Original Article

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

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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...
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 (Anabal Fine Chemicals, Mumbai), egg albumin (Loba Chemie, Mumbai), horse serum (Serum Institute of India Ltd., Pune) and other drugs, dexamethasone, ketotifen fumerate, ibuprofen and EEZJ were freshly prepared as 1% w/v suspension uniform in carboxy methyl cellulose (CMC) prior to administration.

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 were 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 in carboxy 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 eosinocytes 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...
intravenously with 0.5 ml of solution containing mixture (1:1) of Evan’s blue (0.5% w/v) and EA (100 µg) via tail vein, 30 min after the drug/extract treatment. Area of leakage of the blue dye was measured with the help of Vernier Caliper and expressed as mm² (Gautam et al., 1989).

**Effect of EEZJ on serum IgE in rats**

The sensitized rats by the procedure described earlier were randomly divided into four groups (6/group): Group I (induced control) received orally CMC; group II and III, received EEZJ 500 and 1000 mg/kg, orally respectively and group IV dexamethasone (0.27 mg/kg, i.p.) from 4th day onwards up to 10th day. The blood samples of sensitized rats were collected on 6th, 8th and 11th day and serum IgE was assayed.

Serum IgE was determined by sandwich ELISA using anti-rat IgE antibody to capture reagent, and biotin-conjugated anti-rat IgE antibody as detection reagent. Thereafter, peroxidase conjugated extravidin and OPD-H₂O₂ were added. IgE standard used in the concentration range of 0.15-20 ng/ml and samples were diluted to between 1/20 and 1/200. The relative concentration of anti-egg albumin IgE was calculated by comparing with the pooled serum sample of diluted egg albumin-immunized rats to which 10 AU/ml were assigned.

**Active 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 EEZJ (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, dyspnea, cyanosis and mortality were recorded. The severity 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 (12) (Mitra et al., 1999).

**Histamine induced paw edema**

Rats were divided into five groups (6/group) and treated with EEZJ (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 EEZJ 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 (Holsapple et al., 1980). The paw volume was measured prior, at 2 and 3 h using plethysmometer (Orchid scientifics, Nashik).

**Effect of EEZJ on compound 48/80 induced mast cell degranulation in rats**

Thirty rats were sensitized with compound 48/80 (1 mg/kg, s.c.). The sensitized rats were randomly divided into five groups (6/group). Group II induced control, and received (CMC, p.o.). Group II, III, IV received EEZJ (250, 500 and 1000 mg/kg, p.o., respectively) and group V ketotifen fumurate (1 mg/kg, i.p.), daily for seven days. Group I normal untreated control. On the 7th day, rats were injected (i.p.) 10 ml cold phosphate buffered saline. After a gentle abdominal massage, animals were sacrificed; the peritoneal fluid was collected and transferred into siliconized test tube containing 7-10 ml of phosphate buffer (pH 7.4). Mast cell viability was checked by Trypan blue dye (0.4%) exclusion test. Mast cells were purified by percoll density centrifugation method. The pellets of mast cells were resuspended in phosphate buffer solution and incubated with compound 48/80 (5 µg/ml) at 37°C for 10 min. After incubation, cells were centrifuged and stained with Toluidine blue (0.1%). Degranulated and intact mast cells were counted under high power microscopy and percent degranulation was calculated (Andhare et al., 2012).

**Effect of EEZJ on histamine induced contraction of isolated guinea pig ileum preparation**

Fasted guinea pigs were sacrificed and ileum was isolated and mounted in an organ bath containing Tyrode solution, aerated at 37 ± 0.5°C and dose response curves of histamine was studied in the presence and absence of EEZJ and chlorpheniramine maleate and IC₅₀ was calculated.

**Shultz-Dale inhibition test**

Male guinea pigs were sensitized with EA (10 mg/kg, i.p.) on the 1st and 3rd day. On the 14th day, guinea pigs were sacrificed and isolated ileal strip from each animal, suspended in an organ bath containing aerated Tyrode solution at 37°C. Tissue sensitivity was tested with histamine (0.05 µg/ml). EA (10 µg/ml) was added and responses were recorded for 90 s on a Sherrington’s rotating drum. Various concentration of EEZJ (10-150 µg/ml) and disodium cromoglycate (5-10 µg/ml) were added to bath prior to EA and contraction of ileal strips were recorded. The control group induced ileum contraction with EA (10 µg/ml) was 100% considered and compared with contraction of ileum with the test groups (sensitized and treated with EEZJ and disodium cromoglycate). The percent inhibition of ileum contraction was calculated.

**Effect of EEZJ on guinea pig tracheal chain preparation**

Guinea pig trachea was cut into individual rings and tied together in series to form a chain. Tracheal chain was suspended in Kreb’s solution, aerated at 37°C and response curve of acetylcholine (Ach) was studied in the presence and absence of atropine and EEZJ maleate and IC₅₀ was calculated (Chaudhari and Lahiri, 1974).

**Statistical analysis**

The mean ± SEM values were calculated for each group. Analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test was used for statistical analysis. Values of p < 0.05 were considered statistically significance. IC₅₀ values were calculated by the method of Litchfield and Wilcoxon.
Results

Preliminary phytochemical analysis

The preliminary phytochemical analyses of EEZJ showed the presence of saponins, alkaloids, carbohydrates, proteins, and flavonoids and are outlined in Chart 1.

Chart 1
Preliminary physicochemical studies of ethanolic extract of Ziziphus jujuba fruits.

<table>
<thead>
<tr>
<th>Test</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nature and appearance</td>
<td>Semi solid, reddish Brown</td>
</tr>
<tr>
<td>Solubility</td>
<td>Soluble (water, ethanol, methanol), insoluble (toluene, acetone, benzene)</td>
</tr>
<tr>
<td>UV (EtOH) λ max</td>
<td>257, 314</td>
</tr>
<tr>
<td>IR spectra vmax at KBr</td>
<td>EZJ IR values: IR cm⁻¹ 3366 (-OH Stretch), 2927 (-CH stretch), 1733 (-C=O)</td>
</tr>
<tr>
<td>Phytochemicals present</td>
<td>Saponins (++), Alkaloids (++), Proteins (++), flavonoids (++), Triterpenoids (+), Tannins (+)</td>
</tr>
</tbody>
</table>

(+ ) moderate; (+) mild.

Thin layer chromatography of EEZJ

The TLC profile clearly indicates the presence of four phytoconstituents (betulinic acid, oleanolic acid, betulin and lupeol) identified on the basis of color density: oleanolic acid (Rf 0.56) dark violet, betulinic acid (Rf 0.57) light violet, botulin (Rf 0.48) violet and lupeol (Rf 0.72) darker violet (Fig. 1A and 1B).

Acute toxicity study

EEZJ treatment up to 2000 mg/kg orally to mice did not induce mortality or clinical during 96 h observation period. Hence, LD₅₀ was considered to be more than 2000 mg/kg. Based on acute toxicity results, 250, 500 and 1000 mg/kg doses were selected for EEZJ of anti-allergy and anti-anaphylaxis studies.

Anti-allergic activity screening

Effect of EEZJ on milk induced eosinophilia, leukocytosis, and differential leukocytes count

Subcutaneous administration of full milk significantly elevated eosinophils, WBC’s and differential leukocytes (neutrophils and monocytes) in rats. Pretreatment with EEZJ (500 mg/kg and 1000 mg/kg, p.o), dose dependently reduced milk induced eosinophilia, leukocytosis, monocytes, and neutrophils. Dexamethasone treatment showed a greater reduction of blood cells (Table 1).

Abbreviations Phyto-constituents Structure

<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Phyto-constituents</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>samp.</td>
<td>Sample of EZJ</td>
<td></td>
</tr>
<tr>
<td>bet.</td>
<td>betulin (Rf 0.48)</td>
<td></td>
</tr>
<tr>
<td>lp.</td>
<td>lupeol (Rf 0.72)</td>
<td></td>
</tr>
<tr>
<td>b.a.</td>
<td>betulinic acid (Rf 0.57)</td>
<td><img src="image1.png" alt="Structure" /></td>
</tr>
<tr>
<td>o.a.</td>
<td>oleanolic acid (Rf 0.56)</td>
<td><img src="image2.png" alt="Structure" /></td>
</tr>
</tbody>
</table>

Fig. 1 – A, TLC profile of ethanolic extract of Ziziphus jujuba fruits. B, Rf value of phyto-constituents.
Effect of ethanolic extract of *Ziziphus jujuba* fruits on milk induced eosinophilia, leukocytosis and differential leukocytes in mice.

**Table 1**

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Eosinophils per cu. mm (×10³/µl)a</th>
<th>Differential Leukocytes (×10³/µl)b</th>
<th>Total Leukocytes per cu. mmc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (NC)</td>
<td>68.88 ± 7.09</td>
<td>3.20 ± 0.40</td>
<td>6120 ± 28.0</td>
</tr>
<tr>
<td>Induced control (IC)</td>
<td>158.80 ± 13.46</td>
<td>10.60 ± 0.84</td>
<td>9920 ± 50.9</td>
</tr>
<tr>
<td>EZJ (250, p.o.)</td>
<td>120.20 ± 11.67</td>
<td>6.8 ± 0.8</td>
<td>8890 ± 56.4</td>
</tr>
<tr>
<td>EZJ (500, p.o.)</td>
<td>102.00 ± 10.74</td>
<td>7.2 ± 0.6</td>
<td>8690 ± 54.2</td>
</tr>
<tr>
<td>EZJ (1000, p.o.)</td>
<td>94.00 ± 9.90</td>
<td>6.3 ± 0.8</td>
<td>8420 ± 26.0</td>
</tr>
<tr>
<td>Dexamethasone (0.27, i.p.)</td>
<td>89.40 ± 7.71</td>
<td>6.1 ± 0.7+</td>
<td>7980 ± 24</td>
</tr>
</tbody>
</table>

**Effect of EEZJ on anaphylaxis (passive and active cutaneous) and compound 48/80 induced mast cell degranulation (in vivo)**

Pretreatment with EEZJ the reduced dose dependently passive cutaneous reaction. Pretreatment of sensitized rats with EEZJ and dexamethasone ameliorated anaphylactic reactions (decreased the respiratory score) determined by considering respiratory rate, dyspnea, cyanosis, and mortality due to challenge. Rats sensitized with compound 48/80 (1 mg/kg, s.c.), and subsequently treated with EEZJ produced dose dependent protection against mast cell degranulation due to the following challenge. Reference drug (ketotifen fumerate, 1 mg/kg, p.o.) treatment produced a greater protection against mast cell granulation than EEZJ (Table 2).

**Effect of EEZJ on histamine and Ach induced contraction response of isolated guinea pig ileum and tracheal chain preparations respectively**

Histamine elicited a dose dependent contraction in guinea pig ileum preparation and its responses were significantly inhibited in the presence of EEZJ and chlorpheniramine maleate. The IC₅₀ values of histamine responses is chlorpheniramine maleate [0.24 (0.22-0.26) µg/ml] and EEZJ [425 (350-510) µg/ml] were calculated. Acetylcholine produced dose dependent contraction in guinea pig tracheal chain preparation and its responses were dose dependently reduced in the presence of atropine and EEZJ maleate. The IC₅₀ values of Ach responses for atropine maleate [2.85 (2.6-3.4) ng/ml] and EEZJ [265 (241-292) µg/ml] were calculated [Table 3].

**Table 2**

Effect of ethanolic extract of *Ziziphus jujuba* fruits on anaphylaxis (passive and active cutaneous) and compound 48/80 induced mast cell degranulation (in vivo).

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

IC$_{50}$ values of histamine and Ach on isolated guinea pig ileum and tracheal chain preparations respectively.

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>Histamine response in the presence of antagonist in guinea pig ileum</th>
<th>Ach response in the presence of antagonist in guinea pig tracheal chain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC$_{50}$ (µg/ml)</td>
<td>IC$_{50}$ (µg/ml)</td>
</tr>
<tr>
<td>Nili</td>
<td>16 (14.3-17.9) µg/ml</td>
<td>160 (145-176) µg/ml</td>
</tr>
<tr>
<td>Chlorpheniramine maleate</td>
<td>0.24 (0.22-0.26) µg/ml</td>
<td>[100 % contraction]</td>
</tr>
<tr>
<td>Atropine maleate</td>
<td>-</td>
<td>2.85 (2.6-3.4) ng/ml</td>
</tr>
<tr>
<td>EZJ</td>
<td>425 (350-510) µg/ml</td>
<td>265 (241-292) µg/ml</td>
</tr>
</tbody>
</table>

**Effect of EEZJ on serum IgE in rats**

The antibody (IgE) formation was reduced at equally at both doses of EEZJ (500/1000 mg/kg) treatment. The effect may be due to the sealing effect of EEZJ at 500 mg/kg hence, increase in dose may not produce greater effect. The reduction of antibody formation by EEZJ (500/1000 mg/kg) was comparable to that of dexamethasone (0.27 mg/kg, i.p.) effect (Table 4).

**Table 4**

Effect of ethanolic extract of *Ziziphus jujuba* fruits on serum IgE antibodies in rats.

<table>
<thead>
<tr>
<th>Treatment and dose</th>
<th>Serum IgE (µg/ml) on different days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Albumin sensitized control group</td>
<td>4.3 ± 0.12</td>
</tr>
<tr>
<td>EZJ (500 mg/kg, p.o.)</td>
<td>4.0 ± 0.14</td>
</tr>
<tr>
<td>EZJ (1000 mg/kg, p.o.)</td>
<td>4.0 ± 0.11</td>
</tr>
<tr>
<td>Dexamethasone (0.27 mg/kg, p.o.)</td>
<td>3.4 ± 0.1</td>
</tr>
</tbody>
</table>

**Table 5**

Effect of ethanolic extract of *Ziziphus jujuba* fruits on histamine induced hind paw edema in rats and inhibition Shultz-Dale reaction in guinea pig ileum (ex-vivo)

The injection of histamine into the sub plantar region of hind paw of rat induced an edema formation and edema was observed peak at 1 h. The rats pretreated with ibuprofen and EEZJ significantly inhibited paw edema formation at all intervals.

The EA induced contraction response on sensitized guinea pig ileum (ex vivo) was dose dependently inhibited d in the presence of EEZJ as well as disodium cromoglycate in the perfusate (Table 5).

EA 10 mg/ml was taken as 100% contraction and compared with drug treated group.

**Discussions**

Eosinophils are distinguished phenotypically by their bilobed nuclei and large acidophilic cytoplasmic granules and participating also a major effector cells in allergy, asthma and airway function affect. Eosinophil degranulation is an important immunological event responsible for allergic cutaneous inflammation manifested in cow’s milk. Therefore increased eosinophils reflect the overall state of allergic condition. This is supported by the fact that asthma/allergy patients show increased eosinophilia and leukocytosis (Horn et al., 1975; Noga et al., 2003) and can serve a sensitive cellular biomarker. The overall increased eosinophils and milk leukocytosis following administration may be due to the participation of bone marrow and T-cells derived lymphocytes (Lenon et al., 2007). During varied type of allergic manifestation, variety release inflammatory mediators (histamine, leukotrienes C4 and D4, cytokines and basic proteins) which provoke edema formation, vascular dilation and eosinophilic infiltration (Brigden, 1999; Justice et al., 2003). Prevented EEZJ treatment dose dependently cellular immune reactions,(like eosinophilia and leukocytosis) induced by milk in mice may have therapeutic implications in type-I hypersensitivity reaction and asthma.
EEZJ 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 *Z. jujuba* in the treatment of allergy related diseases. EEZJ 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 and anaphylaxis.

**Acknowledgment**

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**References**


