Antti-inflammatory activity of the methanolic extract of Solanum torvum (berries) by formaldehyde induced Rat pow oedema.

Paper subtitle: Solanum torvum as an anti inflammatory plant on formaldehyde induced rat pow oedema model.

Bhagyashree Mahanta\textsuperscript{a}  
Department of Zoology  
Gauhati University  
Assam, India  
E-mail: mahantabhagyashree@yahoo.in

Jogen Ch. Kalita\textsuperscript{a},  
Department of Zoology  
Gauhati University  
Assam, India  
E-mail: jogenck@yahoo.co.in

Abstract—The methanolic extract of the leaves of raw berries of the Solanum torvum was investigated for its anti inflammatory activity in animal model. The extract at different doses (300 and 600 mg/kg b.w) used showed good anti inflammatory activity which has been done significantly, by the formation of oedema induced by formaldehyde. These results were also comparable to indomethacene, the reference drugs used in this study. The results from present study indicate the efficacy of the methanolic extract as a therapeutic agent in acute as well as chronic inflammatory properties. The phytochemical screening revealed the presence of active phytoconstituents i.e. flavonoids and phenolics, which may offer anti-inflammatory conditions. Thus it could be concluded that the methanolic extract of raw berries of Solanum torvum possess significant anti-inflammatory properties.

Keywords - Anti-Inflammation, Solanum Torvum, Indomethacene, Rats, Phytochemicals.

I: INTRODUCTION

Herbal medicine is the oldest form of healthcare known to mankind. India has rich medicinal plants of more than 7500 species. Of these, 4635 species are used commercially on a fairly large scale. Over 50% of all modern clinical drugs are of natural product origin and thus these products play important roles in drug development in the pharmaceutical sciences. The compounds found in the plants that are not required for normal functioning of the body, but have a beneficial effect on health or play an active role in amelioration of diseases. Now-a-days, the demand for more and more drugs from plant sources is continuously increasing. It is therefore essential to evaluate plants of medicinal value systematically for various ailments that are used in traditional medicine.

Inflammation is a disorder involving localized increases in the number of leukocytes and a variety of complex mediator molecules. Prostaglandins are ubiquitous substances that indicate and modulate cell and tissue responses involved in inflammation [1]. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. The research into plants with alleged folkloric use as pain relievers, antiinflammatory agents, should
therefore be viewed as a fruitful and logical research strategy in the search for new analgesic and antiinflammatory drugs [1].

The present study was designed to investigate the anti-inflammatory effects of Solanum torvum in acute and chronic inflammation.

II: MATERIAL AND METHODS:

A. Plant materials

The plant was collected from Mayong circle of Morigaon district, Assam, India. The plant was identified in the Department of Botany, Gauhati University, Guwahati, Assam, India. One voucher specimen was deposited there for future reference. The whole plant was shade dried and extracted in methanol by soxhlet apparatus (MLE, yield 5%). The extracts were suspended in 0.5% Tween-80 in saline water and administered p.o.

B. Experimental Animals

For in vivo experiments, wister rats of both sexes were used. All the animals required for experiments were obtained from the Animal House Facilities of the Department of Zoology, Gauhati University, India. Experiments on animals were approved by the Institutional Animal Ethics Committee (IAEC) and accepted according to veterinary medical practice. Animal of same age group i.e., adult approximately 3 month of age weighing about 190-180 gm were taken for different experiments as per the respective protocol. Animals were housed in wire mesh plastic cages with solid bottom containing sawdust, maintained under uniform condition of natural photoperiod (12 hr light/dark cycle), relative humidity (50-85%) and temperature (25±2°C). A complete hygienic condition of the animal house was ensured before and during the time of experiments. All the rats had free access to water and commercially available animal diet with vitamins and minerals supplements (purchased from Agrivet Farm Care Division, Glaxo Smithkline, Chennai, India) and were fed ad libitum. Body weight and clinical sign were recorded and also a regular health check up of the animals was performed on daily basis throughout the experimental period.

C. Chemicals

Pure sample of Indomethacene was obtained from sigma chemicals, USA. Tween-80 (Polyoxy methylene sorbitan monoleate), Formaldehyde solution were obtained from Merck India Ltd. All chemicals were supplied by local North-East Chemicals Pvt. Ltd., Guwahati, Assam whenever required.

D. Phytochemical analysis

A qualitative phytochemical test was carried out to detect the presence of volatile oils, alkaloids, tannins, saponins, flavonoids, glycosides, steroids, terpenoids and phenols utilizing standard methods of analysis [2] [3] [4]. The intensity of the coloration determines the abundance of the compound present. For tannins one gm plant grinded, then sample was boiled in 20 mL ethanol 70% for 2 min on a hot plate. The mixture was filtered and a portion of the filtrate diluted with sterile distilled water in a ratio of 1:4 and 3 drop of 10% ferric chloride solution added. Blue-black precipitate indicated the presence of tannins. For phenol 2 mL of extract was added to 2 mL of ferric chloride solution (FeCl₃); a deep bluish green solution is formed with presence of phenols. The test for alkaloids was carried out by subjecting 5 g ground plant material extracted with 10 ml ammoniacal chloroform and 5 ml chloroform. After filtration, the solution was shaken with 10 drops aqueous sulphuric acid 0.5 M. Creamish precipitate indicated the presence of respective alkaloids. For steroids Liebermann-Burchard reaction was applied. Two hundred milligram plant material boiled in 10 ml chloroform and the mixture was filtered; a 2 ml filtrate was added to 2 ml acetic anhydride and concentrated H₂SO₄. Blue-green ring indicated the presence of steroids and red color indicated the presence of terpenoids. The alcoholic extract (15 ml, corresponding to 3 g of plant material) was
treated with a few drops of concentrated HCl and magnesium ribbon (0.5 g). Pink-tomato red color indicated the presence of flavonoids. The test for saponin was carried out by subjecting 5 g of the plant powder extracted with 15 ml methanol. After evaporation, residue was shaken vigorously with ethyl ether and 5 ml HCl 2N. Precipitate indicated the presence of saponin.

For detection of volatile oils, 1 g fresh plant sample was boiled in 10 ml petroleum ether, filtered and then 2.0 ml of extract solution was shaken vigorously with ethyl ether and 5 ml HCl 2N. A white precipitate indicated the presence of volatile oils [5].

The extract was also tested for free glycoside. Fehling’s solution (A and B) was added to the extract and the solution was heated on a hot plate and brick-red precipitate indicated the presence of glycosides.

E. Toxicity studies (LD-50)

Wistar rats of both sexes were taken for this experiment. Animals were divided in six groups (n=6) and were given different doses of plant extract (p.o.) (150, 300, 500, 1000, 2000, 3000mg/kg, b.w.) for four consecutive days and their mortality, loss of body wt. and general behaviour was recorded from the first dose up to 72 hours after the last administration of plant extract. One group was taken as control group and was administered with normal saline (p.o.) [6].

F. Anti-inflammatory activity
(Formaldehyde-induced rat paw oedema)

In this method, rats were again divided into four groups of four each. The animals of each groups were pretreated with only vehicle, drug (Indomethacene 10mg/kg b.w.) and methanolic extracts in concentration of (MLE-300mg/kg, and 600mg/kg b.w.) were given by (p.o.) one hour before formaldehyde injection. 0.2ml of 1% w/v formaldehyde was injected into the sub plantar tissue of left hind paw of each rat. Swellings of formaldehyde-injected foot were measured once at 0hr, 1hr, 2hr and 3hr for acute study and another in day1, day2,day3, day4 and day15 for chronic study by using cotton thread [7] [8] [9] [10].

G. Statistical analysis of the data

Values are expressed as Mean ± SEM & significance within the group was analysed using Student’s t test and P <0.05 and P <0.01 was considered to be significant [11].

H. Calculation of inhibitiontions (%) 

Percentage of inhibition of pow volume for each biological parameter was calculated using the following formula:

\[
\text{Inhibition rate} = \frac{(V_t - V_o) C - (V_t - V_o) E}{(V_t - V_o) C}
\]

Where, V_t =left hind paw volume at time t, V_o=left hind paw volume before sub plantar injection, C= control group, E= experimental group [12].

III. RESULT AND DISCUSSION

The results of the preliminary phytochemical analysis showed the presence of alkaloids, tannins, flavonoids, seponin, steroids, volatile oil, phenols, glycosides and sterols. Methanolic extract of Solanum torvum did not produce any toxic symptoms or mortality up to the dose level of 3000 mg/kg body weight in rat, and hence the extract was considered to be safe and non-toxic for further pharmacological screening.

The present study establishes the anti-inflammatory activity of methanolic extract of Solanum torvum berries collected from Mayong, Morigaon district, Assam, India , both in acute (0hr to 3hr) and chronic phase (day1 to day 15).

In the acute phase no significant anti-inflammatory effect was shown by two different dose of plant extract (300 and 600mg/kg body weight) in respect to indomethacene (table II). On the other hand in chronic phase, both the dose showed significant anti-inflammatory effect (table III).

The percentage of inhibition in the pow volume of Rat was not as much satisfying by the both doses of extract in respect to
indomethacene in the acute phase, but in chronic phase 600mg/kg B.W. dose showed a neck to neck result with indomethacene in day2, day3, day4 and day 15 (fig II). In day 3 the percentage of inhibition was greater in 600mg/kg B.W. dose of plant extract (69.45%) then indomethacene(68.06%).

Among the people of Mayong, Morigaon district, Assam, India (Survey and plant collection area for the authors),there is a belief that the berries of Solanum torvum has good utility against inflammation and pain. But this kind of knowledge about traditional medicine is only in the minds of the people of Mayong. There are no any type of documentation are available about traditional medicine. Hence, results of present investigations might give scientific authentication to the traditional claims. Observed results may be due to the presence of phytochemical constituents like phenols, saponins and glycosides. Thus the results from present study indicate the efficacy of the active constituents as a therapeutic agent in chronic inflammatory conditions.

IV. ACKNOWLEDGEMENT

The authors are thankful to the Dept. of Zoology and Dept. of Botany Gauhati University for providing all the necessary supports.

V. REFERENCES:


### Table I: Preliminary phytochemical screening of the plant *Solanum torvum*

<table>
<thead>
<tr>
<th>Plant sample</th>
<th>Phytochemical groups</th>
<th>Detection (+=presence/-=absence)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Solanum torvum</em></td>
<td>Tenin</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Alkaloid</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Saponin</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Terpenoid</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Steroid</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Flavanoid</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Volatile oil</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Phenol</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Glycosides</td>
<td>+</td>
</tr>
</tbody>
</table>

### Table II: Effect of Methanolic extract of *S. torvum* and reference drug on the swelling of rat hind paw induced by Formaldehyde (Acute phase). (Mean ±SEM, n=4)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>0hr</th>
<th>1hr</th>
<th>2hr</th>
<th>3hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Vehicle treated as negative control</td>
<td>2.63±0.048</td>
<td>3.55±0.029</td>
<td>3.5±0.025</td>
<td>3.45±0.029</td>
</tr>
<tr>
<td>II</td>
<td>Indomethacene treated as positive control (10mg/kg body weight)</td>
<td>2.6±0.041&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>3.23±0.111*</td>
<td>3.23±0.095*</td>
<td>3.15±0.087*</td>
</tr>
<tr>
<td>III</td>
<td>Extract treated (300mg/kg body weight)</td>
<td>2.67±0.025&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>3.53±0.048&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>3.4±0.04&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>3.3±0.092&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>IV</td>
<td>Extract treated</td>
<td>2.63±0.048&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>3.47±0.063&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>3.35±0.065&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>3.33±0.048&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Fig 1: Inhibitory effect of *S. torvum* Methanolic extract at different concentration (300 and 600 mg/kg body weight) and Indomethacene against swelling of rat hind paw Induced by formaldehyde. (Acute phase).

**Table III:** Effect of Methanolic extract of *S. torvum* and reference drug on the swelling of rat hind paw induced by Formaldehyde (Chronic phase). (Mean ±SEM, n=4)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Vehicle treated as negative control</td>
<td>3.45±0.029</td>
<td>3.37±0.025</td>
<td>3.35±0.029</td>
<td>3.33±0.025</td>
<td>3.27±0.048</td>
</tr>
<tr>
<td>II</td>
<td>Indomethacene treated as positive control (10mg /kg body weight)</td>
<td>3.15±0.087*</td>
<td>3.03±0.025**</td>
<td>2.83±0.025**</td>
<td>2.77±0.063**</td>
<td>2.67±0.048**</td>
</tr>
<tr>
<td>III</td>
<td>Extract treated (300mg/kg body weight)</td>
<td>3.33±0.092NS</td>
<td>3.15±0.029**</td>
<td>2.97±0.058**</td>
<td>2.97±0.048**</td>
<td>2.93±0.025**</td>
</tr>
<tr>
<td>IV</td>
<td>Extract treated (600mg/kg body weight)</td>
<td>3.30±0.048NS</td>
<td>3.07±0.029**</td>
<td>2.85±0.029**</td>
<td>2.83±0.025**</td>
<td>2.75±0.029**</td>
</tr>
</tbody>
</table>
Student’s t-test: \(*P \leq 0.05; **P \leq 0.01; \text{NS} \): Not Significant.

**Fig II:** Inhibitory effect of *S. torvum* Methanolic extract at different concentration (300 and 600 mg/kg body weight) and Indomethacene against swelling of rat hind paw Induced by formaldehyde. (Chronic phase).


She is currently working as a Research Scholar in Animal Physiology and Biochemistry Lab, Deptt. of Zoology, Gauhati University. Her previous and current research interest is medicinal plant and Reproductive disease.

**Publication:**


M/S Mahanta.

He is currently working as a Associate professor of Zoology, Gauhati University.

Dr. Kalita

Membership: Seminar & Conferences attended (National & International): 45

General Secretary of the Gauhati University Teachers’ Association

(GUTA): For 4 terms
