Demonstration of anti-inflammatory activity of alcoholic and hydroalcoholic extracts of \textit{Tridax procumbens} using the rat paw edema assay.

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INTRODUCTION

Synthetic drugs used in treatment of inflammatory conditions are not free from undesirable side effects and also very expensive to develop. On the contrary medicines of plant origin are believed to be safe and without any adverse effects and also constitute readily available, cost effective and natural source for medicines. Therefore, in the past efforts have been made to screen several plants for their anti-inflammatory activities (Flower et al 1980, Paschapur et al 2009). specimen voucher number is 9107.

\textit{Tridax procumbens} linn is a common weed belonging to Asteraceae family and is found throughout the year. It has been reported to possess antioxidative, immunomodulatory, anti diabetic, hepatoprotective activities and used against jaundice in indigenous medicine. This plant is used as an antifungal, insect repellant, wound healing and against dysentery in traditional medicine. It promotes hair growth and leaf extracts are used to treat infectious skin diseases in folk medicines. (Salandeen et al 1991, Bhagwat et al 2008, Raivkumar et al 2005, Tiwari et al 2004, Saraf et al 1991,Diwan et al 1982). In order to scientifically apprise some of ethnomedicinal uses of \textit{Tridax}, the present study has been undertaken to investigate the anti-inflammatory activity of \textit{Tridax procumbens} leaf extracts using the rat paw edema assay.

MATERIALS AND METHODS:

Plant material: The leaves of \textit{Tridax} (Linn) were collected from the local region of Nagpur; the leaves were identified by the staff of R. T. M. Nagpur University's Botany department as the leaves of \textit{Tridax procumbens} Linn. Voucher specimen of the plant has been deposited in the university's herbarium. The specimen voucher number is 9107.

Preparation of Extracts:
The healthy, fresh, mature leaves were collected washed under tap water and then by distilled water. The leaves were air dried under shade and powder of air dried leaves \textit{T. procumbens} was exhaustively macerated for seven days with hydro alcoholic (1:1) with frequent shaking. The macerate was filtered and concentrated in vaccum under reduced pressure at 35 C, give yield of 9.23 gram % of hydroalcoholic extract. Whereas, crude alcoholic extract was prepared by extracting powder with ethanol in soxhlet apparatus. The above extract concentrated and dried giving yield 12.44 gram %.

Animals:

Received 12^th Jan accepted after revision 29^th May 2011

BBRC ISSN : 0974 - 6455 A Soc Sci Nat India Publication
Wistar albino rats of either sexes, weighing between 150-200 grams were used. The animals were kept in boxes in a condition of temperature (37), with air humidity control, light and dark cycles of 12 hours and were allowed free access to food (Standard pellet diet) and water adlibitum. All animals were fasted for 12 hours but allowed free access to water before commencement of experiment.

**Anti-inflammatory Activity:**
To test anti-inflammatory activity method of winter et al is used (1962). In this study, rats were placed in 5 groups each containing five animals (n = 5). Group – I receiving vehicle only. Group -II animals receiving extract (100mg/kg), Group - III receiving extract (250mg/kg). Group - IV receiving plant extract (500mg/kg). Group – V receiving reference drug Indomethacin (100mg/kg). Same experiment was carried out using alcoholic extracts of *Tridax procumbens* Linn. Acclimatization of rats was done for 60 minutes before any injection. The inflammatory agent carrageenan (Hi- Media) 0.1 ml (1%) in saline was injected to sub plantar region of right hind paw. The paw volumes of the animals were determined using Plethysmometer (Ugo Basile, Italy). The measurement of paw volume were taken before injection and then at hourly intervals for 3 hours after injection of carrageenan. Hydro-alcoholic extract was administered orally 1 hour before carrageenan injection at various doses of 100, 250, 500 mg/kg of body weight. Indomethacin was used as reference drug 100 mg/kg. Same procedure was repeated for alcoholic extract. Anti-inflammatory activity is expressed as percent inhibition of edema.

\[\% \text{Inhibition} = \frac{Co - Ct}{Co} \times 100\]
Where, Co = Average inflammation of control. Ct= Average inflammation of experimental. 
Difference between experimental and control rats was analyzed statistically by students T test. Values for edema (inflammation) was expressed as mean ± S.D. Difference with p value less than 0.05 between experimental group and control animals were considered statistically significant.

**Phytochemical screening:**
Chemical tests were carried out on the powdered sample obtained from alcoholic and hydro-alcoholic extracts using standard procedures for preliminary identification of phytoconstituents as described by Sofowara (1993).

**RESULTS & DISCUSSION:**
Anti-inflammatory activity of orally administered alcoholic and hydroalcoholic extracts at 1st and 3rd hours on carrageenan induced rat hind paw edema is shown in table. Injection of carrageenan in control group induces inflammation with prominent increase in paw thickness for observed period of three hours. Animals in group orally administered with hydro-alcoholic extract at doses of 100, 250 and 500 mg/kg of body weight inhibited the formation of paw edema by 4.54%, 15.28% and 22.2% during first hour whereas, it is 10.82%, 16.80% and 25.4% at the 3rd hour of experiment respectively.

Animals in group treated with reference drug Indomethacin inhibit edema formation by 37.6% and 65.8% during first and third hours respectively. Oral administration of crude alcoholic extract showed dose and time dependent inhibition. 21.66%, 23.88% and 26.11% inhibition observed at first hour for alcoholic extract. Highest edema inhibition for alcoholic extract was 36.78% at concentration of 500 mg/kg of body weight and 30.05% for 100 mg/Kg whereas it is 33.16% for 250 mg/kg of body weight during third hours. Results of both crude extracts are statistically significant compared to control in which continuous increase in edema is noted for observed period. Alcoholic extract of *Tridax procumbens* showed more potent anti-inflammatory activity at all tested concentration compared to hydro-alcoholic extract.

Carrageenan induced paw edema is biphasic event. In the first phase different chemical mediators such as histamine and serotonin play role, while in second phase kinins and prostaglandins are mainly involved (Hernandez et al., 2002, Vinegar et al., 1969). Edema inhibition in our study is continuously increases from 1st hour to 3rd hour, in a dose dependent manner. This finding indicate that leaves extracts probably inhibit different aspects and various chemical mediators of inflammation. Studies have shown that many secondary metabolites from the plants with diverse chemical structure posses anti-inflammatory activity in studied animal models. (Hyun-Ju 2005, Santos et al 1997). Many phytochemicals such as Procumbenitin, (3S)-16,17-didehydrofalcarnil, flavones glycoside: 5,7,4-d and Trihydrax–6,3-dimethasley Falavone 5-0 alpha–L-rhamnopyramoside are isolated and structurally characterised from *Tridax* (Ali et al 2001, Zhelmy et al 2010, Yadava et al., 1998, Raju et al 1994).

Preliminary phytochemical identification of hydro-alcoholic and alcoholic extracts of *Tridax* also showed presence of various secondary metabolites including sterols, sugars, tannins, flavanoids, polyphenols and anti-inflammatory activity may be attributed these bioactive compounds. From the above study it is quite apparent that the *Tridax* leaves extract possesses significant anti-inflammatory activity. The study justifies its use in inflammation. It needs further studies to determine the structure of bioactive compound responsible for studied activity and its mechanism of action.
Table No. 1: Preliminary Phytochemical Screening of extracts of *Tridax procumbens* leaves.

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Plant constituent</th>
<th>Test/Reagent</th>
<th>Alcoholic</th>
<th>Hydoralcoholic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Sterols</td>
<td>Libermann-Buchard’s</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salkovaski</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Alkaloids</td>
<td>Dragendorff’s</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hager’s</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mayer’s</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wagner’s</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tannic acid</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Saponins</td>
<td>Foam test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Anthraquino ne glycoside</td>
<td>Bontrager’s</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Sugars</td>
<td>Molisch’s</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Barfoed’s</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>6.</td>
<td>Cardiac glycoside</td>
<td>Killer-Killiani</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>Tannins</td>
<td>Ferric chloride</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lead acetate</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Potassium dichromate</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>Flavanoid</td>
<td>Shinoda</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>9.</td>
<td>Total Polyphenol</td>
<td></td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

Percent inhibition of carrageenan induced edema by alcoholic extract of *Tridax* leaves at 1st and 3rd hours compared to control.
Percent inhibition of carrageenan induced paw edema by Hydroalcoholic extract of *Tridax* leaves at 1<sup>st</sup> and 3<sup>rd</sup> hours compared to control.

![Graph showing percent inhibition](image)

### REFERENCES


