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

Ghanshyam Parmar [a,*], Kilambi Pundarikakshudu [b], R. Balaraman [a], Girish Sailor [a]

[a] Department of Pharmacy, Sumandeep Vidyapeeth, At post: Piparia, Taluka: Waghodia, Dist: Vadodara-391760 Gujarat, India
[b] Department of Pharmacognosy, L. J. Institute of Pharmacy, Between Sarkhej Circle and Kataria Motors, S. G. Road, Ahmedabad 382210, Gujarat, India

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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

* Corresponding author.
E-mail address: ghanstaurus22@gmail.com (G. Parmar).

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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,

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