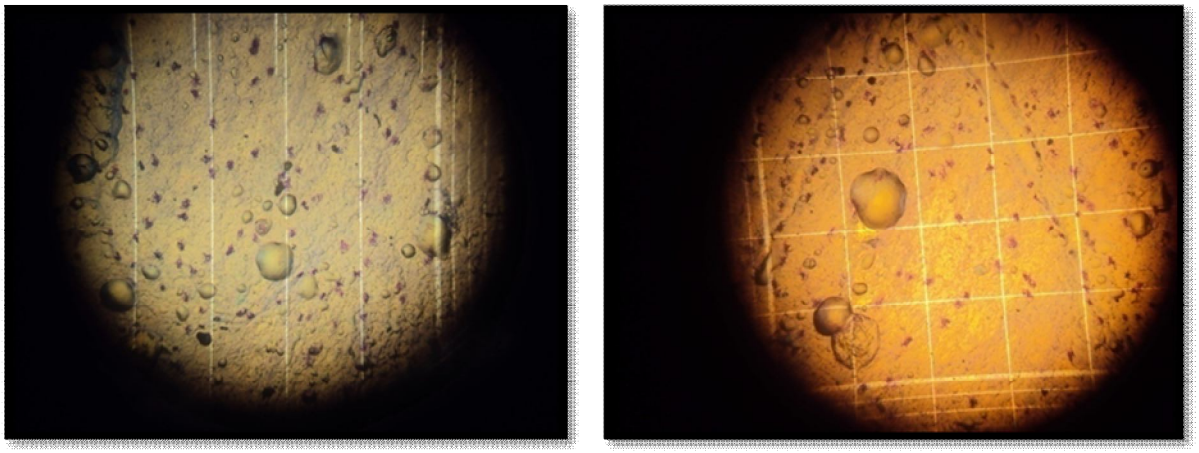

Fig. 5,6 Mast cells as seen under microscope (small bluish dot-like structures)

We used disodium cromoglycate as standard drug because it inhibits degranulation of sensitized mast cells, release of mediators of type I allergic reactions, including histamine and cysteinyl leukotrienes (e.g., slow-reacting substance of anaphylaxis [SRS-A] from sensitized mast cells after exposure to antigens. Mast cell degranulation results in serotonin-induced bronchoconstriction. [19]

When allergen binds to IgE bound cell, chemical mediators such as histamine, leukotrienes, prostaglandins and platelet activating factors are released. This develops the airway inflammation and bronchoconstriction. Mast cells also promote chronic inflammation and the

development of airway remodeling in asthma through cytokines, chemokines, proteases, and interaction with other immune cells (T cells and eosinophils). Thus, targeting the mast cells may be a new treatment modality in treatment and prevention of asthma.[20-22]

In this model an attempt was made to find out whether the extract has ability to decrease the rate of disruption of mast cells. FBHLE offered significant protection against albumin induced mast cell degranulation, and this is ultimately responsible for prevention of airway inflammation and release of mediators.

Table 3 Mast Cell Degranulation In Peritoneal Fluid

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
Negative Control	Saline	229.8 ± 5.294***	100	-
Disease Control	Saline	119.0 ± 6.763	51.78	48.22
Standard (disodium cromoglycate)	20µg/ml	208.3 ± 7.210***	90.64	9.36
Test 1 (FBHLE)	50 µg/ ml	143.3 ± 11.82^{ns}	62.35	37.65
Test 2 (FBHLE)	100 µg/ ml	160.2 ± 9.635**	69.71	30.29
Test 3 (FBHLE)	200 µg/ ml	169.8 ± 2.613***	73.89	26.11

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

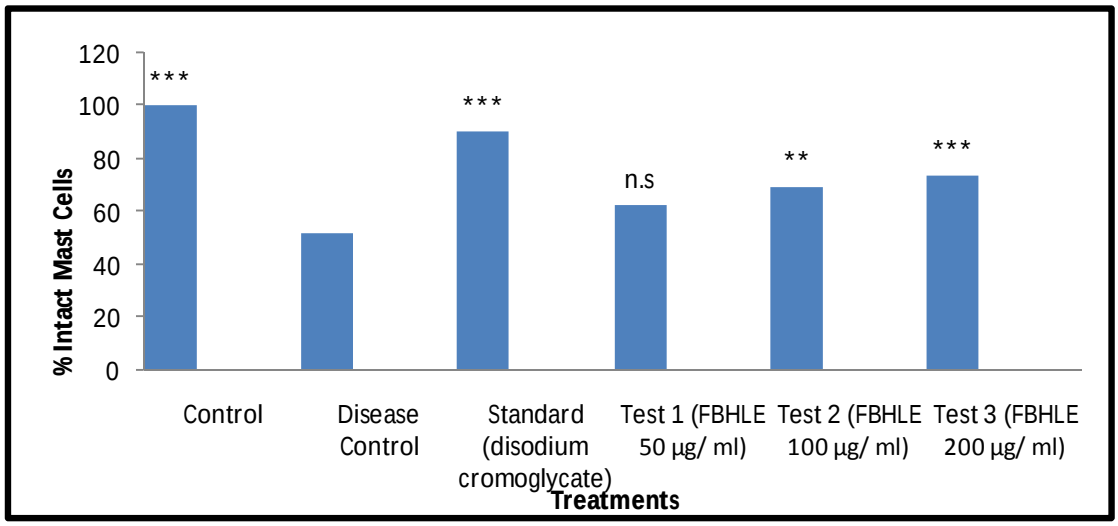

Fig. 7 Mast Cell Degranulation In Peritoneal Fluid (Intact mast cells)

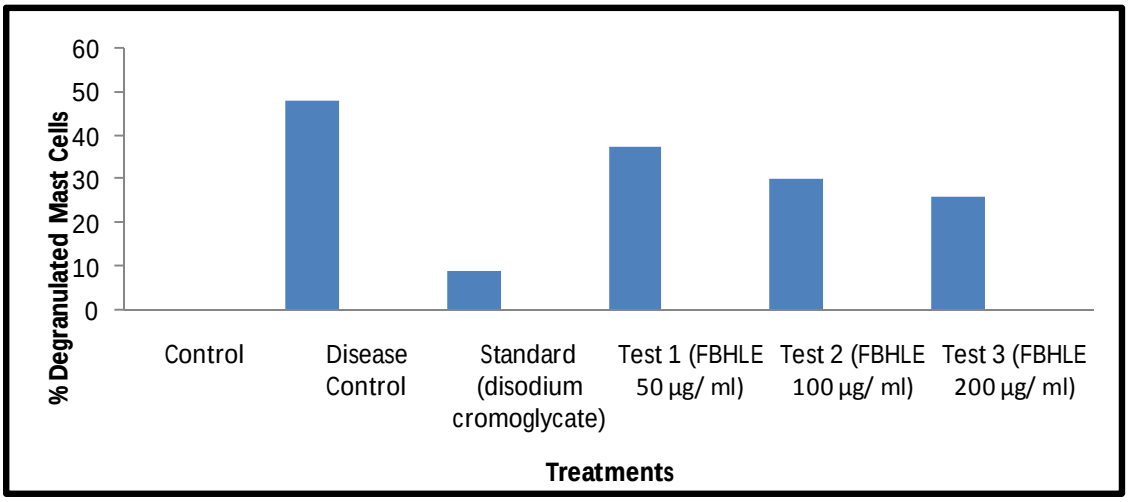

Fig. 8 Mast Cell Degranulation In Peritoneal Fluid (Degranulated mast cells)

In this study mast cell degranulation was studied in the peritoneal fluid obtained from the peritoneal cavities of Wistar rats. Mast cells were degranulated by using albumin as the degranulator. Mast cell degranulation in the fluid by albumin was found to be 48.22% in the Disease Control group. Addition of Disodium cromoglycate inhibited degranulation significantly ($p<0.0001$) and hence amount of intact (non-degranulated) mast cells was 90.64 % when compared with 51.78% of Disease Control. Test doses, 150, 300, 500 mg/kg, of the hydro-alcoholic extract of *Ficus bengalensis*, showed 62.35, 69.71 and 73.89 % of intact mast cells (non-degranulated) respectively. Inhibition of degranulation of mast cells was dose dependent and significant ($p<0.0001$) at the highest dose and non- significant at the lowest dose of the extract. The protection given by Test-1 and Test-2 doses of extract was comparable with that of disodium cromoglycate which is potent mast cell degranulation inhibitor.

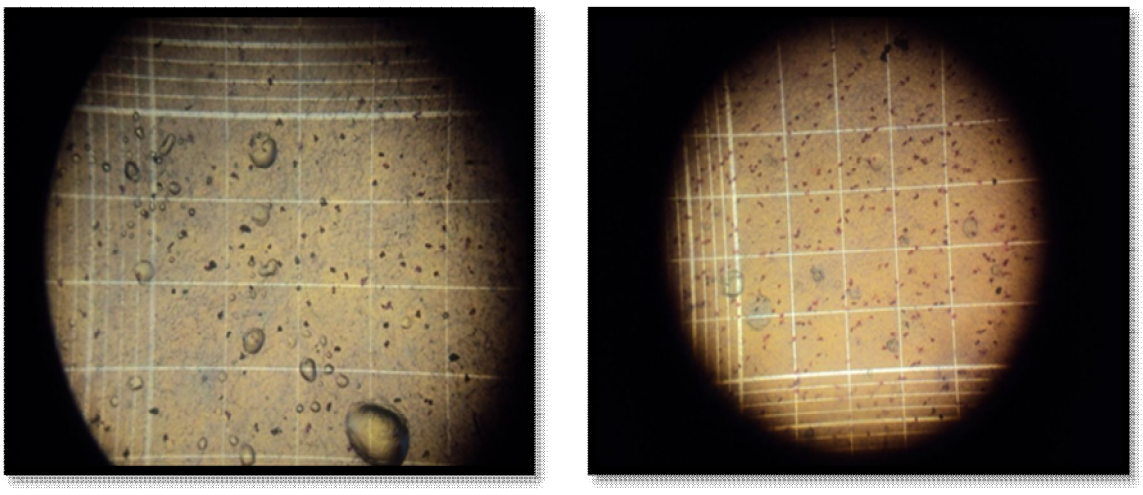

Fig 9,10 Mast cells as seen under microscope (bluish dot-like structures)

Table 4 Spasmolytic Activity Of Isolated Guinea Pig Ileum

ml of histamine	% Inhibition		
	TEST 1 (FBHLE 50 µg/ ml)	TEST 2 (FBHLE 100 µg/ ml)	TEST 3 (FBHLE 200 µg/ ml)
0.1	13.75±5.728	33.75±1.399	55.12±6.329
0.2	24.27±0.9791	34.27±3.59	53.19±5.086
0.4	28.76±2.919	30.87±7.452	58.26±5.123
0.8	29.99±3.543	29.88±4.853	52.40±2.405

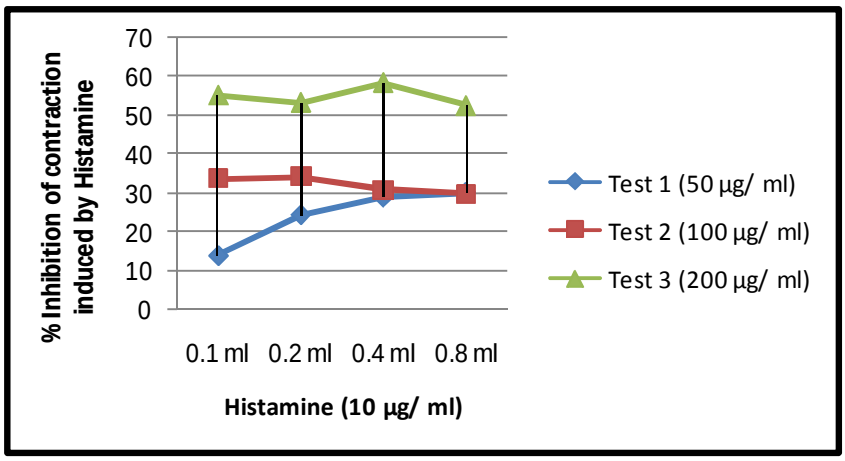

Fig. 11 Spasmolytic Activity of Isolated Guinea Pig Ileum (Inhibition of contraction)

In order to confirm the action of FBHLE on the contraction of smooth muscles, Guinea pig ileum model (invitro) was used. The stimulation of H_1 receptors produces graded dose related contraction of guinea pig ileum. Inhibition of contraction indicates H_1 receptor antagonist effect of the extract. In the present study histamine (20 µg/ ml) produced dose dependent contraction of guinea pig ileum as indicated in the graph. The extent of inhibition of the contractility of tissue by the extract was more at its highest dose (200 µg/ ml).

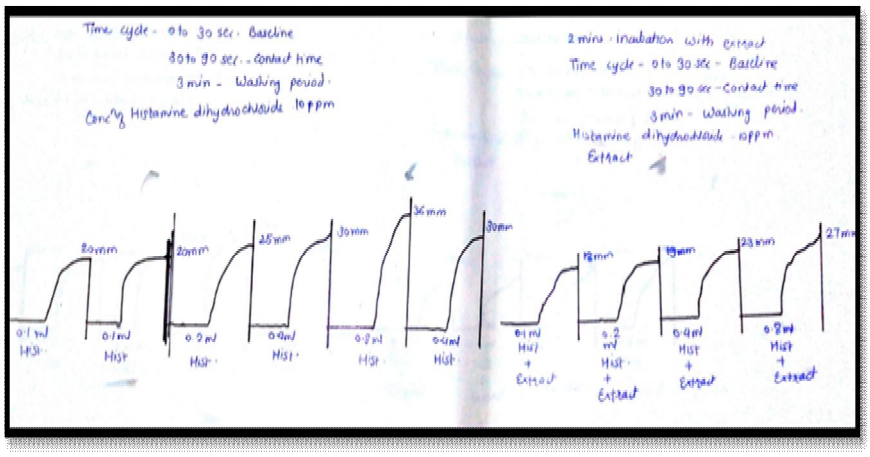

Fig. 12 Kymograph sheet with DRC (Dose Response Curve) on Guinea pig ileum

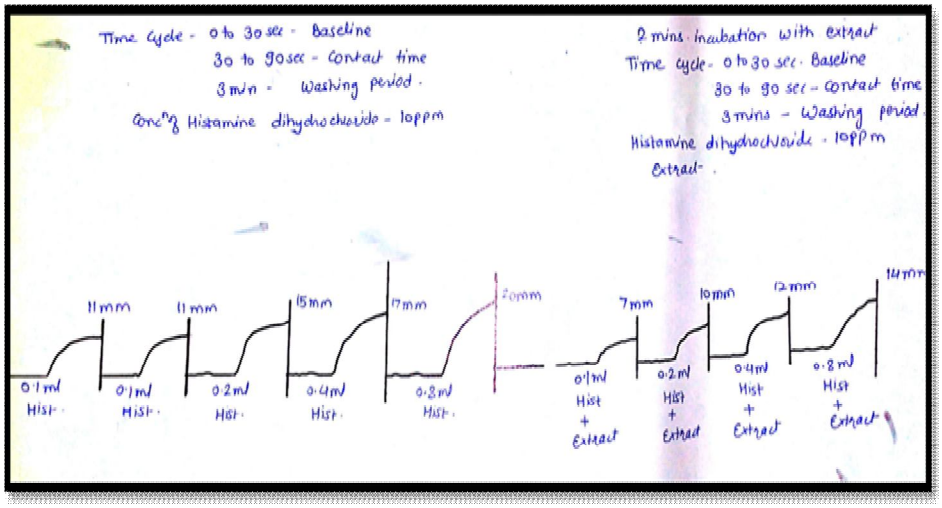

Fig. 13 Kymograph sheet with DRC (Dose Response Curve) on Guinea pig ileum

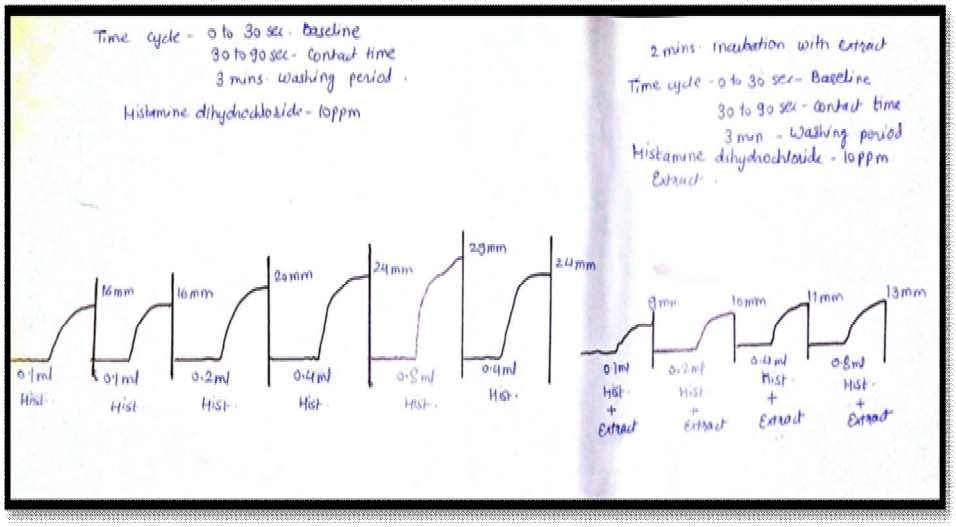

Fig. 14 Kymograph sheet with DRC (Dose Response Curve) on Guinea pig ileum

Table 5 Spasmolytic Activity Of Isolated Goat Trachea.

ml of histamine	% Inhibition		
	TEST 1 (FBHLE 50 µg/ ml)	TEST 2 (FBHLE 100 µg/ ml)	TEST 3 (FBHLE 200 µg/ ml)
0.1	21.30±7.151	26.72±10.81	52.61±4.202
0.2	32.56±7.432	37.98±4.070	57.75±1.285
0.4	42.7±6.619	37.04±2.534	55.02±2.619
0.8	44.26±4.083	47.80±10.79	59.41±0.7663

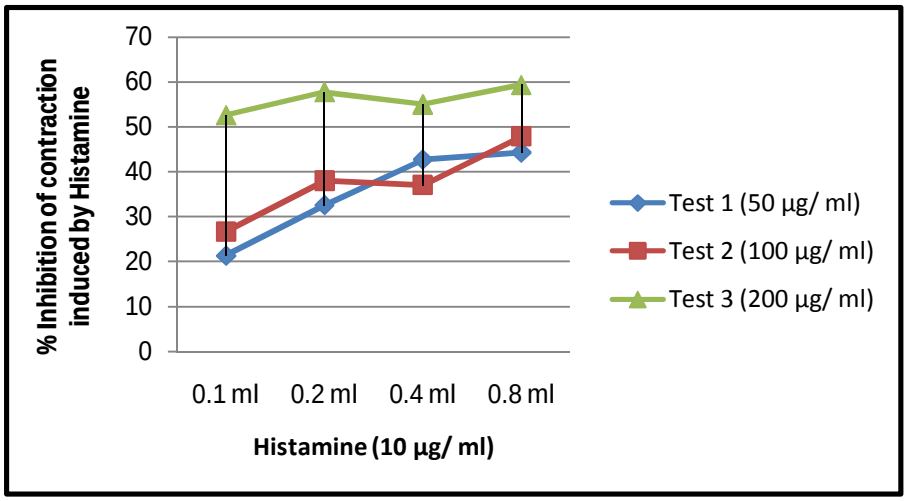

Fig. 15 Spasmolytic Activity Of Isolated Goat Trachea (Inhibition of tracheal tissue contraction)

Goat trachea (tracheal rings) was used for the screening of anti-histaminic activity and the effect of FBHLE on the trachea, one of the important anatomical structures involved in bronchoconstriction. The stimulation of H_1 receptors produces graded dose related contraction of tracheal rings. Inhibition of contraction indicates H_1 receptor antagonist effect of the extract. In the present study histamine (20 µg/ ml) produced dose dependent contraction of tracheal rings as indicated in the graph. The extent of inhibition of the contractility of tissue by the extract was more at its highest dose (200 µg/ ml).

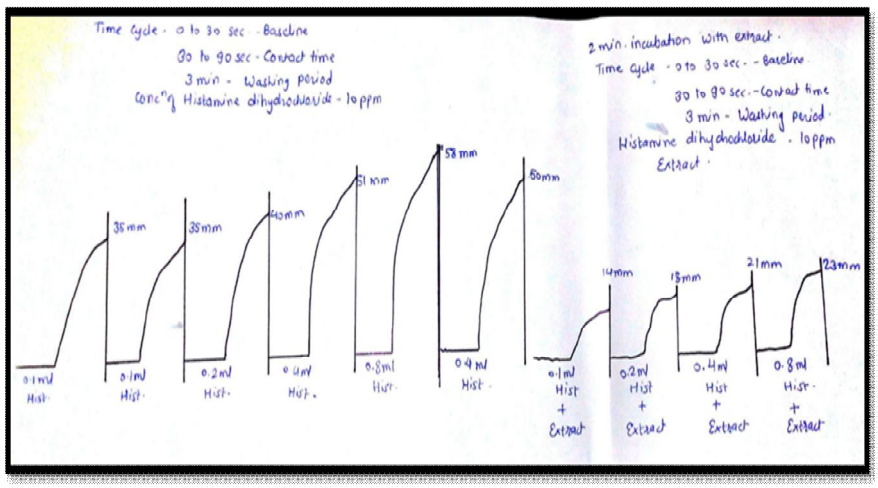

Fig. 16 Kymograph sheet with DRC (Dose Response Curve) on Goat trachea.

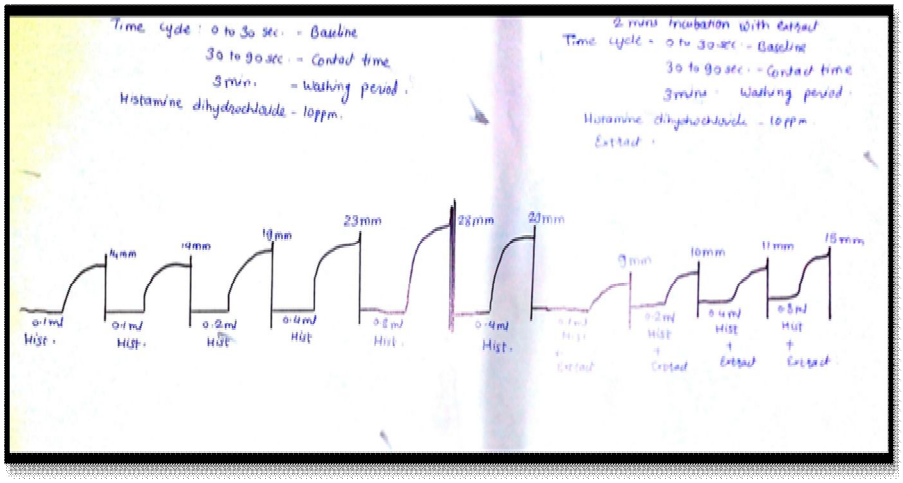

Fig. 17 Kymograph sheet with DRC (Dose Response Curve) on Goat trachea.

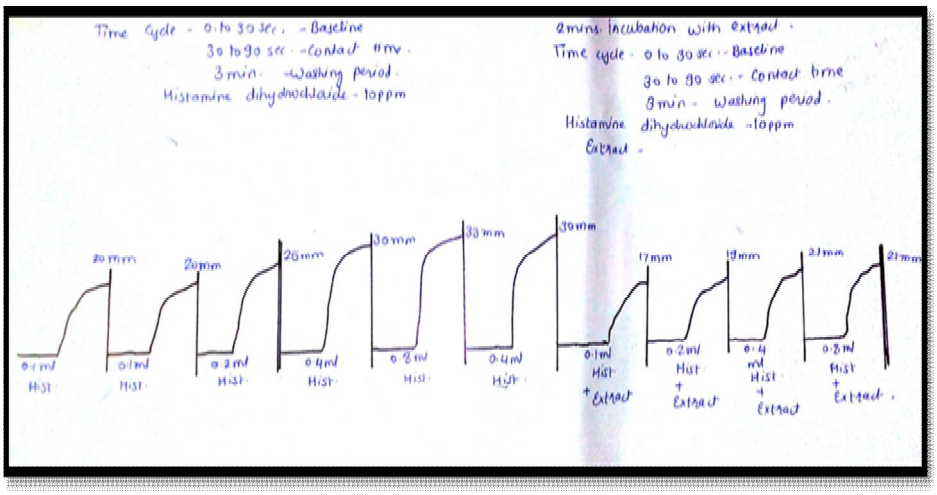

Fig. 18 Kymograph sheet with DRC (Dose Response Curve) on Goat trachea.

Approach towards this model is more useful because goat trachea is a representative of respiratory smooth muscles. This method is useful for checking the antispasmodic effect of extract on bronchial musculature. The goat trachea was found to contain H1 (excitatory), scanty population of H2 (inhibitory), 5- HT and also muscarinic excitatory receptors, since the Acetylcholine induced contractions were effectively blocked by atropine. [23] Histologically the trachea and bronchi possess a common type of cartilage and muscle and pharmacologically both trachea and bronchi react in the same way. Stimulation of histaminergic receptor causes the activation of VIP (Vasozctive Intestinal Peptide) in cerebral cortex, which is responsible for the release of histamine from sensory neurons. [24]

The present study was carried out to find out the suitability of goat tracheal chain preparation for the assay of an antagonist against spasmogen induced contraction on tracheal muscle. [25]

The results show significant percent inhibition of contraction, which provides the data for spasmolytic activity of FBHLE.

CONCLUSION

W.H.O has identified 'Allergic Diseases' as the 4th largest form of chronic diseases residing amongst human population. Pollution in the environment, and considering the evolution of drug resistant allergens, ever-growing danger of allergic diseases in the world will only rise from now. Allergy gives rise to other diseases, one of them being asthma. This study was undertaken to evaluate the anti-allergic and hence, anti-asthmatic potential of the leaf extract of *Ficus bengalensis*.

In this study, *Ficus bengalensis* hydroalcoholic leaf extract of (FBHLE), has shown potent anti-asthmatic activity. This property was established by using different animal models; Histamine induced bronchospasm in guinea pigs, Mast cell degranulation in mesentery of Wistar rats, Mast cell degranulation in peritoneal fluid, Spasmolytic activity of isolated guinea pig ileum and Spasmolytic activity of isolated goat trachea.

From the results and discussion, it can be summarized that, 500 mg / kg of *Ficus bengalensis* hydroalcoholic leaf extract of (FBHLE) significantly ($p<0.01$) inhibited the contraction induced by the histamine on guinea pig ileum and bronco-constriction. Similarly, it shows significant ($p<0.01$) inhibition at 500 mg/kg in invivo mast cell degranulation; and significant ($p<0.01$) and very significant ($p<0.0001$) inhibition at 100µg/ml and 200µg/ml respectively, in invitro mast cell degranulation. This reduces the release of histamine which is one of the reasons for spasmogenic response in bronchi. Over 50% inhibition of contraction was exhibited at the highest test dose of 200µg/ml of *Ficus bengalensis* hydroalcoholic leaf extract of (FBHLE) in guinea pig ileum and goat tracheal tissue.

By summarizing the data, it can be concluded that, 500 mg/kg (invivo) and 200 µg/ml of (invitro) dose of *Ficus bengalensis* hydroalcoholic leaf extract of (FBHLE) show a good spasmolytic activity, bronchodilation, and mast cell degranulation inhibition in the preclinical study involving guinea pigs, rats, and the isolated tissues of goat.

With better application of advanced techniques of isolation of active contents of the *Ficus bengalensis* leaves, isolation of quercetin is possible. Synthetic drugs like sympatho-mimetic, have been providing symptomatic relief to asthmatics over the years. However, they have not

been able to completely eradicate the disease. Combination of anti-histaminic (anti-allergic) and sympathomimetic drugs is the future of Asthma management.

CONFLICT OF INTEREST

There is no conflict of interest associated with the authors of this paper.

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