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Original Article

Evaluation of anti-allergic and anti-anaphylactic activity of ethanolic extract of *Zizyphus jujuba* fruits in rodents

Suresh R. Naik[a,*], *Sushant Bhagat*[b], *Priyank D. Shah*[a], *Abhishek A. Tare*[a], *Deepa Ingawale*[a], *Raju R. Wadekar*[a]

[a]*Sinhgad Technical Education Society's, Sinhgad Institute of Pharmaceutical Sciences, Lonavala, Pune, India*
[b]*Sinhgad College of Pharmacy, Vadgaon, Pune, India*

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ABSTRACT

The present study reports the anti-allergic activity of ethanolic extract of *Zizyphus jujuba* Mill., Rhamnaceae, and its possible mode of action. The effect of extract of *Z. jujuba* at different doses (250, 500 and 1000 mg/kg, orally) was simulated on studied animal models of asthma and allergy: a) milk induced eosinophilia and leukocytosis; b) compound 48/80 induced mast cell degranulation; and, c) active and passive cutaneous anaphylaxis. In addition, extract of *Z. jujuba's* effect on sensitized guinea pig ileum (*ex vivo*) and tracheal chain preparations (*in-vitro*) were investigated. Treatment with extract of *Z. jujuba* at all doses significantly: prevented the milk-induced eosinophilia and compound 48/80 induced degranulation of mesenteric mast cells; decreased passive cutaneous and active anaphylactic reactions. In addition, extract of *Z. jujuba* inhibited acetylcholine as well as histamine induced tracheal chain contraction, and also antigen induced contraction of sensitized guinea pig ileum (Shultz-Dale inhibition test). Furthermore, it exhibited also free radicals scavenging activity *(in vitro)*. The observed anti-allergic and anti-anaphylactic activity of extract of *Z. jujuba* may be largely through the stabilization of mast cells by the membrane presence of phytoconstituents (steroidal saponins and flavonoids).

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Introduction

The allergy is an immune disease origin which includes asthma, rhinitis, atopic eczema and dermatitis syndrome (Xie and He, 2005). Anaphylaxis is a hyper response to antigen cross-linking of IgE bound to mast cells, its causes degranulation leading to the release of mediators such as histamine, prostaglandins and later on, proteases, leukotrienes and several pro-inflammatory and chemotactic cytokines (Kalesnikoff and Galli, 2008) triggering smooth muscle contraction, vasodilatation, increased vascular permeability and mucous hyper secretion (Cavalher-Machado et al., 2008).

Zizyphus jujuba Mill, called as Red date, Chinese date, and Bera (Pushto) belongs to family Rhamnaceae which constitute

*Corresponding author.
E-mail: srnaik5@rediffmail.com (S.R. Naik)

fifty genera and more than 900 species. The *Z. jujuba* fruit has been described to the ''fruit of life" and used in traditional Chinese medicine. In addition, it is also used in the treatment of various physiological functions of the body viz. digestive disorders, weakness, urinary tract infections, diabetes, skin infections, loss of appetite, fever, pharyngitis, bronchitis, anemia, hyperlipidemia, and diarrhea (Bown, 1995; Him-Che, 1985). The fresh leaves of *Z. mauritiana* L (one of the species) are used in jaundice (Gul et al., 2009), and it contains therapeutically active phytochemicals, vitamin C, phenolic compounds, flavonoids, triterpenic acids, and polysaccharides. Documented reports of jujuba fruits also shown to produce anti-inflammatory, anti-obesity, immune-stimulating, antioxidant, gastrointestinal and hepatoprotective effect and inhibit foam cell formation in macrophages (Gao et al., 2013) and jujuba containing herbal formulation found to exhibit anticancer activity (Saif et al., 2010).

Z. jujuba grows in Ahamadnagar, Maharashtra State and commonly called the Indian date and fruits of this plant are edible and used for the treatment of various diseases such as pharyngitis, bronchitis, inflammation, liver diseases, and skin infections by local tribal people. Although, *Z. jujuba* is traditionally used for allergy disorder, there is no scientific data available on allergy related disorders. Therefore, objective of the study was evaluation of anti-allergic and anaphylactic activity of ethanolic extract of *Zizyphus jujuba* fruit in animals models and *in-vitro* condition and also to understand its mode of action.

Materials and methods

Animals

Wistar rats (150-200 g), Swiss albino mice (20-25 g), Dunkin-Hartley guinea pigs (350-400 g) of either sex were purchased from Indian Toxicological Institute, Pune. Animals were maintained in our animal house under conditions [temperature (24 ± 1°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 (SCOP/IAEC/Approval/2008-09/13).

Drugs, chemicals and reagents

Compound 48/80 (Sigma Aldrich, USA), histamine hydrochloride (Analab Fine Chemicals, Mumbai), egg albumin (Loba Chemie, Mumbai), horse serum (Serum Institute of India Ltd., Pune) and other drugs, dexamethasone, ketotifen fumerate (Cipla Healthcare Ltd., India) were procured.

Preparation of ethanolic extract of Z. jujuba fruits (EEZJ)

The fruits of *Zizyphus jujuba* Mill, Rhamnaceae, were collected in the month of December from local supplier, Pune. The plant materials was authenticated in the Botany Department of Agharkar Research Institute, Pune, Maharashtra India (voucher specimen number, Auth. 09-02) and deposited. The fruits were shade dried, powdered and sieved through 40# mesh. The dried powder (1000 g) of fruits was extracted by cold maceration for 72 h with ethanol (95%). The ethanolic extract was filtered and concentrated in rotary vacuum evaporator to yield semi solid extract (yield-7.13% w/w) and preserved at 10°C.

Phytochemical analysis of EEZJ

The phytochemical analysis of EEZJ for the presence of alkaloid, saponins, flavonoids, tannins and proteins and spectral studies, FT-IR and UV were performed.

Thin layer chromatography of EEZJ

TLC was performed on pre-coated plates of silica gel 60 F254 (Merck) using solvent system (benzene:ethyl acetate:formic acid, 36:12:5). The phytoconstituents separated on chromatographic plate by running reference compounds (Betulinic acid, oleanolic acid, betulin and lupeol) were identified by spraying the mixture of anisaldehyde and sulfuric acid and scanned at 314 nm and spotted.

Drug preparation

Dexamethasone, ketotifen fumerate, ibuprofen and EEZJ were freshly prepared as 1% w/v suspension uniform incarboxy methyl cellulose (CMC) prior to administration.

Acute toxicity study

Acute toxicity of EEZJ was performed by oral route in mice as per OECD guidelines 423.

Anti-allergic activity evaluation

Effect of EEZJ on milk-induced leukocytosis and eosinophilia in mice

Swiss albino mice (20-25 g) were randomly divided into five groups (6/group). Group-I received CMC (10 ml/kg, *p.o.*), administered group II boiled and cooled milk [4 ml/kg, subcutaneously (*s.c.*)] and groups, III, IV, V and VI received EEZJ (250, 500 and 1000 mg/kg, *p.o.*) and dexamethasone (0.27 mg/kg, *i.p.*) respectively. One hour later, all groups of mice received boiled and cooled milk (4 ml/kg, *s.c.*). Blood samples were collected before and 24 h after milk administration from retro-orbital plexus, under light ether anesthesia and full eosinophils and leukocytes were counted (Bhargava and Singh, 1981).

Passive cutaneous anaphylaxis in rats

Rats were sensitized with 100 µg of egg albumin (EA) adsorbed on 12 mg of aluminum hydroxide (adjuvant) *s.c.* on 1st, 3rd and 5th day. On 11th day, the blood samples were collected and serum was separated. The rats were randomly divided into five groups (6/group). The rat homologous antiserum (100 µl) was injected *s.c.* into the shaved dorsal skin of rats. After 24 h, group I (induced control) received CMC suspension (1% w/v 10 ml/kg, *p.o.*), group II, III, and IV received EEZJ (250, 500 and 1000 mg/kg, *p.o.*, respectively) and group V dexamethasone (0.27 mg/kg, *i.p.*). The rats of all groups were injected

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