

Author's Accepted Manuscript

Anti-allergy and anti-tussive activity of *Clitoria ternatea* L. in experimental animals

Niraj Kumar Singh, Debapriya Garabadu, Priyanka Sharma, Sushant Kumar Shrivastava, Pradeep Mishra



PII: S0378-8741(17)34077-1
DOI: <https://doi.org/10.1016/j.jep.2018.05.026>
Reference: JEP11372

To appear in: *Journal of Ethnopharmacology*

Received date: 10 November 2017

Revised date: 17 April 2018

Accepted date: 18 May 2018

Cite this article as: Niraj Kumar Singh, Debapriya Garabadu, Priyanka Sharma, Sushant Kumar Shrivastava and Pradeep Mishra, Anti-allergy and anti-tussive activity of *Clitoria ternatea* L. in experimental animals, *Journal of Ethnopharmacology*, <https://doi.org/10.1016/j.jep.2018.05.026>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting galley proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Anti-allergy and anti-tussive activity of *Clitoria ternatea* L. in experimental animals

Niraj Kumar Singh¹, Debapriya Garabadu^{1*}, Priyanka Sharma², Sushant Kumar Shrivastava² and Pradeep Mishra³

¹Division of Pharmacology and ³Division of Pharmaceutical Chemistry, Institute of Pharmaceutical Research, GLA University, Mathura-281 406, Uttar Pradesh, India

²Department of Pharmaceutical Engineering and Technology, Indian Institute of Technology (Banaras Hindu University), Varanasi-221 005, Uttar Pradesh, India

***Corresponding author:** Dr. Debapriya Garabadu, Associate Professor Division of Pharmacology, Institute of Pharmaceutical Research, GLA University, Mathura, 281406, India. Tel.: +91-8853139229; Fax: +91-566-2242687

E-mail: debapriya.garabadu@gla.ac.in;
debapriya.2007@gmail.com

Abstract**Ethnopharmacological relevance**

Clitoria ternatea flower is traditionally used in the treatment of respiratory disorders including bronchitis and is one of the ingredients in different Ayurvedic preparations that are used in respiratory disorders. However, till date there is no scientific report on the anti-asthmatic activity of this flower.

Aim of the study

Ethanollic extract of *Clitoria ternatea* flowers (ECT) was evaluated for its anti-allergy and anti-tussive potential in experimental animals. Additionally, the anti-inflammatory potential of ECT was carried out to draw a plausible mechanism of action of the drug.

Materials and Methods

In-vitro anti-asthmatic activity of ECT was evaluated in goat tracheal chain and isolated guinea pig ileum preparations. Acute and chronic anti-asthmatic activity of ECT (100, 200 and 400 mg/kg; p.o.) was estimated in histamine aerosol exposed guinea pigs and in OVA sensitized and challenged mice respectively. Anti-tussive activity of ECT (100, 200 and 400 mg/kg; p.o.) was evaluated against sulfur dioxide- and citric acid-induced cough in experimental animals. Moreover, the anti-inflammatory activity of ECT (100, 200 and 400 mg/kg; p.o.) was evaluated against carrageenan- and acetic acid-induced inflammation in rats.

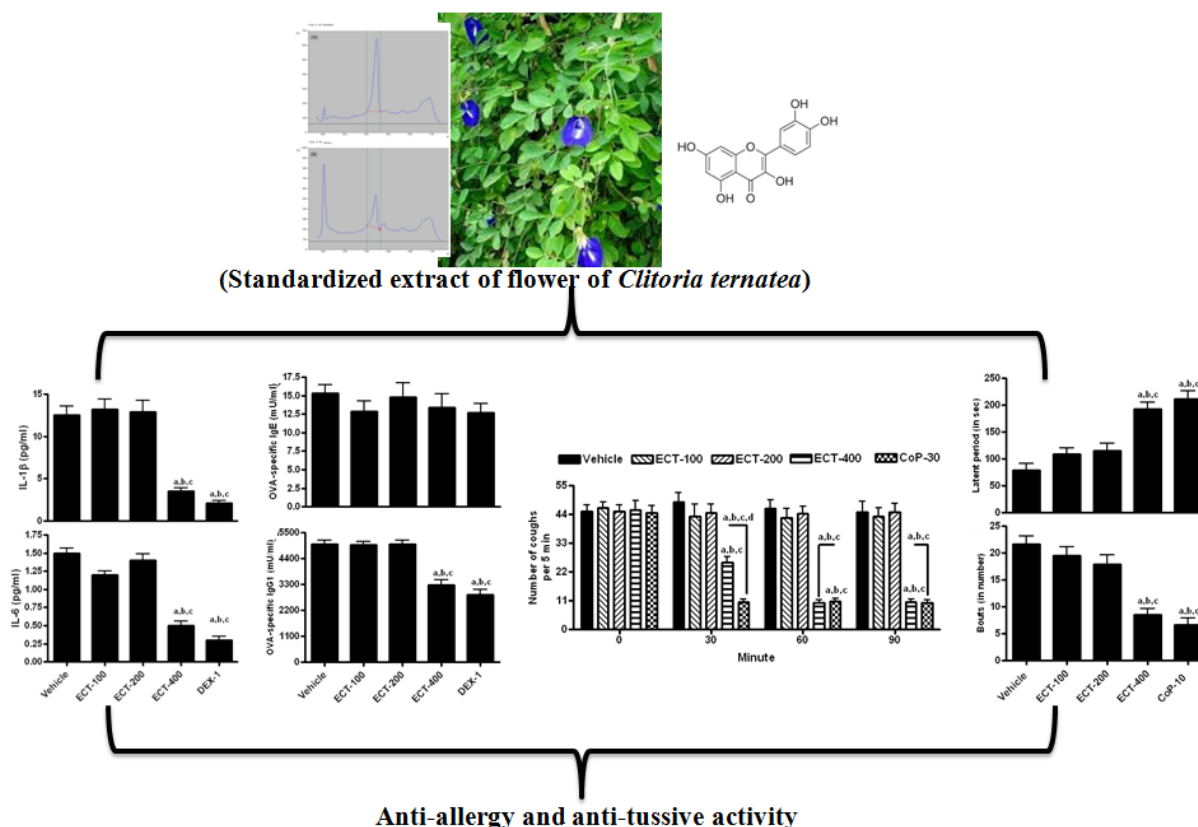
Results

: ECT attenuated histamine-induced contraction in both goat tracheal chain and isolated guinea pig ileum preparations. ECT (400 mg/kg) attenuated histamine-induced dyspnoea and OVA-induced changes in differential cell count in bronchoalveolar fluid, levels of interleukins (IL-1 β and IL-6) and immunoglobulin (OVA-sensitive IgG1) in animals. ECT (400 mg/kg) further ameliorated sulfur dioxide- and citric acid-induced cough in experimental animals. Additionally, ECT (400 mg/kg) attenuated inflammation in carrageenan and acetic acid challenged rodents.

Conclusions

Standardized ECT could be considered as a potential therapeutic alternative in the management of allergy-induced asthma.

Graphical abstract



Abbreviations:

ANOVA, Analysis of variance ; BALF, Bronchoalveolar Lavage Fluid; ECT , Ethanolic Extract of *Clitoria ternatea* Flowers; CMC , Carboxy Methyl Cellulose; CF , Charles-Foster; CP , Chlorpheniramine Maleate; CPCSEA , Committee for the Purpose of Control and Supervision of Experiments on Animals ; CT , *Clitoria ternatea*; DEX, Dexamethasone; HPTL, High Performance Thin Layer Chromatography; IND , Indomethacine; IL , Interleukins; Ig , Immunoglobulin; OECD, Organization for Environmental Control Development ; OVA , Ovalbumin; PB , Peribronchial; PV, Perivascular; PBS, Phosphate Buffer Saline; PCT , Preconvulsive Time; UV, Ultra Violet

Keywords: *Clitoria ternatea*; Allergy; Asthma; Interleukin; Immunoglobulin.

1. Introduction

Asthma is considered as a chronic inflammatory disorder of respiratory system that is highly prevalent in world population (Masoli et al., 2004; Bousquet et al., 2005). There are several predisposing factors including allergens play a significant role in the pathogenesis of asthma (Custovic et al., 2010). Epidemiological, clinical and experimental studies suggest that allergenic triggers such as indoor (house dust mites, molds, pets, cockroaches, and rodents) and outdoor (pollens and molds) allergens aggravate the severity of asthma (Bloemen et al., 2007). Thus, allergy gains critical attention in pathophysiology of asthma.

Allergen-induced asthma is characterized by reversible airway obstruction, airway hyperresponsiveness, mucus hypersecretion and airway remodeling (Agrawal and Shao, 2010). Mucus plugging and epithelium denudation is considered as one of the important pathological characteristics in allergen-sensitive asthma (Wenzel et al., 1999; Dweik et al., 2010). Mast cells predominantly localized in mucosal tissue and its activation by allergens produce a plethora of soluble mast cell-derived mediators such as histamine and cytokines including interleukin (IL)-1 and IL-6 (Weller et al., 2011; Chung et al., 2014; Carr et al., 2016; Piliponsky et al., 2002; Piliponsky et al., 2003). These cytokines initiate infiltration of several types of cells including eosinophils, neutrophils, lymphocytes and phagocytes into the respiratory airways and thus initiates inflammation. It has also been postulated that eosinophil degranulation can damage epithelial cells, modulate the function of smooth muscles of the airways and at molecular level it increases the level of IgE in respiratory airways (Bradley et al., 1991; Kritas et al., 2014; Jatakanon et al., 1999; Gibson et al., 2001; Baines et al., 2010; Wilson et al., 2013; Miranda et al., 2004; Bittar et al., 2015). It has also been suggested that

under allergic conditions, allergens induce the aggregation of the high-affinity IgE receptor which is expressed on mast cell surface (Chung et al., 2014; Carr et al., 2016). Moreover, it is well established that allergens such as pollen, dust mites, mold or animal dander can initiate an acute immune response (Agrawal and Shao, 2010). Thus, immunity and inflammation play a vicious cycle in the pathogenesis of allergen-sensitive asthma.

Pharmacotherapy of asthma includes anti-allergic drugs, bronchodilators, and corticosteroids (Cumming and Mitchell, 1997; Guilbert et al., 2006). These drugs are clinically limited due to their severe unwanted side effects such as hyperglycemia, hyperlipidemia and susceptibility to pathogens (Tan, 1991). Ayurvedic medicines, the Indian Traditional System of medicines, include several medicinal plants in its formulations for the treatment of asthma and allergic disorders. Further, experimental studies report minimal adverse effects of these herbal medicines. Thus, it can be assumed that the herbal drug therapy could be a potential alternative in the pharmacotherapy of allergen-induced asthma.

Clitoria ternatea (CT; family: Fabaceae) also known as 'Aparajita', is a perennial climber, strongly persistent, and sparsely pubescent legume herb mentioned in ancient system of medicine (Gomez and Kalamani, 2003). It is widely distributed throughout the tropical regions of Africa, Asia and Central America. Literature review suggests that CT possesses several bioactive properties including antioxidant (Kamkaen and Wilkinson, 2009), local anesthetic (Mukherjee et al., 2008), anthelmintic (Khadatkar et al., 2008), antipyretic and anti-inflammatory (Parimaladevi et al., 2003). Preclinical studies also suggest that the roots of CT possess anti-asthmatic and anti-histaminergic activity in experimental animal models (Taur and Patil, 2010; Taur and Patil, 2011). However, it is well established that harvesting roots for the purpose of medicinal use significantly disturbs the ecosystem than aerial parts of the plant (Alves and Ros, 2007). Moreover, CT flower is traditionally used in the treatment of respiratory disorders including bronchitis (Parrotta, 2001; Prajapati and Sharma, 2003; Khare,

2004). In addition, CT flower is one of the ingredients in different Ayurvedic preparations that are used in respiratory disorders (Patil and Patil, 2011). However, till date there is no scientific report on the anti-asthmatic activity of CT flower.

Hence, in the present study, anti-allergic activity of standardized ethanolic extract of flower of CT was investigated in histamine- and ovalbumin (OVA)-induced asthma. Further, anti-tussive and anti-inflammatory activities were evaluated in several experimental animal models.

2. Materials and methods

2.1 Chemicals and reagents

Quercetin and Ovalbumin (OVA; Grade V) was procured from Merck Pvt. Ltd. and Sigma-Aldrich, India respectively. All other chemicals and reagents of high-performance liquid chromatography and analytical grade were procured from Merck Pvt Ltd., New Delhi and Himedia Laboratories Pvt Ltd., Mumbai, India.

2.2 Plant material and extraction procedure

The flowers from *C. trenatea* were collected from medicinal garden of GLA University, Mathura, Uttar Pradesh, India in September 2011. A voucher specimen was identified and deposited in the Birbal Sahni Institute of Palaeobotany, Lucknow, India (Voucher specimen No.: 13387). The dried and powered plant material was extracted exhaustively using Soxhlet apparatus with ethanol 98% at 1:7 plant material/solvent (w/v) five consecutive times at room temperature, which was then sonicated in ultrasound (30 min/day). The solvent was removed under reduced pressure using rotary evaporator and lyophilized to yield dried ethanolic extract of flowers (ECT; 9%, w/w).

2.3 Phytochemical analysis of *C. trenatea* flower

2.3.1 Preliminary phytochemical screening and estimation of total phenolic and flavonoid content

The ECT was subjected to identification tests for flavonoids, alkaloids, tannins, cardiac glycosides anthraquinones, saponins and phenols using standard protocols (Ajikumaran et al., 2006).

2.3.2 Estimation of total phenolic content

Assaying method of Singleton and Rossi was adopted for estimation of total phenolics content in ECT using Folin-Ciocalteu reagent with slight modification. About 100 μ l of ECT (1 mg/ml) were prepared in a 100 ml volumetric flask and added 0.75 ml of Folin-Ciocalteu reagent (previously diluted in 1:10 ratio with deionised water) was thoroughly mixed and allowed to incubate for 5-8 min at room temperature (25 ± 1 °C) condition. Thereafter, 0.75 ml of Na_2CO_3 solution (60 g/L) was added to mixture and allowed it to stand at room temperature (25 ± 1 °C) for 2 h intervals. Absorbance was measured against blank using Double-Beam UV-Vis Spectrophotometer (Simadzu-1800) at λ_{max} 725 nm respectively. Gallic acid was used as a standard to construct calibration curve (0.02-0.1 mg ml⁻¹) and readings taken in triplicate to get accurate results. The total phenolic content was expressed as milligrams of gallic acid equivalent/ g extract.

2.3.3 Estimation of total flavonoid content

Total flavonoid content was determined by the aluminium trichloride (AlCl_3) colorimetric assay procedure of Zhishen et al. (1999). Briefly, 1.5 ml of 2% AlCl_3 was added to 1.5 ml ECT solution (500 ppm in 80% MeOH). After incubation of 1 hour at room temperature, the absorbance was estimated against blank using Double-Beam UV-Vis Spectrophotometer at 420 nm against a blank. Quercetin was used as a standard to plot the calibration curve. The amount of total flavonoid was expressed as quercetin equivalents (mg quercetin/g extract). All the samples and the standards were analyzed in triplicate.

2.3.4 Standardization of ECT to quercetin by HPTLC method

The ECT was standardized to quercetin using high performance thin layer chromatography (HPTLC) method. Mobile phase for developing the chromatogram was composed of chloroform:methanol and formic acid mixture in the ratio 7.5:1.5:1 (v/v/v). The concentration of quercetin was taken as 0.5 mg/mL, while that of the ECT was taken as 5 mg/mL in methanol. The study was carried out using Camag-HPTLC instrumentation (Camag, Muttenz, Switzerland) equipped with Linomat V sample applicator, Camag TLC scanner 3, Camag TLC visualizer, and WINCATS 4 software for data interpretation. The R_f values were recorded and the developed plate was screened and photo documented at ultra violet range with wavelength ($\lambda_{\max}$) of 254 nm.

2.4 Animals

Inbred adult male Guinea Pig (450-500 g), Charles-Foster (CF) albino rats (150-200 g) and albino mice (25-30 g) were obtained from Central Animal House, Institute of Pharmaceutical Research, GLA University, Mathura and were used in the study. The animals were grouped and housed in Poly-acrylic cages lined with husk under standard condition (24 ± 2 °C temperature, 45-55% relative humidity and 12 h light: 12 h dark cycle) respectively. Animals were allowed to freely feed their standard pellet diet (Lipton India, Ltd., Mumbai) and water *ad libitum*. Fasted animals were deprived with their food for 16-18 h before experimentations but allowed to water *ad libitum*. All the experimental procedures utilized were performed in accordance with the approval of the Institutional Animal Ethics Committee (1260/ac/09/CPCSEA) under strict compliance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for the experimental studies.

2.5 Acute toxicity study

An acute oral toxicity study was performed according to “Organization for Environmental Control Development” guidelines (OECD: Guidelines 420; Fixed Dose Method) for oral administration of ECT (suspended in 0.5% carboxy methyl cellulose; CMC). Albino male

rats (N = 6, 150-200 g), overnight fasted for 18 h were used for the experiment. The ECT was administered in increasing dose of 5, 50, 300 and maximum dose up to 2000 mg/kg to the experimental animals.

2.6 Evaluation of in-vitro anti-asthmatic activity

2.6.1 Isolated goat tracheal chain preparation

Isolated adult goat tracheal tissue was obtained from slaughter house. Trachea was cut into individual rings and tied together in series to form a chain. Trachea was suspended in bath of Kreb's solution and was continuously aerated at 37 ± 0.5 °C. Dose response curves of histamine in plain Kreb's solution and in Kreb's solution containing ECT (10 mg/ml) were performed (Chaudhari and Lahiri, 1974). Percent of maximum contractile responses were plotted to record dose response curves of histamine in the absence and presence of ECT.

2.6.2 Isolated guinea pig ileum preparation

The guinea pigs (overnight fasted) were sacrificed and the ileum was mounted in an organ bath containing Tyrode solution, which was continuously aerated at 37 ± 0.5 °C. Dose response curve of histamine in plain Tyrode solution and in Tyrode solution containing ECT (10 mg/ml) were performed (Vogel and Vogel, 1998). Percentage maximum contractile response was plotted to generate dose response curve of histamine, in the absence and presence of the plant extract.

2.7 Evaluation of in-vivo anti-asthmatic activity

2.7.1 Estimation of acute effect of ECT (100, 200 and 400 mg/kg) in guinea pigs exposed to histamine aerosol

The guinea pigs were randomly allocated to five groups of six each and namely: Vehicle, ECT-100, ECT-200, ECT-400 and CP-2. Each animal was exposed to 0.2% histamine aerosol, of particle size 5 μ m through a compressed nebulizer (Rolex Pvt. Ltd., India) at a

flow rate of 0.4 mL/min, in a glass histamine chamber (60 × 36 × 60 cm). The preconvulsive time (PCT) was determined from the time of exposure to onset of dyspnoea leading to the appearance of preconvulsive dyspnoea (PCT₁). Thereafter, the animals were kept in their respective cages with fresh airs. The vehicle group received CMC (0.5 %) while rest of the groups administered with ECT (100, 200 and 400 mg/kg) and CP (2 mg/kg) respectively after 30 min to first exposure of histamine aerosol. After 1 hr to drug or vehicle administration, the second exposure of histamine aerosol was made following the earlier procedure and the onset of dyspnoea (PCT₂) was measured for each animal. The mean exposition time was calculated as the difference between PCT₂ and PCT₁. Further, the percentage of protection was calculated using the formula = $[(1 - (PCT_1/PCT_2)) \times 100]$.

2.7.2 Estimation of chronic effect of ECT (100, 200 and 400 mg/kg) in OVA sensitized and challenged mice

The asthmatic model was established by sensitization to OVA. Specifically, all of the mice in the Vehicle, ECT-100, ECT-200, ECT-400 and DEX-1 groups were sensitized on days 0, 7, and 14 by intraperitoneal injection of 20 µg OVA emulsified in 2 mg aluminum hydroxide gel (Invivo-Gen, San Diego, CA, USA) in a total volume of 200 µL. These sensitized mice were exposed to aerosolized 5% OVA in sterile saline for 7 days beginning on the 15th day of the experiment at a time gap of 24 hr for 30 min at each time. The mice were placed in a glass chambers (51 × 31 × 21 cm) that were connected to a jet nebulizer (Rolex Pvt Ltd., India) to create a whole-body inhalation system. ECT (100, 200 and 400 mg/kg), DEX (1 mg/kg), and Vehicle (0.5% CMC) were administered orally 30 min before each OVA challenge. The mice were sacrificed at 24 h after the last challenge, and bronchoalveolar lavage fluid (BALF) and lung tissues were collected for analysis.

2.7.2.1 BALF Collection and Differential Cell Counts

Briefly, the mice were anesthetized by intraperitoneal injection of pentobarbital sodium (70 mg/kg) at 24 h after the final challenge. BALF was collected by lavage with ice-cold phosphate buffer saline (PBS, 400 μ L $\times$ 3; 85–90% of the lavage volume was recovered) via a tracheal catheter. The lavage samples from each mouse were centrifuged at 1000 rpm for 10 min at 4 °C. The total number of inflammatory cells in the BALF was counted using a hemocytometer. Differential cell counts were performed using Wright's staining on the basis of morphological criteria. The number of cells in the BALF was determined by two independent investigators in a single-blind study, and at least 200 cells each were analyzed from three different random locations using a microscope. Further, the cytokine proteins such as IL-1 β (ab100704) and IL-6 (ab100713) were quantified in BALF using ELISA test kit (Abcam Pvt. Ltd., India).

2.7.2.2 Quantitative estimation of antigen-specific IgE and IgG1 antibodies

OVA-specific IgE (ab157718) and IgG1 (ab133045) antibodies were measured in serum of the mice using ELISA test kit (Abcam Pvt. Ltd., India).

2.7.2.3 Lung histology

After BALF samples were collected, a 20 mL syringe equipped with an 18G needle was used to inject 10–15mL PBS slowly into the right ventricle. Then the lungs were inflated with 4% paraformaldehyde under 20 cm pressures by a tracheal catheter and placed in 4% paraformaldehyde fixative for paraffin embedding. A series of microsections (5 μ m) were cut with a microtome and stained with hematoxylin and eosin (H&E) to assess inflammatory cell infiltration. The inflammation score was determined as follows: grade 0: no inflammation; grade 1: occasional cuffing with inflammatory cells; and grades 2, 3, and 4: most bronchi or vessels which were surrounded by a thin layer (1-2 cells: grade 2), a moderate layer (3–5 cells: grade 3), or a thick layer (>5 cells: grade 4) of inflammatory cells,

respectively. The total inflammation score was calculated by the addition of the peribronchial (PB) and perivascular (PV) inflammation scores.

2.8 Evaluation of anti-tussive activity

2.8.1 Sulfur dioxide (SO₂)-induced cough

Healthy mice were divided into several groups and drug treatment was continued for 7 days. NaHSO₃ solution in water (500 mg/kg, 2.0 ml) was placed at the base of a specially designed desiccator and 0.2 ml of sulfuric acid was added using a pipette which resulted in the formation of sulfur dioxide. After 15 s, each mouse was placed on the stage in the desiccator and exposed to SO₂ for 45 s. The mice were then removed and placed in a clear glass chamber for counting of bouts of cough for 5 min (Gupta et al., 2009).

2.8.2 Citric acid-induced cough

Antitussive assay was carried out as per earlier report (Chung et al., 2003). Unrestrained conscious guinea pigs were individually placed in a transparent 20 cm × 10 cm × 10 cm airtight Plexiglass chamber and exposed to 17.5% (w/v) citric acid aerosol produced by an ultrasonic nebulizer for 1 min with a flow rate of 0.6 mL/min, the aerosol particles had a mass median aerodynamic diameter of 0.9 µm. During the 10 min observation period, the guinea pigs were continuously observed, and the latent period of cough and numbers of coughs were simultaneously noted.

2.9 Evaluation of anti-inflammatory activity

2.9.1 Anti-inflammatory activity in carrageenan-induced paw oedema test

Overnight fasted animals were divided into five groups (n = 6). 0.1 ml of 1% carrageenan solution was injected into the sub plantar region of the right hind paw of the rats, 1 h after the administration of vehicle, Indomethacine (IND; 10 mg/kg, p.o.) and ECT (100, 200 and 400 mg/kg). The paw volume was measured with a micrometer screw gauge (SMC-20326, Sterling Manufacturing Company, Ambala Cantt, India) at 1, 2, 3, 4 and 6 h after

administration of either standard or test drug. The difference between the left and right hind paw volumes (indicating the degree of inflammation) was determined in comparison to the control animals (Moody et al., 2006).

2.9.2 Acetic acid-induced vascular permeability

This test was carried out by the method described by Whittle (1964) with a slight modification. 5 groups of mice ($n = 6$) were used for the study. Group I served as vehicle control, groups II, III, and IV were treated with extracts 100, 200, and 400 mg/kg, respectively, whereas group V received indomethacin 10 mg/kg orally. One hour after the treatment, mice were injected with 1% acetic acid solution (10 mL/kg) in saline intraperitoneally. Just after 2% Evans blue solution (10 mL/kg) in normal saline was injected intravenously through tail vein. 30 min later, mice were sacrificed by cervical dislocation and the peritoneal cavity was washed with normal saline (5 mL). The washings were collected and made volume up to 10 mL into heparinized test tubes and subsequently centrifuged at 2,000 rpm for 10 min. The dye content in the supernatant was measured spectrophotometrically (Shimadzu 1800, Japan) at a wavelength of 610 nm. The vascular permeability effects were expressed as the total amount of dye leaked into the intraperitoneal cavity based on their absorbance.

2.9.3 Cotton-pellet induced granuloma in rats

Sterile cotton-pellets (10 mg each) were implanted subcutaneously in the back axilla regions of each rat under pentobarbital sodium (50 mg/kg, i.p.) anaesthesia. The animals in the two test groups were treated orally with ECT extract at 200 or 400 mg/kg daily for 7 consecutive days; while the control group received either normal saline (vehicle control) or IND (10 mg/kg body weight). Scarification was made after 8th day and the cotton-pellets were removed, freed from extraneous tissues and the dry weight of each pellet was recorded. The

percentage of inhibition with respect to the dry weight of the cotton-pellet was calculated and compared with the control groups (Debprasad et al., 2012).

2.10 Statistical analysis

The results were expressed as Mean $\pm$ standard error of mean (S.E.M.). Statistical analysis was performed using Graph Pad Prism 4.2[®] software. The difference between groups was assessed by analysis of variance (ANOVA), followed, when necessary, by the suitable post-hoc test. The significance level for rejection of the null hypothesis was always $\geq 5\%$.

3 Results

3.1 Phytochemical analysis of *C. trenatea* flower

Preliminary phytochemical screening suggested the presence of flavonoids, alkaloids, tannins, cardiac glycosides anthraquinones, saponins and phenols in ECT. Further, the spectrophotometric determination revealed that the total phenolic and flavonoid content was found to be 223.4 ± 10.5 (mg gallic acid equivalent/g extract) and 25.8 ± 2.1 (mg quercetin/g extract). Further, the HPTLC analysis suggested the presence of quercetin (4.3%, Fig-1).

3.2 Selection of dose of standardized ECT

The acute oral toxicity study revealed that the ECT in increasing dose of 5, 50, 300 and maximum dose up to 2000 mg/kg was found tolerable in the experimental animals. Low dose: 100 mg/kg (half of one-tenth of the maximum lethal dose), Median dose: 200 mg/kg (one-tenth of the maximum lethal dose) and High dose: 400 mg/kg (twice that of one-tenth dose) were selected for evaluation of pharmacological activities. Moreover, the rats did not show any gross behavioral, neurological or autonomic toxic effects after first 3 h of ECT administration and for any lethality after 24 to 72 h till 14 days.

3.3 Effect of standardized ECT on isolated goat tracheal chain and guinea pig ileum preparation

Fig-2 illustrates the effect of ECT and vehicle on histamine-induced contraction in isolated goat tracheal chain (A) and guinea pig ileum (B). Two-way ANOVA revealed that there were significant differences in histamine-induced isolated goat tracheal chain and guinea pig ileum contraction among treatments ($[F(1, 20) = 240.7, p < 0.05]$ and $[F(1, 20) = 134.8, p < 0.05]$ respectively) and concentrations ($[F(4, 20) = 49.4, p < 0.05]$ and $[F(4, 20) = 75.9, p < 0.05]$ respectively). Further, there was a significant interaction between treatment and concentration in histamine-induced isolated goat tracheal chain $[F(4, 20) = 7.0, p < 0.05]$ and guinea pig ileum $[F(4, 20) = 3.1, p < 0.05]$ contraction. Post-hoc test showed that ECT significantly decreased the histamine-induced contraction in both isolated goat tracheal chain and guinea pig ileum at all concentrations of histamine.

3.4 Effect of ECT (100, 200 and 400 mg/kg) on histamine-induced onset of dyspnoea in guinea pigs

The effect of ECT (100, 200 and 400 mg/kg) on histamine-induced onset of dyspnoea in guinea pigs is depicted on Table-1. One-way ANOVA revealed that there were significant differences in mean exposition time among groups $[F(4, 25) = 142.0; p < 0.05]$. Post-hoc test showed that ECT (100 and 200 mg/kg) did not cause any significant change in the mean exposition time compared to vehicle administered histamine treated guinea pigs. However, ECT (400 mg/kg) and standard drug significantly increased the mean exposition time in histamine treated guinea pigs compared to vehicle administered animals.

3.5 Effect of ECT (100, 200, 400 mg/kg) on OVA sensitized and challenged alterations in differential cell counts in BALF in mice

Table-2 illustrates the effect of ECT (100, 200 and 400 mg/kg) on OVA sensitized and challenged alterations in total leukocytes, neutrophils, eosinophils, macrophages and lymphocytes in BALF in mice. One-way ANOVA revealed that there were significant differences in total leukocytes $[F(4, 25) = 25.4; p < 0.05]$, neutrophils $[F(4, 25) = 21.8;$

$p < 0.05$], eosinophiles [$F(4, 25) = 13.3$; $p < 0.05$], macrophages [$F(4, 25) = 25.1$; $p < 0.05$] and lymphocytes [$F(4, 25) = 17.3$; $p < 0.05$] in mice BALF among groups. Post-hoc test showed that ECT (100 and 200 mg/kg) did not cause any change in the levels of total differential counts of cells in OVA sensitized and challenged mice compared to vehicle treated animals. However, ECT at highest dose level and standard drug significantly mitigated the levels of total differential counts in cells in OVA sensitized and challenged mice compared to vehicle treated animals.

3.6 Effect of ECT (100, 200, 400 mg/kg) on OVA sensitized and challenged alterations in levels of IL-1 beta and IL-6 in BALF in mice

The effect of ECT (100, 200 and 400 mg/kg) on OVA sensitized and challenged alterations in the levels of IL-1 β (A) and IL-6 (B) in BALF in mice is depicted in Fig-3. One-way ANOVA revealed that there were significant differences in IL-1 β [$F(4, 25) = 31.6$; $p < 0.05$] and IL-6 [$F(4, 25) = 68.7$; $p < 0.05$] in mice BALF among groups. Post-hoc test showed that ECT (100 and 200 mg/kg) did not cause any change in the levels of IL-1 β and IL-6 in OVA sensitized and challenged mice compared to vehicle treated animals. On contrary, ECT (400 mg/kg) and DEX significantly reduced the levels of both cytokines in OVA sensitized and challenged mice compared to vehicle treated animals.

3.7 Effect of ECT (100, 200, 400 mg/kg) on OVA sensitized and challenged alterations in levels of antigen-specific IgE and IgG1 in BALF in mice

Fig-4 illustrates the effect of ECT (100, 200 and 400 mg/kg) on OVA sensitized and challenged alterations in the levels of IgE (A) and IgG1 (B) in BALF in mice. One-way ANOVA revealed that there were significant differences in IL-6 [$F(4, 25) = 31.2$; $p < 0.05$] in mice BALF among groups. However, there were no significant differences in the level of IgE [$F(4, 25) = 0.6$; $p < 0.05$] in mice BALF among groups. Post-hoc test showed that ECT (100

and 200 mg/kg) did not cause any change in the levels of IgG1 in OVA sensitized and challenged mice compared to vehicle treated animals. On contrary, ECT (400 mg/kg) and DEX significantly reduced the level of IgG1 in OVA sensitized and challenged mice compared to vehicle treated animals.

3.8 Effect of ECT (100, 200, 400 mg/kg) on OVA sensitized and challenged alterations in lung histology in mice

The effect of ECT (100, 200 and 400 mg/kg) on OVA sensitized and challenged alterations in the cytoarchitecture of lung tissue (Fig-5) and inflammation score (Fig-6) in mice. One-way ANOVA revealed that there were significant differences in PB [$F(4, 25) = 23.1$; $p < 0.05$], PV [$F(4, 25) = 49.3$; $p < 0.05$] and total inflammation [$F(4, 25) = 65.4$; $p < 0.05$] score in mice lung section among groups. Post-hoc test showed that ECT (100 and 200 mg/kg) did not cause any change in the score of PB, PV and total inflammation in OVA sensitized and challenged mice compared to vehicle treated animals. On contrary, ECT (400 mg/kg) and DEX significantly reduced the score of PB, PV and total inflammation in OVA sensitized and challenged mice compared to vehicle treated animals.

3.9 Effect of ECT (100, 200 and 400 mg/kg) on Sulphur Dioxide-induced cough in mice

Fig-7 illustrates the effect of ECT (100, 200, 400 mg/kg) on sulphur dioxide-induced cough in mice. Repeated measures of two-way ANOVA revealed that there were significant differences in number of coughs per 5 min among treatment [$F(4, 100) = 87.8$; $p < 0.05$] and time [$F(3, 100) = 30.4$; $p < 0.05$]. Further, there was a significant interaction between treatment and time [$F(12, 100) = 10.7$; $p < 0.05$]. Post-hoc test showed that ECT (100 and 200 mg/kg) did not cause any significant change in number of coughs at any time point in mice compared to vehicle treated animals. However, ECT (400 mg/kg) and standard drug (codeine phosphate

30 mg/kg) significantly decreased the number of coughs in mice compared to vehicle treated animals at 30 min and this effect was persisted up to 90 min.

3.10 Effect of ECT (100, 200 and 400 mg/kg) on citric acid evoked cough in guinea pigs

Effect of ECT (100, 200 and 400 mg/kg) on latent period to cough (A) and number of bouts (B) in citric acid exposed guinea pigs are illustrated in Fig-8. One-way ANOVA revealed that there were significant differences in latent period to cough [$F(4, 25) = 18.8, p < 0.05$] and number of bouts [$F(4, 25) = 21.6, p < 0.05$] among the groups. Post-hoc analysis showed that ECT (100 and 200 mg/kg) did not cause any significant change in latency to cough and number of bouts in guinea pigs compared to vehicle administered animals. However, ECT (400 mg/kg) and standard drug (codeine phosphate 100 mg/kg) significantly increased and decreased the latency to cough and number of bouts in animals compared to other group guinea pigs respectively.

3.11 Effect of ECT (100, 200 and 400 mg/kg) carrageenan-induced paw edema in rats

Table-3 depicts the effect of ECT (100, 200, 400 mg/kg) on carrageenan-induced paw edema at different time points in rats. Repeated measures of two-way ANOVA revealed that there were significant differences in the volume of paw among treatment [$(4, 125) = 435.4; p < 0.05$] and time [$(4, 125) = 239.0; p < 0.05$]. Further, there was a significant interaction between treatment and time [$(16, 125) = 27.8; p < 0.05$]. Post-hoc test showed that standard drug but not ECT (100, 200 and 400 mg/kg) significantly decreased the volume of paw at 1 hr in mice compared to vehicle treated animals. Moreover, ECT (400 mg/kg) and standard drug significantly decreased the volume of paw in mice compared to vehicle treated animals at 2 hr. Further, the extent of decrease in the volume of paw in standard drug treated animals were significantly higher compared to ECT (400 mg/kg) administered rats. In addition, it has been observed that both ECT (400 mg/kg) and IND were significantly decreased the volume of

paw compared to vehicle treated animals. This effect was continued up to 6 hr of the experimental design.

3.12 Effect of ECT (100, 200, and 400 mg/kg) on acetic acid-induced vascular permeability in mice

Fig-9 illustrated the effect of ECT (100, 200, and 400mg/kg) on acetic acid-induced vascular permeability in terms of leakage of dye in rats. One-way ANOVA revealed that there were significant differences in dye leakage among the groups [$F(4, 25) = 20.5$, $p < 0.05$]. Post-hoc analysis showed that ECT (100 and 200 mg/kg) did not cause any significant change in terms of dye leakage in mice compared to vehicle administered animals. However, ECT (400 mg/kg) and IND significantly decreased the dye leakage in animals compared to vehicle treated mice.

3.13 Effect of ECT (100, 200 and 400 mg/kg) on cotton pellet-induced granuloma formation in rats

Fig-10 illustrates the effect of ECT (100, 200, and 400 mg/kg) on cotton pellet-induced granuloma formation in terms of weight of cotton pellet in rats. One-way ANOVA revealed that there were significant differences in weight of cotton pellet among the groups [$F(4, 25) = 26.1$; $p < 0.05$]. Post-hoc analysis showed that ECT (100 and 200 mg/kg) did not cause any significant change in weight of cotton pellet compared to vehicle administered rats. However, ECT (400 mg/kg) and IND significantly decreased the weight of cotton pellet compared to other groups of the experimental protocol.

4. Discussion

We for the first time report the potential therapeutic activity of highest dose level of ECT against histamine- and OVA-induced asthma in animals. In addition, ECT (400 mg/kg) exhibited anti-tussive and anti-inflammatory activities in experimental animals. Taken into

consideration of the above facts, it can be assumed that standardized ECT may be considered as a potential therapeutic option in the management of allergy-induced asthma.

In the present *in vitro* study, ECT at 10 mg/ml concentration significantly attenuated histamine-induced contraction of goat trachea and guinea pig ileum. These observations indicate that standardized ECT probably exhibited anti-histaminergic activity. Additional *in vivo* study revealed that ECT (400 mg/kg) delayed onset of histamine-induced dyspnoea in guinea pigs. These reports further strengthen the assumption that the standardized ECT possesses significant anti-histaminergic activity. In support to our results, earlier reports suggest that roots of CT exhibit anti-histaminergic activity in experimental animals (Parimaladevi et al., 2003; Taur and Patil, 2010). Hence, it could be presumed that the therapeutic active constituent(s) may be common to root and flower of CT.

It has been well accepted that OVA sensitization followed by its challenge develops asthmatic symptoms in the experimental animals. There is a characteristic histological alteration in the airways of the animals (Kucharewicz et al., 2008). Moreover, OVA sensitization increases histamine- and cytokine-mediated infiltration of eosinophils into the mucosa of airways (Lukacs, 2001; Song, 2016). Our results demonstrate that ECT attenuated OVA-induced asthmatic symptoms such as airway inflammation and mucus hypersecretion in the animals. Further, ECT attenuated immune responses especially IL-1 β and IL-6-mediated inflammatory response and mucus hypersecretion in the rodents. It has been well documented that cytokines such as IL-1 β and IL-6 trigger the differentiation of airway epithelial cells into mucus-producing goblet cells that culminate into the production of more mucus into the airways (Dabbagh et al., 1999). In other reports, it has been suggested that histamine is an important mediator in the initiation and the development of antigen-induced airway responses and inflammation (Shimizu et al., 2000). Considering the above facts, it can be assumed that ECT could be a therapeutic option in the management of allergen-induced asthma.

Citric acid-induced cough is considered as one of the best model for assessing the anti-tussive effects of medicinal plants and synthetic compounds. The inhalation of citric acid causes bronchial hyper-responsiveness and hypersensitivity to histamine-induced microvascular leakage. It has also been suggested that citric acid stimulates histaminergic receptors that are located in the larynx and upper airways of the respiratory tract by opening the pH gated ion channels to induce bronchial contraction and cough (Canning, 2006). ECT attenuated the latent period and number of bouts in the rodents. These observations could be correlated to the anti-histaminergic and anti-allergy effects of ECT, and thus this therapeutic alternative can be considered as an anti-tussive agent however subject to further investigations. SO₂ induced cough model is also used for evaluation of antitussive potential of a traditional candidate medicine (Wang et al., 2011; Han et al., 2010). ECT also attenuated the latent period and number of bouts in experimentally induced cough in mice using SO₂. Determination of underlying mechanism of beneficial effect is major topic requiring further comprehensive investigation.

It has been well suggested that histamine plays a significant role in the pathogenesis of inflammation (Amin, 2012). As the present study demonstrates the anti-histaminergic activity of ECT and carrageenan is one of the mediators to modulate the activity of histamine and can induce inflammation, we carried out the anti-inflammatory activity of ECT in carrageenan-induced paw edema in rats. The carrageenan-induced paw edema is a well defined model of acute inflammation that includes several inflammatory mediators in the development of time-dependent edema (Vinegar et al., 1987). In the first phase (1-2 hr after carrageenan administration) histamine is first detected and in the later phase (2-3 hr after carrageenan administration) being attributed to serotonin, kinins and prostaglandins (Necas and Bartosikova, 2013). Subsequently, carrageenan injection also provokes the release of certain important pro-inflammatory cytokines such as IL-1 β and IL-6 and cytokine-induced

neutrophil chemo-attractant (Cuzzocrea et al., 1999; Mansouri et al., 2015; Boughton et al., 1993). Cotton pellet induced granuloma model is considered a reliable *in vivo* model for studying chronic inflammatory condition. Efficacy of drug in this model clearly highlights inhibitory activity on leucocytes infiltration, aggregation and release pro-inflammatory cytokine including IL1- β and IL-6 (Kumar et al., 2016). Based on the above observations, it can be postulated that ECT could be considered as a potential anti-allergy and anti-inflammatory and thus anti-asthma candidate.

In conclusion, ECT exhibited beneficial effect against histamine- and OVA-induced asthma in animals. Moreover, ECT exhibited anti-tussive and anti-inflammatory activities in experimental animals. Taken into consideration of the above facts, it can be assumed that standardized ECT could be considered as a potential therapeutic alternative in the management of allergy-induced asthma.

Acknowledgements

NS is thankful to GLA University, Mathura for the financial assistance.

Conflict of interest

The authors declare no conflict of interest.

Authors contributions

NS designed hypothesis, carried out the study, analyzed results and wrote the manuscript.

DG designed hypothesis, analyzed results and wrote the manuscript.

PS and SKS conducted the chromatographic analysis and wrote the manuscript.

PM designed hypothesis and wrote the manuscript.

All authors read and approved the final manuscript.

References:

- Agrawal, D.K., Shao, Z., 2010. Pathogenesis of allergic airway inflammation. *Curr. Allergy Asthma Rep.* 10, 39-48.
- Ajikumaran, N.S., Shylesh, B.S., Gopakumar, B., Subramoniam, A., 2006. Anti-diabetes and hypoglycaemic properties of *Hemionitis arifolia* (Burm.) Moore in rats. *J. Ethnopharmacol.* 106, 2, 192-197.
- Alves, R.R.N., Ros, I.M.L., 2007. Biodiversity, traditional medicine and public health: where do they meet? *J. Ethnobiol. Ethnomed.* 3, 14.
- Amin, K., 2012. The role of mast cells in allergic inflammation. *Respir. Med.* 106, 1, 9-14.
- Baines, K.J., Simpson, J.L., Bowden, N.A., Scott, R.J., Gibson, P.G., 2010. Differential gene expression and cytokine production from neutrophils in asthma phenotypes. *Eur. Respir. J.* 35, 522-531.
- Bittar, H.E.T., Yousem, S.A., Wenzel, S.E., 2015. Pathobiology of severe asthma. *Ann. Rev. Pathol.* 10, 511-545.
- Bloemen, K., Verstraelen, S., Heuvel, Van Den R., Witters, H., Nelissen, I., Schoeters, G., 2007. The allergic cascade: review of the most important molecules in the asthmatic lung. *Immunol. Lett.* 113, 1, 6-18.
- Boughton, S.N.K., Deakin, A.M., Follenfant, R.L., Whittle, B.J.R., Garland, L.G., 1993. Role of oxygen radicals and arachidonic acid metabolites in the reverse passive Arthus reaction and carrageenin paw oedema in the rat. *Br. J. Pharmacol.* 110, 896-902.

- Bousquet, J., Bousquet, P.J., Godard, P., Daures, J.P., 2005. The public health implications of asthma. *Bull. World Health Organ.* 83, 548-554.
- Bradley, J.B.L., Azzawi, M., Jacobson, M., Assoufi, B., Collins, J.V., Irani, A.M., Schwartz, L.B., Durham, S.R., Jeffery, P.K., Kay, A.B., 1991. Eosinophils, T-lymphocytes, mast cells, neutrophils, and macrophages in bronchial biopsy specimens from atopic subjects with asthma: comparison with biopsy specimens from atopic subjects without asthma and normal control subjects and relationship to bronchial hyperresponsiveness. *J. Allergy Clin. Immunol.* 88, 4, 661-674.
- Canning, B.J., 2006. Anatomy and neurophysiology of the cough reflex: ACCP evidence-based clinical practice guidelines. *Chest.* 129, 1, 33S-47S.
- Carr, T.F., Berdnikovs, S., Simon, H.U., Bochner, B.S., Rosenwasser, L.J., 2016. Eosinophilic bioactivities in severe asthma. *World Allergy Organ. J.* 9, 21, 1-7.
- Chaudhari, K.N., Lahiri, C., 1974. Role of Goat Trachea for an Isolated Tracheal Chain Preparation. *Indian J. Pharmacol.* 6, 149-151.
- Chung, H.S., Hon, P.M., Lin, G., But, P.P., Dong, H., 2003. Antitussive activity of *Stemona* alkaloids from *Stemona tuberosa*. *Planta Med.* 69, 914-920.
- Chung, K.F., Wenzel, S.E., Brozek, J.L., Bush, A., Castro, M., 2014. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur. Respir. J.* 43, 2, 343-373.
- Cumming, R.G., Mitchell, P., Leeder, .S.R., 1997. Use of inhaled corticosteroids and the risk of cataracts. *N. Engl. J. Med.* 337, 8-14.
- Custovic, A., Simpson, A., Bardin, P.G., Souef, Le P., 2010. Allergy is an important factor in asthma exacerbation: a pro/con debate. *Respirology.* 15, 1021-1027.
- Cuzzocrea, S., Sautebin, L., De Sarro, G., Costantino, G., Rombola, L., Mazzon, E., Lalenti, A., De Sarro, A., Ciliberto, G., Di Rosa, M., Caputi, A.P., Thiemermann, C.H., 1999. Role of IL-6 in the pleurisy and lung injury caused by carrageenan. *J. Immunol.* 163, 5094-5104.
- Dabbagh, K., Takeyama, K., Lee, H.M., Ueki, I.F., Lausier, J.A., Nadel, J.A., 1999. IL-4 induces mucin gene expression and goblet cell metaplasia in vitro and in vivo. *J. Immunol.* 162, 6233-6237.
- Debprasad, C., Hemanta, , Paromita, B., Durbadal, O., Kumar, K.A., Shanta, D., Kumar, H.P., Tapan, C., Ashoke, S., Sekhar, C., 2012. Inhibition of NO₂, PGE₂, TNF- α and iNOS

- expression by *Shorea robusta* L.: an Ethnomedicine used for Antiinflammatory and Analgesic activity. Evid. Based Complement Alternat Med. 1-14.
- Dweik, R.A., Sorkness, R.L., Wenzel, S., Hammel, J., Curran-Everett, D., Comhair, S.A., 2010. Use of exhaled nitric oxide measurement to identify a reactive, at risk phenotype among patients with asthma. Am. J. Respir. Crit. Care Med. 181, 1033-1041.
- Gibson, P.G., Simpson, J.L., Saltos, N., 2001. Heterogeneity of airway inflammation in persistent asthma: evidence of neutrophilic inflammation and increased sputum interleukin-8. Chest. 119, 1329-1336.
- Gomez, S.M., Kalamani, K., 2003. Butterfly pea (*Clitoria ternatea*): a nutritive multipurpose forage legume for the tropics-An overview. Pakistan J. Nutr. 2, 6, 374-379.
- Guilbert, T.W., Morgan, W.J., Zeiger, R.S., Mauger, D.T., Boehmer, S.J., Szeffler, S.J., 2006. Long-term inhaled corticosteroids in preschool children at high risk for asthma. N. Engl. J. Med. 354, 1985-1997.
- Gupta, Y.K., Katyal, J., Kumar, G., Mehla, J., Katiyar, C.K., Sharma, N., Yadav, S., 2009. Evaluation of antitussive activity of formulations with herbal extracts in sulphur dioxide (SO₂) induced cough model in mice. Indian J. Physiol. Pharmacol. 53, 61-66.
- Han, N., Chang, C., Wang, Y., Huang, T., Liu, Z., Yin, J., 2010. The in vivo expectorant and antitussive activity of extract and fractions from *Reineckia carnea*. J. Ethnopharmacol. 131. 220-223.
- Jatakanon, A., Uasuf, C., Maziak, W., Lim, S., Chung, K.F., Barnes, P.J., 1999. Neutrophilic inflammation in severe persistent asthma. Am. J. Respir Crit. Care Med. 160, 1532-1539.
- Kamkaen, N., Wilkinson, J.M., 2009. The antioxidant activity of *Clitoria ternatea* L. flower petal extracts and eye gel. Phytother. Res. 23, 11, 1625-1626.
- Khadatkar, S.N., Manwar, J.V., Bhajipale, N.S., 2008. In-Viro anthelmintic activity of root of *Clitoria ternatea* Linn. Pharmacogn. Mag. 4, 13, 148-150.
- Khare, C.P., 2004. Encyclopedia of Indian medicinal plants, Springer publication. 153-154.
- Kritas, S.K., Saggini, A., Cerulli, G., Caraffa, A., Antinolfi, P., Pantalone, A., Saggini, R., Frydas, S., Rosati, M., Tei, M., Speziali, A., Pandolfi, F., Conti, P., 2014. Interrelationship between IL-3 and mast cells. J. Biol. Regul. Homeost Agents. 28, 1, 17-21.
- Kucharewicz, I.I., Anna Bodzenta-Łukaszyk, A.B., Buczek, W., 2008. Experimental asthma in rats. Pharmacol. Rep. 60, 783-788.

- Kumar, R., Gupta, Y.K., Singh, S., 2016. Anti-inflammatory and antigranuloma activity of *Berberis aristata* DC. In experimental models of inflammation. Indian J. Pharmacol. 48, 2, 155-161.
- Lukacs, N.W., 2001. Role of chemokines in the pathogenesis of asthma. Nat. Rev. Immunol. 1, 108-116.
- Mansouri, M.T., Hemmati, A.A., Naghizadeh, B., Ali Mard, S., Rezaie, A., Ghorbanzadeh, B., 2015. A study of the mechanisms underlying the anti-inflammatory effect of ellagic acid in carrageenan induced paw edema in rats. Indian J. Pharmacol. 47, 3, 292-298.
- Masoli, M., Fabian, D., Holt, S., Beasley, R., 2004. The global burden of asthma: executive summary of the gina dissemination committee report. Allergy. 59, 469-468.
- Miranda, C., Busacker, A., Balzar, S., Trudeau, J., Wenzel, S.E., 2004. Distinguishing severe asthma phenotypes: role of age at onset and eosinophilic inflammation. J. Allergy Clin. Immunol. 113, 101-108.
- Moody, J.O., Robert, V.A., Connolly, J.D., Houghton, P.J., 2006. Anti-inflammatory activities of the methanol extracts and an isolated furanoditerpene constituent of *Sphenocentrum jollyanum* Pierre (menispermaceae). J. Ethnopharmacol. 104, 87-91.
- Mukherjee, K.P., Kumara, V., Satheesh, N.K., Micheal, H., 2008. The Ayurvedic medicine *Clitoria ternatea* L-From traditional use to scientific assessment. J. Ethnopharmacol. 120, 291-301.
- Necas, J., Bartosikova, L., 2013. Carrageenan: a review. Veterinarni Medicina. 58, 187-205.
- Parimaladevi, B., Boominathan, R., Mandal, S.C., 2003. Anti-inflammatory, analgesic and antipyretic properties of *Clitoria ternatea* root. Fitoterapia. 74, 4, 345-349.
- Parrotta, J. A., 2001. Healing plants of peninsular India, C.A.B.I. publication. 382-383.
- Patil, A.P., Patil, R.V., 2001. *Clitoria ternatea* Linn. An Overview. Int. J. Pharm. Res. 3, 1, 20-23.
- Piliponsky A.M., Gleich, G.J., Bar, I., Levi-Schaffer, F., 2002. Effects of eosinophils on mast cells: A new pathway for the perpetuation of allergic inflammation. Mol. Immunol. 38, 1369-1372.
- Piliponsky, A.M., Gleich, G.J., Nagler, A., Bar, I., Levi-Schaffer, F., 2003. Non-Ige-dependent activation of human lung- and cord blood-derived mast cells is induced by eosinophil major basic protein and modulated by the membrane form of stem cell factor. Blood. 101, 1898-1904.

- Prajapati, P., Sharma, A., 2003. Handbook of medicinal plants a complete source book, Agrobios publication. 154-155.
- Shimizu, T., Hirano, H., Majima, Y., Sakakura, Y., 2000. A Mechanism of antigen-induced mucus production in nasal epithelium of sensitized rats. *Am. J. Respir. Crit. Care Med.* 161, 1648-1654.
- Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* 16, 3, 144-158.
- Song, W., 2016. meso-Dihydroguaiaretic acid attenuates airway inflammation and mucus hypersecretion in an ovalbumin-induced murine model of asthma. *Int. Immunopharmacol.* 31, 239-247.
- Tan, W.C., 1991. Rational drug strategy in bronchial asthma, *Ann. Acad. Med. Singapore.* 20, 165-171.
- Taur, D.J., Patil, R.Y., 2010. Antihistaminic activity of *Clitoria ternatea* L. roots. *J. Basic Clin. Pharm.* 2, 1, 41-44.
- Taur, D.J., Patil, R.Y., 2011. Evaluation of antiasthmatic activity of *Clitoria ternatea* L. roots, *J. Ethnopharmacol.* 136, 2, 374-376.
- Vinegar, R., Truax, J.F., Selph, J.L., Johnston, P.R., Venable, A.L., McKenzie, K.K., 1987. Pathway to carrageenan-induced inflammation in the hind limb of the rat. *Fed. Proc.* 46, 1, 118-126.
- Vogel, G.H., Vogel, W.H., 1998. Drug Discovery and Evaluation. Berlin: Springer Verlag.
- Wang, D., Zhu, J., Wang, S., Wang, X., Ou, Y., Wei, D., 2011. Antitussive, expectorant and anti-inflammatory alkaloids from Bulbus *Fritillariae Cirrhosae*. *Fitoterapia.* 82, 1290-1294.
- Weller, C.L., Collington, S.J., Williams, T., Lamb, J.R., 2011. Mast cells in health and disease. *Clin. Sci. (Lond).* 120, 11, 473-84.
- Wenzel, S.E., Schwartz, L.B., Langmack, E.L., Halliday, J.L., Trudeau, J.B., Gibbs, R.L., 1999. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am. J. Respir. Crit. Care Med.* 160, 1001-1008.
- Whittle, B.A., 1964. The use of changes in capillary permeability in mice to distinguish between narcotic and nonnarcotic analgesics. *Br. J. Pharmacol. Chemother.* 22, 246-253.

- Wilson. S.J., Rigden, H.M., Ward, J.A., Lavolette, M., Jarjour, N.N., Djukanovic, R., 2013. The relationship between eosinophilia and airway remodelling in mild asthma. Clin. Exp. Allergy. 43, 134-1350.
- Zhishen, J., Mengcheng, T., Jianming, W., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64, 4, 555-559.

Figure-1: HPTLC chromatograms of standard quercetin (A) and ethanolic extract of *C. trenatea* flower (ECT). X-axis represents absorbance unit (AU) and Y-axis represents retention factor (R_f) for each chromatogram.

Figure-2: Effect of ECT (2.5, 5 and 10 mg/ml) on maximum effective dose (E_{max}) of histamine (Hist)-induced contraction in isolated goat tracheal chain (A), E_{max} of Hist- and acetylcholine (ACh)-induced contraction in isolated tracheal chain (B and C respectively) and ileum (D and E respectively) preparation of guinea pig. All values are Mean $\pm$ SEM (n = 6). * $P < 0.05$ and [#] $P < 0.05$ compared to Hist and Hist + ECT (2.5 mg/ml) respectively (One-way ANOVA followed by Student Newmann Keuls Post-hoc test).

Figure-3: Effect of ECT (100, 200 and 400 mg/kg) on ovalbumin (OVA) sensitized and challenged alterations in the levels of interleukin (IL)-1 β (A) and IL-6 (B) in BALF in mice. All values are Mean $\pm$ SEM (n = 6). ^a $P < 0.05$, ^b $P < 0.05$ and ^c $P < 0.05$ compared to Vehicle, ECT-100 and ECT-200 group respectively (One-way ANOVA followed by Student Newmann Keuls Post-hoc test).

Figure-4: Effect of ECT (100, 200 and 400 mg/kg) on OVA sensitized and challenged alterations in the levels of immunoglobulin E (IgE; A) and IgG1 (B) in BALF in mice. All values are Mean $\pm$ SEM (n = 6). ^a $P < 0.05$, ^b $P < 0.05$ and ^c $P < 0.05$ compared to Vehicle, ECT-100 and ECT-200 group respectively (One-way ANOVA followed by Student Newmann Keuls Post-hoc test).

Figure-5: Histopathological observation of treatment effect of ECT (100, 200 and 400 mg/kg) on OVA sensitized and challenged alterations in the cytoarchitecture of mice lung tissue (haematoxylin and eosin staining). Representative histological section of mice lung of Vehicle (A), ECT-100 (B), ECT-200 (C), ECT-400 (D) and DEX (E). (Arrow marks indicate: Grey: inflammation in perivascular area and Black: inflammation in peribronchial area).

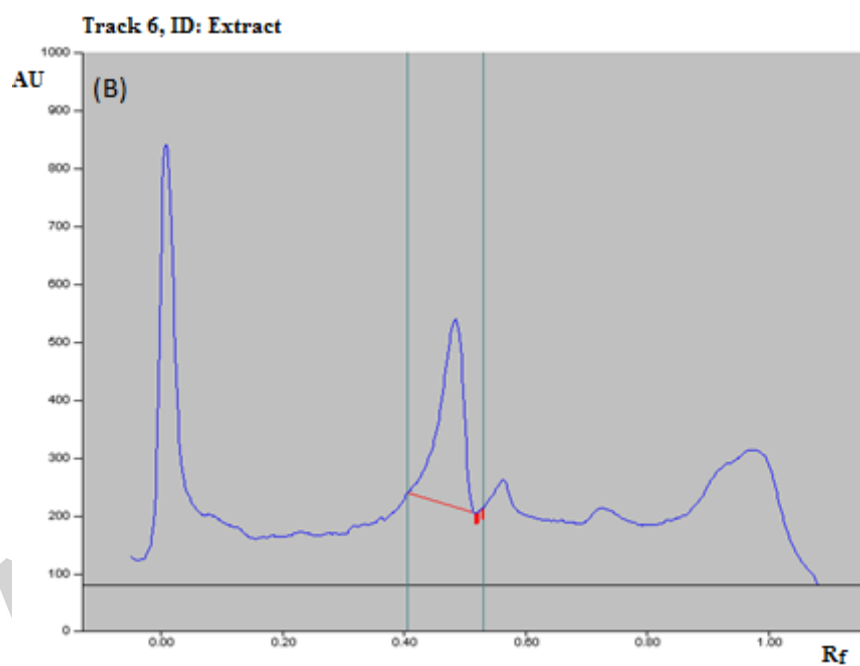
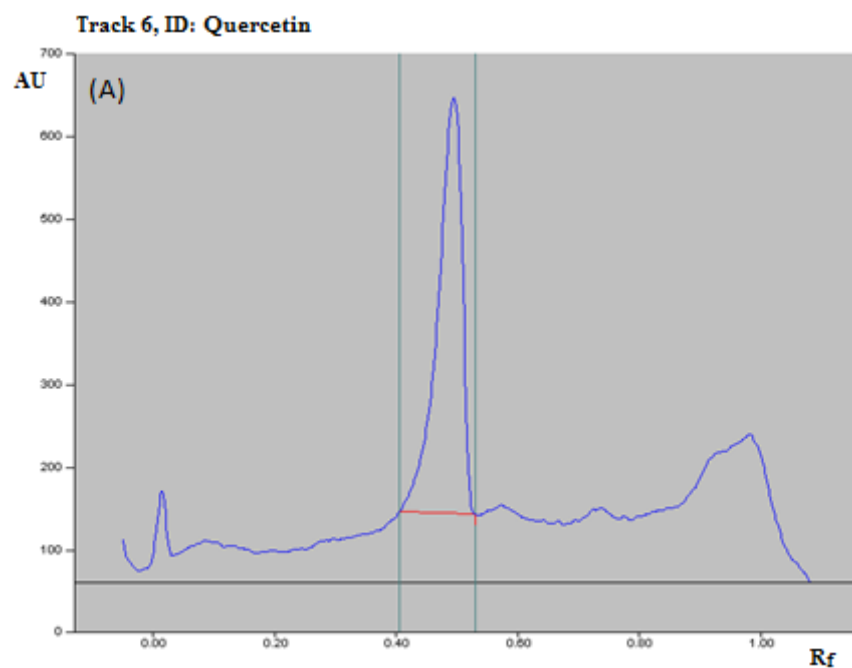
Figure-6: Effect of ECT (100, 200 and 400 mg/kg) on OVA sensitized and challenged alterations in the inflammation score in peribronchial (A), perivascular (B) and total tissue section (C) of mice lung. All values are Mean $\pm$ SEM (n = 6). ^aP<0.05, ^bP<0.05 and ^cP<0.05 compared to Vehicle, ECT-100 and ECT-200 group respectively (Kruskal-Wallis test followed by Dunns multiple comparison test).

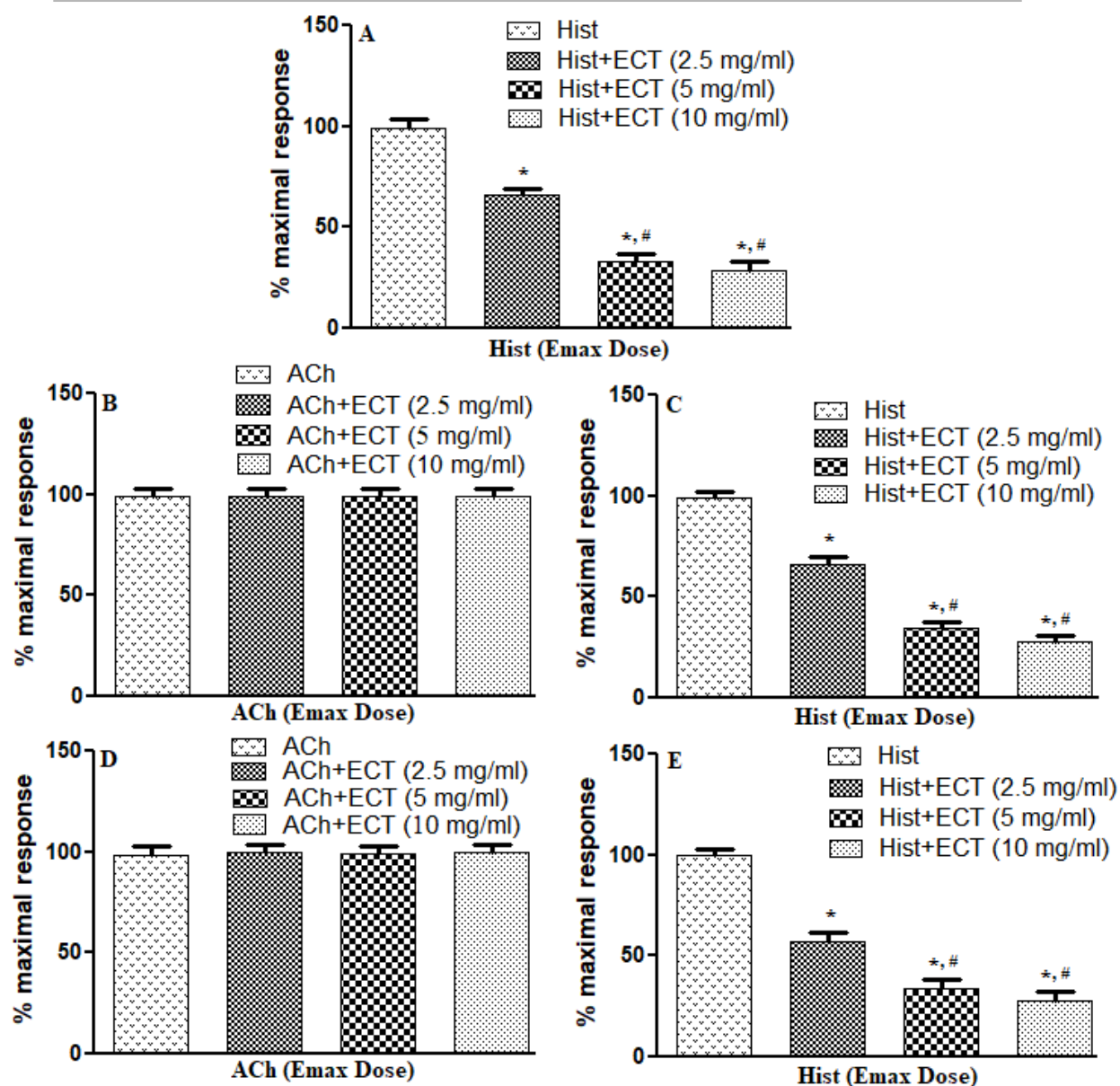
Figure-7: Effect of ECT (100, 200, 400 mg/kg) on sulphur dioxide-induced cough in mice. All values are Mean $\pm$ SEM (n = 6). ^aP<0.05, ^bP<0.05, ^cP<0.05 and ^dP<0.05 compared to Vehicle, ECT-100, ECT-200 and ECT-400 group respectively (Repeated measures two-way ANOVA followed by Bonferroni Post-hoc test).

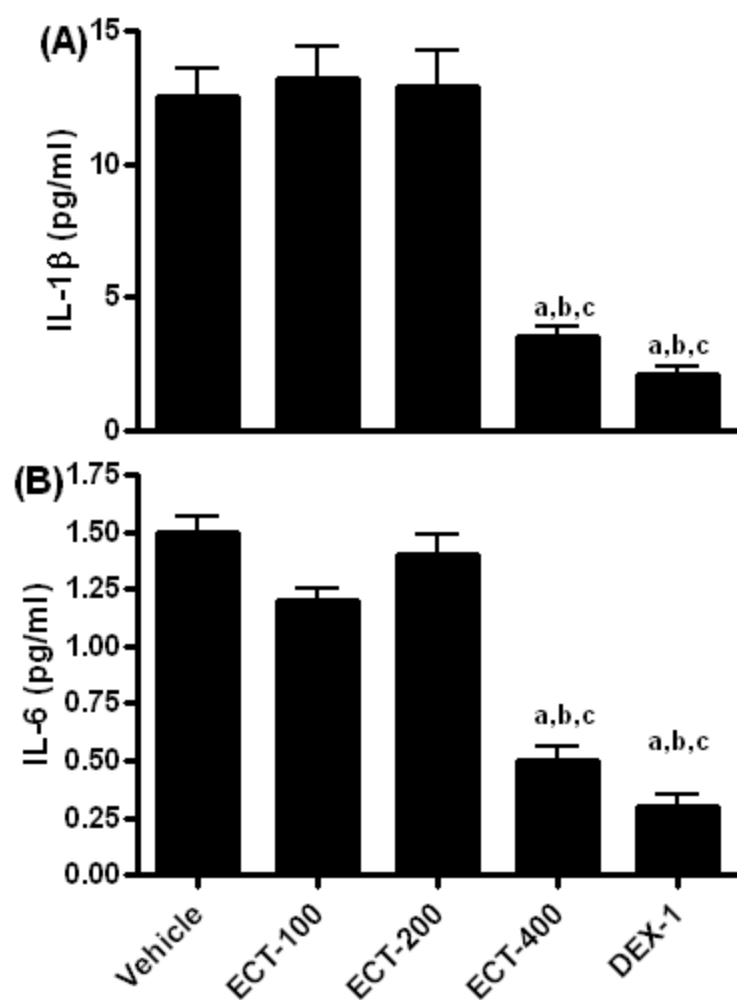
Figure-8: Effect of ECT (100, 200 and 400 mg/kg) on latent period to cough (A) and number of bouts (B) in citric acid exposed guinea pigs. All values are Mean $\pm$ SEM (n = 6). ^aP<0.05, ^bP<0.05 and ^cP<0.05 compared to Vehicle, ECT-100 and ECT-200 group respectively (One-way ANOVA followed by Student Newmann Keuls Post-hoc test).

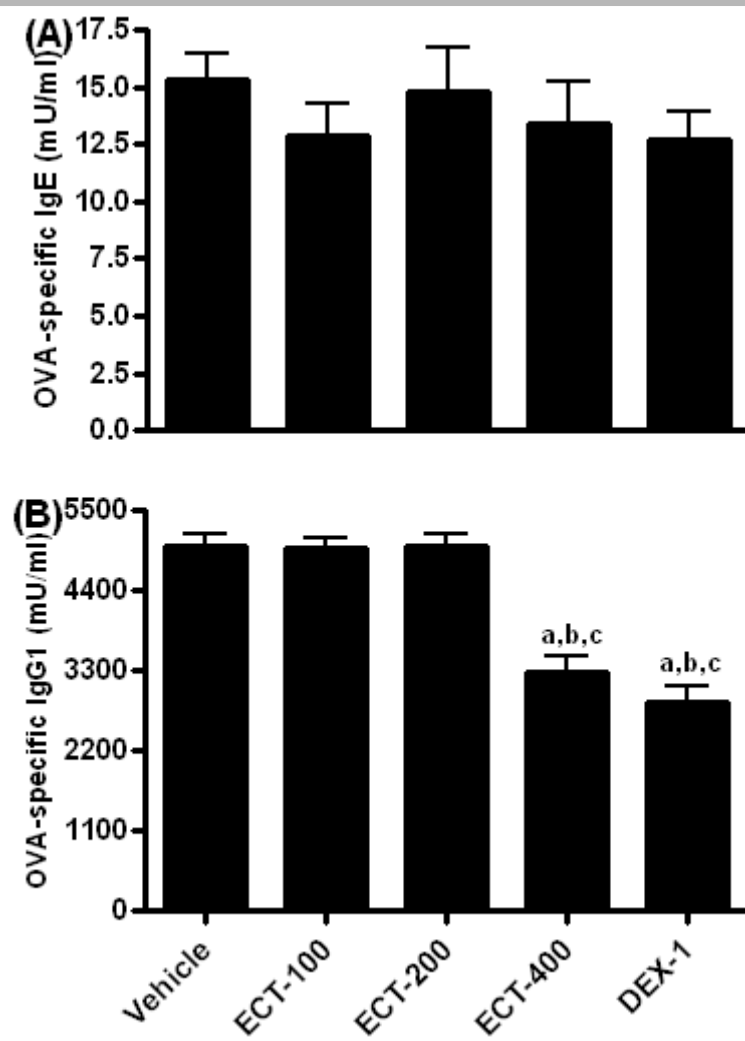
Figure-9: Effect of ECT (100, 200 and 400 mg/kg) on acetic acid-induced vascular permeability in terms of leakage of dye in rats. All values are Mean $\pm$ SEM (n = 6). ^aP<0.05, ^bP<0.05 and ^cP<0.05 compared to Vehicle, ECT-100 and ECT-200 group respectively (One-way ANOVA followed by Student Newmann Keuls Post-hoc test).

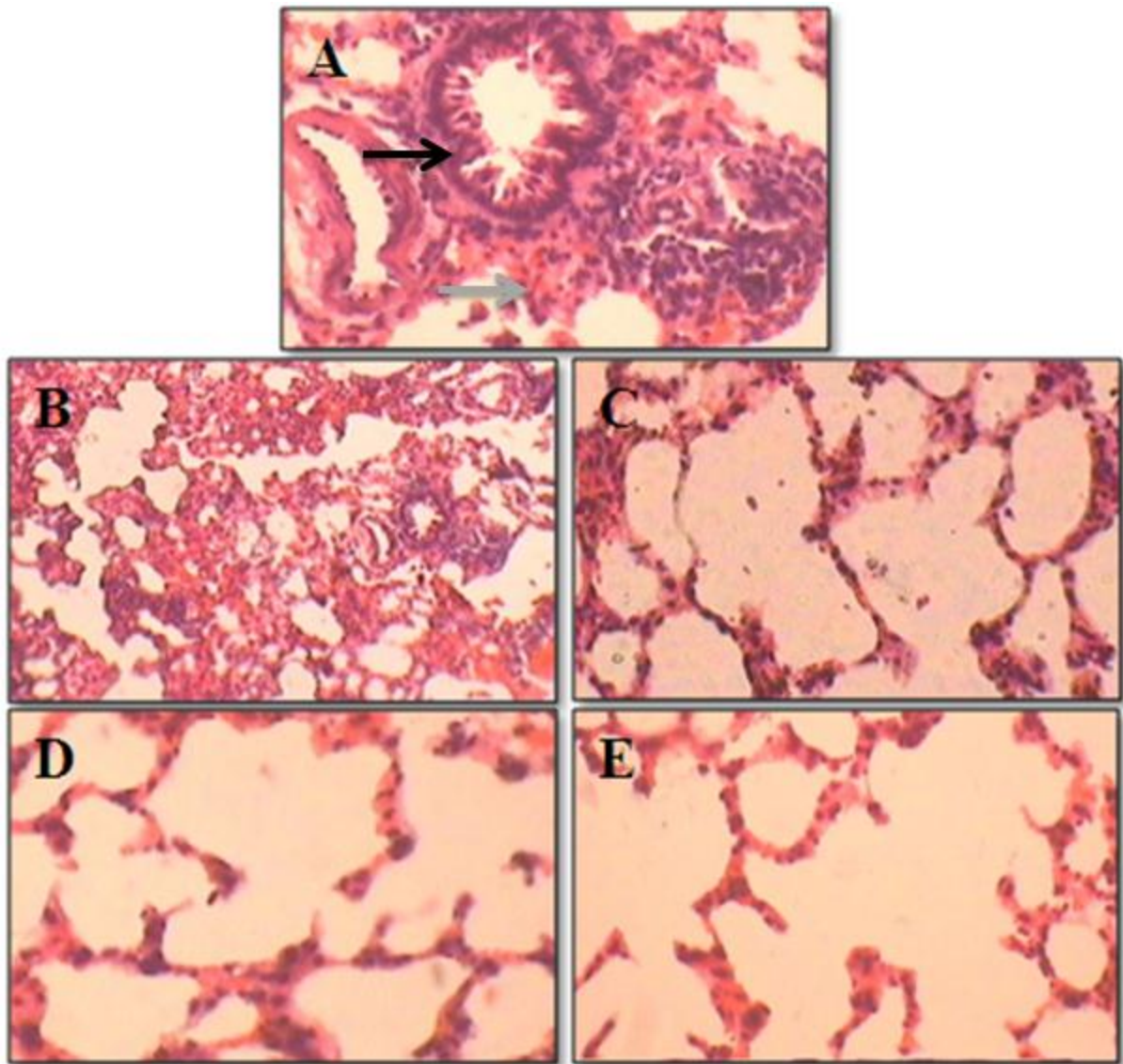
Figure-10: Effect of ECT (100, 200 and 400 mg/kg) on cotton pellet-induced granuloma formation in terms of weight of cotton pellet in rats. All values are Mean $\pm$ SEM (n = 6). ^aP<0.05, ^bP<0.05 and ^cP<0.05 compared to Vehicle, ECT-100 and ECT-200 group respectively (One-way ANOVA followed by Student Newmann Keuls Post-hoc test).

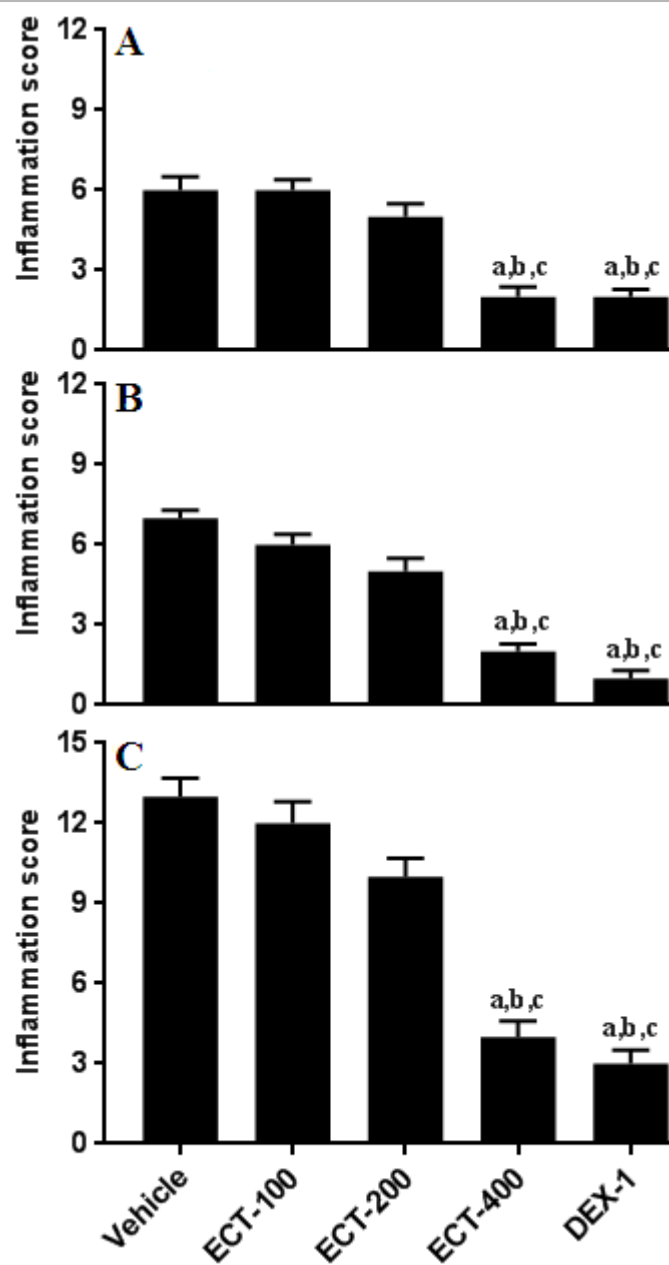


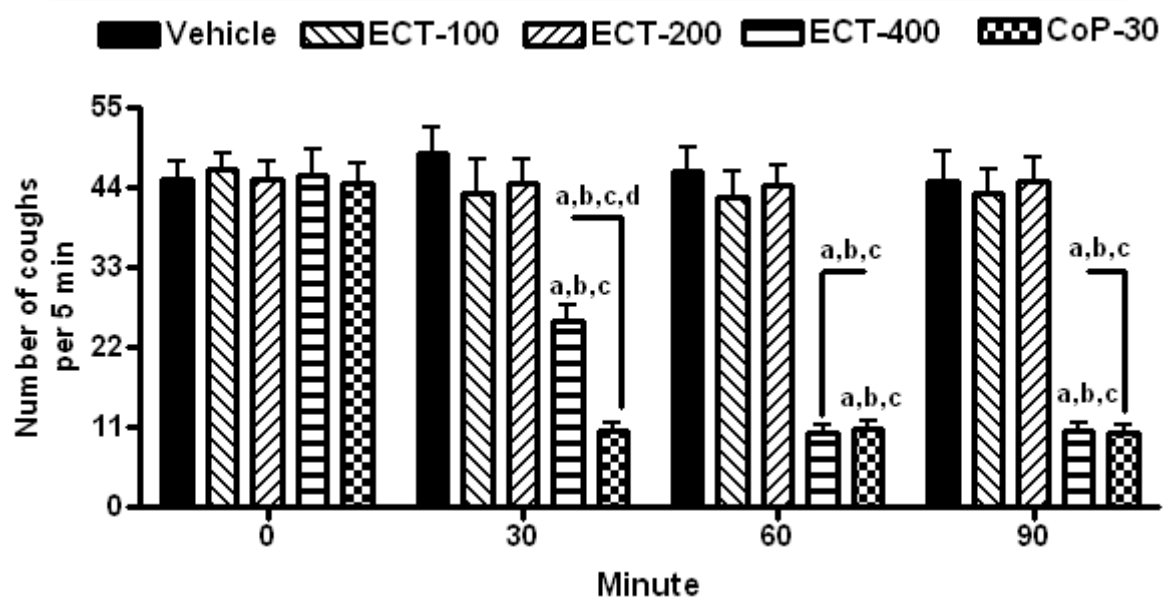


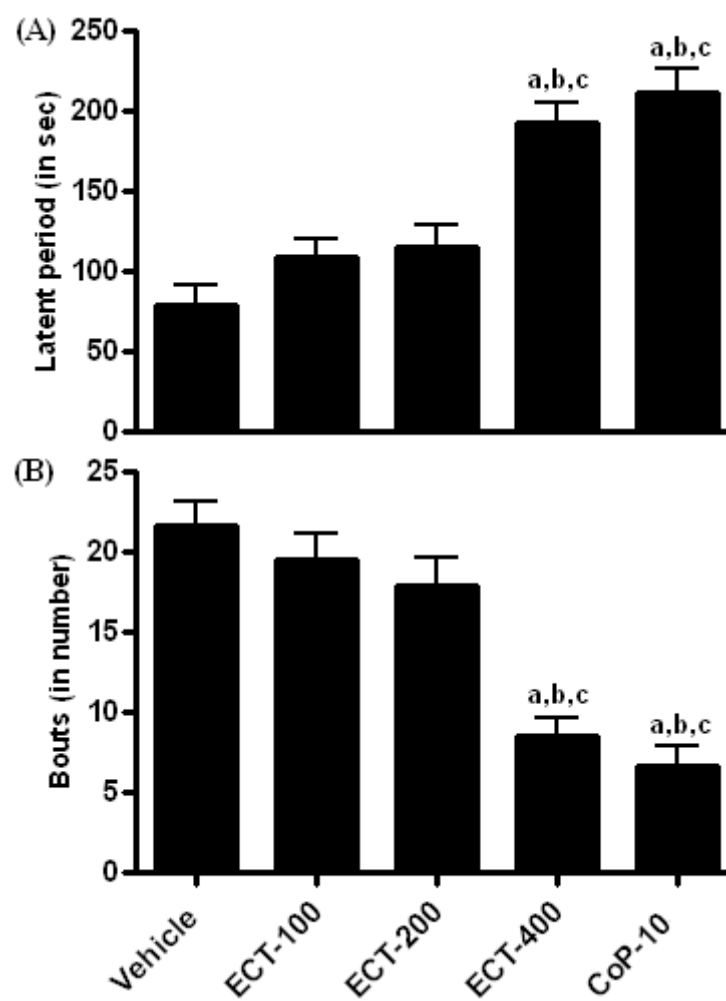


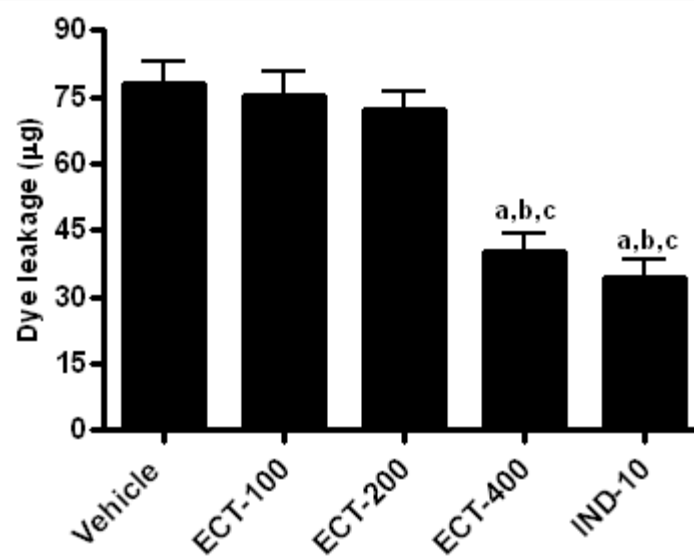












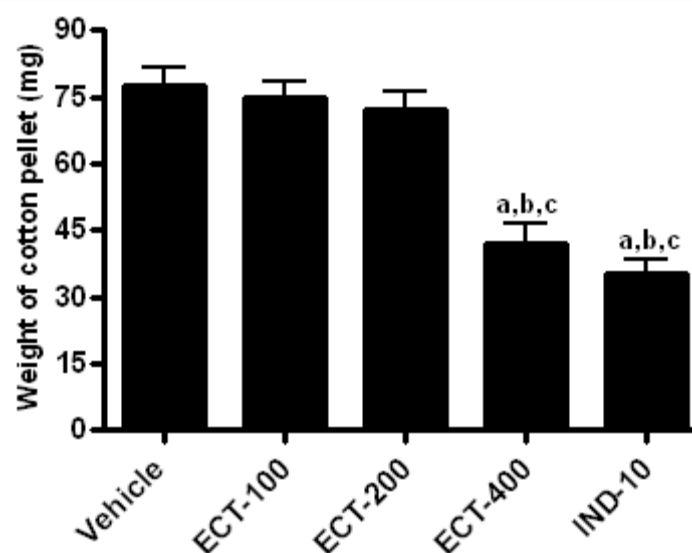


Table-1: Effect of ECT (100, 200 and 400 mg/kg) on histamine-induced onset of dyspnoea in guinea pig

Group	PCT ₁ (sec)	PCT ₂ (sec)	Mean exposition time (sec)	% Protection
Vehicle	115.6 ± 13.6	116.8 ± 29.8	1.5 ± 0.2	1.2
ECT-100	117.2 ± 14.2	118.5 ± 27.3	2.7 ± 0.4	2.3
ECT-200	110.6 ±	139.4 ±	25.4 ± 5.5	18.7

	12.9		31.8		
ECT-400	114.8	±	487.8	±	386.3 ± 29.4 ^{a,b,c}
	15.8		37.6		78.5
CPM-2	119.6	±	590.7	±	473.4 ± 31.7 ^{a,b,c}
	17.6		35.4		80.3

All values are Mean ± SEM (n = 6). ^ap<0.05 compared to Vehicle, ^bp<0.05 compared to ECT-100 and ^cp<0.05 compared to ECT-200 (One-way ANOVA followed by Student Newmann Keuls Post-hoc test).

Table-2: Effect of ECT (100, 200 and 400 mg/kg) on OVA-induced alterations in differential cell counts in BALF in mice

Group	Total leukocytes (cu/mm)	Neutrophiles (cu/mm)	Eosinophiles (cu/mm)	Macrophages (cu/mm)	Lymphocytes (cu/mm)
Vehicle	18326.2 ± 924.4	839.1 ± 78.4	925.4 ± 68.5	8587.3 ± 513.3	235.3 ± 22.1
ECT-100	17565.4 ± 1210.7	755.3 ± 79.2	852.3 ± 79.7	8641.4 ± 631.4	156.2 ± 20.6
ECT-200	16879.3 ± 1205.6	710.2 ± 74.5	825.5 ± 71.6	8379.8 ± 684.1	147.3 ± 18.4
ECT-400	8674.3 ± 1150.3 ^{a,b,c}	246.4 ± 42.8 ^{a,b,c}	431.2 ± 62.2 ^{a,b,c}	4231.4 ± 231.5 ^{a,b,c}	58.4 ± 14.8 ^{a,b,c}
DEX-1	6854.5 ± 860.2 ^{a,b,c}	185.3 ± 41.7 ^{a,b,c}	398.5 ± 59.8 ^{a,b,c}	3784.2 ± 221.3 ^{a,b,c}	49.5 ± 15.4 ^{a,b,c}

All values are Mean ± SEM (n = 6). ^ap<0.05 compared to Vehicle, ^bp<0.05 compared to ECT-100 and ^cp<0.05 compared to ECT-200 (One-way ANOVA followed by Student Newmann Keuls Post-hoc test).

Table-3: Effect of ECT (100, 200 and 400 mg/kg) on Carrageenan-induced paw edema in the rats

Groups	Increase in paw volume in mL at				
	1 hr	2 hr	3 hr	4 hr	6 hr
Vehicle	0.70 ± 0.03	1.22 ± 0.05	1.80 ± 0.07	2.10 ± 0.06	2.50 ± 0.08
ECT-100	0.69 ± 0.04	1.12 ± 0.05	1.73 ± 0.09	1.99 ± 0.09	2.45 ± 0.09
ECT-200	0.64 ± 0.07	1.10 ± 0.08	1.68 ± 0.08	1.89 ± 0.08	2.41 ± 0.07
ECT-400	0.60 ± 0.06	0.62 ± 0.07 ^{a,b,c}	0.55 ± 0.08 ^{a,b,c}	0.53 ± 0.07 ^{a,b,c}	0.59 ± 0.08 ^{a,b,c}
IND	0.12 ± 0.03 ^{a,b,c,d}	0.30 ± 0.03 ^{a,b,c,d}	0.50 ± 0.04 ^{a,b,c}	0.50 ± 0.03 ^{a,b,c}	0.60 ± 0.04 ^{a,b,c}

All values are Mean ± SEM (n = 6). ^ap<0.05 compared to Vehicle, ^bp<0.05 compared to ECT-100 and ^cp<0.05 compared to ECT-200 (Repeated measures two-way ANOVA followed by Bonferroni Post-hoc test).