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Amelioration of anaphylaxis, mast cell degranulation and bronchospasm by *Euphorbia hirta* L. extracts in experimental animals

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ABSTRACT

The current investigation was aimed to assess anti-anaphylactic, mast cell stabilizing and anti-asthmatic activity of methanol and aqueous extract of *Euphorbia hirta* L. (Euphorbiaceae) on experimental animals. Anaphylaxis was induced by administration of horse serum and triple antigen vaccine subcutaneously in albino Wistar rats. Extracts of *E. hirta* (EH) were administered to the rats in dose of 250 and 500 mg/kg b. w. 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 tumour necrosis factor (TNF)-α were measured by ELISA commercial kit from Broncho alveolar lavage fluid (BALF). Histopathological changes of lungs were observed. Anti-asthmatic activity of extracts of EH 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 EH 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 EH extracts. The pre-treatment with extracts of EH displayed significant reduction in degranulation of mesenteric mast cell numbers. The treatment with extracts of EH significantly increased in time of pre-convulsive dyspnoea (PCD). Thus, these findings concluded that *E. hirta* could be effectively used in the treatment of anaphylaxis and asthma.

1. Introduction

Asthma is a worldwide public health problem affecting about 300 million people and asthma prevalence increases globally by 50% every decade (Masoli et al., 2004). Asthma is a "chronic inflammatory disorder of the airway with reversible form of airway obstruction, either spontaneously or with treatment". Airway inflammation in asthma patients involves multiple components and is orchestrated by numerous cell types, particularly mast cells, eosinophils, macrophages, CD4 + T lymphocytes and epithelial cell (Bousquet et al., 2006).

Allergen-specific Th2 lymphocytes are the key orchestrators of this inflammation, starting and engendering inflammation through the arrival of their cytokines interleukin (IL)-4, IL-5 and IL-13. IL-5 recruits and activates eosinophils, which help airway inflammation and discharge eosinophil cationic protein (ECP) which has turned into a standout amongst the most critical markers of the illness (Casolaro et al., 1989; Humbert et al., 1996; Stone et al., 2010). This also leads to synthesis of allergen-specific IgE that binds to high-affinity IgE receptors (FcεRI) on the surface of the mast cells. Exposure of IgE-coated mast cells to the same antigen causes cross-linking of IgE and triggers rapid activation and degranulation of the mast cells, which then releases numerous pro-inflammatory mediators (histamine, prostaglandins, leukotrienes, platelet activating factor, TNF-α, cytokines etc.) into the tissue surrounding the cells (Jarjour et al., 1997; Liu et al., 1991).

The accessible treatment choices for upper and lower respiratory tract hypersensitive disorders have significant impediments because of low adequacy, related unfavourable events and agreeability issues. In this respect, Ayurveda, an Indian system of medicine, has represented a few medications from indigenous plant sources in the treatment of bronchial asthma and allergic disorders.

The medicinal plant *Euphorbia hirta* L. (Family Euphorbiaceae) known as *Dudheli*, grows widely in most parts of India and in other tropical countries, especially on roadsides and on wasteland (Linfang et al., 2012). The *E. hirta* (EH) traditionally used to treat

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gastrointestinal diseases like diarrhoea, dysentery, and intestinal parasitosis (Anonymous, 2003). *In vivo* studies from literature survey emphasized that ethanolic extract of EH extract possesses antibacterial activity and antifungal activity against pathogens (Andhare et al., 2012; Mohamed et al., 1996; Suresh, 2008). The preclinical claims of aqueous and methanol extracts of aerial part of EH exhibited anticancer (Anonymous, 2003), antimalarial (Liu et al., 2007), antidiarrheal (Galvez et al., 1993), anxiolytic, anti-inflammatory, analgesic and antipyretic activities (Lanhers et al., 1990; Martínez Vázquez Mariano and Ramírez Apan Teresa, 1999). It has been also reported that ethanol extract of *E. hirta* has also been shown to inhibit polysorbate 80-induced degranulation of isolated peritoneal mast cells *in vitro* (Ramesh and Padmavathi, 2010). Phytochemical screening of *E. hirta* revealed the presence of several chemicals such as afzelin, quercitrin, myricitrin, euphorbin-A, euphorbin-B, euphorbin-C, euphorbin-D including flavonoids like rutin, quercitin which may be responsible for its strong anti-oxidative (Basma et al., 2011) and antidiabetic activity (Kumar et al., 2010). However, there is lack of scientific data regarding anti-asthmatic potential of *E. hirta* extracts. Hence, this investigation was aimed to evaluate the antiasthmatic potential of methanol and aqueous extract of *E. hirta* using *in vivo* and *in vitro* experimental animal models for anaphylaxis, mast cell degranulation, and bronchospasm.

2. Material and methods

2.1. Reagents

Horse serum was procured from HIMEDIA Chemicals (Mumbai, India), Triple antigen (DPT Vaccines) was purchased from local market (Mfg. by Serum Institute of India Pvt. Ltd.). Histamine and Compound 48/80 were purchased from Sigma Aldrich, St. Louis, MO, USA. Ketotifen fumarate was procured from Torrent Research Centre, Ahmedabad, India. O-toludine blue, other reagents and chemicals used in the experiment were of analytical grade purchased from Merck (India).

2.2. Plant Material

Fresh aerial parts of EH was gathered during the September 2013 from Amargadh town, Taluka, locale of Rajkot, Gujarat, India. The taxonomical confirmation of the plant was confirmed and voucher specimen No. DP/SVU/PHCOG/Herb/02 was kept at CSIR-NISCAIR, New Delhi, India. The collected aerial part was washed under running tap water to clear the soil and sun dried.

2.3. Extraction

The collected aerial parts were sun dried, pulverized and passed through sieve #40. The cold maceration for 72 h was performed to prepare methanol (EHM) and aqueous (EHW) extracts of EH. The extracts were concentrated using a water bath (Mack, Ahmedabad, India) and subsequently lyophilized and stored at −20 °C until further use. The % yield of EHM and EHW extracts was 18.56% and 8.45% (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 standard rat pellet diet (Pranav Agro Ltd. Vadodara, India) and had 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. Triple antigen and horse serum-induced active anaphylaxis in rats

Forty-two 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 (Gupta, 1974). The sensitized animals were divided into 7 groups of 6 animals each. Group I served as normal control (NC) received normal diet and drinking water *ad libitum*, group II served as sensitized control (SC) received 0.5% CMC (1 mL/kg b.w, p.o.), group III was administered standard prednisolone (10 mg/kg b.w., p.o.), group IV and V were administered EHW (250 mg/kg and 500 mg/kg b.w., p.o. respectively) while group VI and VII were received EHM (250 mg/kg and 500 mg/kg b.w., p.o. respectively), once a day for 14 days. All extracts and prednisolone were suspended in 0.5% CMC solution and administered orally be gastric gavages. At the end 14 days, 2 h after treatment, all the animals were challenged by i.v. injection of 0.25 mL of horse serum in saline through tail vein except Group I. The antigen challenged animals were monitored to 1 h for onset of anaphylactic symptoms. The severity of symptoms was scored as follows: No visual symptoms- 0, increased respiratory rate- 2, increased respiratory rate with immobility- 4, dyspnoea for 10 min- 6, cyanosis for 10 min- 8, dyspnoea with cyanosis for 10 min- 10 and respiratory failure and death- 12 (Gupta et al., 1968).

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

On day 14th, after the aforementioned treatment, the blood was collected from *retro*-orbital plexus under light ether anaesthesia and stored with or without ethylene diamine tetra acetate for estimation of WBC, total leukocyte, eosinophil count, and % polymorphs count. The serum was separated (using EDTA) and stored at −70 °C and analyze for IgE levels. For the collection of BALF, 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 supernatant was analyze for TNF-α, IL-4 and IL-5.

2.5.2. Quantification of serum IgE

Serum IgE was quantified with an ELISA kit (#: 555248, BD Biosciences Pharmingen, San Diego, CA) according to the manufacturer's protocol and results were reported in ng/mL for protein (Brusselle et al., 1994; Minami et al., 2016).

2.5.3. 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 (Holgate et al., 2005; Minami et al., 2016).

2.5.4. Lung Histopathology

After the collection of BALF, the lungs from rats were isolated, and immersed in 10% formalin. The paraffin-embedded lungs were cut in thin sections (5 μm thickness) with the help of microtome. The thin sections were then 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 (Culling, 1974).

2.6. The mast cell stabilization activity in rats

The adult albino Wistar rats ware sacrificed with excess anesthetic diethyl ether and mesentery were separated carefully,

placed in Petri plate containing Ringer-Locke solution for 30 min and grouped as follows: Petri plate I: Ringer Locke solution (sensitized control), Petri plate II: 0.1 mL of Ketotifen (20 μg/mL), Petri plate III and IV: 0.1 mL of EHW (100 and 200 μg/mL in 0.5% CMC, respectively) and Petri plate V and VI: 0.1 mL of EHM (100 and 200 μg/mL in 0.5% CMC, respectively). Each Petri plate was incubated for 15 min at 37 °C, then challenged with compound 48/80 (10 μg/mL) and again incubated for 10 min. The pieces of intestinal mesentery were mounted on slides, air dried followed by staining 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 (Norton, 1954). Percentage inhibition of mast cell degranulation (MCD) for each treated group was calculated by following formula: % inhibition of MCD = (1-Number of degranulated cells/total number of mast cells) × 100.

2.7. Histamine-induced bronchospasm in guinea pigs

Bronchospasm was experimentally induced by exposing guinea pig to histamine aerosol as per the method described by Taur et al., 2017. Briefly, guinea pigs of either sex were selected and grouped as follows (n = 5); Group I and II received EHW 250 mg/kg and 500 mg/kg b.w. orally, respectively. Group III and IV received EHM 250 mg/kg and 500 mg/kg b.w. orally, respectively. Animals showed progressive dyspnoea 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 dyspnoea (PCD) recorded as pre-convulsive time (PCT). The moment the PCD occurred, animals were immediately relocated in the fresh air. The required for the onset of PCD was taken as day 0 value for treatment groups. On 5th day, the PCT was recorded 2 h after the last dose of EH extracts. Percentage increase in PCT was estimated using equation: % PCT = (1 – T_1/T_2) × 100 Where T_1 = time for PCD onset on day 0, T_2 = time for the PCD onset on day 5.

3. Statistical analysis

The values were expressed as the mean ± SEM for each group. A statistic software Graph Pad Prism version 6 (GraphPad Software, Inc. USA) was used to evaluate the statistical difference between different groups using ANOVA followed by Tukey multiple comparison post hoc test. $p \leq .05$ indicate statistical significance difference.

4. Results

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

Intravenous administration of horse serum on 14 th day induced active anaphylactic shock symptoms characterized by increased respiratory rate, dyspnoea, cyanosis & mortality as compared to normal control rats ($p < .001$). The rats were pre-treated with EHM 250 mg/kg, EHM 500 mg/kg and EHW 500 mg/kg caused significant ($p < .001$) delay in the onset of anaphylactic reaction along with reduced symptomatic score post antigen challenge on 14th 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 EHM 500 mg/kg was more effective than EHW 250 mg/kg b.w. ($p < .05$) while no statistical difference was observed among other treatment groups (Fig. 1).

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

Elevated levels of total WBCs, monocytes, neutrophils and eosinophils and reduced level of lymphocytes count in antigen challenged animals were significantly ($p < .001$) ameliorate by treatment with EHM and EHW in dose dependent manner compared with sensitized control animals (Table 1). Moreover, these animals also showed a significant rise in monocytes, neutrophils and eosinophils count ($p < .001$) while reduction in lymphocytes count ($p < .001$) when compared with that of normal control. Intervention with EHM and EHW significantly reduced total WBCs ($p < .001$ for treatment groups), eosinophils ($p < .001$ for all treatment groups), neutrophils ($p < .001$ for all treatment groups) and increased lymphocyte count ($p < .001$ for all treatment groups) in

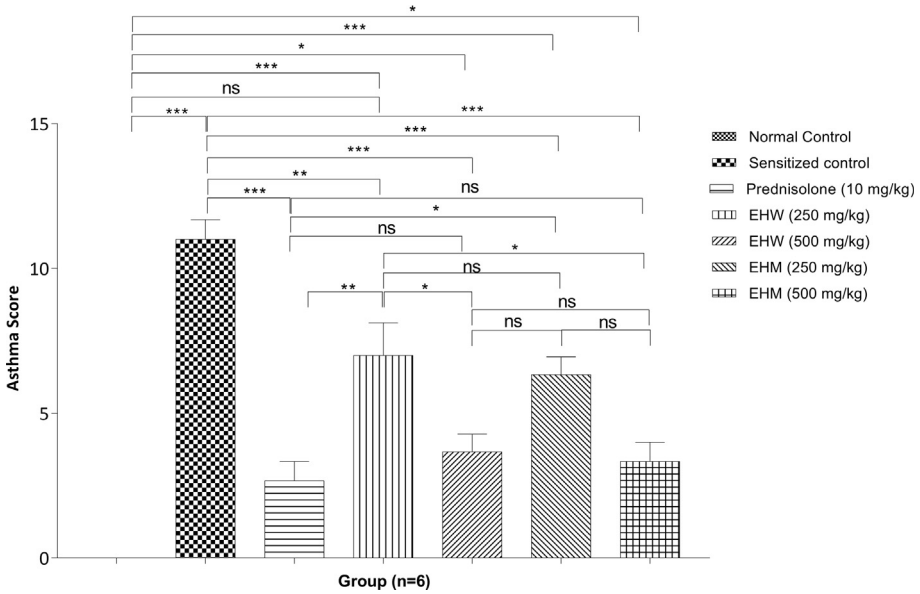

Fig. 1. Effect of *Euphorbia hirta* extracts on triple antigen and horse serum induced anaphylaxis in rats. The each column represents the asthma score are expressed as Mean ± S.E.M. (n = 6). Statistical analysis was performed using ANOVA followed by Tukey multiple comparison post hoc test. Significance between vehicle and drug/extract treated rats denoted by *p < .05, **p < .01, ***p < .001 when compared between the each group.

Table 1
Effect of EH extract on blood total cell count and differential count in triple antigen induced anaphylaxis in rats.

Parameters	NC	SC	Prednisolone 10 mg/kg	EHW 250 mg/kg	EHW 500 mg/kg	EHM 250 mg/kg	EHM 500 mg/kg
Total cell (cells/mL)	4960 ± 3.64	10200 ± 20.0$^{\$}$	6010 ± 15.18$^{\$,\#}$	9120 ± 73.48$^{\$,\#,\dagger,\#}$	7850 ± 12.41$^{\$,\#,\ \dagger,\#,\ b,\#}$	7960 ± 22.00$^{\$,\#,\ \dagger,\#,\ b,\#}$	6550 ± 14.53$^{\$,\#,\ \dagger,\#,\ d,\#}$
Lymphocytes (%)	69.7 ± 2.1	30.2 ± 3.3$^{\$}$	61.8 ± 3.3$^{\#}$	40.8 ± 2.2$^{\$,\ \dagger,\#}$	50.5 ± 1.0$^{\$,\#,\ \dagger,*,}$	40.5 ± 1.1$^{\$,\ \dagger,\#}$	58.3 ± 3.1$^{\dagger,\#,\ a,\#,\ d,\#}$
Monocytes (%)	1.5 ± 1.1	10.1 ± 1.0$^{\$}$	3.6 ± 1.3**	7.36 ± 1.42†	7.22 ± 1.3†	7.5 ± 1.0†	4.7 ± 1.1$^{\dagger,\ *}$
Neutrophils (%)	26.91 ± 1.2	38.50 ± 1.5$^{\$}$	27.20 ± 1.7$^{\#}$	34.20 ± 1.7$^{\dagger,\ \dagger,*}$	29.80 ± 1.6**	37.20 ± 1.4$^{\$,\ \dagger,\#,\ a,*}$	28.50 ± 1.1$^{\#,\ d,**}$
Eosinophils (%)	1.89 ± 1.5	21.2 ± 1.3$^{\$}$	7.4 ± 1.5$^{\#}$	17.64 ± 1.0$^{\$,\ \dagger,\#}$	12.48 ± 0.2$^{\$,\ \#}$	14.8 ± 1.3$^{\$,\ *,\ \dagger,**}$	8.5 ± 1.7$^{\dagger,\ \#,\ a,\#,\ c,*}$

Values are expressed as 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 $^{\dagger}p < .05$, $^{\ddagger}p < .01$, $^{\$}p < .001$ when compared with NC, $^{*}p < .05$, $^{**}p < .01$, $^{\#}p < .001$ when compared with SC, $^{\dagger,*}p < .05$, $^{\dagger,**}p < .01$, $^{\dagger,\#}p < .001$ when compared with Prednisolone, $^{a,*}p < .05$, $^{a,**}p < .01$, $^{a,\#}p < .001$ when compared with EHW 250 mg/kg, $^{b,*}p < .05$, $^{b,**}p < .01$, $^{b,\#}p < .001$ when compared with EHW 500 mg/kg, $^{c,*}p < .05$, $^{c,**}p < .01$, $^{c,\#}p < .001$ when compared with EHM 250 mg/kg, $^{d,*}p < .05$, $^{d,**}p < .01$, $^{d,\#}p < .001$ when compared with EHM 500 mg/kg.

blood when compared with vehicle treated sensitized control animals. In case of monocytes, only reference drug prednisolone at a dose of 10 mg/kg b.w. could significantly reduce blood monocytes count when compared with disease control ($p < .01$).

4.3. The effect of EH extracts on serum IgE in horse serum-induced anaphylaxis in rats

Increase in serum IgE level in horse serum challenged rats was significantly ($p < .001$) suppressed by treatment with EHM 500 mg/kg and EHW 500 mg/kg by 40.50% and 28.86% respectively when compared with sensitized control (Fig. 2). The efficacy EHM and EHW treatment at higher dose was almost comparable to that of standard drug prednisolone 10 mg/kg b.w. ($p < .001$).

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

As shown in Fig. 3, the levels of TNF-α, IL-4, and IL-5 were significantly ($p < .001$) increased in BALF in horse serum challenged rats as compared with normal control group. Animals treated with EH extracts and prednisolone showed a significant ($p < .001$) decline in TNF-α, IL-4, and IL-5 levels in BALF when compared with sensitized control group. The inhibitory effect of EH extracts against TNF-α, IL-4 and IL-5 was dose dependent between 250 and 500 mg/kg with highest inhibition to 57.91%, 54.95%, and 47.98% respectively.

4.5. The effect of EH 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 EHM (100 and 200 μg/mL), EHW (100 and 200 μg/mL) or ketotifen (20 μg/ml) showed significant ($p < .001$) protection against antigen induced degranulation in respective treatment groups. In the present study, EHM at 200 μg/mL was more effective in preventing mast cell degranulation when compared with EHM 100 μg/mL ($p < .001$) and EHW 200 μg/mL ($p < .01$) (Fig. 4).

4.6. The effect of EH extracts on histamine-induced bronchospasm in guinea pigs

Treatment with EHM and EHW 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 EHM (500 mg/kg b.w.) and EHW (500 mg/kg b.w.) was found to be 80.28% and 76.76% respectively. The EHM at a dose of 500 mg/kg b.w. showed significant increase in PCT when compared with EHW 250 mg/kg b.w. treated guinea pigs ($p < .05$), 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 (Fig. 5).

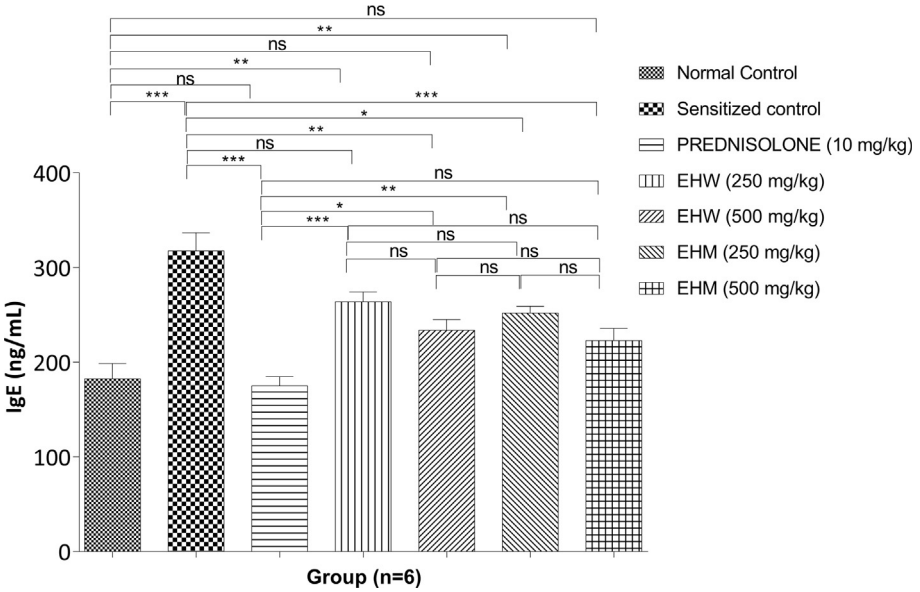

Fig. 2. Effect of *Euphorbia hirta* extracts on triple antigen and horse serum induced anaphylaxis in rats. The IgE levels in serum are expressed as Mean ± S.E.M. (n = 6). Statistical analysis was performed using ANOVA followed by Tukey multiple comparison post hoc test. Significance between vehicle and drug/extract treated rats denoted by $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$ when compared between the each group.

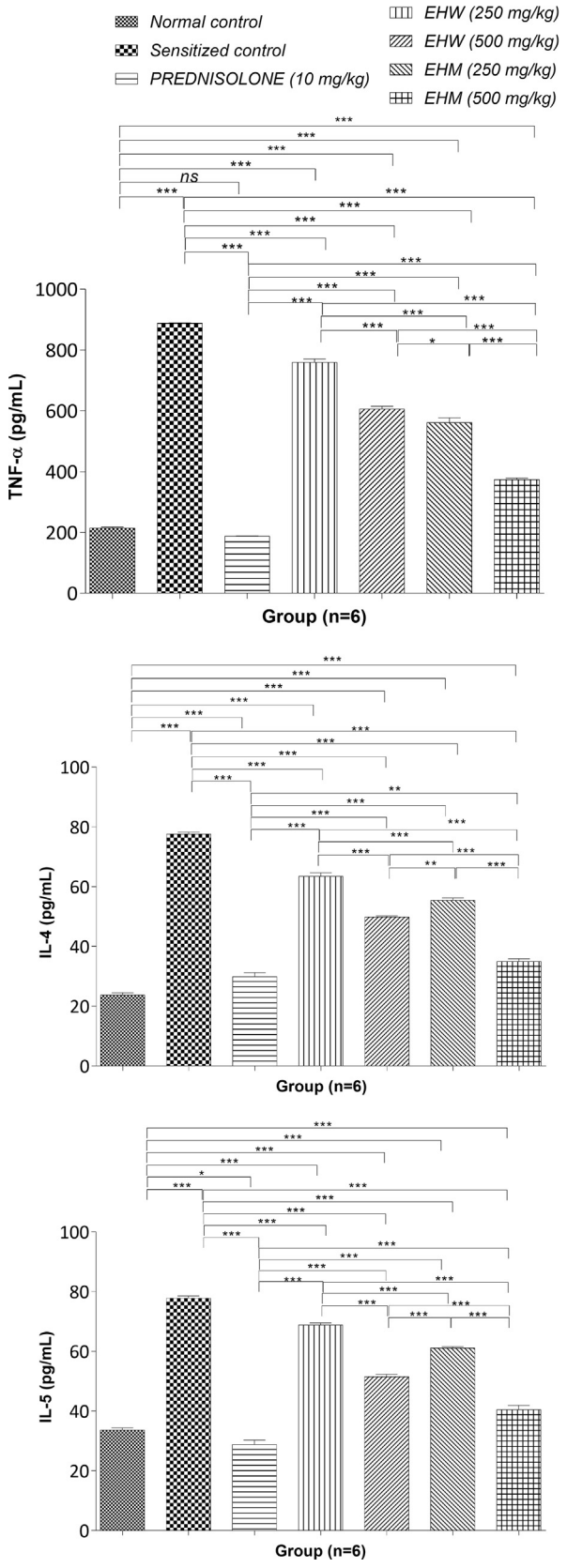

Fig. 3. Effect of *Euphorbia hirta* extracts on triple antigen and horse serum induced anaphylaxis in rats. The TNF-α, IL-4 and IL-5 levels from BALF are expressed as Mean ± S.E.M. (n = 6). Statistical analysis was performed using ANOVA followed by Tukey multiple comparison post hoc test. Significance between vehicle and drug/extract treated rats denoted by *p < .05, **p < .01, ***p < .001 when compared between the each group.

4.7. Histopathology of lungs

Fig. 6a represents lung section of normal animal with no change in cellular architecture. The lung section of sensitized control animal showing abnormal changes in airway lumen (Av), thickened pseudostratified columnar epithelium (#), increased infiltration of lymphocytes ($), severe eosinophilic edema (∗) and parenchymal inflammation observed (Fig. 6b). Fig. 6c–g represents lung section of animals treated with prednisolone 10 mg/kg, EHM 250 mg/kg, EHM 500 mg/kg, EHW 250 mg/kg, EHW 500 mg/kg and EHM 250 mg/kg b.w. respectively showed a significant protection against triple antigen and horse serum induced inflammatory changes in lungs as compared to sensitized control group suggesting protective role of methanol and aqueous EH extracts as anti-inflammatory and anti-anaphylactic in asthma.

5. Discussion

The present investigation was attempt to evaluate the antiasthmatic potential of methanol and aqueous extract of *E. hirta* in triple antigen and horse serum-induced active anaphylaxis in rats and histamine induced bronchospasm in Guinea pigs. Furthermore, *in vitro* mast cell stabilization activity was also assessed to evaluate anti-allergic potential of *E. hirta* extract.

In the current experimentation, anaphylactic shock was induced by subcutaneous administration of triple antigen vaccine followed by sensitization with horse serum. A globulin protein present in horse serum fraction responsible for triggering anaphylactic response in rats (Dale and Hartley, 1916). The present investigation shows the dose dependent amelioration of active anaphylactic reactions, marked by respiratory symptoms such as dyspnoea, cyanosis, and death (Andhare et al., 2012) in triple antigen and horse serum induced anaphylaxis in rat. The treatment with EH extracts exerted significant protection from mortality which substantiate its anti-allergic potential. Moreover, treatment with both EHM 500 mg/kg and EHW 500 mg/kg b.w. significantly ameliorated respiratory symptoms in antigen challenged animals. The protection afforded by EH extracts could be attributed to inhibition of histamine release from antigen activated mast cell (Youssouf et al., 2007) as well as anti-inflammatory activity of EH (Ekpo and Pretorius, 2008).

The phenomena of an allergic response: an immediate hypersensitivity; intervened by the IgE–FcεRI complex on mast cells surface leads allergic reaction in different organs. This crosslinking is responsible for the highly cytokinergic activation of early phase of allergic reaction like mast cell (degranulation) and synthesis of cytokines and chemokines. These lipid mediators recruits and activate inflammatory cells, which is the main pathway that commands the setting of anaphylaxis (Gould and Sutton, 2008; Holgate, 1999). In the present investigation the serum level of IgE in horse serum sensitized rats was significantly reduced by administration of EH extracts. Hence it appears EH extracts has potential to regulate anti-allergic activity or events associated with IgE-mediated anaphylactic reactions probably due to stabilization of mast cell membrane and thereby inhibit the release of inflammatory mediators (Ramesh and Padmavathi, 2010).

Ample of evidences suggest that cytokines secreted by mast cell disruption, particularly IL-4 and IL-5 have a specific role in the development of allergic diseases (Essayan et al., 1996; Humbert et al., 1996; Ying et al., 1997). In our study, BALF collected from antigen sensitized animals showed significantly high levels of TNF-α, IL -4 and IL- 5 when compared with normal control animals. Administration of extracts EHM 500 mg/kg and EHW 500 mg/kg b.w. significantly lowered pro-inflammatory cytokines levels in BALF suggesting protective effect of extracts against allergen induced inflammatory responses in lungs and airways of sensitized

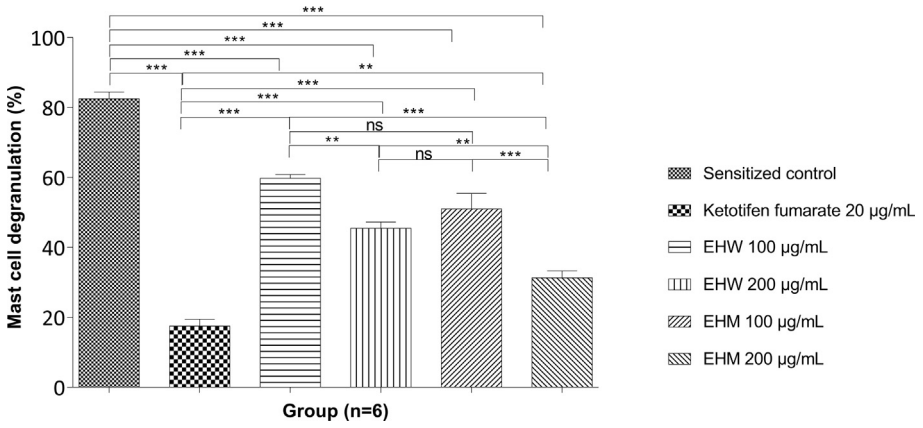

Fig. 4. Effect of *Euphorbia hirta* extracts on compound 48/80 induced mast cell degranulation in rats. The percentage mast cell degranulation are expressed as Mean ± S.E.M. (n = 6). Statistical analysis was performed using ANOVA followed by Tukey multiple comparison post hoc test. Significance between vehicle and drug/extract treated rats denoted by *p < .05, **p < .01, ***p < .001 when compared between the each group.

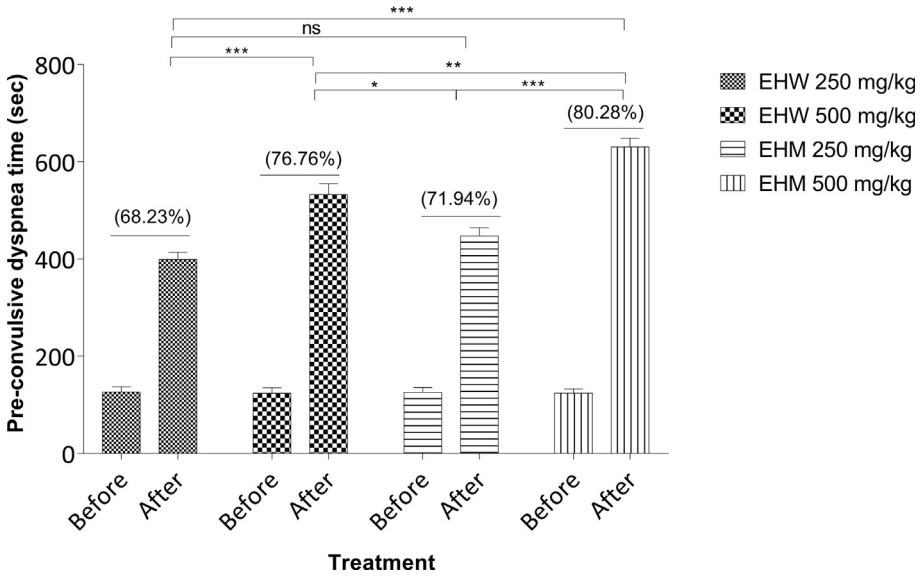

Fig. 5. Effect of *Euphorbia hirta* extracts on histamine-induced bronchospasm in Guinea pigs. The pre-convulsive dyspnoea time are expressed as Mean ± S.E.M. (n = 5). The percent increase in PCT after treatment is mentioned in bracket. Statistical analysis was performed using ANOVA followed by Tukey multiple comparison test post hoc test. Significance between vehicle and drug/extract treated rats denoted by *p < .05, **p < .01, ***p < .001 when compared between the each group.

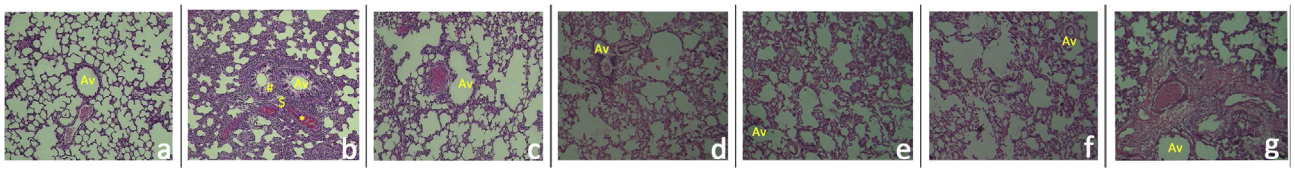

Fig. 6. Qualitative evaluations of anti-anaphylactic and antiasthmatic effects of aqueous and methanolic extracts of EH in rats for 14 days. Histological study of lung sections (representative H&E-stained) from: (a) NC group, (b) SC group; triple antigen and horse serum challenged group treated with saline solution, (c) Prednisolone 10 mg/kg, (d) EHW extract 250 mg/kg, (e) EHW extract 500 mg/kg, (f) EHM extract 250 mg/kg, (g) EHM extract 500 mg/kg. Objective lens 100×. 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.

animals. The observed effect could be attributed to the ability of EH extracts to modulate/suppress the function of B-lymphocytes and eosinophils during inflammatory response.

Mast cells play vital role as an activator to trigger allergic reaction via cross linking with a high affinity IgE receptor (FCεRI) (Gohil and Mehta, 2011) and release various cytokines and eicosanoids and secretory granules (Amin, 2012). Many researchers have reported that degranulation of mast cells is one of the crucial events during allergen induced asthma due to release of several bioactive molecules such as histamine, leukotrienes and various cytokines/chemokines which plays an important role in activating underlying mechanisms of asthma (Casolaro et al., 1989; Galli et al., 2005; Norton, 1954; Stone et al., 2010).

To study these effects, rat mesenteries excised from antigen challenged animals were exposed to antigenic compound 48/80, which promotes histamine release by activation of G-proteins and

influx of Ca^{2+} ions into the mast cell, ability to induce mast cell degranulation (Paul and Seder, 1994). Treatment with EH exerts, especially EHM and EHW at a dose of 500 mg/kg b.w. showed significant protection against compound 48/80 induced mast cell degranulation. The observed effect of EH extracts could be attributed to the ability of phytoconstituents to control the release of inflammatory mediators by virtue of their ability to modify antigen-antibody reaction, thereby preventing degranulation of mast cells (Youssouf et al., 2007). Another probable explanation for suppressive action of EHM 500 and EHW 500 on mast cells degranulation could be attributed to inhibition of calcium ions entry into the cell membrane from the extracellular fluid after compound 48/80 challenge (Baruah and Gupta, 1998; Pepy, 1973). This finding evident that EH extracts may exert anti-asthmatic potential with similar mechanism outlined for the reference drug.

Bronchoconstriction is considered as a vital in asthma, results from antigen-induced airway blockade via H_1 receptor (Matsumoto et al., 1996). The histamine-induced bronchospasm in guinea pigs is a conventional model used to study anti-asthmatic effect. The results of the present study confirmed the protective effect of EH extracts in asthma based on the observations that EHM and EHW at a dose of 500 mg/kg b.w. significantly prolonged the latent period of convulsions (PCT) following exposure to histamine aerosol. The observed protective effect of EH extracts could be due to antihistaminic potential of their phytoconstituents (Gupta and Tripathi, 1973; Taur and Patil, 2011). Furthermore, Youssouf et al., 2007 have reported that antigen (compound 48/80) induced histamine release is also inhibited by flavonoids. Preliminary phytochemical screening of EHM and EHW showed the presence of various phytoconstituents such as saponins, flavonoids, polyphenols and glycosides (Parmar and Pundarikakshudu, 2017). Therefore, it is suggested that these constituents might have contributed synergistically in exerting overall protection by one or more mechanisms in antigen induced asthma.

6. Conclusion

Our findings revealed that EH extracts have significant anti-anaphylactic and anti-inflammatory activities which might be due their inhibitory/ modulatory actions on levels of IgE, cytokines and pro-inflammatory mediators in experimental models. Moreover, the anti-allergic effect of both plant extracts (methanol and aqueous) could be due to their stabilizing effect on antigen-induced degranulation of the mast cell membrane and prolongation in latent period of convulsion. The present study confirms the therapeutic usefulness of EH extracts in the treatment of allergen induced asthma since it showed anti-allergic, anti-inflammatory, mast cell stabilizing and bronchial smooth muscle relaxant effects in *in vivo* and *in vitro* models of asthma. Furthermore, present investigation also revealed that EHM 500 mg/kg w.b. was most potent as an asthmatic agent among the treatment groups and can be considered as a potential candidate in the management of asthma.

7. Declaration of interest

The authors report no declarations of interest.

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