Antiasthmatic activity of aqueous extract of *pistacia integerrima* galls

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Pistacia integerrima (Fam. Anacardiaceae) is called as Karkatakashringi in Ayurveda and Indian traditional medicine is used as a folk medicine in the treatment of allergies, asthma, coughs etc. Present study was undertaken to study the activity of aqueous extract of the galls against the mast cell stabilization in rats, histamine-induced bronchospasm in guinea pigs and spasmodytic activity in isolated guinea pig tracheal chain preparation. The extract was subjected to phytochemical screening and found to contain essential oils, volatile oils, tannins, phenolics, flavonoids, carbohydrates and resinous matters. The rats were pretreated with the extract (27 and 54 mg kg−1 p.o.) and the antiasthmatic effect was compared with that of Prednisolone (10 mg kg−1 p.o.) on disruption rate of actively sensitized mesenteric mast cells of albino rats when challenged with antigen (horse serum along with triple antigen vaccine) and the extract (23.25 and 46.50 mg kg−1 p.o.) and the antiasthmatic effect was compared with that of Ketotifen (1 mg kg−1 p.o.) on histamine aerosol-induced bronchospasm in guinea pigs. The results emphasize that the aqueous extract of *P. integerrima* galls treatment for ten days resulted in significant effect on disruption rate of actively sensitized mesenteric mast cells of albino rats when challenged with antigen and significant protection against histamine aerosol-induced bronchospasm in guinea pigs and showed the spasmodytic activity against histamine induced contractions in isolated guinea pig tracheal chain preparation. Antiasthmatic activity of aqueous extract of *Pistacia integerrima* galls may be possibly due to the membrane stabilizing potential, suppression of antibody production and inhibition of antigen induced histamine release.

Key words: *Pistacia integerrima*, antiasthmatic activity, sensitization, mast cell disruption.

INTRODUCTION

Pistacia integerrima belong to family anacardiaceae. It is found in eastern Himalayan range from Indus to Kumaon (Uddin et al., 2012) at a height of 12000 to 8000 feet (Anonymous, 1998). *P. integrrima* is a medium sized deciduous tree that can attain a height of forty feet. Hard, horn-shaped, rugose, hollow galls like excrescences are formed on the leaves and petioles of the plant by an insect of *Pemphigus* species (Chopra et al., 1965). Dry powdered galls have a very astringent and slightly bitter taste and terebinthineodour.

The galls of *Pistacia integerrima* are aromatic, astringent and expectorant and are valued in Indian Medicine as a remedy for asthma, phthisis and other ailments for the respiratory tract; they are also useful in dysentery, antiinflammatory, antidiabetic agent, a blood purifier, a remedy for gastrointestinal disorders, chronic bronchitis, hiccough, vomiting of children, skin diseases, psoriasis, fever, to increase appetite and to remove bed humors. In India it is used as an herbal remedy for ailments such as cough, asthma (Padulosi et al., 1996; Ahmad et al., 2008) fever, vomiting and diarrhea (Pant and Samant, 2010; Ghias et al., 2012).

In Pakistan, galls of *P. integrrima* are used for treatment of hepatitis and other liver disorders (Uddin et al., 2012). Galls of *P. integrrima* are used as herbal drug for diarrhea in northern India (Ahmad et al., 2010) for Infections, diabetes, pain, inflammatory conditions, and fever (Uddin et al., 2012, Ahmad et al., 2008). Sushruta prescribed the galls in combination with other drugs for the treatment of snake bite and scorpion sting. Monoterpenes (Monaco et al., 1974; Ansari and Ali, 1996; Ansari et al., 1993; Ansari et al., 1994a; Ansari et
al., 1994), triterpenoids (Caputo and Mangoni, 1970; Tabacik-Wlotzka et al., 1967) sterols (Hiroi, 1966), dihydromvalval acid (Vickery, 1981) and flavonoids (Kalidhar and Sharma, 1985) have been isolated from the different parts of Pistacia species (Uddin et al., 2011). Phytochemical investigation of the galls of Pistacia integerrima yielded three new phytoconstituents characterized as n-decan-3′-ol-yl-n-eicosanoate, n-octadecan-9,11-diol-7-one and 3-oxo-9β-lanost-1,20(22)-dien-26-oic acid along with the known compound β-sitosterol. The structures of these phytoconstituents have been elucidated on the basis of spectral data analysis and chemical reactions (Chung, 2005).

It is believed that up to 10% of adults and 20% of children are affected globally. National Institutes of Health report indicate an estimated 500,000 hospitalizations and 5000 deaths annually. International asthma mortality is reported as high as 0.86 deaths per 100,000 persons in some countries. In allopathic system of medicine, they depend mainly on anti asthmatics, anti allergics, corticosteroids, bronchospasm relaxants which give very fast relief.

Treating asthma is tough, since the respiratory treat cannot take rest and is also exposed to irritants which hinder the healing process. By using herbal plants, besides treating the disease, it helps in improving body’s defense mechanism fight against various disease conditions, general resistance, also to avoid recurrences. Prevention is first care better than cure to reduce conditions, general resistance, also to avoid recurrences. By using herbal plants, besides treating the disease, it helps in improving body’s defense mechanism fight against various disease conditions, general resistance, also to avoid recurrences.

Fasting is one of the key components of the healing process. Fasting helps in improving body’s defense mechanism fight against various disease conditions, general resistance, also to avoid recurrences.

**Materials and Methods**

**Plant extract, chemicals and drugs**

Aqueous extract of *Pistacia integerrima* galls (gift sample) was obtained from Ansar Industry, Surat, Gujarat, India, authenticated by the Taxonomist of Gujarat Technical University and a herbarium specimen and evidences was deposited in the Department of pharmacology, Nargund College of Pharmacy, Bangalore for future reference. Triple Antigen Vaccine (Containing 20,000 thousand organisms of *Bordetellapertusis*) (Company name: Serum institute of India Ltd, Pune.), Horse Serum, Toluidine Blue, Xylene, Acetone, Histamine hydrochloride or Histamine diphosphate salt (Company name: Sigma-Aldrich), Histamine chamber, Ultrasound nebulizer, Stop watch, Organ bath, Carbogen, Krebs solution and all other chemicals and reagents used in this study were of analytical grade.

**Experimental animals for mast cell stabilizing activity in rats (Tripathi and Das, 1977; Mitra, 1999; Padmalata, 2000)**

Albino wistar rats of either sex weighing 120 to 160g were fed with standard diet and water ad-libitum. Of the five groups six animals were taken in each group and maintained under standard laboratory conditions. All experimental procedures were followed in strict accordance with the guidelines prescribed by the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA), Proposal No. IAEC/NCP/09/09.

The rats were sensitized by 0.5ml of subcutaneous injection of horse serum along with 0.5ml of triple antigen vaccine (20 thousand million organisms of *Bordetellapertusis*). Then sensitized rats were divided into 4 groups and treatment was started on the 7th day of the sensitization for 14 days according to following dose schedule. One group kept as saline control without sensitization.

Group I: 0.5 ml horse serum +0.5 ml triple antigen vaccine per s.c (Sensitized control)
Group II: 0.5 ml of 0.9% (w/v) saline per orally (Normal control without sensitization)
Group III: Aqueous extract of *Pistacia integerrima galls* 27 mg/kg b.w. p.o (Mitra, 1999).
Group IV: Aqueous extract of *Pistacia integerrima galls* 54 mg/kg b.w. p.o (Mitra, 1999).
Group V: Prednisolone 10 mg/kg b.w.p.o (Das, 1977).

On 14th day, 2 hours after the last dose treatment rats were sacrificed and intestinal mesenteries were isolated for the study of mast cells. Mesenteries of sacrificed rats along with intestinal pieces were kept in a Ringer-Locke’s solution at 37°C. Then mesenteric pieces were challenged with 5% horse serum for 10 minutes. Pieces of mesentery were stained supravitally with toluidine blue by the following method.

Tissue was first immersed in 0.1% toluidine blue in 4% aqueous formal saline for 10 minutes. The tissue was then transferred to xylene for 5 to 10 minutes. Finally it was rinsed with acetone twice, placed on microscope slide and stretched with the help of needles. The intestinal pieces were cut and removed. The tissue was examined under a microscope. The numbers of intact, disrupted and partially disrupted mast cells per high power field, 10X, 40X, 100X were counted. The cells in at least more than 10 such randomly selected fields from each tissue were counted.

**Statistical analysis**

The results of various studies were expressed as mean ± SEM and analyzed statistically using one-way ANOVA,
followed by Dunnet’s Multiple Comparison Test to find out the level of significance. P < 0.05 was considered statistically significant. The analysis was performed using Graph pad Prism software.

Experimental animals for histamine-induced bronchospasm in guinea pigs (Tripathi and Das, 1977; Mitra, 1999; Padmalata, 2000; Nagore, 2009; Singh, 2009; Abraham 1992; Ahirwar et al., 2008)

Albino guinea pigs of either sex weighing 200 to 450 g were fed with standard diet and water ad-libitum. Of the four groups six animals were taken in each group and maintained under standard laboratory conditions. All experimental procedures were followed in strict accordance with the guidelines prescribed by CPCSEA Proposal No. IAEC/NCP/09/09.

Bronchospasm was induced in guinea pigs by exposing them to 1% histamine aerosol under constant pressure (160 mm Hg) in an aerosol chamber (28 x 28 x 14) made up of perplex glass (Figure1). Of the two groups of six animals each, group I served as control and group II received aqueous extract of Pic acid galls 23.25 mg/kg b.w. p.o. (low dose), group III received aqueous extract of Pic acid galls 46.50 mg/kg b.w.p.o. (high dose) and group IV received Ketotifen 1 mg/kg b.w.p.o. used as standard drug once a day for 10 days.

Group I: Control (0.5 ml of 0.9% saline p.o.)
Group II: Aqueous extract of Pistacia integerrima galls 23.25 mg/kg b.w. p.o.
Group III: Aqueous extract of Pistacia integerrima galls 46.50 mg/kg b.w.p.o.
Group IV: Ketotifen 1 mg/kg b.w. p.o.

The animals were exposed to 1% histamine aerosol under constant pressure (160 mm Hg) in an aerosol chamber on day 0 without any treatment. The end point, preconvulsive dyspnea (PCD) was determined from the time of aerosol exposure to onset of dyspnea leading to the appearance of convulsions. As soon as PCD commenced the animals were removed from the chamber and exposed to fresh air. This PCD was taken as day 0 value. On day 10, 2 h after administration of aqueous extract of Pic acid galls, the time for the onset of PCD was recorded as on day 0. The protection offered by the treatment was calculated by the following formula:

Percentage protection = \[1 - \frac{T_1}{T_2}\] x 100

Where:
T_1 is time for PCD onset on day 0 and
T_2 is time for PCD onset on day 10

The results of various studies were expressed as mean ± SEM and analysed using Students paired-T-test to find out the level of significance. P < 0.05 was considered statistically significant. The analysis was performed using Graph pad Prism software.

Spasmolytic activity in isolated guinea pig tracheal chain preparation (Zelalem, 2009)

Guinea pigs of either sex, weighing 250-300 g were sacrificed by cervical dislocation and carotid bleeding. The trachea was dissected out and transferred to a dish containing kerb’s solution (composition (g/l): NaCl (6.8), KCl (0.35), CaCl_2 (0.28), MgSO_4·7H_2O (0.25), NaHCO_3 (2.1), KH_2PO_4 (0.16) and glucose (2.0)) and cut transversely between the segments of the cartilage so as to give a number of rings of the trachea. About 5 to 6 rings these were tied to form a chain of approximately 4-5 cm length, which was in kerb’s solution, contained in an organ bath maintained at 37°C and continuously aerated with carbogen (95% O_2+5% CO_2). One end of the tracheal chain was attached to a tissue holder at the base of organ bath and the other end to a frontal lever; the responses were recorded on a slow moving kymograph. The suspended tracheal was allowed to stabilize for at least 30 minutes. During stabilization, the bath was supplied with fresh kerb’s solution ones per every 15 minutes. Then cumulative concentration response to histamine in the absence and presence of aqueous extract of Pic acid galls were recorded with a slow moving kymograph.

RESULTS AND DISCUSSION

The effect of aqueous extract of Pistacia integerrima galls on the mast cell stabilizing activity was studied following active anaphylaxis. Aqueous extract of Pic acid galls resulted in marked protection against the mast cell...
### Table 1. Mast cell stabilizing activity in rats against aqueous extract of *Pistacia integerrima* galls

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mast cells (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Intact</td>
<td>Disrupted</td>
</tr>
<tr>
<td>I. Sensitized Control</td>
<td>(0.5ml horse serum + 0.5ml triple antigen vaccine)</td>
<td>2.048 ± 0.582</td>
<td>90.75 ± 1.812</td>
</tr>
<tr>
<td>II. Control</td>
<td>(0.5ml of 0.9% NaCl)</td>
<td>79.89 ± 0.725***</td>
<td>8.88 ± 0.678***</td>
</tr>
<tr>
<td>III. <em>P. integerrima</em> Treated</td>
<td>(23.25mg/kg b.w.p.o.)</td>
<td>26.11 ± 4.840***</td>
<td>30.73 ± 1.420***</td>
</tr>
<tr>
<td>IV. <em>P. integerrima</em> Treated</td>
<td>(46.50mg/kg b.w.p.o.)</td>
<td>56.16 ± 2.073***</td>
<td>22.46 ± 2.640***</td>
</tr>
<tr>
<td>V. Pred. Treated</td>
<td>(10mg/kg b.w. p.o.)</td>
<td>68.01 ± 2.008***</td>
<td>17.65 ± 1.127***</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, n = 6 in each group. (Data analysed by One-way ANOVA Followed by Dunnet's Multiple Comparison Test), significantly different from Sensitized Control group ***P < 0.001, ns- non significant.

### Table 2. Histamine induced bronchospasm in guinea pigs

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>PCD Time (sec) Before Treatment on Day 0</th>
<th>PCD Time (sec) After Treatment on Day 10</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td><em>P. integerrima</em> Treated (23.25 mg/kg b.w. p.o.)</td>
<td>70.50 ± 3.274</td>
<td>178.7 ± 11.31***</td>
<td>60.55</td>
</tr>
<tr>
<td>II</td>
<td><em>P. integerrima</em> Treated (46.50 mg/kg b.w. p.o.)</td>
<td>75.83 ± 2.822</td>
<td>211.7 ± 14.93***</td>
<td>64.19</td>
</tr>
<tr>
<td>III</td>
<td>Ketotifen Treated (10mg/kg b.w.p.o)</td>
<td>96.17 ± 7.560</td>
<td>279.3 ± 13.93***</td>
<td>65.57</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, n = 6 in each group. Significantly different from Control group. (Data analysed by Students paired –T-test), ***P < 0.001.

### Table 3. Data showing the effect of aqueous extract of *Pistacia integerrima* galls on histamine induced contractions in isolated guinea pig tracheal chain preparation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0.2ml hist.</th>
<th>0.1ml Ex. + 0.2ml hist.</th>
<th>0.2ml Ex. + 0.2ml hist.</th>
<th>0.4ml Ex. + 0.2ml hist.</th>
<th>0.2ml hist.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean contraction response in mm</td>
<td>8</td>
<td>6.0</td>
<td>4.5</td>
<td>0</td>
<td>7.5</td>
</tr>
<tr>
<td>Percentage(% inhibition in contraction response)</td>
<td>-</td>
<td>25</td>
<td>43.75</td>
<td>100</td>
<td>-</td>
</tr>
</tbody>
</table>

Hist. = Histamine hydrochloride, Ex. = Aqueous extract of *Pistacia integerrima*galls

Disruption followed by antigen challenge in sensitized animals. (Data and percentage intact and disrupted mast cells can be seen in Table 1 and Figures 3, 5 and 7) The protection offered by aqueous extract of *P. integerrima* galls on the mast cells may be attributable by the presence of various components responsible for their mast cell stabilizing potential against antigen-antibody reaction. (The intact, disrupted and partially disrupted mast cells can be seen as in Figure 2, 4, 6).

Aqueous extract of *P.integerrima* galls prolonged the latent period of convulsions in guinea pigs following histamine aerosol. This may be suggestive of a spasmolytic activity following treatment with aqueous extract of *P. integerrima* galls. Moreover, protection against anaphylactic shock-induced bronchospasm in guinea pigs was observed. This anti-anaphylactic effect may be due to stabilization of mast cell membrane, inhibition of mast cell induced histamine release or non-
Figure 2. Intact mast cells.

Figure 3. Percentage of Intact mast cells in different groups of rats in mast cell stabilizing activity model.

Figure 4. Disrupted mast cells.

Figure 5. Percentage of Disrupted mast cells in different groups of rats in mast cell stabilizing activity model.

Figure 6. Partially disrupted mast cells.

Figure 7. Percentage of Partially disrupted mast cells in different groups of rats in mast cell stabilizing activity model.
availability of antibodies on the mast cell surface in aqueous extract of *Pistacia integerrima* galls treated animals (Table 2 and Figures 8, 9, 10). Aqueous extract of *P. integerrima* galls showed complete antagonism against histamine induced contractions in guinea pig tracheal chain preparation (Figure 11). Histamine induced contractions could be blocked completely and reversed by using higher concentration of histamine (Table 3).

The disruption of mast cells is an important feature of anaphylaxis. An attempt was made to find out whether aqueous extract of *P. integerrima* galls has any effect on the rate of disruption of mast cells following horse serum along with triple antigen vaccine which are agents that causes histamine release. It has been assumed that the process leading to histamine secretion may be mediated by calcium release from an intracellular store of mast cells. In this study, aqueous extract of *P. integerrima* galls offered significant protection against horse serum along with triple antigen vaccine induced mast cell disruption.

All these findings reveal the antiasthmatic activity of aqueous extract of *P. integerrima* galls may be due to the mast cell stabilizing potential, suppression of antibody production and inhibition of antigen-induced histamine release by some of the components of the extract.

**Conclusion**

The aqueous extract of *Pistacia integerrima* galls shows mast cell stabilizing activity against horse serum and triple antigen vaccine induced mast cell disruption in rats, protection against histamine induced bronchospasm in guinea pigs and inhibition of histamine induced contractions in isolated tracheal chain preparation. Further studies are required to find out mast cell stabilizing activity, bronchial smooth muscle relaxation mechanism at molecular level.
REFERENCES


