IN-VITRO ANTIHISTAMINIC AND ANTISPASMODIC POTENTIAL OF METHANOLIC EXTRACT OF LEAVES OF TRIDAX PROCUMBENS LINN.

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ABSTRACT

Background: Tridax procumbens, commonly known as coatbuttons or tridax daisy, is a species of flowering plant in the daisy family. The plant bears daisy like yellow-centered white or yellow flowers with three-toothed ray florets. The leaves are toothed and generally arrowhead-shaped. Its fruit is a hard achene covered with stiff hairs and having a feathery, plume like white pappus at one end. Traditionally, Tridax procumbens has been in use in India for wound healing and as an anticoagulant, anti-inflammatory, antifungal, and insect repellent. The present study was undertaken to evaluate in-vitro antihistaminic and anti-spasmodic activity of methanolic extract of leaves of Tridax procumbens L. by using isolated goat tracheal chain and chicken ileum preparation. The cumulative concentration response to histamine and Acetyl choline in the absence and presence of methanolic extract were recorded with a kymograph. The methanolic extract decreases the goat tracheal contraction and ileum contractions induced by histamine and Acetyl choline in dose dependent manner as compared with standard drug Chlorpheniramine Maleate and Atropine Sulphate respectively. This revealed that methanolic extract of leaves of Tridax procumbens L. possess a high degree of antihistaminic and spasmylytic activity by blocking histaminic and cholinergic receptors.

KEYWORDS: Anti-histaminic, Anti-spasmodic, Tridax procumbens, Histamine, Chlorpheniramine maleate.
INTRODUCTION
Herbal medicines are being increasingly utilized to treat a wide variety of diseases, though the knowledge about their mode of action is relatively insufficient. Interest regarding the pharmacological evaluation of various plants used in traditional system of medicine is relatively increasing.\(^1\) The use of traditional medicine is expanding to newer horizons and plants still remain as the novel source of structurally important compounds that lead to the development of innovative drugs. Naturally occurring compounds from plants are still used in pharmaceutical preparations in pure or extracted forms.\(^2\) Traditionally, Tridax procumbens belonging to family ‘Asteraceae’ has been used for wound healing and as an anticoagulant, anti-inflammatory, antifungal, and insect repellent.\(^3\) The different parts of the plant were have been reported for antioxidant\(^4\), anti-inflammatory\(^5\), antibacterial\(^6\), antifungal.\(^6\)

Thus taking into the consideration the traditional claims and reported pharmacological activities of *Tridax procumbens* (*L*) the present study was undertaken to evaluate anti-histaminic and antispasmodic potential of methanolic extract of *Tridax procumbens* *L* (TP) which may further contribute in allergic disorders.

MATERIALS AND METHODS

Procurement of Plant
The fresh leaves of *Tridax procumbens* *L* were collected locally from Sangli District of Maharashtra, India and were authentified by Dr. S. M. Shendage, Balwant College, Vita, India. A voucher specimen has been kept in the herbarium (SKY 001- 2016-17) at Department of Botany.

Preparation of methanolic extract
The extractions of leaves of *Tridax procumbens* (*L*) was carried by using soxhlation method. After 5-days of drying, the dried leaves were powdered by grinding and sieved with a 40# sieve. In this method 500 gm. of leaves powder was extracted with 95% methanol. It was then filtered and concentrated in vacuum under reduced pressure using a rotary evaporator and concentrated to obtain the methanolic extract of *Tridax procumbens* (*L*) (TP). The % yield obtained from leaves was 25% w/w.
Procurement of Animals

Isolated adult goat tracheal and chicken tissue was collected from slaughter house. Trachea was collected in the ice cold oxygenated Krebs’ solution and chicken tissue was collected in the ice cold oxygenated Tyrode solution.

METHODOLOGY

Histamine induced contraction of isolated goat trachea preparation \(^{[7,8, \text{and } 9]}\)

The goat tracheal tissue was obtained immediately after slaughter of animals. Pieces of trachea were collected in freshly prepared ice-cold oxygenated Kreb’s solution (Composition mM: NaCl, 115; KCl, 4.7; CaCl\(_2\), 2; NaHCO\(_3\), 25; KH2PO\(_4\), 1.2; Mg\(_2\)SO\(_4\), 1.19; glucose, 11.5). Goat trachea was then cut into individual rings and tied together in series to form a chain. It was suspended in bath containing Kreb’s solution and maintained at 37 ± 0.5 °C, a stream of air was bubbled through the organ tube (1 bubble/sec). One end of the tracheal muscle was attached to S-shaped aerator and the other attached to isotonic frontal writing lever to a drum. The tissue was allowed to equilibrate for 45 min under a load of 1g. The contractile responses of tracheal strip to histamine (30μg/ml) with doses of 0.1ml, 0.2ml, 0.4ml, 0.8ml and 1.6ml were recorded in absence and presence of methanolic extract of *Tridax procumbens* (L) (TP) (100 μg/ml) by using Sherrington’s Recording Drum with a frontal writing lever. The similar concentration-effect curve was taken in presence of standard drug Chlorpheniramine Maleate (1 μg/ml). The height of response curve was measured to express percentage inhibition. The graph was plotted by taking log dose verses height of response curve.

Histamine induced contraction of chicken ileum preparation \(^{[8,9 \text{and } 10]}\)

Chicken ileum was suspended in bath containing Tyrode solution (Composition mM: NaCl, 136.7; KCl, 2.68; CaCl\(_2\), 1.8; NaHCO\(_3\),11.90; NaH2PO\(_4\), 0.42; MgCl\(_2\), 1.05; glucose, 5.55) maintained at 37±0.5°C. A stream of air was bubbled through the organ tube (1bubble/sec). One end of the ileum was attached to S-shaped aerator and the other attached to isotonic frontal writing lever to a drum. The tissue was allowed to equilibrate for 45 min under a load of 500 mg. Contact time of 60 sec, and base line of 30sec time cycle were opted for proper recording. Cumulative concentration-effect curves were recorded on kymograph for Acetyl choline (1μg/ml) in absence and presence of methanolic extract of *Tridax procumbens* (L) (TP) (100μg/ml) on Kymograph by using Sherrington’s Recording Drum. The same procedure was carried for concentration-effect curve of Ach in presence of Atropine sulphate.
as a standard drug. The percentage inhibition of extract and standard drug was calculated and graph was plotted by taking log dose verses height of response curve.

RESULTS AND DISCUSSION

Preliminary phytochemical evaluation of extract showed presence of saponins, flavonoids, tannins, glycosides and carbohydrates etc. The percentage yield was obtained about 6.7% w/w. The antihistaminic effect of methanolic extract of *Tridax procumbens* L (TP) was carried out using histamine induced contraction on goat tracheal strip preparation. Histamine contracts the tracheabronchial muscle of guinea pig, goat, horse, dog and man. Goat tracheal chain is much more sensitive and easier to handle. Histamine produced dose dependent increase in contraction of goat tracheal chain and thus subsequent increase in height response curve which was significantly inhibited by aqueous extract *Tridax procumbens* L (TP) of as compared to standard drug Chlorpheniramine Maleate (Table 1). The antagonistic effect of aqueous extract towards histamine was indicated from the right shift of cumulative dose response curve of histamine in presence of methanolic extract (Figure 1) which may be due to blocking H1 receptors.

Table: 1 Antihistaminic effect of methanolic extract of *Tridax procumbens* L (TP) on goat tracheal chain

<table>
<thead>
<tr>
<th>Dose of Histamine (ml)</th>
<th>Height of DRC (mm)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Histamine</td>
<td>CPM + Histamine</td>
<td>TP + Histamine</td>
</tr>
<tr>
<td>0.1</td>
<td>5</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>0.2</td>
<td>8</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>0.4</td>
<td>11</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>0.8</td>
<td>16</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>1.6</td>
<td>20</td>
<td>10</td>
<td>14</td>
</tr>
</tbody>
</table>

Figure: 1 Antihistaminic effect of methanolic extract of TP
Similarly, spasmolytic (antispasmodic) effect of methanolic extract of *Tridax procumbens* *L* (TP) was also evaluated by observing its decrease in Ach induced ileal contractions (Table 2) and shift the dose response curve of Atropine (Figure 2) to right side. These indicate that *Tridax procumbens* *L* (TP) has spasmolytic activity on gastrointestinal smooth muscles. This effect may be contributes by anticholinergic action.

**Table: 2 Antispasmodic effect of methanolic extract of *Tridax procumbens* *L* (TP) on chicken ileum.**

<table>
<thead>
<tr>
<th>Dose of Ach (ml)</th>
<th>Height of DRC (mm)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ach</td>
<td>Atropine + Ach</td>
<td>TP + Atropine</td>
</tr>
<tr>
<td>0.1</td>
<td>6</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>0.2</td>
<td>10</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>0.4</td>
<td>14</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>0.8</td>
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<td>11</td>
<td>17</td>
</tr>
<tr>
<td>1.6</td>
<td>23</td>
<td>15</td>
<td>20</td>
</tr>
</tbody>
</table>

**Figure: 2 Antagonistic effect of TP against Ach**

**CONCLUSION**

Our present study showed that, methanolic extract of *Tridax procumbens* *L* (TP) is capable of inhibiting the response of wide range of contractile stimuli such as Histamine and Ach on tracheal and intestinal smooth muscles. Therefore possessing wide range of antihistaminic and spasmolytic action which may be useful in the treatment of respiratory and GIT disorders. Further study regards to isolation, purification, mechanisms and pharmacological screening of the active principles responsible for the activity is to be carried out.
ACKNOWLEDGEMENTS
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REFERENCES