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Anti-anaphylactic and antiasthmatic activity of *Euphorbia thymifolia* L. on experimental animals

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ABSTRACT

In Ayurveda, *Euphorbia thymifolia* L. (Euphorbiaceae) prescribed in the treatment of various ailments like bronchial asthma, cough, diarrhea and bleeding piles. The present study was investigated to evaluate antianaphylactic, mast cell stabilizing and antiasthmatic activity of methanol and aqueous extract of *E. thymifolia* (ET) on experimental animals. Anaphylaxis was induced by administration of horse serum and triple antigen vaccine intraperitoneal (i.p.) in albino Wistar rats. Extracts of ET were administered to the rats in dose of 250 and 500 mg/kg orally for 14 days. At the end of treatment, asthma score was measured and various blood parameters like differential count (DC), total WBC count and IgE were estimated. Interleukin (IL)-4, IL-5 and TNF-α were measured by ELISA commercial kit from BALF. Histopathological changes of lungs were observed. Antiasthmatic activity of extracts of ET was also studied on histamine-induced bronchospasm in guinea pigs. *In vitro* mast cell stabilizing activity of extracts was evaluated on compound 48/80 challenged rat intestinal mesenteric mast cells. The treatment with extracts of ET produced significant decrease in asthma score and they also brought to normalcy the increased total WBC, DC counts, serum IgE, TNF-α, IL-4 and IL-5 in BALF. The histopathological study further supported the protective effect of ET extracts. The pretreatment with extracts of ET displayed significant reduction in degranulation of mesenteric mast cell numbers. The treatment with extracts of ET significantly increased in time of PCD. Thus, these findings concluded that *E. thymifolia* could be effectively used in the treatment of anaphylaxis and asthma.

1. Introduction

Anaphylaxis is a life-threatening form of allergy and it is characterized by respiratory distress, bronchoconstriction, vascular collapse, shock, and death.[1] The blood eosinophilia and mast cells release histamine which is responsible for the production of IgE. This immune response plays central event in the type-I hypersensitivity of allergy.[2] The degranulated mast cell-released many biologically active molecules, which include histamine, prostaglandins, leukotriene, platelet activating factor, tumor necrosis factor (TNF)-α, and cytokines etc.[3] These results, in turn, can cause symptoms like visceral muscle contraction, vasodilation, up-regulation of vascular permeability and excess mucus production.[4] The entry of leukocytes into the lungs along with numerous blood cells such as lymphocytes (B and T helper cells Type 2 cells), eosinophil, macrophages, basophils and endothelial cells could cause hypersensitive inflammation in asthma.[5] The allergen-specific T helper Type 2 (Th2) lymphocytes play a vital role in starting and engendering of inflammation via the infiltration of Th2 mediated cytokines such as interleukin (IL)-4, IL-5, IL-6 and IL-13 in tissues.[6] These cytokines initiate the synthesis of immunoglobulin E (IgE) and activation of mast cells, lymphocytes, and eosinophil.[7] Interleukin-5 activates the recruitment of eosinophil which initiates airway inflammation and discharge ECP (Eosinophil cationic protein) and this turned out to be the most critical markers of the illness.[8]

The accessible treatment choices for upper and lower respiratory tract hypersensitive disorders have significant impediments because of low adequacy, related unfavorable events, and agreeability issues.[9] Ayurveda, a traditional Indian system of medicine,

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has represented a few medications from the traditional indigenous plants in the management of asthma and allergy.

The medicinal plant *Euphorbia thymifolia* L. (Euphorbiaceae) commonly known as *nani dudheli* in vernacular language (Gujarati). The *E. thymifolia* (ET) is traditionally used as an antiviral in bronchial asthma bronchitis and hay fever.[10] The reported pharmacological indications of ET are antifungal, antispasmodic, anti-herpes, diuretic, anthelmintic, hepatoprotective, antioxidant and anti-arthritic activity.[11–18] *Charaka* prescribed *E. thymifolia* in diarrhea and piles. It is believed to also uses as antidiarrheal, antimalarial and antidysentery.[19] The ET contains hydrolysable tannins,[20] flavonoids,[21] triterpenoids[10] and essential oils.[22]

The present investigation is considered to be the first of its kind to explore the molecular mechanism as anti-anaphylaxis and antiasthmatic activity of this medicinal plant. However, the work has been carried out on this plant, but molecular mechanism has not been elucidated yet. Although, no scientific study is reported on anti-anaphylaxis, mast cell stabilization and antiasthmatic activity of this plant. Hence, this investigation was conducted to evaluate the antianaphylaxis and antiasthmatic effect of methanol and aqueous extract from *E. thymifolia* on immunological and non-immunological models of anaphylaxis.

2. Material and methods

2.1. Reagents

The horse serum (HIMEDIA Chemicals, Mumbai, India), triple antigen (DPT vaccines, Serum Institute of India Pvt. Ltd.), histamine and compound 48/80 (Sigma aldrich, USA), Ketotifen fumarate (Torrent Research Centre, Ahmedabad, India) were used in the all experiments. Other analytical grade chemicals and reagents used in the experiment were purchased from Merck (India).

2.2. Plant material

Fresh aerial part of ET was collected during the September 2013 from Amargadh village, Taluka, the district of Rajkot, Gujarat, India. The taxonomical authentication of the plants was confirmed and the voucher specimen no. DP/SVU/PHCOG/Herb/03 was deposited at CSIR-NISCAIR, New Delhi, India. The collected entire plant was washed under running tap water to remove the soil debris and dried under shade.

2.3. Extraction

The collected aerial parts were sun dried, pulverized and sieved through sieve #40. The cold maceration method was utilized to prepare methanol (ETM) and aqueous (ETW) extracts of ET for 72 h. Extracts were concentrated using a water bath (Mack, Ahmedabad, India) at a temperature of 70 °C and subsequently lyophilized and stored at −20 °C and used for future. The % yield of ETM and ETW extracts was 16.34% and 6.25% (w/w) respectively.

2.4. Experimental animals

Healthy adult male Wistar albino rats (180–200 g, Zydus Cadila Healthcare, Ahmedabad), were kept in standard polypropylene cages (4 per cage) with all standard laboratory conditions. The rats were fed a standard rat pellet diet (Pranav Agro Ltd. Vadodara, India) and had free access to water *ad libitum*. The protocol was approved by Ethical Committee of Sumandeep Vidyapeeth (Reg. No. SVU/DP/IAEC/2013/10/17).

2.5. The acute oral toxicity study

OECD guideline (2001)[23] was followed to carried out acute toxicity study for the extracts of ET. Methanol and aqueous extracts of ET (2500 and 5000 mg/kg) were dispersed with 0.5% carboxy methyl cellulose (CMC), administered to overnight fasted, healthy rats (n = 3). They were observed up to 14 days.[24]

2.6. Animal grouping and dosing

In triple antigen and horse serum-induced anaphylaxis model, animals were grouped in to seven groups (n = 6) as follows. All dried extracts and prednisolone suspended in 0.5% CMC solution and administered via oral route to sensitized rats. All the test drugs and vehicle were given orally through oral gavages. Group I served as normal control (NC, received normal diet and water only), group II served as sensitized control (SC, 0.5% CMC p.o.), group III was administered standard prednisolone (10 mg/kg p.o.), group IV and V were administered 250 mg/kg and 500 mg/kg (p.o.) ETM respectively, group VI and VII were given 250 mg/kg and 500 mg/kg (p.o.) ETW respectively.

2.7. Triple antigen and horse serum-induced active anaphylaxis in rats

Albino Wistar rats (Male, 200–250 g) were sensitized by subcutaneous injection of 0.5 mL of horse serum followed by 0.5 mL of triple antigen vaccine containing 2×10^{10} *Bordetella pertussis* organisms per mL in all groups except normal control group.[25] The entire experiment model was carried out for 14 days. At the end of treatment, 0.25 mL of horse serum in saline was challenged via intravenous injection in tail vein. The challenged animals with antigen were monitored to 1 h for onset of symptoms. The severities of symptoms were scored as follow: No visual symptoms 0, increased respiratory rate-2, increased respiratory rate with immobility-4, dyspnoea-6 for 10 min, cyanosis-8 for 10, dyspnea with cyanosis-10 for 10 min and respiratory failure and death-12.[26]

2.7.1. Collection of blood and bronchoalveolar lavage fluid (BALF)

On day 14th, after the aforementioned treatment, the blood was collected from retro-orbital plexus and serum was separated. It was stored at −70 °C and analyses for IgE levels, WBC, total leukocyte, eosinophil count, and % polymorphs count. A tracheal cannula was inserted via mid cervical incision and lavage with 1 mL of cold phosphate buffer saline (PBS), pH = 7.4. The BALF was centrifuged for 10 min and the supernatant was analyses for TNF-α, IL-4 and IL-5.

2.7.2. Quantification of serum IgE

Serum IgE was quantified with an enzyme linked immunosorbent assay (ELISA) kit (#: 555248, BD Biosciences Pharmingen, San Diego, CA) according to the manufacturer's protocol and results were reported in ng/mL for protein.[27,28]

2.7.3. Quantification of TNF-α, IL-4 and IL-5 from BALF

TNF-α (#: 560479), IL-4 (#. 555198) and IL-5 (#. 555236) were estimated in BALF by ELISA kits (BD Biosciences Pharmingen, San Diego, CA) according to the prescribed methods and results were noted in pg/mL for each protein.[28,29]

2.7.4. Lung histopathology

After the collection of BALF, the lungs of rats were isolated, and then immersed in 10% formaldehyde. The paraffin-embedded lungs were cut in thin sections (5 μm thickness) with the help of microtome. The thin sections were stained with hematoxylin and eosin

(H&E) and observed under a microscope for the histopathological changes in airway lumen, columnar epithelium, accumulation of lymphocytes, and parenchymal inflammation.[30]

2.8. The mast cell stabilization activity in rats

The adult albino Wistar rats ware sacrificed with excess anesthetic diethyl ether and the mesentery were separated out, and kept in Ringer-Locke solution in Petri plate for 30 min and then divided for treatment as: petri dish I: Ringer Locke solution (sensitized control), petri dish II: 0.1 mL of Ketotifen (20 μg/mL), petri dish III and IV: 0.1 mL of ETM (100 and 200 μg/mL in 0.5% CMC, respectively) and Petridis V and VI: 0.1 mL of ETW (100 and 200 μg/mL in 0.5% CMC, respectively). Each Petri dish was incubated for 15 min at 37 °C. Then each petri dish was challenged with compound 48/80 (10 μg/mL) and incubated for 10 min again. The pieces of intestinal mesentery were mounted on slides. All slides were air dried, then stained with 0.1% *O*-toluidine blue, at room temperature for 5 min. Mast cells were rapidly identified by their metachromatic cytoplasmic granules under light microscopy.[31] Percentage inhibition of mast cell degranulation (MCD) for each treated group was calculated by following formula: % inhibition of MCD = (1- Number of degranulated cells/the total number of mast cells) × 100.

2.9. Histamine-induced bronchospasm in Guinea pigs

Taur et al.[32] suggested the method for experimentally induced bronchospasm by histamine aerosol exposing to guinea pig. Briefly, guinea pig of either sex was selected and grouped (n = 5). Animals were treated with extracts of ET as shown in Table 2. Animals showed progressive dyspnea when they were challenged with 1% w/v histamine aerosol at 40 mmHg pressure from nebulizer of histamine chamber (M/s Inco Ambala, India). The time of onset of pre-convulsive dyspnea (PCD) recorded as pre-convulsive time (PCT). The moment when the PCD were recorded immediately the animals were relocated in the fresh air. This time of PCD was taken as day 0 value for the treatment. On day 5th, the PCT was recorded after 2 h of administration of ET extracts. Percent increase PCD was estimated using equation: % PCD = $(1 - T_1/T_2) \times 100$ Where T_1 = time for PCD onset on day 0, T_2 = time for the PCD onset on day 5^{th}.

3. Statistical analysis

A statistic software Graph Pad Prism version 6 (GraphPad Software, Inc. USA) was used to evaluate the statistical difference between different groups. The values are expressed as the mean ± SEM for each group. The statistical significance was performed using One-Way ANOVA followed by, post hoc Bonferroni-multiple comparison test. A *P*-value ≤ 0.05 was considered to be statistically significant.

4. Results

4.1. Acute oral toxicity study

The aqueous and the methanol extract of ET did not show any mortality, morbidity and any significant changes in the general behavior of rats up to a dose of 2500–5000 mg/kg. Therefore, the dose of extracts selected was one tenth of the highest tolerable dose (2500–5000 mg/kg) for the present study.

4.2. The effect of ET extracts on triple antigen and horse serum-induced anaphylaxis in rats

The symptomatic score of the animals challenged with 0.25 mL of horse serum was calculated. On day 14th after the 2 h of administration of treatment the sensitized rats were challenged with intravenous administration of horse serum induced active anaphylaxis symptoms characterized by increased respiratory rate, dyspnoea, cyanosis & mortality as compared to normal control rats ($P < 0.001$). The rate pre-treated with, ETM 500 mg/kg and ETW 500 mg/kg caused significant ($P<0.001$) delay in the onset of anaphylactic reaction along with reduced symptomatic score post antigen challenge on 14^{th} day compared with that of sensitized control animals. It was observed that animals treated with standard drug prednisolone showed highest protection against anaphylactic shock symptoms when compared with disease control group. Comparison among the treatment groups showed that ETM 500 mg/kg was more effective than ETW 500 mg/kg b.w. ($P < 0.05$) while ETM and ETW 250 mg/kg showed lesser statistical difference (Fig. 1).

4.3. The effect of ET extracts on serum WBC and differential count (DC) in horse serum-induced anaphylaxis in rats

The serum revealed different types of White Blood Cells (WBC). Moreover, differential leukocyte count revealed that the absolute number of each type of leukocytes (eosinophils; lymphocytes; monocytes; and neutrophils) in the asthmatic control group was significantly ($P<0.001$) higher than that in normal control group. Sensitized rats treated with prednisolone showed a significant ($P<0.001$) decrease in the elevated eosinophil, lymphocyte, and monocytes count as compare to sensitized control. However, there was no significant change in the neutrophil counts in sensitized control rats. The treatment with ETM and ETW showed a significant

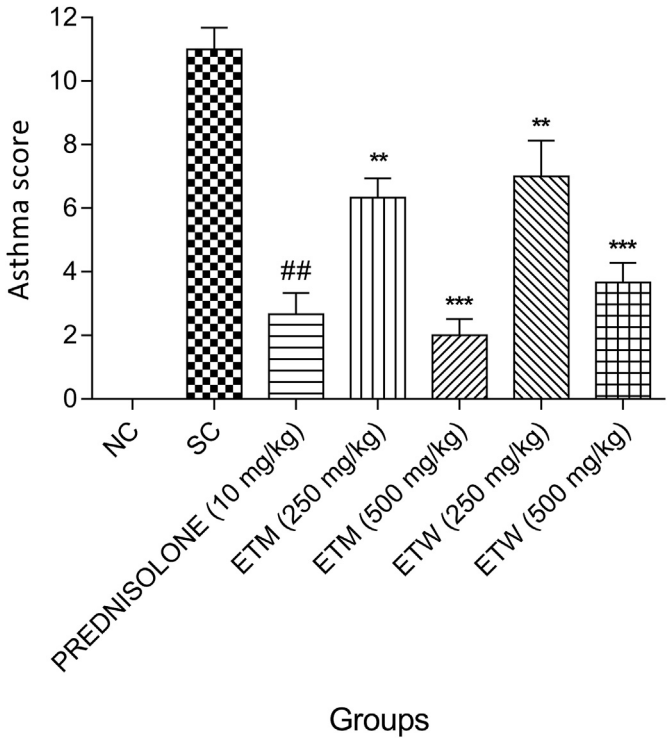

Fig. 1. Asthma score of ET treated animals. Values are expressed as mean ± S.E.M. of 6 animals. #$P < 0.05$ compared to NC, **$P < 0.01$ and ***$P < 0.001$ compared to SC.

($P<0.001$) reduction in the levels of eosinophil, lymphocytes, monocytes and neutrophils count as compared to sensitized animals (Table 1).

4.4. The effect of ET extracts on serum IgE in BALF of horse serum-induced anaphylaxis in rats

Increase in serum IgE level in horse serum challenged rats was significantly ($p < 0.001$) suppressed by treatment with ETM 500 mg/kg and ETW 500 mg/kg by 30.02% and 26.40% respectively (% Inhibition was calculated by dividing difference of IgE on 14th and 0 day with IgE level on 14th day of the same group and subsequently subtracting it from 100) when compared with sensitized control (Table 1). The efficacy ETM and ETW treatment at higher dose was almost comparable to that of standard drug prednisolone 10 mg/kg b.w. ($P < 0.001$).

4.5. The effect on, ET extracts on TNF-α, IL-4 and IL-5 levels in BALF of horse serum induced anaphylaxis in rats

The determination of the TNF-α, IL-4, and IL-5 levels in BALF were estimated by using ELISA (iMark, Biorad, USA), and the results of different groups were compared. As shown in Table 1, the levels of TNF-α, IL-4, and IL-5 were significantly ($P < 0.001$) increased in BALF in horse serum challenged rats as compared with normal control group. Animals treated with ET extracts showed a significant protective effect as compared to the sensitized group. TNF-α, IL-4, and IL-5 levels were significantly decreased in treatment groups. However, treatment with ETM (500 mg/kg) showed highest percentage inhibition of TNF-α, IL-4 and IL-5 (51.11%, 41.86%, and 37.44% respectively) as compared to other extracts.

4.6. The effect of ET extracts on mast cell degranulation

The rat intestinal mesentery when challenged with antigen (compound 48/80) caused significant degranulation of mast cells *in vitro*. Pre-treatment with ETM (100 and 200 μg/mL), ETW (100 and 200 μg/mL) or kitotifen (20 μg/mL) showed significant ($P < 0.001$) protection against antigen induced degranulation in respective treatment groups. In the present study, ETM at 200 μg/mL was more effective in preventing mast cell degranulation when compared with ETM 100 μg/mL ($P < 0.001$) and ETW 200 μg/mL ($P < 0.01$) (Table 2).

4.7. The effect of ET extracts on histamine-induced bronchospasm on Guinea pigs

Treatment with ETM and ETW showed a significant and dose-dependent delay in the onset of PCD following exposure to histamine aerosol when compared with their 0 day PCT. The % increase in PCT after treatment with ETM (500 mg/kg b.w.) and ETW (500 mg/kg b.w.) was found to be 72% and 70.90% respectively. The ETM at a dose of 500 mg/kg b.w. showed significant increase in PCT when compared with ETW 250 mg/kg b.w. treated guinea pigs ($P < 0.05$), whereas no statistically significant difference was observed among other treatment groups indicating equipotency in delaying onset of PCD in histamine induced bronchospasm in guinea pigs (Table 3).

4.8. Histopathology of lungs

There was no change in normal animal (Fig. 2a). In contrast, a histological section of sensitized control lung showed significant changes, in airway lumen (Av), thickened pseudostratified columnar epithelium (#), the high accumulation of lymphocytes ($), severe eosinophilic edema fluid deposition (*) and parenchymal inflammation observed [Fig. 2b]. Treatment with ET showed a significant histopathological change as compared to sensitized control groups (Fig. 2c–g).

5. Discussion

Allergic asthma is a chronic airway inflammatory disease. Asthma is mostly phenotype featured by the influx of leukocytes into lung and airway.[5] According to Ayurveda, *Euphorbia thymifolia* possess several activities, including anti-inflammatory, antiviral, anti-anthelmintic and also used in dysentery, diarrhea. The current investigation was performed to evaluate the effect of extract of ET on the murine model of allergic asthma. In addition, mast cell degranulation and histamine-induced bronchospasm were also used to study the protective effect this plants in asthma.

Phytochemical screening of ETM and ETW showed the presence of similar phytoconstituents such as saponins, flavonoids, polyphenols and glycoside. In an earlier study, it is reported that saponins possess antiallergic, antihistaminic and mast cell stabilizing activities.[33] Flavonoids present in many plants also exhibited smooth muscle relaxant and bronchodilator activity.[34] Furthermore, histamine releasing induced by comp 48/80 was also inhibited by flavonoids.[35] All these constituents present in the ETM and ETW may exert a protective effect in the sensitized rat by several mechanisms.

In the present investigation, the anaphylactic shock was induced by triple antigen followed by injection of horse serum. The euglobulin fraction of horse serum is responsible for triggering the allergic response.[36] In the present study, the triple antigen and horse serum was administered subcutaneously to sensitize the rats. This consequence of active anaphylactic shock is manifested by respiratory symptoms such as dyspnea, cyanosis, and death.[37] The result of the present study indicates that the treatment with ET exhibit a significant protection from mortality as well as

Table 1
Effect of ET extract on triple antigen induced anaphylaxis in rats.

Parameters	NC[a]	SC[a]	Prednisolone[a] 10 mg/kg	ETW[a] 250 mg/kg	ETW[a] 500 mg/kg	ETM[a] 250 mg/kg	ETM[a] 500 mg/kg
Total cell (cells/mL)	4960 ± 3.64	10200 ± 20.0	6010 ± 15.18*	9340 ± 10.23*	8100 ± 11.67*	7950 ± 20.23*	6780 ± 14.34*
Lymphocytes (%)	69.7 ± 2.1	30.2 ± 3.3	61.8 ± 3.3*	42.90 ± 2.6*	49.25 ± 3.1*	40.43 ± 2.7*	58.43 ± 2.4*
Monocytes (%)	1.5 ± 1.1	10.1 ± 1.0	3.6 ± 1.3*	12.11 ± 1.2*	10.10 ± 1.1*	9.56 ± 1.1*	7.2 ± 1.3*
Neutrophils (%)	26.91 ± 1.2	38.50 ± 1.5	27.20 ± 1.7*	35.78 ± 1.5*	27.98 ± 1.4*	33.45 ± 1.8*	26.67 ± 1.6*
Eosinophil (%)	1.89 ± 1.5	21.2 ± 1.3	7.4 ± 1.5*	19.23 ± 1.4*	12.67 ± 1.2*	16.56 ± 1.5*	9.7 ± 1.1*
IgE (ng/mL)	182.3 ± 16.21	317.5 ± 19.09	175.0 ± 9.66*	263.8 ± 10.26*	233.8 ± 11.25*	251.8 ± 7.09*	222.3 ± 12.89*
TNF-α (pg/mL)	214.0 ± 4.00	887.8 ± 1.96	188.0 ± 0.63*	744.0 ± 6.78*	606.0 ± 9.27*	706.6 ± 7.78*	434.0 ± 12.88*
IL-4 (pg/mL)	23.68 ± 0.82	77.56 ± 0.67	29.87 ± 1.32*	65.86 ± 1.96*	54.95 ± 1.54*	58.04 ± 2.06*	45.09 ± 1.84*
IL-5 (pg/mL)	33.60 ± 0.81	77.80 ± 0.73	28.80 ± 1.45*	70.47 ± 1.23*	57.65 ± 0.69*	65.54 ± 1.09*	48.67 ± 2.37*

[a] Mean ± S.E.M. (n = 6), Statistical analysis was performed using ANOVA followed by Tukey multiple comparison post hoc test. Significance between vehicle and drug/extract treated rats denoted by *$P < 0.001$ when compared with sensitized control.

Table 2
Effect of ET extracts on compound 48/80 induced mast cell degranulation in rats.

Groups	% Granulated mast cells[a]	% Degranulated mast cells[a]
Sensitized control	17.50 ± 1.96	82.50 ± 1.96
Ketotifen fumarate 20 μg/mL	82.50 ± 1.96	17.50 ± 1.96*
ETW 100 μg/mL	57.17 ± 1.22	42.83 ± 1.22*
ETW 200 μg/mL	49.50 ± 1.68	50.50 ± 1.68*
ETM 100 μg/mL	40.83 ± 0.74	59.17 ± 0.74*
ETM 200 μg/mL	38.00 ± 1.33	62.00 ± 1.33*

[a] Mean ± SEM; n = 6, Statistical analysis was performed using ANOVA followed by Tukey multiple comparison post hoc test. Significance between vehicle and drug/extract treated rats denoted by *$P < 0.001$ when compared with sensitized control.

Table 3
Effects of ET extracts on histamine-induced bronchospasm in guinea pigs.

Groups	Preconvulsive dyspnea time (sec)		
	Before treatment (control)[a]	After treatment[a]	% increase in the time of PCD
ETW 250 mg/kg	127.8 ± 20.33	352.50 ± 24.56*	63.74
ETW 500 mg/kg	127.6 ± 18.45	438.57 ± 27.56*	70.90
ETM 250 mg/kg	128.0 ± 18.56	379.38 ± 19.33*	66.26
ETM 500 mg/kg	126.9 ± 15.89	465.59 ± 27.57*	72.20

[a] Mean ± SEM; n = 5, Statistical analysis was performed using ANOVA followed by Tukey multiple comparison post hoc test. Significance between vehicle and drug/extract treated rats denoted by *$P < 0.001$ when compared with control.

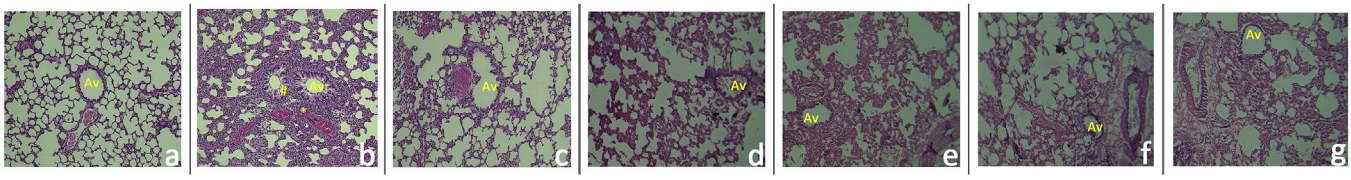

Fig. 2. Qualitative evaluations of anti-anaphylactic and antiasthmatic effects of aqueous and methanolic extracts of ET in rats for 14 days. Histological study of lung sections (representative H&E-stained) from: (a) NC group, (b) SC group; triple antigen and horse serum challenged group treated with saline solution, (c) Prednisolone 10 mg/kg, (d) ETW extract 250 mg/kg, (e) ETW extract 500 mg/kg, (f) ETM extract 250 mg/kg, (g) ETM extract 500 mg/kg. Objective lens X100. Av indicates airway lumen; # indicates thickened pseudo-stratified columnar epithelium; $ indicates the high accumulation of lymphocytes and * indicates parenchymal inflammation. Only a representative picture is shown for each group.

amelioration of active anaphylactic reactions. It also confirms its anti-allergic activity. Furthermore, antigen challenge resulted in a significant increase in the eosinophils, total leukocytes and monocytes counts while a decrease in lymphocytes counts. Treatment with ET significantly reversed the change in blood cell induced by antigen challenge.

The IgE plays an important role in the type-I allergic reaction in asthma. It causes disruption of mast cells to induce the release of histamine, leukotriene, and various cytokines/chemokine. In view of this study on the effect of ET on IgE levels. It was found that both treatments were able to decrease the serum levels of IgE when it was challenged with horse serum. Furthermore, cytokines secreted by mast cell disruption, particularly IL-4 and IL-5 have a specific role in the development of allergic diseases. IL-4 demonstrates a vital function in immune response as it triggers the synthesis of IgE in B cells.[38] Treatment with ET significantly altered in the increased levels of IL-4 found in the BALF of sensitized animals. However, in the airway inflammation IL-5 plays a vital role in maturation and recruitment of eosinophil.[39] It was found that IL-5 strongly elevated in BALF of sensitized animals. The results of the present study indicated inhibitory effects of ET on IL-5 production in BALF. These results suggest that ET could contribute the protection in airway inflammation via modulation of the eosinophil function.

TNF-α is an important cytokine which plays a key role in the immediate host defense before activation of the adaptive immune system. The possible role of TNF-α contributes to inflammatory response supported by the observation that TNF-α, mRNA and protein levels were increased in allergic asthma.[40] As described earlier, the release of mediators from mast cells plays a crucial role in the airway hypersensitivity and bronchospasm in asthma. TNF-α induced release of histamine from mast cells directly contributes in the positive autocrine loop that influences the mast cell cytokine secretion.[41] The raised levels of TNF-α in BALF were significantly ameliorated by ET compared to sensitized animals.

Mast cells synthesize, store and activation of the release of several biologically active molecules. In allergic asthma, allergen-induced mast cell activation causes release mediators to induce disordered airway function. Treatment with ET exerts the protective effect of the mast cell from degranulation by comp 48/80 as a result of the inhibition of IgE synthesis. Due to the effect of ET, the antigen-antibody reaction appears to have been modifying their by the release of mediators are controlled. On the other hand, the protective effect of ET could be explained by inhibition of the calcium entries into the cell membrane from the extracellular fluid of degranulated mast cells that was induced by comp 48/80.[42,43]

The histamine-induced bronchospasm is a conventional model of an antigen-induced airway blockade. Inhalation of histamine causes hypoxia which leads to convulsion and causes smooth muscle contraction. The prominent effect of histamine showed a severe bronchoconstriction in animals. The results of the present study confirmed the protective effect of ET extracts in asthma via significant prolongation in the latent period of convulsions followed by exposure to histamine aerosol.

6. Conclusion

Our finding revealed that ET have a significant anaphylactic activity which might be due controlling the levels of IgE, cytokines and pro-inflammatory mediators in experimental models. Furthermore, the antiallergic effect of both plants possesses stabilizing effect in an antigen-induced degranulation of the mast cells membrane and prolonged the latent period of convulsion. In conclusion, ET is effective in the treatment of asthma since it shows antiallergic potential. However, results proved that ETM 500 mg/kg possesses a more significant antiasthmatic effect than the all extracts.

Declaration of interest

The authors report no declarations of interest.

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References

1. Boyce AJ, Austen KF. Allergies, anaphylaxis, and systemic mastocytosis. In: *Harrison's Principles of Internal Medicine*. fifteenth ed. New York. USA: McGraw-Hill Education; 2015.
2. Bousquet J, Aubier M, Sastre J, et al. Comparison of roflumilast, an oral anti-inflammatory, with beclomethasone dipropionate in the treatment of persistent asthma. *Allergy Eur J Allergy Clin Immunol*. 2006;61(1):72–78. https://doi.org/10.1111/j.1398-9995.2005.00931.x.
3. Kuby J. *Immunology*. third ed. New York. USA: W. H. Freeman and Company; 1997.
4. Kay a B. Overview of "allergy and allergic diseases: with a view to the future". *Br Med Bull*. 2000;56(4):843–864. https://doi.org/10.1258/0007142001903481.
5. Rivera DG, Hernández I, Merino N, et al. *Mangifera indica* L. extract (Vimang) and mangiferin reduce the airway inflammation and Th2 cytokines in murine model of allergic asthma. *J Pharm Pharmacol*. 2011;63(10):1336–1345. https://doi.org/10.1111/j.2042-7158.2011.01328.x.
6. Heib V, Becker M, Taube C, Stassen M. Advances in the understanding of mast cell function. *Br J Haematol*. 2008;142(5):683–694.
7. Doria Rossi MI, De Oliveira Barreto E, Pires ALA, et al. Long-term exacerbation by interleukin 13 of IgE-mediated eosinophilia in rats. *Int Immunopharm*. 2005;5(9):1353–1364. https://doi.org/10.1016/j.intimp.2005.02.014.
8. Stone KD, Prussin C, Metcalfe DD. IgE, mast cells, basophils, and eosinophils. *J Allergy Clin Immunol*. 2010;125(2 suppl 2). https://doi.org/10.1016/j.jaci.2009.11.017.
9. Salib RJ, Drake-Lee a, Howarth PH. Allergic rhinitis: past, present and the future. *Clin Otolaryngol Allied Sci*. 2003;28(4):291–303. https://doi.org/10.1046/j.1365-2273.2003.00706.x.
10. Khare CP. *Indian Herbal Remedies: Rational Western Therapy, Ayurvedic and Other Traditional Usage*. Springer; 2004:43–45. https://doi.org/10.1007/978-3-642-18659-2.
11. Garipelli N, Runja C, Potnuri N, Pigili RK. Anti-inflammatory and anti-oxidant activities of ethanolic extract of *Euphorbia thymifolia* Linn whole plant. *Int J Pharm Pharmaceut Sci*. 2012;4(SUPPL.3):516–519.
12. Kane SR, Apte VA, Todkar SS, Mohite SK. Diuretic and laxative activity of ethanolic extract and its fractions of *Euphorbia thymifolia* Linn. *Int J ChemTech Res*. 1(2):974–4290.
13. Sandeep L, Kane R, Mohite SK, Shete JS. Antihelmintic activity of aqueous and methanolic extracts of *Euphorbia thymifolia*. *Int J PharmTech Res*. 1(3):666–669.
14. Khan NH, Rahman M, Nur-e-Kamal MS. Antibacterial activity of *Euphorbia thymifolia* Linn. *Indian J Med Res*. 1988;87:395–397.
15. Lin CC, Cheng HY, Yang CM, Lin TC. Antioxidant and antiviral activities of *Euphorbia thymifolia* L. *J Biomed Sci*. 2002;9(6 Pt 2):656–664. doi:67281 [pii].
16. Mamatha G, Prabhakar T, Madhuri V. Anti-arthritic activity of *Euphorbia thymifolia* Linn. *World J For*. 2014;3:1323–1331.
17. Aamir S, Thippeswamy BS, Kulkarni VH, Karunakar H. Hepatoprotective effect of *Euphorbia thymifolia* whole plant extract on CCl_4 induced hepatic damage in rats. *Int J Res Ayurveda Pharm*. 2011;2(2):681–686.
18. Tona L, Kambu K, Mesia K, et al. Biological screening of traditional preparations from some medicinal plants used as antidiarrhoeal in Kinshasa, Congo. *Phytomedicine*. 1999;6(1):59–66. https://doi.org/10.1016/S0944-7113(99)80036-1.
19. Gupta B, Srivastava RS, Goyal R. Therapeutic uses of *Euphorbia thymifolia*: a review. *Pharmacogn Rev*. 2017;1(2):299.
20. Lee SH, Tanaka T, Nonaka G ichiro, Nishioka I. Hydrolysable tannins from *Euphorbia thymifolia*. *Phytochemistry*. 1990;29(11):3621–3625. https://doi.org/10.1016/0031-9422(90)85288-Q.
21. Kainsa S, Singh R, Singh R, Singh R. Flavan-3-ol isomers isolated from *Euphorbia thymifolia* Linn. *Pharmacogn Commun*. 2016;6(1):28–33. https://doi.org/10.5530/pc.2016.1.5.
22. Prasad K, Bisht G. Evaluation of nutritive minerals and antioxidants values of *Euphorbia thymifolia* Linn. *Curr Res Chem*. 2011;3(2):98–105. https://doi.org/10.3923/crc.2011.98.105.
23. *Test No. 423: Acute Oral Toxicity - Acute Toxic Class Method*. OECD Publishing; 2002. https://doi.org/10.1787/9789264071001-en.
24. Turner R. *Screening Methods in Pharmacology*. first ed. New York. USA: Academic Press; 1965.
25. Gupta S. Some observations on the anti-asthmatic effect of the saponins of *Gardenia latifolia*. *Asp Allergy Appl Immunol*. 1974;7(1):198–204.
26. Gupta SS, Paresh RM, Ram AK. Development of anti-allergic and antihistaminic activity in relation to histamine releasing effects of a plant saponin form *Clerodendron serratum*. *Asp Allergy Appl Immunol*. 1968;2:133–142.
27. Brusselle GG, Kips JC, Tavernier JH, et al. Attenuation of allergic airway inflammation in IL-4 deficient mice. *Clin Exp Allergy*. 1994;24(1):73–80. https://doi.org/10.1111/j.1365-2222.1994.tb00920.x.
28. Minami M, Konishi T, Jiang Z, Arai T, Makino T. Effect of Shin'iseihaito on murine allergic reaction induced by nasal sensitization. *J Tradit Complement Med*. 2016;6(3):252–256. https://doi.org/10.1016/j.jtcme.2015.06.001.
29. Holgate S, Casale T, Wenzel S, Bousquet J, Deniz Y, Reisner C. The anti-inflammatory effects of omalizumab confirm the central role of IgE in allergic inflammation. *J Allergy Clin Immunol*. 2005;115(3):459–465. https://doi.org/10.1016/j.jaci.2004.11.053.
30. Culling C. *Handbook of Histopathological and Histochemical Techniques: Including Museum Techniques*. third ed. London: Butterworth; 1974.
31. NORTON S. Quantitative determination of mast cell fragmentation by compound 48/80. *Br J Pharmacol Chemother*. 1954;9(4):494–497. https://doi.org/10.1111/j.1476-5381.1954.tb00867.x.
32. Taur DJ, Patil RN, Patil RY. Antiasthmatic related properties of *Abrus precatorius* leaves on various models. *J Tradit Complement Med*. 2017. https://doi.org/10.1016/j.jtcme.2016.12.007.
33. Gupta SS, Tripathi RM. Effect of chronic treatment of the saponin of *Clerodendron serratum* on disruption of the mesenteric mast cells of rats. *Asp Allergy Appl Immunol*. 1973;6:177–188.
34. Taur DJ, Patil RY. Evaluation of antiasthmatic activity of *Clitoria ternatea* L. roots. *J Ethnopharmacol*. 2011;136(2):374–376. https://doi.org/10.1016/j.jep.2011.04.064.
35. Youssouf MS, Kaiser P, Tahir M, et al. Anti-anaphylactic effect of *Euphorbia hirta*. *Fitoterapia*. 2007;78(7-8):535–539. https://doi.org/10.1016/j.fitote.2007.06.003.
36. Dale HH, Hartley P. Anaphylaxis to the separated proteins of horse-serum. *Biochem J*. 1916;10(3):408–433.
37. Andhare RN, Raut MK, Naik SR. Evaluation of antiallergic and anti-anaphylactic activity of ethanolic extract of *Sanseveiria trifasciata* leaves (EEST) in rodents. *J Ethnopharmacol*. 2012;142(3):627–633. https://doi.org/10.1016/j.jep.2012.05.007.
38. Holloway J, Rorke S, Beghé B, Holgate S. Genetic influences on asthma severity. In: Szefler S, Leung D, eds. *Severe Asthma: Pathogenesis and Clinical Management*. New York. USA: Marcel Dekker; 2001:19–38.
39. Lee JS, Yang EJ, Yun CY, Kim DH, Kim IS. Suppressive effect of *Petasites japonicus* extract on ovalbumin-induced airway inflammation in an asthmatic mouse model. *J Ethnopharmacol*. 2011;133(2):551–557. https://doi.org/10.1016/j.jep.2010.10.038.
40. Berry M, Brightling C, Pavord I, Wardlaw A. TNF-alpha in asthma. *Curr Opin Pharmacol*. 2007;7(3):279–282. https://doi.org/10.1016/j.coph.2007.03.001.
41. Coward WR, Okayama Y, Sagara H, Wilson SJ, Holgate ST, Church MK. NF-kappa B and TNF-alpha: a positive autocrine loop in human lung mast cells? *J Immunol*. 2002;169(9):5287–5293. https://doi.org/10.4049/jimmunol.169.9.5287.
42. Baruah CC, Gupta PP, Nath A, Patnaik LGK, Dhawan BN. Anti-allergic and anti-anaphylactic activity of picroliv—a standardised iridoid glycoside fraction of *Picrorhiza kurroa*. *Pharmacol Res*. 1998;38(6):487–492. https://doi.org/10.1006/phrs.1998.0397.
43. Pepy J. Asthma physiology. In: Austen KF, Lichtenstein LM, eds. *Immunopharmacology and Treatment*. New York. USA: Academic Press; 1973:280–310.