Anti-asthmatic activity of methanolic extract of leaves of Tamarindus Indica Linn.

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ABSTRACT

Tamarindus indica Linn. (Caesalpiniaceae), is a medicinal plant, used in folk medicine for treating asthma, dysentery, vaginal and uterine complaints, inflammation and variety of other condition. In this study methanolic extract of leaves of Tamarindus indica Linn at the doses of 175, 350 and 700 mg/kg, p.o., exhibited significant (p<0.01) mast cell stabilizing activity against clonidine induced mast cell degranulation in rats and significantly inhibited (p<0.01) the milk induced leucocytes and eosinophilia in mice. Clonidine, a \( \alpha_2 \) adrenoreceptors agonist induces dose dependent catalepsy in mice, which is inhibited by histamine H\(_1\) receptor antagonists. Prior treatment with methanolic extract of Tamarindus indica Linn at the dose of 250, 500 and 1000 mg/kg, p.o., significantly inhibited (p<0.01) the clonidine-induced catalepsy in mice. Thus the present study revealed that the methanolic extract of leaves of Tamarindus indica Linn exhibited significant antihistaminic, adaptogenic and mast cell stabilizing activity in the laboratory animals.

Keywords: Asthma, Tamarindus indica, Catalepsy, Antihistaminic, Adaptogenic.

INTRODUCTION

For more than a country, asthma has been a major cause of morbidity and mortality. Bronchial hyper responsiveness (BHR) is a characteristic feature in most asthmatic patients, which correlates with the severity of the disease (Durham, 1985), although its precise mechanism remain unclear. Mast cell and derivative mediators are believed to be crucial in the development of asthma. After antigen cross linking, mast cell produces mediators including tryptase, leukotrienes and platelet activating factor (Schleimer and Peters, 1986., Casale and Wood, 1987., Gomez and Corrado, 1993) which can potentially induce BHR. Furthermore mast cell contains and produces several pro-inflammatory mediators like cytokines, granulocyte-macrophage colony stimulating factor, tumor necrosis factor, interleukin (IL)-3, IL-4, and IL-5, which may contribute to eosinophil recruitment to the airway and BHR (Gordan and Burd, 1990., Galli and Wershil, 1991).

Research on medicinal plant has increased recently all over the world. Medicinal plants have been used in various systems, as they have potential against numerous diseases. Tamarindus indica Linn (Caesalpiniaceae), is chosen in this investigation. Tamarindus indica Linn (Caesalpiniaceae), is a commonly found plant in moist waste ground, lawns and open plantation. It is cultivated throughout India, self sown in waste places and forest lands in central India, Madhya Pradesh, and also planted along roadsides throughout India. The methanolic extract of Tamarindus indica Linn is used in the treatment of diarrhoea, dysentery, fever and fistula (Kirtikar, 1933). The phytochemical characterization shows the presence of tannins, steroids, alkaloids, triterpenes and flavonoids (Gokhale and Saraf, 2002). According to Ayurveda, Tamarindus indica Linn is used in the treatment of biliousness, vaginal and uterine complaints, inflammations, burning sensation, asthma and other conditions. The methanolic extract of leaves contain ascorbic acid and \( \alpha \)-carotene is proven to be anti-lipoperoxidant and anti-hepatotoxic. Some studies have reported immunomodulatory effect of Tamarindus indica Linn (Joyeux and Mortier, 1995).

However no scientific data are available regarding the effect of Tamarindus indica Linn in the treatment of asthma. The present study is to evaluate the action of the Tamarindus indica Linn on various aspects of asthma like H\(_1\) receptor antagonist, eosinophilia, leucocytosis and mast stabilizing activity using various animal models.

MATERIALS AND METHODS

1. Plant material

The leaves of Tamarindus indica Linn were collected from local Nursery in Pimpri, Pune, India. As well as in agricultural areas
around Pune city region. The plant specimen was authenticated by Mr. P.G. Diwakar (Scientist ‘D’ for Joint Director) at “Botanical Survey of India” Pune, India, (Voucher specimen no. TYP1).

2. Extraction

The leaves of *Tamarindus indica* Linn were air-dried. After 10 days of drying, the leaves were powdered using a mixer and passes through sieve no. 40#. The methanolic extract was prepared by maceration method. The extract was concentrated and dried at 60°C, (yield 8.7 %) (Rangari, 2004).

3. Experimental animal

All experimental procedures were carried out in strict accordance with the guidelines prescribed by the Committee for the Purpose of Control and Supervision on Experimentation on Animals (CPCSEA) and were approved by the Institutional Animal Ethics Committee. Adult albino rats of Wistar strain weighing between 120 to 180 g and Albino Swiss mice weighing between 25 to 30 gm. were used. The above animals of either sex were purchased from National Toxicological Center, Pune. Animals had free access to standard pellet diet (Amrut laboratory animal feed, Sangli-Maharashtra) and water. They were housed in groups of five under standard laboratory conditions of temperature (25 ± 2°C) and 12/12 hr light/dark cycle. The distribution of animals in the groups along with the sequence of trials and the treatment allotted to each group were randomized throughout the experiment. Separate groups of fresh animals were used for each experiment.

4. Clonidine-induced mast cell degranulation in rats

Rats were divided in five groups, (n=5). The seven days drug treatment schedule was followed. Group-I received Distilled water (10 ml/kg p.o.). Group-II was treated with sodium cromoglycate (0.5 mg/kg, intraperitonialy). Groups-III, IV and V were treated with methanolic extract of *Tamarindus indica* Linn 175, 350 and 700 mg/kg, p.o., respectively. On 7th day, 2 hours after the assigned treatment mast cells were collected from the peritoneal cavity (Lakashmana et al, 2001, Lakadawala et al 1980).

The rats were anesthetized with ether and were injected 10 ml of normal saline solution into peritoneal cavity. The abdomen was gently massaged for 90 seconds. The peritoneal cavity was carefully opened and the fluid containing mast cells were aspirated and collected in siliconised test tube containing 7 to 10 ml of RPMI-1640 Medium (pH 7.2- 7.4).

The mast cells were then washed three times by centrifugation at low speed (400-500 rpm) and the pallet of mast cells was taken from the medium. Then 0.5 ug/ml of clonidine solution was added to the mast cell suspension (approximately 1 x 10⁹ /ml) and incubated at 37°C in a water bath for 10 min. Later they were stained with 1 % toludine blue (Dye) and observed under high power microscope field (400 X). A total 100 cells were counted from different visual areas and percent protection against - induced mast cell degranulation was calculated.

5. Milk-induced leukocytosis and Eosinophilia in mice

Mice were divided into five groups (n=5). Animals belonging to Group-I received distilled water 10 ml/kg, p.o. Animals belonging to Group II, III, IV and V received boiled and cooled milk in dose of 4 ml/kg, (subcutanously). Animals belonging to groups III, IV and V received methanolic extract of *Tamarindus indica* Linn 250, 500 and 1000 mg/kg, p.o., respectively. 1 hr before milk injection. Blood samples were collected from each mouse from the retro orbital plexus, under light ether anesthesia. Total leukocyte count was recorded in each group before drug administration and 24 hrs after milk injection. Differences in total leucocytes and eosinophil count before and after 24 hrs. drug administration were calculated (Brekhman and Dardymov 1969, Bhargava and Singh, 1981).

6. Clonidine-induced catalepsy in mice

Bar test was used to study the effect of test drugs on clonidine-induced catalepsy. Mice were divided into five groups (n=5). Animals belonging to group I served as control and were administered the vehicle (10 ml/kg, p.o.). Animals belonging to Group II received chlorpheniramine maleate (10 mg/kg, intraperitoniotal). Animals belonging to groups III, IV and V received *Tamarindus indica* Linin doses (250, 500 and 1000 mg/kg, p.o.) respectively.

The forepaws of mice were placed on a horizontal bar (1 cm in diameter, 3 cm above the table) and the time required to remove the paws from bar was noted for each animal. All the groups received clonidine (1 mg/kg, subcutaneously), 1 hr after the drug administration and the duration of catalepsy was measured at 15, 30, 60, 90, 120, 150 and 180 min (Brigden, 1999, Dhanlakshami et al 2001).

7. Statistical Analysis

All observations were presented as Mean ± SEM (Standard error of mean). The data was analyzed by Student’s t-test or one-way ANOVA followed by Dunnet’s test. P<0.05 was considered as significant.

RESULTS AND DISCUSSION

1. Mast cell stabilizing potential of *Tamarindus indica* Linn

Clonidine challenge resulted in significant mast cell degranulation. Pretreatment of animal with methanolic extract of *Tamarindus indica* Linn(175, 350 and 700 mg/kg, p.o.) resulted in significant reduction (p<0.01) in degranulation of mast cells when challenged with clonidine. The percent protection was found to be 47.92, 60.74 and 67.42 respectively. Sodium cromoglycate (0.5 mg/kg, intraperitoniotal) significantly inhibited (p<0.01) clonidine induced mast cell degranulation and the percent protection was found to be 70.42.
2. Effect of methanolic extract of *Tamarindus indica* Linn on milk-induced leucocytosis in Mice

Subcutaneous injection of milk in dose of 4 ml/kg, (subcutaneously) produced a significant increase (p<0.001) in the leukocyte count after 24 hr of its administration. Mice pretreated with methanolic extract of *Tamarindus indica* Linn at dose of 250, 500 and 1000 mg/kg, p.o. exhibited significant inhibition (p<0.01) of milk induced leucocytosis.

3. Effect of methanolic extract of *Tamarindus indica* Linn on milk-induced eosinophilia in mice.

Significant increase (p<0.001) in total eosinophil count was seen in animals treated with milk 4 ml/kg, (s.c.) the groups pretreated with *Tamarindus indica* Linn at the dose of 250, 500 and 1000 mg/kg, p.o., there was significant inhibition (p<0.05 and p<0.01) of milk induced eosinophilia.

4. Clonidine-induced catalepsy in mice

Clonidine (1 mg/kg, s.c.) produced catalepsy in mice, which remained for 3 hr. The vehicle treated group showed maximum duration of catalepsy (216.8 ± 7.76 sec.) at 150 minuteafter the administration of clonidine. There was significant inhibition (p<0.05 and p<0.01) clonidine induced catalepsy in the animals pretreated with *Tamarindus indica* Linn (250, 500 and 1000 mg/kg, p.o.). Chlorpheniramine maleate, (10 mg/kg, i.p.) treated group significantly reversed (p< 0.01) induced catalepsy in mice.

Values are mean ±SEM. @ p<0.001. Group-II was compared with Group-I (Student’s ‘t’ test) *p<0.05, **p<0.01, Group-III, Group-IV and Group-V were compared with Group-II. (ANOVA followed by Dunnet’s Test).

### Table 1: Effect of *Tamarindus indica* Linn on milk-induced leucocytosis and eosinophilia in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Diff. in no of leucocytes count (per cu mm) (Mean ± SEM)</th>
<th>Diff. in eosinophil count (per cu mm) (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Distilled water (10 ml/kg, p.o.)</td>
<td>84.4 ± 2.97</td>
<td>21.2 ± 2.05</td>
</tr>
<tr>
<td>II</td>
<td>Distilled water (10 ml/kg, p.o.) + milk (4 ml/kg, s.c.)</td>
<td>4678 ± 16.74@</td>
<td>164.4 ± 7.09@</td>
</tr>
<tr>
<td>III</td>
<td><em>Tamarindus indica</em> Linn (250 mg/kg, p.o.) + milk (4 ml/kg, s.c.)</td>
<td>2920 ± 34.84**</td>
<td>141.4 ± 3.98**</td>
</tr>
<tr>
<td>IV</td>
<td><em>Tamarindus indica</em> Linn (500 mg/kg, p.o.) + milk (4 ml/kg, s.c.)</td>
<td>2237 ± 12.81**</td>
<td>129.4 ± 3.70**</td>
</tr>
<tr>
<td>V</td>
<td><em>Tamarindus indica</em> Linn (1000 mg/kg, p.o.) + milk (4 ml/kg, s.c.)</td>
<td>1724 ± 14.51**</td>
<td>101.2 ± 3.63**</td>
</tr>
</tbody>
</table>

Values are mean ±SEM. @ p<0.001. Group-II was compared with Group-I (Student’s ‘t’ test) *p<0.05, **p<0.01, Group-III, Group-IV and Group-V were compared with Group-II. (ANOVA followed by Dunnet’s Test).

### Table 2: Effect of *Tamarindus indica* Linn on clonidine-induced catalepsy in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Duration of catalepsy (sec) at Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>28.4±1.91</td>
</tr>
<tr>
<td>II</td>
<td>14.0±0.70*</td>
</tr>
<tr>
<td>III</td>
<td>21.2±2.13*</td>
</tr>
<tr>
<td>IV</td>
<td>20.4±0.87*</td>
</tr>
<tr>
<td>V</td>
<td>17.0±1.58*</td>
</tr>
</tbody>
</table>

Where, n= 5: Group-I = Distilled water (10 ml/kg, p.o.); Group-II = Chlorpheniramine maleate (10 mg/kg, i.p.); Group-III = *Tamarindus indica* Linn extract (250 mg/kg, p.o.); Group-IV = *Tamarindus indica* Linn extract (500 mg/kg, p.o.); Group-V = *Tamarindus indica* Linn extract (1000 mg/kg, p.o.); Group II, III, IV, V compared with Group I (ANOVA followed by Dunnet’s test). *p<0.05, **p<0.01

### Table 3: Effect of *Tamarindus indica* Linn on mast cell degranulation in rat

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mast cell %</th>
<th>Intact (Mean ± SEM)</th>
<th>Disrupted (Mean ± SEM)</th>
<th>Percent Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td></td>
<td>21.8 ± 0.92</td>
<td>78.2 ± 0.92</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>Sodium cromoglycet (0.5 mg/kg, i.p.)</td>
<td>71.2 ± 1.80**</td>
<td>28.8 ± 1.80**</td>
<td>70.42</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td><em>Tamarindus indica</em> Linn (175 mg/kg, p.o.)</td>
<td>53.2 ± 1.85**</td>
<td>46.8 ± 1.81**</td>
<td>47.92</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td><em>Tamarindus indica</em> Linn (350 mg/kg, p.o.)</td>
<td>59.1 ± 1.72**</td>
<td>40.9 ± 1.72**</td>
<td>60.74</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td><em>Tamarindus indica</em> Linn (700 mg/kg, p.o.)</td>
<td>67.2 ± 1.36**</td>
<td>32.8 ± 1.36**</td>
<td>67.42</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ±SEM. **p<0.01. Group II, III, IV, V compared with Group I (ANOVA followed by Dunnet’s test); **p<0.01

2. Effect of methanolic extract of *Tamarindus indica* Linn on milk-induced leucocytosis in Mice

Mice pretreated with methanolic extract of *Tamarindus indica* Linn at dose of 250, 500 and 1000 mg/kg, p.o. exhibited significant inhibition (p<0.01) of milk induced leucocytosis.

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Mice pretreated with methanolic extract of *Tamarindus indica* Linn at dose of 250, 500 and 1000 mg/kg, p.o. exhibited significant inhibition (p<0.05 and p<0.01) of milk induced eosinophilia.

4. Clonidine-induced catalepsy in mice

Mice pretreated with clonidine (1 mg/kg, s.c.) produced catalepsy in mice, which remained for 3 hr. The vehicle treated group showed maximum duration of catalepsy (216.8 ± 7.76 sec.) at 150 minuteafter the administration of clonidine. There was significant inhibition (p<0.05 and p<0.01) clonidine induced catalepsy in the animals pretreated with *Tamarindus indica* Linn (250, 500 and 1000 mg/kg, p.o.). Chlorpheniramine maleate, (10 mg/kg, i.p.) treated group significantly reversed (p< 0.01) induced catalepsy in mice.
products, cause vascular and other tissue reactions similar to those of inflammatory processes. In the rat mast cell granules the histamine concentration has been calculated to be around 0.3 M. (Padmalatha and Roopa, 2001). Both clonidine and compound 48/80 act through the dynamic expulsion of granules without causing any damage to the cell wall. Clonidine releases histamine from mast cells in a similar way to a selective liberator like compound 48/80 (Padmalatha and Roopa, 2001). It is known that sodium cromoglycate a standard mast cell stabilizer prevents degranulation of mast cells by raising the cyclic adenosine monophosphate (Padmalatha and Roopa, 2001). It is known that all pharmacological agents that increase intracellular levels of cAMP relax airway smooth muscle and inhibit the release of autacoids from the tissue and basophils (Padmalatha and Roopa, 2001). The groups of animal pretreated with methanolic extract of Tamarindus indica Linn extract exhibited a significant reduction (p<0.01) in degranulation of mast cells and offered 47.92, 60.74 and 67.42 % protection respectively when challenged with clonidine.

Herbal formulations used in the treatment of asthma include some antistress herbs that enable adaptation to stress since excessive stress or nervous debility may aggravate symptoms of asthma. The normalization effect of an adaptogen can be observed in milk- induced leucocytosis (increase in leukocyte count) after parenteral administration of milk.

In the present study, the distilled water treated group of mice, after parental administration of milk showed significant increase in leukocyte count, where as the group in which methanolic extract of Tamarindus indica Linn was administered, showed a normal leukocyte count. This indicates the adaptogenic activity of the Tamarindus indica Linn.

Most allergic and non-allergic asthmatics, including those with mild asthma, have bronchial eosinophilia and there is a significant association between eosinophil activation and asthma severity as well as bronchial hyper responsiveness (Thakur and Tupe., 1999). The total eosinophil count reflects asthmatic activity and is useful in the management of bronchial asthma. In the present study it has been found that, parenteral administration of milk (4 ml/kg, s.c.) to the distilled water treated group significantly increased the eosinophil count after 24 hour, where as, the drug treated groups i.e. methanolic extract of Tamarindus indica Linn significantly inhibited milk induced eosinophilia in mice.

Clonidine produced catalepsy in mice. The vehicle treated group has shown maximum duration of catalepsy at 150 minuteafter the administration of clonidine. There was significant inhibition ofclonidine induced catalepsy in the animals pretreated with Tamarindus indica Linn. This indicates the anti-histaminic($H_1$) activity of the Tamarindus indica Linn.

CONCLUSION

Thus, it can be concluded from the results obtained in the present investigation that Tamarindus indica Linn possess significant antiasthmatic activity. The anti-asthmatic activity of Tamarindus indica Linn can be attributed to bronchodilating, antihistaminic ($H_1$ - antagonist) and anti-inflammatory, suggestive of its potential in treatment and prophylaxis of asthma. further detailed study needs to be conducted to evaluate the clinical efficacy in the treatment of asthmatic patients.

REFERENCES


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