Phytochemical Screening and Investigation of Antiulcer Activity of Tridax Procumbens

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Abstract

Tridax procumbens {Asteraceae} is a plant currently used for the treatment of stomach ailments. The present study was performed to evaluate the anti-ulcerogenic activity of the hydroalcoholic, chloroform and petroleum ether combined extracts of the plant Tridax procumbens against gastric ulceration induced by fasting and administration of Indomethacin in rats. Gastric ulceration was induced in 48 hrs starved rats. The rats were given the plant extract at the doses of 250 mg/kg and 500 mg/kg orally for group III and IV respectively. The positive controls (group II) received Ranitidine at the dose of 30 mg/kg, while negative control (group I) received distilled water. Five minutes after drug treatment, 1 ml of the ulcerative agent Indomethacin (30 mg/kg b.wt.) was given to each rat orally. Rats were sacrificed and the ulcer areas of the gastric walls were determined. The results show that the whole plant extract of Tridax procumbens has protective properties against Indomethacin induced ulcers in rats. The gastric mucosal protection against irritant substances can be mediated by a number of mechanisms that include enhanced gastric mucosal defence through increased mucus production, reducing the volume of gastric acidity. Tridax procumbens (250 mg/kg) significantly inhibited gastric ulceration when compared to control group and Tridax procumbens (500 mg/kg) caused significant reduction in ulcer when compared to standard.

Keywords: Tridax procumbens; Antiulcer; Rats.

Introduction: Natural products research continues to be an important part of the drug discovery process. The main advantage of natural products as a source of lead compounds is the tremendous molecular diversity found in nature. Advances in the methodologies used to evaluate extracts, fractions and pure compounds for biological activity have
enabled the miniaturization and automation of extremely specific biochemical tests. The net result of these advances is that screening has again become a cost-effective way of discovering new lead compounds. When coupled with new chemical methods, it is now possible to isolate and identify interesting biologically active compounds on a submilligram scale. Natural products must be able to compete effectively with these sources for the limited amount of money available for screening, in order for the field to remain of interest.

*Tridax procumbens* is a semi-prostrate annual or short-lived perennial, with stems up to 50cm long. It is a weed of pastures and a wide range of perennial crop types [1-5]. The persistent pappus enables the achenes to be carried by wind over a wind range. The plant bears daisy like yellow-centered white or yellow flowers with three-toothed ray florets [6]. 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. This weed can be found in fields, meadows, croplands, disturbed areas, lawns, and roadsides in areas with tropical or semi-tropical climates.

In traditional Ayurvedic system of medicine, several medicinal properties have been attributed to this plant. Recent pharmacological studies have established the Hepatoprotective activity, Immunomodulatory activity, wound healing activity, Ant diabetic activity, and Antimicrobial activity [7-10].

The present study was undertaken to determine the anti ulcer potential of the combined hydroalcoholic, chloroform and petroleum ether extracts of whole plant *Tridax procumbens* using an experimental gastric ulcer model namely fasting and Indomethacin induction [11-15].

**Materials and methods:**

**Experimental Animals:**

Albino rats of Wistar strain of male sex weighing 250 gms were used. They were housed in polypropylene cages at room temperature. In this experiment the animals were divided into four groups each group containing 4 animals.

**Preparation of the plant extract:**

*Tridax procumbens* plants were collected from HITS COLLEGE OF PHARMACY, Bogaram. They were dried under shade and powdered. The powdered plant material was subjected for extraction using three solvents viz. petroleum ether, chloroform, hydroalcoholic mixture (50:50). About 300 gms of plant material was used for the extraction process [16-19]. The yield of total extract was found to be 36 gms. The plant was first macerated in these solvents separately for 36 hours.
and the marc was subjected to further extraction using Soxhlet apparatus for 48 hours at a temperature of 50-60°C the extracts were concentrated at a temperature of 45-50°C and were subjected to Preliminary phytochemical screening (Figure 1).

Figure 1: Soxhlet Extraction of Plant extract.

**Phytochemical screening:**

The use of the test of Shinoda help to determine the presence of flavonoids, Liebermann-Buchard’s test revealed the existence of sterols. While presence of saponins, alkaloids, carbohydrates, tannins, proteins were revealed by respective tests (Table 1) [20-29].

**Table 1 Qualitative Chemical Examination.**

<table>
<thead>
<tr>
<th>S. no</th>
<th>Test</th>
<th>Hydroalcholic extract</th>
<th>Chloroform extract</th>
<th>Petroleum ether extract</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Detection of <strong>alkaloids:</strong> To the extract dilute HCl was added and filtered.</td>
<td>Cream color precipitate was observed</td>
<td>Cream color precipitate was observed</td>
<td>Cream color precipitate was not observed</td>
<td>Alkaloids are present in hydro alcholic and chloroform extract and absent in petroleum ether extract.</td>
</tr>
<tr>
<td>A.</td>
<td>To the filtrate Mayer’s reagent was added.</td>
<td>Reddish brown precipitate was observed</td>
<td>Reddish brown precipitate was observed</td>
<td>Reddish brown precipitate was not observed</td>
<td>Alkaloids are present in hydroalcoholic and chloroform extract and absent in petroleum ether extract.</td>
</tr>
<tr>
<td>B.</td>
<td>To the filtrate Dragendorff’s reagent was added.</td>
<td>Brown color precipitate was observed</td>
<td>Brown color precipitate was observed</td>
<td>Brown color precipitate was not observed</td>
<td>Alkaloids are present in hydroalcoholic and chloroform extract and absent in petroleum ether extract.</td>
</tr>
<tr>
<td>C.</td>
<td>To the filtrate Wagner’s reagent was added</td>
<td>Cream color precipitate was not observed</td>
<td>Brown color precipitate was not observed</td>
<td>Brown color precipitate was not observed</td>
<td>Alkaloids are present in hydroalcoholic and chloroform extract and absent in petroleum ether.</td>
</tr>
</tbody>
</table>
To the filtrate Hager’s reagent was added and yellow color precipitate was observed. Yellow color precipitate was observed and yellow color precipitate was not observed.

Alkaloids are present in hydroalcoholic and chloroform extract and absent in petroleum ether extract.

Detection of carbohydrates:
A. Molich’s test: To the extract α-napthol in sodium hydroxide was added and few drops of concentrated sulphuric acid were added from the sides of the test tube. A violet color ring was observed at the junction of two layers. A violet color ring was not observed at the junction of two layers. A violet color ring was not observed at the junction of two layers. Carbohydrate are present in hydroalcoholic extract and absent in chloroform extract and petroleum ether extract.

Detection of reducing sugars:
Fehling’s test: To the extract Fehling’s solution A and B were added and warmed on water bath for 5 min. Brick red precipitate was observed. Brick red precipitate was not observed. Brick red precipitate was not observed. Reducing sugars are present in hydroalcoholic extract and absent in chloroform and petroleum ether extract.

Tests for Flavonoids
A. Shinoda test: To the extract Magnesium ribbon and HCl solution were added and heated. Magenta color was observed. Magenta color was observed. Magenta color was observed. Flavonoids are present in hydroalcoholic, chloroform and petroleum ether extract.

B. Ferric chloride test: To the extract few drops of neutral Ferric chloride was added. Blackish red color was observed. Blackish red color was observed. Blackish red color was observed. Flavonoids are present in hydroalcoholic, chloroform and petroleum ether extract.

Detection of Saponins:
The extract was shaken with distilled water. Foaming was observed. Foaming was observed. Foaming was not observed. Saponins are present in hydroalcohol and chloroform extract. And absent in petroleum ether extract.

Detection of Tannins:
A. Lead acetate test: To the extract 10% Lead acetate solution was added. White precipitate was observed. White precipitate was not observed. White precipitate was not observed. Tannins are present in hydroalcoholic extract and absent in chloroform and petroleum ether extract.

B. Ferric chloride test: To the extract ferric chloride solution was added. Green color precipitate was observed. Green color precipitate was not observed. Green color precipitate was not observed. Tannins are present in hydroalcoholic extract and absent in chloroform and petroleum ether extract.
Detection of proteins:
Million’s test: To the extract Million’s reagent was added.
Biuret’s test: To the extract copper sulphate solution was added followed by the addition of sodium hydroxide solution.

Detection of sterols:
Liebermann-Burchard test: To the extract concentric Sulphuric acid, few drops of glacial acetic acid, followed by addition of acetic anhydride.

<table>
<thead>
<tr>
<th>6. A.</th>
<th>7. B.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pink color was observed</td>
<td>Pink color was observed</td>
</tr>
<tr>
<td>Violet color was observed</td>
<td>Violet color was observed</td>
</tr>
<tr>
<td>Green color was not observed</td>
<td>Green color was observed</td>
</tr>
<tr>
<td>Pink color was observed</td>
<td>Pink color was observed</td>
</tr>
<tr>
<td>Violet color was observed</td>
<td>Violet color was observed</td>
</tr>
<tr>
<td>Green color was observed</td>
<td>Green color was observed</td>
</tr>
</tbody>
</table>

Plant extract: *Tridax procumbens*

The three extracts- hydroalcoholic, chloroform and petroleum ether of *Tridax procumbens* were combined and used as test drug for detection of anti ulcer activity on experimental animals.

**Preparation of the Test drug (plant extract):**

Preparation of the test drug T₁: *Tridax procumbens* 250 mg/kg b. wt: About 3.125 g of combined plant extract was prepared as a suspension in distilled water which gives a dose of 62.50 mg/ml.

Preparation of the test drug T₂: *Tridax procumbens* 500 mg/kg b. wt: About 6.250 g of combined plant extract was prepared as a suspension in distilled water which gives a dose of 125 mg/ml.

**Preparation of the standard drug:** Ranitidine 30 mg/kg b. wt: Ranitidine (150mg) tablet powder quantity equivalent to 375 mg was weighed and prepared as a suspension in distilled water which gives a dose of 7.5mg/ml, used as a standard drug in the experiment.

**Control:** Distilled water

**Induction of ulcers in experimental animals**

**Fasting and Indomethacin:** Gastric ulceration was induced in 48 hrs starved (deprived of food and water) rats using Indomethacin at a dose of 30 mg/kg body weight.
Preparation of Indomethacin: About 750 mg of Indomethacin was dissolved in 100 ml of 1% Tween 80 prepared using distilled water which gives a dose of 7.5 mg/ml.

Experimental protocol

**Group I**: Rats served as normal-control and received the vehicle (1 ml distilled water/rat) orally.

**Group II**: Rats served as standard and received Ranitidine 30 mg/kg b. wt in distilled water as a fine aqueous suspension orally.

**Group III**: Rats served as test (T1) and received a dose 250 mg/kg b. wt. in distilled water as fine aqueous suspension orally.

**Group IV**: Rats served as test (T2) and received a dose 500 mg/kg b. wt. in distilled water as fine aqueous suspension orally.

**Fasting-Indomethacin induced ulcer:**

Gastric ulceration was induced in 48 hrs starved (deprived of food and water) rats. The rats were given the plant extract at the doses of 250 mg/kg and 500 mg/kg orally for group III and IV respectively. The positive controls (group II) received Ranitidine at the dose of 30 mg/kg, while negative control (group I) received distilled water. Five minutes after drug treatment, 1 ml of the ulcerative agent Indomethacin (30 mg/kg b. wt.) was given to each rat orally. The rats were killed after 24 hrs using chloroform, and the stomach removed and observed for ulcers in the glandular region (Figures 2-5). The surface area of each lesion was measured and scored. The ulcer index of each rat was taken as the mean ulcer score. The percentage ulcerated surface was calculated as the total area covered by all lesions expressed as a proportion of the total corpus mucosal surface area. The percentage of inhibition (%I) was calculated (Figure 6). 

$$\%I = \frac{(Usc-ust).100}{Usc}$$

**Ulcer Index:**

The stomach was removed and fixed on a cork plate and the number and severity of the ulcers was registered with a stereo-microscope using the following scores

**Score:**

0 = Normal colored stomach.

0.5 = Red coloration

1 = Spot ulcer
1.5 = Hemorrhagic streaks.
2 = Ulcer >3 but < 5,
3 = Ulcer >5

Results and discussion:

Oral administration of Indomethacin produced characteristic lesions in the glandular portion of the rat stomach, with a total surface 76.66 mm$^2$. The whole plant extract of *Tridax procumbens* produced dose-dependent inhibition of gastric ulceration ranging from 89% at the dose of 250 mg/kg to 94% at the dose of 500mg /kg with a respective ulcer surface area of 10 and 4.5 mm$^2$ (Table 2).

Table 2: Effect of *Tridax procumbens* (ulcer index) in Fasting- Indomethacin induced ulceration.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Ulcer index</th>
<th>U S area (mm$^2$)</th>
<th>% I</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>--</td>
<td>7.5</td>
<td>76.66</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>Ranitidine</td>
<td>30</td>
<td>1</td>
<td>6.0</td>
<td>92.17</td>
</tr>
<tr>
<td>III</td>
<td>Extract</td>
<td>250</td>
<td>1.5</td>
<td>10</td>
<td>86.95</td>
</tr>
<tr>
<td>IV</td>
<td>Extract</td>
<td>500</td>
<td>0.75</td>
<td>4.5</td>
<td>94.12</td>
</tr>
</tbody>
</table>

Number of rats per group=4
I=Inhibition
U S= Ulcerated Surface
These results show that the whole plant extract of *Tridax procumbens* has protective properties against Indomethacin induced ulcers in rats. The gastric mucosal protection against irritant substances can be mediated by a number of mechanisms that include enhanced gastric mucosal defense through increased mucus production, reducing the volume of gastric acidity. *Tridax procumbens* (Test 1) significantly inhibited gastric ulceration when compared to control group and *Tridax procumbens* (Test 2) caused significant reduction in ulcer when compared to standard.

Conclusion:

*Tridax procumbens* is known for several potential therapeutic activities like antiviral, anti oxidant, antibiotic efficacies, wound healing activity and insecticidal. The powdered plant material was subjected for extraction using three solvents viz. petroleum ether, chloroform, hydroalcholic mixture (50:50) and was subjected to Preliminary phytochemical
The hydro alcoholic, chloroform, and petroleum ether extracts gave the positive test for flavonoids, proteins.

The hydro alcoholic, chloroform extracts gave the positive test for alkaloids, saponins. The chloroform, petroleum ether extracts gave the positive test for tannins, sterols.

The rats were given the plant extract at the doses of 250 mg/kg and 500 mg/kg orally for group III and IV respectively.

The whole plant extract of *Tridax procumbens* produced dose-dependent inhibition of gastric ulceration ranging from 89% at the dose of 250 mg/kg to 94% at the dose of 500 mg/kg with a respective ulcer surface area of 10 and 4.5 mm$^2$.

Significant anti ulcer activity was found by the combined extract (hydro alcohol, petroleum ether, chloroform) of *Tridax procumbens*.

**Figures:**

**Effect of *Tridax procumbens* Extract on Fasting Ulceration with Indomethacin.**

![Figure 2A: Control group](image1.jpg)  ![Figure 2B: Control group](image2.jpg)  ![Figure 2C: Control group](image3.jpg)

*Figure 2: Exposed stomach region of Control group rats*
Figure 3A: Ranitidine (30 mg/kg)  
Figure 3B: Ranitidine (30 mg/kg) 

Figure 3: Exposed stomach region of Standard group rats

Figure 4A: Plant Extract T\textsubscript{1} (250 mg/kg)  
Figure 4B: Plant Extract T\textsubscript{1} (250 mg/kg) 

Figure 4: Exposed stomach region of Test -1 group rats

Figure 5A: Plant Extract T\textsubscript{2} (500 mg/kg)  
Figure 5B: Plant Extract T\textsubscript{2} (500 mg/kg) 

Figure 5: Exposed stomach region of Test-2 group rats.
Figure 6: Effect of Tridax procumbens extract on indomethacin induced ulcers.

Group 1: Control
Group 2: Ranitidine (30 mg/kg)
Group 3: Plant Extract (250 mg/kg)
Group 4: Plant Extract (500 mg/kg)

References:


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