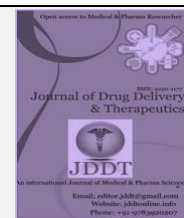
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Research Article

## Evaluation of the Antiasthmatic Activity of Methanolic Extract of *Trigonella Foenum Graecum* on Experimental Models of Bronchial Asthma

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### ABSTRACT

The present study deals with the phytochemical screening and evaluation of antiasthmatic activity of methanolic extract of *Trigonella foenumgraecum* on experimental models of bronchial asthma and anaphylaxis. The antiasthmatic activity was studied on histamine-induced bronchospasm in guinea pig (Dunkey-Hartley) for respiratory parameters such as maximum airflow, minimum airflow, tidal volume, respiratory rate, minute volume, specific airway resistance determination on double chambered whole body plethysmography on un-anesthetized guinea pigs, for mast cell degranulation by compound 48/80 (*in vitro*) was done using rat (Albino Wistar) peritoneal fluid. *Trigonella foenum graecum* treated result indicated significant protection against histamine-induced bronchospasm in guinea pigs at highest dose i.e. 400mg/kg. The bronchodilatory effect of *Trigonella foenum graecum* was found comparable to the protection offered by the standard drug Salbutamol on respiratory parameters in double chambered whole body plethysmography, Treatment with *Trigonella foenum graecum* at a dose of 400mg/kg showed a significant decrease in degranulation rate of actively and passively sensitized mast cells of sensitized rats when challenged with antigen. *Trigonella foenum graecum*. Possess significant anti-asthmatic activity due to its potential anti inflammatory, antioxidant and the antihistaminic activity, which reflects as anti-degranulating effect on mast cells and on respiratory parameters.

**Keywords:** *Trigonella foenum graecum*; asthma; mast cell; compound 48/80; histamine

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### INTRODUCTION:

Asthma is a serious condition in which the bronchial tubes become swollen, hyperproduction of mucus results in a reduction in size, which is characterized by airway inflammation and difficulty in breathing.<sup>1</sup> All of these pathological changes in asthma are mediated by common inflammatory mediators like histamine, tryptase, and other neutral proteases, leukotrienes C4 & D4 and prostaglandins which are released by infiltrating of inflammatory cells, including eosinophils, mast cells, and lymphocytes responsible for the early reaction (immediate bronchoconstriction).<sup>2</sup> Asthma is a complex disease in which the repeated exposure of many types of allergens leads to aggravation of asthma attack some of them are dust particles, pollens, exercise, pets in houses, insects like- dust mites in mattresses, pillows, carpets, curtains smoke from tobacco, wood, agarbattis, changes in weather foods drugs like aspirin,  $\beta$ -blockers and viral respiratory infections.<sup>3</sup> Asthma affects over 20 million individuals in the US and over 60 million individuals worldwide. It affects over 5-10% of the

population in industrialized countries.<sup>4,5</sup> In India 57.5 estimated total deaths (2,000); 5.1 estimated deaths per 100000 population; 277 DALYs (disability-adjusted life-year) per 100,000; 6.5 age-standardized deaths per 100,000; constitutes 0.2% of all deaths and 0.5% of National burden of diseases.<sup>14,5</sup> Common symptoms which observed in asthma are a shortness of breath, wheezing, coughing and a tight feeling in the chest.<sup>6</sup> Even though research in respiratory medicine is in advanced stages, respiratory diseases are still one of the perpetrators of global health. Despite the availability of a wide range of drugs such as steroids,  $\beta_2$ -agonists, anticholinergic and phosphodiesterase (PDE) inhibitors, the relief offered by them is mainly symptomatic, short-lived and have numerous adverse effects. For example, longer use of corticosteroids produces a deficiency in bone mineral density, cardiovascular effects, osteoporosis and osteonecrosis, the risk of cataract, panniculitis, migraine or a migraine-like headache, pharyngitis and sore throat and renal deterioration. Most recently, it has been reported to cause serious pneumonia also. The long-acting  $\beta_2$ -adrenoreceptor agonists produce myocardial ischemia and

osteoporosis. Anticholinergics cause papillary dilation, blurred vision, and acute glaucoma. PDE inhibitors like theophylline cause gastrointestinal symptoms to palpitations, arrhythmias, hypocalcemia, nausea, diarrhea, and headache. Hence the search for a new drug is still the need of the day.<sup>7,8</sup> *Trigonella foenum graecum*. (*Fabaceae*) is commonly known as "Methi" in Hindi, and is used as a vegetable and cultivated throughout India, Sri Lanka, and many other tropical countries.<sup>9</sup> The plant contains steroids, saponins,  $\beta$ -sitosterol, flavonoids, terpenoids, glycosides, alkaloids, phenolic compounds, betalains, tannins, amino acids, lysine and tryptophan, and 4-hydroxyisoleucine. Steroidal saponins (fenugreek in diosgenin) flavonoids as flavone glycosides (apigenin, luteolin, quercetin), Coumarins.<sup>10, 11</sup> Tribal people use decoction (15-20 ml) of the whole plant thrice daily in allergic bronchitis and asthma as folk medicine.<sup>11</sup> However, no scientific studies are carried out to investigate the anti-asthmatic and anti-anaphylactic effect of seeds of *Trigonella foenum graecum*. Hence the present study is carried out to evaluate the anti-asthmatic activity of seeds of *Trigonella foenum graecum*.<sup>7,8</sup> Therefore, need to develop the novel treatment for asthma with fewer side effects, and for this purpose, *Trigonella foenum-graecum* which is natural product can be used as new therapy. However, its exact effects on allergic asthma have been still rarely studied, and need to be verified. In the present study, we aimed to investigate the effects of *Trigonella foenum-graecum* on airway inflammation of allergic asthma in Histamine-induced bronchospasm in guinea pigs, Restrained double chamber body plethysmography measurements, Compound 48/80-induced Mast cell degranulation.

## MATERIALS AND METHODS:

### Plant material:

The plant was collected from a local area of Chopda in Maharashtra state in the month of March and May. The plant was authenticated by Dr. S. R. Kshirsagar, Reader, P.G. Department of Botany, S.S.V.P.S Science College, Dhule- 425 405 (MS). The Seeds were dried under shade and then powdered. The powder was then extracted with 70% methanol by using Soxhlet apparatus.

### Phytochemical investigations:

The obtained methanolic extract was tested for various chemical constituents with the help of qualitative chemical tests.<sup>[12]</sup>

### Animals:

Adult Guinea pig (Dunkey-Hartley) of body weight 500-700 g and Mice (Swiss Albino) of body weight 25-30 gm mice of either sex obtained from Wokhart institute, Aurangabad was used for the experiment.

Rat (Albino Wistar) of body weight 200-250 g were obtained from in-house animal house facility of the institute. Standard conditions of temperature ( $22 \pm 2^\circ\text{C}$ ), relative humidity ( $60 \pm 5\%$ ) and light (12 hrs light/dark cycles) were maintained, standard feed and water were given *ad libitum*. The study was approved by the Institutional Animal Ethics Committee (IAEC) of R.C.P.I.P.E.R, Shirpur, India and according to CPCSEA guideline. Registration no: 651/02/c/CPCSEA.

### Histamine-induced bronchospasm in guinea pigs

Histamine-induced bronchospasm in guinea pigs was carried out by the reported method<sup>13-15</sup> Adult Guinea pigs of weight around 500-700 gm of either sex were divided into 5 groups (From I to V) Control group receives 1ml of saline solution while standard group receives Chlorpheniramine maleate 2 mg/kg and the remaining 3 groups receives 200mg/kg, 300mg/kg, and 400mg/kg respectively. Twenty-five guinea pigs then were exposed to 0.5 % histamine diphosphate H-7375 (Sigma chemicals 23297-93-0) with constant pressure 40mm/Hg in an aerosol chamber to induce experimental bronchial asthma. This PCT was considered as T<sub>1</sub> value. These animals were subjected to histamine challenge two and a half hr. after receiving the drug. The PCT was noted. This PCT was considered as T<sub>2</sub> value. Animals which withstood exposure to histamine aerosol for 15 min were considered to be completely protected. The protection offered by the treatment was calculated by the following formula.

$$\text{Percentage protection} = \left\{ 1 - \frac{T_1}{T_2} \right\} \times 100$$

Where; T<sub>1</sub> is a time in second for PCT before treatment T<sub>2</sub> is the time in second for PCT after treatment.

### Restrained double chamber body plethysmography measurements for histamine-induced bronchoconstriction in unanesthetized guinea pigs

Awaken guinea pigs were divided into different groups as shown in table no. 1 and individually placed in a body plethysmograph box (HSE type 855, Hugo Sachs Elektronik, Germany) and were exposed to histamine in a concentration of 0.5% solution for 10 seconds.<sup>16</sup> The histamine aerosol was generated by a jet nebulizer (PARI Jet-Nebulizer requires an operating pressure of approx. 1.5 bar., 100% of the particles are below 10  $\mu\text{m}$ ) are delivered to the head chamber of the plethysmograph and respiratory frequency and amplitude were recorded. Respiratory function in animals was evaluated by measurement of ventilatory parameters [respiratory rate (RR), tidal volume (VT) and minute ventilation, specific airway resistance measurement (RV)].

**Table 1:** Experimental design for histamine induced bronchospasm.

Groups (n)	Name of group	Drug received	Dose (p.o.)
Group I (5)	Control	Normal saline	1 mL
Group II (5)	Test drug	<b>TFME</b>	200 mg/kg
Group III (5)	Test drug	<b>TFME</b>	300mg/kg
Group IV (5)	Test drug	<b>TFME</b>	400mg/kg
Group V (5)	Standard drug (Std.)	Chlorpheniramine maleate	2 mg/kg

**Compound 48/80-induced Mast cell degranulation:**

Wistar rats of weight around 150-180 g of either sex were divided into six groups containing six animals in each. Normal control group receives only methylcellulose suspension 0.1ml while all remaining groups receive compound 48/80 (10 µg/ml) standard treated group receives Ketotifen (10 mg/ml) Test I, Test II and Test III receives TFME 200mg/kg, 300mg/kg and 400mg/kg respectively<sup>17, 18</sup>. The suspensions were further incubated for 10 min at 37°C. The peritoneal mast cells were stained with toluidine blue and examined microscopically for intact and disrupted mast cells. A minimum of 100 cells was counted for the number of intact and degranulated mast cells.

**Statistical analysis:**

The results of various studies were expressed as mean ± SEM and analyzed statistically using One-way ANOVA followed by *Dunnett's* multiple comparative post-test to find out the level of significance.  $P < 0.05$  was considered statistically significant. The analysis was performed using the GraphPad Prism software package (version 5.0).

**RESULTS****Phytochemical Screening:**

TFME showed the presence of amino acids, proteins, Carbohydrates, Alkaloids and saponins like steroids and triterpenoids while showed the absence of Tannins, Flavonoids.

**% yield:**

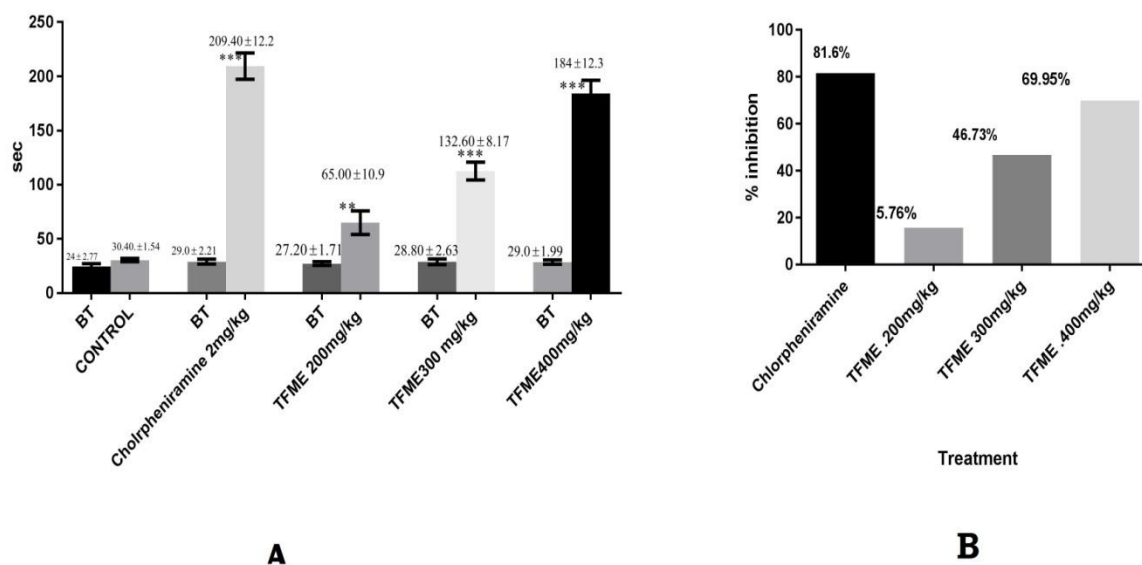
The yield of TFME was found to be 8.77% w/w.

**Acute toxicity study:**

**LD50** was reported<sup>20</sup> Acute Oral Toxicity > 5gm/kg Acute Dermal Toxicity > 2gm/kg

**Bronchial hyperactivity:****Histamine-induced bronchospasm in guinea pigs**

Preconvulsion time is directly proportional to bronchodilator and antihistaminic activity. The dyspnea was evoked within  $30.40 \pm 1.54$  seconds in the control group on exposure of histamine aerosol. In case of standard (Chlorpheniramine), it was  $209.40 \pm 12.20$  seconds. While after the treatment with *T. foenum graecum* at a dose of 200, 300, 400 mg/kg, p.o., of extract PCT was prolonged up to  $65.00 \pm 10.9$ ,  $132.60 \pm 8.17$ ,  $184 \pm 12.3$  seconds under same conditions. The result indicates that *T. foenum graecum* at 200, 300 and 400 mg/kg showed significant protection when compared with chlorpheniramine maleate.

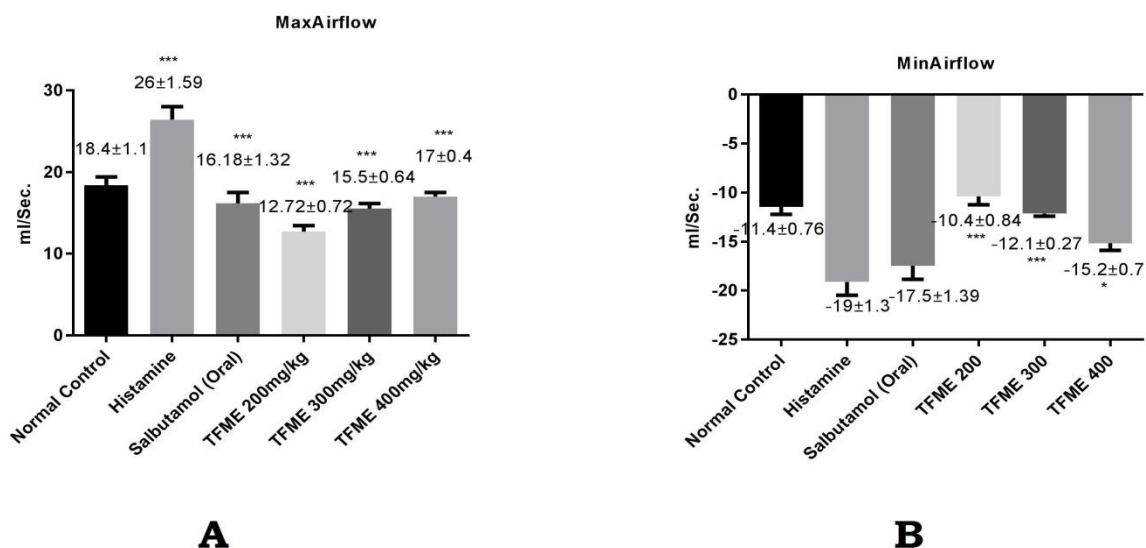


**Figure 1:** A. Effect of methanolic extract of *T. foenum graecum* on histamine aerosol-induced bronchospasm B. Effect of methanolic extract of *T. foenum graecum* Protection against histamine-induced bronchospasm Value represents, Mean ± S.E.M. (n=5) ANOVA: *Dunnett's* Multiple Comparative test \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  as Compared with Control.

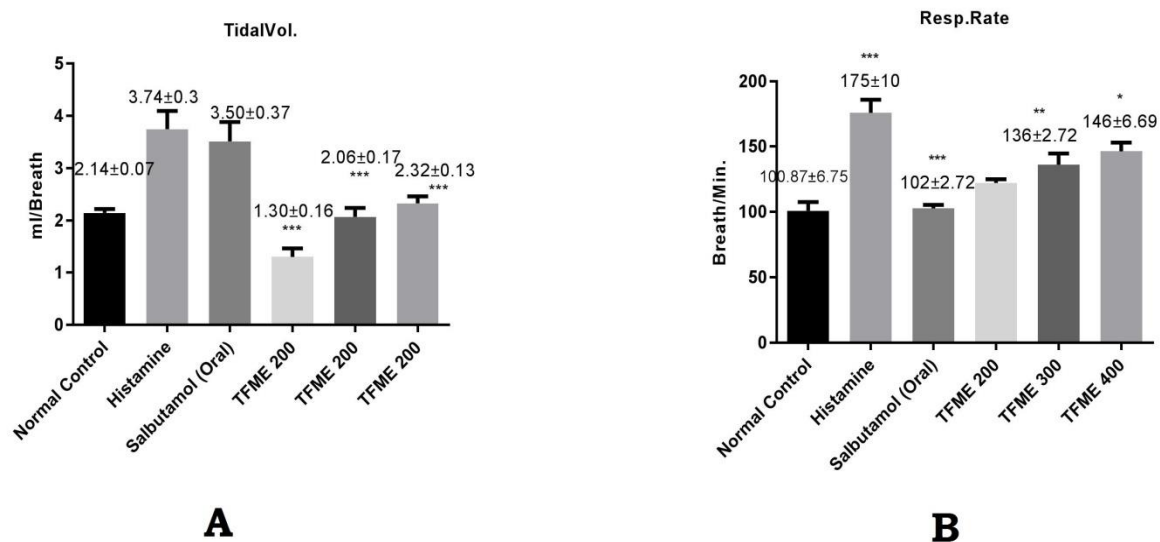
**Restrained double chamber whole body plethysmography measurements for histamine-induced bronchoconstriction in unanesthetized guinea pigs:-**

Histamine inhalation in double chamber whole body plethysmography shows decreased in a respiratory amplitude (diminished respiratory volume due to

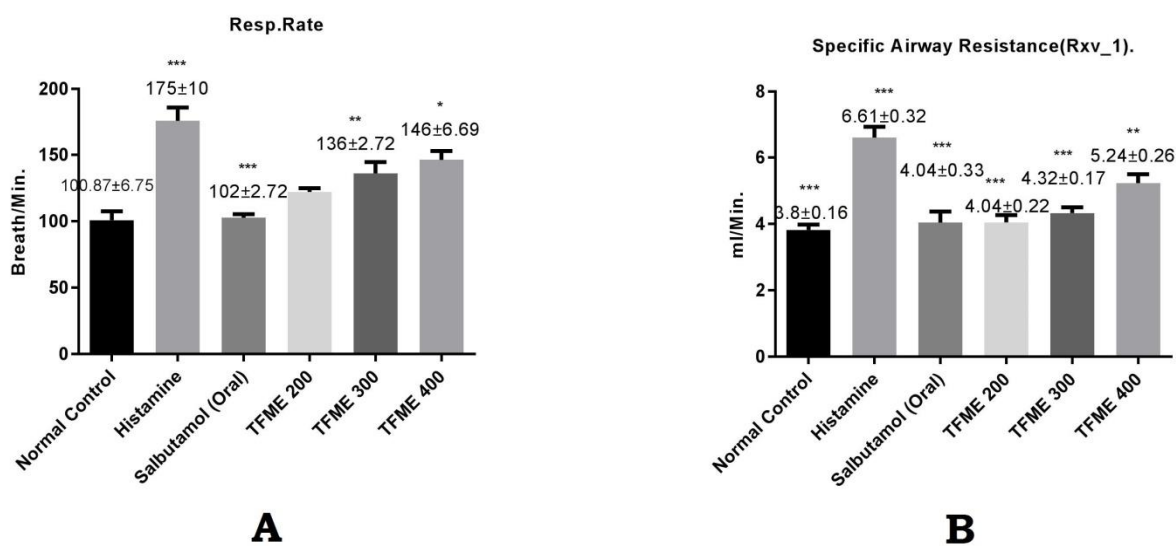
bronchoconstriction) and the reflexory increase of respiratory frequency, this seems to be attenuated by *T. foenum graecum* at the concentration of 200, 300 and 400 mg/kg showed elevations in Respiratory function by measurement of ventilatory parameters [respiratory rate (RR), tidal volume (VT) and minute ventilation, specific airway resistance measurement (RV)].



**Figure 2:** Effect of methanolic extract of *T.foenum graecum* on (A) Maximum airflow (B) Minimum Airflow.



**Figure 3:** Effect of methanolic extract of *T.foenum graecum* on (A) Tidal Volume (B) Respiratory Rate.



**Figure 4:** Effect of methanolic extract of *T.foenum graecum* on (A) Minute volume (B) Specific Airway Resistance (Rsv\_1). Values are expressed as mean ± SEM (n=6), One way ANOVA: Dunnett's Multiple Comparative Test. #p<0.01 when compared with normal control group, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 when compared with Histamine.

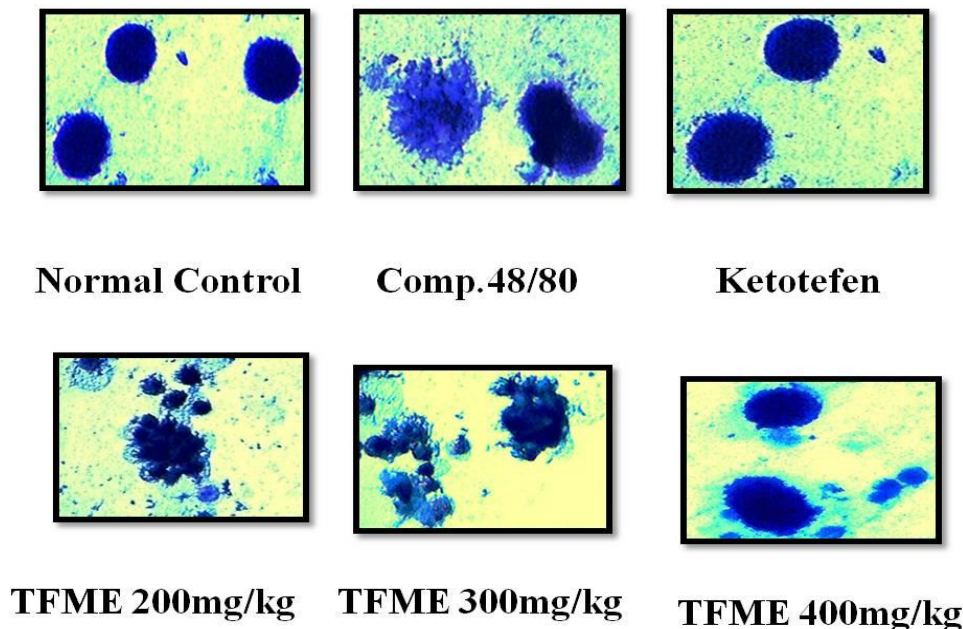


**Evaluation of Mast Cell Stabilization Activity:-**

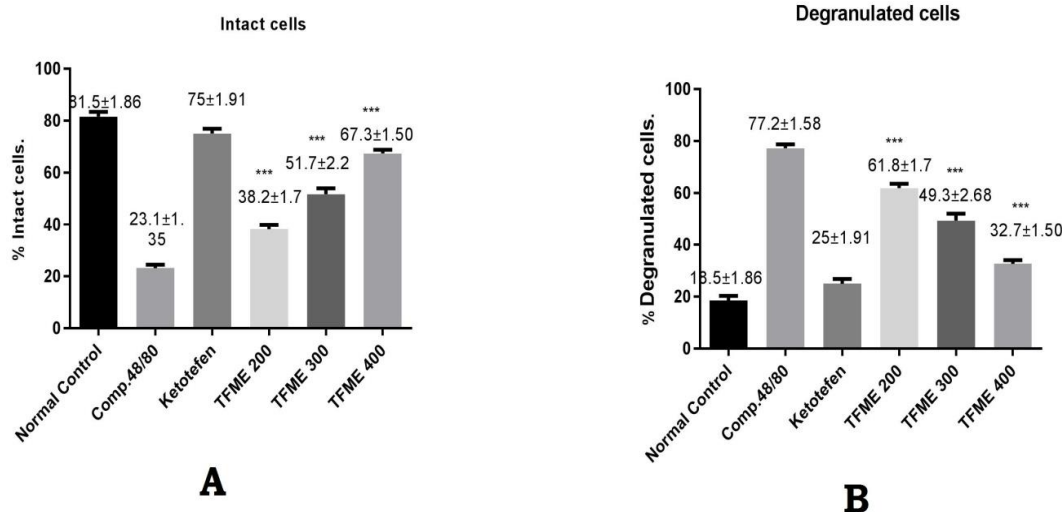
**Compound 48:80-induced Mast cell degranulation:**

Compound 48/80 (10 µg/ml) produced about 77.2% degranulation of mast cells as compared to normal control group. Groups treated with standard drug Ketotifen (0.1 mg/ml) showed 75±1.91 (p<0.01) intact mast cells whereas *T. foenum graecum* showed significant (p<0.01) and dose-

dependent protection against the mast cell degranulation compared to Compound 48/80 group. *T. foenum graecum* at the concentration of 200, 300 and 400 mg/kg showed 38.2±1.7, 51.7±2.2 and 67.3±1.50 % intact mast cells respectively. Standard reference Ketotifen (0.1 mg/ml) showed comparative mast cell stabilization with *T. foenum graecum*.



**Figure 5:** Images of granulated & de-granulated mast cells.



**Figure 6:** Effect of methanolic extract of *T.foenum graecum* on compound 48/80 induced Mast cell degranulation: (A) Intact Cells (B) Degranulated cells Values are expressed as mean ± SEM (n=6), One Way ANOVA: Dunnett’s Multiple Comparative Test. #p<0.01 when compared with normal control group, \*p< 0.05, \*\*p< 0.01, \*\*\*p<0.001 when compared with Sensitized Control.

**DISCUSSION**

The bronchoconstriction appeared in the early stage of asthma is caused due to the release of inflammatory mediators like histamine, leukotrienes, prostaglandins, tryptase, and acetylcholine.<sup>15</sup> *T. foenum graecum* seems to be a promising plant for treatment of bronchial asthma because the plant is a rich source of different phytoconstituents with a variety of potential biological activities and for bronchodilation activity. *T. foenum graecum* proved Anti-inflammatory activity. The plant contains steroidal Saponins

which inhibits the release of several mediators of the phlogistic agents such as prostaglandins, histamine, serotonin, and bradykinin by inhibiting the biosynthetic pathways of inflammatory mediators. Evidence strongly suggests that steroidal Saponins present in the extracts obtained from *T. foenum graecum* may interfere with lipoxygenase and/or cyclo-oxygenase pathway, Histamine is an important mediator of immediate allergic and inflammatory reaction and it induce bronchoconstriction, it acts through H1, receptors through the IP3-DAG pathway,

releases Ca<sup>2+</sup> from intracellular stores and protein kinase-C activation.<sup>21</sup> In the present study histamine, 0.5% solution acts as spasmogens in the form of aerosols for 10 Sec. to cause significant bronchoconstriction in and it results into an overall reduction in airway due to bronchoconstriction in guinea pigs. In the reference standard group, we used the standard drug Chlorpheniramine maleate and Salbutamol against histamine-induced bronchospasm. The crude methanolic extract at a dose of 200mg/kg, 300mg/kg, and 400mg/kg orally 30 min. before the histamine challenge has shown significant bronchoprotection against spasmogens as compared to control the significant effect produced may be due to the inhibition of IP3-DAG pathway or inhibition of releases of Ca<sup>2+</sup> from intracellular stores or inhibition of protein kinase-C activation. The bronchodilatory effect of *T.foenum graecum* was found comparable to the protection offered by the standard drug Chlorpheniramine maleate and salbutamol.

Asthma is an inflammatory disease of the lungs characterized by increased infiltration of leukocytes into the airways and reduced respiratory function. The inflammation leads to bronchoconstriction, increased airway hyperreactivity, and mucus production<sup>22</sup> after antigen challenge, inflammatory leukocytes produce various chemical mediators which are thought to be involved in both the immediate and the late asthmatic responses. During the LAR infiltration of eosinophils, neutrophils, lymphocytes, monocytes into the airways have been identified in BALF from the asthmatics<sup>23</sup> Mast cells, which are constituents of virtually all organs and tissue, are important mediators of inflammatory responses such as allergy and anaphylaxis. Mast cell degranulation can be elicited non-immunologically by the most potent synthetic secretagogue compound 48/80, which is a condensation product of N-methoxyphenylamine with formaldehyde and a high dose of which induces almost a 90% release of histamine from mast cells. To study the mechanism of anaphylaxis Compound 48/80 has been used [17, 18] it increases the permeability of the lipid bilayer membrane of the cell by causing a perturbation in the membrane and initiates the activation of a signal transduction pathway, which leads to degranulation and histamine release<sup>17</sup> The intracellular calcium (Ca<sup>2+</sup>) pathways are critical to the degranulation of mast cells. *T. foenum graecum* attenuated rate of mast cell degranulation dose-dependently as observed microscopically by toluidine blue staining.

## CONCLUSION

Thus, it can be concluded from the results obtained in the present pharmacological investigation that *T. foenum graecum* possess: Anti-asthmatic activity, Anti-histaminic activity Anti-degranulating activity Anti-allergic activity this may be attributed to Mast cell stabilization, inhibiting the release of histamine from the mast cells. Further study is needed to identify, isolate, purify and evaluate active constituent present in the *T. foenum graecum* for its anti-asthmatic activity.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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