



EVALUATION OF ANTI-ALLERGIC AND ANTI-ANAPHYLACTIC ACTIVITY OF ETHANOLIC EXTRACT OF SEEDS OF *LINUM USITATISSIMUM*

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ABSTRACT

To investigate the anti-anaphylactic and anaphylactic properties of Ethanolic extract of Seeds of *Linum Usitatissimum* The anti-anaphylactic activity of was studied by using models such as passive cutaneous anaphylaxis, passive paw anaphylaxis and its anaphylactic activity Castor oil-induced diarrhea in rats albino rats was also investigated using ketotifen and Loperamide as reference drugs. A dose-dependent beneficial effect was observed on leakage of evans blue dye in skin challenged with antigen and on paw anaphylaxis induced by antiserum. The ethanolic extract of Seeds of *Linum Usitatissimum* also exhibited significant ($P<0.01$) inhibition of rat paw edema and granuloma tissue formation, including significant protection of RBC against the haemolytic effect of hypotonic solution. The results of this investigation revealed that *Linum Usitatissimum* contains pharmacologically active substances with anaphylactic properties. These attributes may provide the rationale for the use of *Linum Usitatissimum* in diarrhoea management by traditional healers. Further research is needed to fractionate the ethanol extract and isolate the molecule(s) responsible for the anaphylactic activity observed. Anti-anaphylactic activity of ethanolic extract of Seeds of *Linum Usitatissimum* may be possibly due to inhibition of the release of various inflammatory mediators. Anaphylactic activity of ethanolic extract of Seeds of *Linum Usitatissimum* may be pharmacologically active substances with anaphylactic properties.

INTRODUCTION

Allergic disorders are in rise every year and stated as an endemic disease of the 21st century. Some of the allergic disorders, which may be caused by an allergen originating from immune system, environment, and by genes, are, asthma, eczema, hay fever, anaphylaxis, autoimmune disorders (1).

A number of plants are described in Ayurveda for use in the treatment of allergic disorders, namely psoriasis, eczema, bronchial asthma, etc. But only a few have been studied for their antiallergic activity. On activation, mast cells immediately released the preformed and the de novo synthesized mediators such as histamine, proteases, leukotrienes, prostaglandins, and cytokines (2)

As a consequence, the acute reactions such as vasodilation, increased vascular permeability, and bronchoconstriction were induced. In addition, allergic responses also trigger the influx and activation of a variety of inflammatory cells including eosinophils and lymphocytes. Rapidly released mediators and numerous cytokines produced by mast cells are strongly believed to induce and sustain these responses, which may contribute to chronic inflammation. Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemicals or microbiological agents. Inflammation is body's response to inactivate or destroy the invading organisms, remove irritants and set stage for tissue repair (3).

Inflammation is triggered by the release of chemical mediators from the injured tissues and migrating cells. The specific chemical mediators vary with the type of inflammatory process and include amines such as histamine, serotonin, lipids such as prostaglandins and small peptides such as kinins (4).

Conventional anaphylactic drugs such as steroidal anaphylactic drugs (SAID) and nonsteroidal anaphylactic drugs (NSAID) are used in the treatment of most of the acute and chronic pain and inflammatory disorders including rheumatoid arthritis. However, long-term use of these agents may produce serious adverse effects. Thus, it is worth developing new plant-derived anaphylactic agents with fewer adverse effects. *Linum Usitatissimum* is one such plant, which is reputed to have numerous applications in traditional medicine. The plant has been mentioned in ancient literature as anaphylactic, ant-allergic, diuretic, carminative, laxative, anti-ulcer, and useful in treating leprosy and ascites, and also possesses cytotoxic activity and anti-microbial activity (5).

In the present paper we report the anti-anaphylactic, and anaphylactic of the isolated ethanolic extract of Seeds of *Linum Usitatissimum*.

MATERIALS AND METHODS

ANIMALS

Wistar rats (150-200 g), of either sex were arranged from IIMT College of pharmacy. Animal's were maintained in our animal house under conditions [temperature ($24 \pm 1^\circ\text{C}$), relative humidity (45-55%), light (12 h) and dark (12 h) cycle] and free access to ready-made food pellets and water *ad libitum*. The

experimental protocols were approved by the Institutional Animal Ethics Committee of IIMT college of Pharmacy.

DRUGS, CHEMICALS AND REAGENTS

Histamine hydrochloride (Analab Fine Chemicals, Mumbai), egg albumin (Loba Chemie, Mumbai), horse serum (Serum Institute of India Ltd., Pune) and other drugs, dexamethasone, ketotifen fumarate (Cipla Healthcare Ltd., India) were procured

PLANT MATERIALS

The plant seeds specimen for the proposed study was collected from the local market of Dadri UP It was identified and authenticated by Dr. Ashwani Kumar Goyal, HoD (department of botany) Govt. Post Graduate College Noida Gautam Budh Nagar.

EXTRACTION

Extraction procedure Dried and powdered seeds (500 g) were extracted using a soxhlet extractor with solvents of increasing polarity beginning with petroleum ether followed by ethanol then water and finally chloroform, each extraction was carried out for 8-10 hours continuously. The solvents were removed using a rotary vacuum evaporator at 40°C to give concentrated extracts which were frozen and freeze-dried until use (6)

ACUTE TOXICITY STUDY

Acute toxicity study-up and down procedure was carried out as per the guidelines by Organization for Economic Co-operation and Development (OECD) 423. Mice (6/group) were divided into six groups. The first 5 groups received oral doses of 100, 200, 300, 400 and 500 mg/kg of isolated ethanolic extract of Seeds of *Linum Usitatissimum*. The sixth group received saline (10 mL/kg) orally. Mortality was assessed 24 h after administration. The animals were also observed for toxic symptoms and mortality was determined 24 h after treatment. (7)

ANTI-ALLERGIC ACTIVITY

STUDY ON PASSIVE CUTANEOUS ANAPHYLAXIS

The Wistar rats of either sex were injected intraperitoneally with 0.2 mL, 10% egg albumin, 0.2 mL of bordetella pertusis vaccine on day 1, 3, and 5. After 21 days of first immunization, blood was collected from orbital plexus under light ether anesthesia.

The blood was allowed to clot and serum was separated by centrifugation at 1 500 rpm. The separated serum was stored at 20 °C until it was used for the experiment.

Rats (6 per group) were divided into five groups. The first 3 groups received oral doses of 10, 25 and 50 mg/kg of the ethanolic extract of Seeds of *Linum Usitatissimum*. The 4th and 5th groups were treated orally with ketotifen (5 mg/kg *p.o.*) as a reference drug and saline (10 mL/kg) as control, respectively. The anti ovalbumin serum was injected intradermally on the dorsal skin of the animal. Drug/extract was administered to animal according to their group for three consecutive days from the day of sensitization. After treatment, 1 mL of 0.5% evans blue solution containing 20 mg of egg albumin was injected intravenously through tail vein. Because of antigen-antibody reaction there was increased vascular permeability and dye would penetrate that tissue area. This area of skin was removed after sacrificed. The skin portion was transferred to the solution of 70% acetone for 24 h. The dye was extract out in the acetone and evans blue dye was measured colorimetrically at 620 nm. The amount of dye penetrating in the skin area reflects the severity of hypersensitivity reaction. The % inhibition was calculated by using the formula: $(C-T/C) \times 100$. (8)

STUDY ON ACTIVE CUTANEOUS ANAPHYLAXIS IN RATS

Thirty rats were sensitized by injecting (s.c.) 0.5 ml of horse serum/rat. Sensitized rats were randomly divided into five groups (6/group). Group II served the induced control received CMC (10 ml/kg, *p.o.*). Groups III, IV and V were administered EELU (250, 500 and 1000 mg/kg, *p.o.*) respectively, and group VI with dexamethasone (0.27 mg/kg, *i.p.*) daily for ten days. Group I did not receive any treatment and served the usual control. On 10th day, 2 h after the treatment, the rats were challenged with 0.25 ml (diluted 1:1 in standard saline) horse serum/rat by intravenous injection. The symptoms of anaphylactic reactions like

respiratory distress, increased respiratory rate, dyspnoea, cyanosis and mortality were recorded. The severities of anaphylactic reactions were respiratory scored on a scale (0-20). The scoring system was: increased respiratory rate (2), dyspnea for 10 min. (4), dyspnea and cyanosis for 10 min (8) and respiratory collapse and death(9)

HISTAMINE INDUCED PAW EDEMA

Rats were divided into five groups (6/group) and treated with EELU (500 and 1000 mg/kg, *p.o.*) and ibuprofen (50 mg/kg, *p.o.*) and vehicle control (CMC, *p.o.*). Normal control group did not receive any treatment. The EELU and ibuprofen were administered 30 min prior to the injection of 0.1 ml histamine (1% w/v) into the planter region of the right paw of each rat (Hollisopple et al., 1980). The paw volume was measured prior, at 2 and 3 h using plethysmometer (Orchid scientifics, Nashik).¹⁰

RESULTS

PRELIMINARY PHYTOCHEMICAL ANALYSIS

Preliminary Phytochemical Analysis for Flax seed Powder showed the presence of glycerides, saponins, alkaloids and flavonoids

ACUTE TOXICITY STUDIES

Acute toxicity study showed that the ethanolic extract of Seeds of *Linum Usitatissimum* possessed high safety profile as no death was observed at oral doses of 100-2000 mg/kg in mice.

ANTI-ANAPHYLACTIC ACTIVITY

In the study on passive cutaneous anaphylaxis model, ethanolic extract of Seeds of *Linum Usitatissimum* produced a significant dose dependent decrease in the amount of evans blue dye leaked at site when compared with control. Standard drug also produced significant decrease in the amount of evans blue dye leaked at site. In passive paw anaphylaxis model, ethanolic extract of Seeds of *Linum Usitatissimum* produced a significant dose dependent decrease in the paw volume induced by antiserum.

Table 1: Passive and active cutaneous anaphylaxis

| Treatment (mg/kg, orally) | mm3 of blueing reaction | Onset of behavioral symptoms in min (% mortality) | Respiratory score (% mortality) |
|---------------------------|-------------------------|---|---------------------------------|
| Normal control (NC) | 0 | Normal | 0 (0) |
| Induced control (IC) | 76.35 ± 6.64 | Respiratory distress/collapse, 3-5 min (70) | 8.80 ± 7.70(60) |
| EZJ (250) | 65.50 ± 5.74 | Dyspnea, 4-8 min (40) | 6.40 ± 4.55(50) |
| EZJ (500) | 59.38 ± 6.60 | Dyspnea, 10-15 min (20) | 5.20 ± 6.57(30) |
| EZJ (1000) | 54.35 ± 5.46 | Normal (0) | 2.40 ± 1.30(20) |
| Dexamethasone (0.27) | 46.40 ± 6.50 | Normal (0) | 2.00 ± 1.23(10) |
| Ketotifen (1) | - | - | - |

Table 2: Effect of ethanolic extract of *Linum Usitatissimum* on histamine induced hind paw edema

| Treatment (mg/kg, p.o.) | Paw edema (ml) at different time interval | | |
|----------------------------|---|-------------|-------------|
| | Initial | 1 h | 3 h |
| Normal control | 0.85 ± 0.04 | 0.86 ± 0.05 | 0.86 ± 0.03 |
| Induced control | 0.87 ± 0.02 | 1.70 ± 0.08 | 1.60 ± 0.05 |
| EEJUJ (500 mg/kg, p.o.) | 0.83 ± 0.01 | 1.50 ± 0.03 | 1.42 ± 0.03 |
| EEJU (1000 mg/kg, p.o.) | 0.86 ± 0.03 | 1.32 ± 0.06 | 1.30 ± 0.02 |
| Ibuprofen (50 mg/kg, p.o.) | 0.87 ± 0.04 | 1.30 ± 0.03 | 1.26 ± 0.04 |
| EEJU(20/50/100 g/ml) | - | - | - |

CONCLUSION

The present study was undertaken for the evaluation of anti-anaphylactic activity and anaphylactic property of a ethanolic extract of Seeds of *Linum Usitatissimum*. The anti-anaphylactic activity was done using passive cutaneous anaphylaxis for evaluation of ethanolic extract of Seeds of *Linum Usitatissimum* on immediate hypersensitivity reaction.

EEJU exert potent anti-allergic activity via inhibition of a) IgE formation and b) chemical mediators released from mast cells by different mechanism(s) by there support traditional uses of *Linum Usitatissimum* in the treatment of allergy related diseases. EEJU can be developed to an anti-allergic herbal medicine by isolating active phytoconstituents individually or in combination and conducting

clinical trials on human subjects to establish themselves its efficacy in the management of allergy.

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