Evaluation of the Anti-Inflammatory Activity of Desmodium Triangulare (Retz.) Merr. Root

R.S.Jayaseelan1*, Fijesh P.Vijayan1, Brindha2, Suresh .V1 Jose Padikkala2
1Department of pharmacology, J. k. k.Munirajaha medical research foundation college of pharmacy, B.komarapalayam-638183, Namakkal (DT) Tamil nadu India.
2Department of Plant Biotechnology Amala cancer research Centre Amala Nagar, thrisssur, kerala, India.

Keywords: Desmodium triangulare, Anti-inflammatory, carrageenan, dextran, formaldehyde-induce edema

Abstract

Desmodium triangulare, belonging to the family fabaceae is a medicinal plant use in the present study, the anti-inflammatory effect of Desmodium triangulare was studied using both model acute and chronic inflammatory models. carrageenan and dextran and induced acute paw edema. formalin induced chronic paw edema in swiss albino mice. Diclofenac at a dose of 10mg /kg body weight severe as standard drug. the anti-inflammatory activity estimated volumetrically by measuring the mean mice with the help of a vernier caliper at different time of paw intervals. The administration of Desmodium triangulare extract at dose 50,250mg /kg body weight given by oral administration. inhibited by carrageenan after 3 hour 50mg/kg(0.29±0.007)(47.06) 250mg kg(0.20±0.018)(64.70%)dextran with reduced after 5hour inhibition50mg/kg (0.27±0.244)(30.84%)250mg/kg (0.235±0.274)(53.86%)formaline reduced the 6th day interval 50mg(1.64±0.72 )(49.38%) 250mg/kg ( 1.15±0.47)(64.50%) of inhibition activity. The experimental data demonstrated that Desmodium triangulare extract possess remarkable anti-inflammatory activity.

Keywords: Desmodium triangulare, Anti-inflammatory, carrageenan, dextran, formaldehyde-induce edema

Introduction

Medicinal plant is believed to be an important source of new chemical substances with potential therapeutic effects. Herbalism is a traditional medicinal or folk medicine practice based on the used plant and plant extracts.(Acharya et al.,2008) many plant synthesize substance that are use full synthesis and maintenance of the health human for animals these include the aromatic substances. Most of which are phenol are there oxygen-substituted derivates such as tannis. Many herbs spices of used to human spices used to human season food yield used the medicinal compounds (lai 2004; tapsell,2006) Inflammation is a physiologic process in response to tissue damage resulting from microbial pathogen infection, chemical irritation, and/or wounding ( Philip et al.,2004). At the very early stage of inflammation, neutrophils are the first cells to migrate to the inflammatory sites under the regulation of molecules produced by rapidly responding macrophages and mast cells prostational in tissues (Cousens and, Werb2002)(Nathan2002). As the inflammation progresses, various types of leukocytes, lymphocytes, and other inflammatory cells are activated and attracted to the inflamed site by a signaling network involving a great number of growth factors, cytokines, and chemokines (Cousens and, Werb2002)(Nathan2002). Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can aggravate many diseases. (Sosa et al.,2002) The current management of inflammatory diseases is limited to the use of anti-inflammatory drugs whose chronic administration is associated with several adverse effects. Therefore, development of newer and more anti-inflammatory drugs with lesser side effects is necessary.

Material and Method

Animals

Male Swiss albino mice of 8-10 week old weighing 25-28 g, were selected from inbred group maintained under standard condition of temperature (25±5) and humidity. Animals were provided with food and water ad libitum. All experiments in this study were carried out with the prior approval of the Institutional Animal Ethics Committee (IAEC) and were conducted strictly adhering to the guidelines of Committee for the Purpose of Control and Supervision of Experiments...
on Animals (CPCEA) constituted by the Animal Welfare division of government of India.

Evaluation of acute oral toxicity of *Desmodium triangulare* extract

Acute oral toxicity studies were done according to OECD guidelines (Guidelines no: 423). 15 animals are randomly selected and divided into 5 groups (3 animals each), marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The extracts at doses 5, 50, 300 and 2000 mg/kg body weight were used for the study. The extract was administered in a single dose by gavage using a stomach tube or a suitable intubation canula. Animals were observed individually for mortality and moribundity, after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days. The additional observations such as changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity, behaviour pattern and attention to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.fare Division of Government of India.

Evaluation of Anti-Inflammatory Activities

*Carrageenan induced acute inflammation*

The anti-inflammatory activity was evaluated by the carrageenan-induced paw edema test in the mice. Male Swiss albino mice (22–28 g) were injected subplantarily into right hind paw with 0.02 ml of 1% suspension of carrageenan in 0.9% normal saline. Paw volume was measured 1h prior and for 5 h after carrageenan administration using a Vernier Caliper. The 70% methanol extract of *D. triangulare*. at dosages 50 mg/kg body and 250 mg/kg was administered (oral) 1 h prior to carrageenan injection. Diclofenac (10 mg/kg) was used as standard reference drug. The control group received equivalent volume of the vehicle. The percentages of inhibition were calculated according to the following formula.

\[
\text{Percent inhibition} = \left(\frac{V_T - V_o}{V_T - V_o}\right) \times 100
\]

Were, VT - Paw oedema at various time intervals
V0 - Initial paw oedema

Group 1: Control, injected with 1% carrageenan.
Group 2: Standard, administered with 10 mg/ml Diclofenac + 1% carrageenan injection
Group 3: The 70% methanol extract of *D. triangulare* extract 50 mg/kg body weight + 1% carrageenan injection.
Group 4: The 70% methanol extract of *D. triangulare* extract 250 mg/kg body weight + 1% carrageenan injection.

**Dextran induced acute inflammation**

The inflammatory was induced by using dextran in Swiss albino mice. All the animals were injected subplantarily into right hind paw with 0.02 ml of 1% suspension of dextran in 0.1% carboxy methyl cellulose. Paw volume was measured 1h prior and for 5 h after dextran administration using a vernier caliper. The 70% methanol extract of *D. triangulare*. extract at dosages 50 mg/kg body and 250 mg/kg was administered (oral) 1 h prior to dextran injection. Diclofenac (10 mg/kg) was used as standard reference drug. The control group received equivalent volume of the vehicle. The percentages of inhibition were calculated according to the following formula.

\[
\text{Percent inhibition} = \left(\frac{V_T - V_o}{V_T - V_o}\right) \times 100
\]

Were, VT - Paw oedema at various time intervals
V0 - Initial paw oedema

Group 1: Control, injected with 1% dextran.
Group 2: Standard, administered with 10 mg/ml Diclofenac + 1% dextran injection
Group 3: The 70% methanol extract of *D. triangulare* extract 50 mg/kg body weight + 1% dextran injection.
Group 4: The 70% methanol extract of *D. triangulare* extract 250 mg/kg body weight + 1% dextran injection.

**Formalin induced acute inflammation**

The inflammatory was induced by using formalin in Swiss albino mice. All the animals were injected subplantarily into right hind paw with 0.02 ml of 1% solution of formalin. Paw volume was measured 1h prior and for 6 days after formalin administration using a vernier caliper. The 70% methanol extract of *D. triangulare*. extract at dosages 50mg/kg body and 250 mg/kg was administered (oral) 1 h prior to dextran injection. Diclofenac (10 mg/kg) was used as standard reference drug. The control group received equivalent volume of the vehicle. The percentages of inhibition were calculated according to the following formula.

\[
\text{Percent inhibition} = \left(\frac{V_T - V_o}{V_T - V_o}\right) \times 100
\]

Were, VT - Paw oedema at various time intervals
V0 - Initial paw oedema

Group 1: Control, injected with 1% formalin.
Group 2: Standard, administered with 10 mg/ml Diclofenac + 1% formalin injection
Group 3: The 70% methanol extract of *D. triangulare* extract 50 mg/kg body weight + 1% formaline injection.
Group 4: The crude methanol extract of *D. triangulare* extract 250 mg/kg body weight + 1% formalin injection.

**Statistical Analysis**

The values are presented as mean ± SD. Differences between group's means were estimated using a one way analysis of variance followed by Dunnett test, using GraphPad Instat Software. The results were considered stastically significant when \(P<0.05\).
Table: 1. Effect of *D. triangulare* extract on Carrageenan induced paw edema in Swiss albino.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial</th>
<th>0th hour</th>
<th>1st hour</th>
<th>2nd hour</th>
<th>3rd hour</th>
<th>4th hour</th>
<th>5th hour</th>
<th>24th hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.20 ± 0.019</td>
<td>0.32 ± 0.012</td>
<td>0.34 ± 0.015</td>
<td>0.37 ± 0.011</td>
<td>0.37 ± 0.034</td>
<td>0.33 ± 0.035</td>
<td>0.30 ± 0.028</td>
<td>0.22 ± 0.010</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>0.20 ± 0.018</td>
<td>0.35 ± 0.012**</td>
<td>0.38 ± 0.008**</td>
<td>0.37 ± 0.011</td>
<td>0.27 ± 0.011**</td>
<td>0.24 ± 0.022**</td>
<td>0.22 ± 0.021**</td>
<td>0.20 ± 0.010</td>
</tr>
<tr>
<td><em>D. triangulare</em></td>
<td>0.20 ± 0.013</td>
<td>0.32 ± 0.011</td>
<td>0.32 ± 0.011</td>
<td>0.33 ± 0.013**</td>
<td>0.29 ± 0.007**</td>
<td>0.26 ± 0.011*</td>
<td>0.22 ± 0.017**</td>
<td>0.21 ± 0.010</td>
</tr>
<tr>
<td><em>D. triangulare</em></td>
<td>0.20 ± 0.018</td>
<td>0.33 ± 0.012</td>
<td>0.34 ± 0.012</td>
<td>0.30 ± 0.043**</td>
<td>0.26 ± 0.032**</td>
<td>0.23 ± 0.025**</td>
<td>0.22 ± 0.0148**</td>
<td>0.20 ± 0.013</td>
</tr>
</tbody>
</table>

Values are Mean ± SD, for 6 animals in each group. *P < 0.01; **P < 0.05 when compared to control.

Table: 2. Inhibition of carrageenan induced paw edema volume in mice by *D. triangulare* treatment on 3rd hour.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Doses</th>
<th>Initial Paw thickness (cm)</th>
<th>Paw thickness on 3rd hour (cm)</th>
<th>Increase in paw thickness</th>
<th>% of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>0.20 ± 0.019</td>
<td>0.37 ± 0.034</td>
<td>0.170015</td>
<td>—</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>10 mg/kg body weight</td>
<td>0.20 ± 0.018</td>
<td>0.27 ± 0.011</td>
<td>0.06993</td>
<td>58.86</td>
</tr>
<tr>
<td><em>D. triangulare</em></td>
<td>50 mg/kg body weight</td>
<td>0.20 ± 0.013</td>
<td>0.29 ± 0.007</td>
<td>0.08999</td>
<td>47.06</td>
</tr>
<tr>
<td><em>D. triangulare</em></td>
<td>250 mg/kg body weight</td>
<td>0.20 ± 0.018</td>
<td>0.26 ± 0.032</td>
<td>0.0600</td>
<td>64.70</td>
</tr>
</tbody>
</table>

Values are Mean ± SD, for 6 animals in each group. *P < 0.01; **P < 0.05 when compared to control.

The carrageenan induced acute inflammation. The sub plantar injection of carrageenan into the mice hind paw elicited an inflammation (swelling and erythema) and a time-dependent increase in paw oedema that was maximal at 3rd hour after injection of carrageenan (0.27±0.011)(58.86) (P < 0.001). The inflammatory response to subplantar carrageenan, i.e. edema, was significantly reduced.

Table: 3. Effect of *D. triangulare* extract on dextrone induced paw edema in swiss albino.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Doses</th>
<th>Initial Paw thickness (cm)</th>
<th>Paw thickness on 3rd hour (cm)</th>
<th>Increase in paw thickness (cm)</th>
<th>% of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>0.17 ± 0.011</td>
<td>0.31 ± 0.151</td>
<td>0.25 ± 0.280</td>
<td>0.23 ± 0.257</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>10 mg/kg body weight</td>
<td>0.18 ± 0.0205</td>
<td>0.32 ± 0.0830**</td>
<td>0.25 ± 0.211</td>
<td>0.21 ± 0.011</td>
</tr>
<tr>
<td><em>D. triangulare</em></td>
<td>50 mg/kg body weight</td>
<td>0.18 ± 0.01491</td>
<td>0.31 ± 0.1475**</td>
<td>0.27±0.0244</td>
<td>0.20 ± 0.0205</td>
</tr>
<tr>
<td><em>D. triangulare</em></td>
<td>250 mg/kg body weight</td>
<td>0.20 ± 0.0290</td>
<td>0.3140 ± 0.08616**</td>
<td>0.23 ± 0.274</td>
<td>0.22 ± 0.01461</td>
</tr>
</tbody>
</table>

Values are Mean ± SD, for 6 animals in each group. *P < 0.01; **P < 0.05 when compared to control.

Table: 4. Inhibition of dextrone induced paw edema volume in mice by *D. triangulare* treatment on 3rd hour.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Doses</th>
<th>Initial Paw thickness (cm)</th>
<th>Paw thickness on 3rd hour (cm)</th>
<th>Increase in paw thickness (cm)</th>
<th>% of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>0.17 ± 0.011</td>
<td>0.30 ± 0.011</td>
<td>0.13</td>
<td>—</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>10 mg/kg body weight</td>
<td>0.18 ± 0.0205</td>
<td>0.24 ± 0.237</td>
<td>0.05981</td>
<td>53.99</td>
</tr>
<tr>
<td><em>D. triangulare</em></td>
<td>50 mg/kg body weight</td>
<td>0.18 ± 0.01491</td>
<td>0.27 ± 0.0214</td>
<td>0.0899</td>
<td>30.84</td>
</tr>
<tr>
<td><em>D. triangulare</em></td>
<td>250 mg/kg body weight</td>
<td>0.20 ± 0.0290</td>
<td>0.26 ± 0.0325</td>
<td>0.05997</td>
<td>53.86</td>
</tr>
</tbody>
</table>

Values are Mean ± SD, for 6 animals in each group. *P < 0.01; **P < 0.05 when compared to control.

Acute oral toxicity studies

The acute oral toxicity of the methanolic extract of Desmodium triangulare was carried out by guidelines as OECD 423 – guidelines (Acute toxic class method). The acute toxicity studies revealed that LD<sub>50</sub> > 2000mg/kg for this extract.

by *D. triangulare* at doses of 50mg/kg and 250mg/kg given orally 1 hour prior to carrageenan, but the time course of the anti-oedema effect varied among two different doses. Thus, at *D. triangulare* doses of 250mg/kg, the inhibition of oedema formation by the drug was treated at and 3rd hour time points post-carrageenan and PTX given at the above doses reduced the paw oedema response.
by (0.29±0.007)(47.06) or by 0.20±0.018 (64.70)%, respectively, 3rd hour following carrageenan. (Tab.1,2)

**Dextran induced paw edema**

In the control group, the paw thickness increased to 0.307±0.011, three hours after injection of dextran, representing increase in paw thickness. The oedema response was significantly reduced at 3rd hour by

Table: 5, Effect of D. triangulare extract on formaline induced paw edema in Swiss albino.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial</th>
<th>0th day</th>
<th>1st day</th>
<th>2nd day</th>
<th>3rd day</th>
<th>4th day</th>
<th>5th day</th>
<th>6th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.20±0.0250</td>
<td>0.304±0.0134</td>
<td>0.336±0.1140</td>
<td>0.372±0.0113</td>
<td>0.392±0.0216</td>
<td>0.39±0.00707</td>
<td>0.38±0.0254</td>
<td>0.378±0.0178</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>0.20±0.1303</td>
<td>0.304±0.0134</td>
<td>0.336±0.0158</td>
<td>0.352±0.0228</td>
<td>0.372±0.0286</td>
<td>0.352±0.0189**</td>
<td>0.317±0.0170**</td>
<td>0.275±0.02081**</td>
</tr>
<tr>
<td>D. triangulare</td>
<td>0.21±0.0447</td>
<td>0.30±0.0141</td>
<td>0.334±0.0151</td>
<td>0.358±0.0148</td>
<td>0.356±0.0114*</td>
<td>0.374±0.0114</td>
<td>0.332±0.01483**</td>
<td>0.316±0.0070**</td>
</tr>
<tr>
<td>D. triangulare</td>
<td>0.206±0.2073*</td>
<td>0.298±0.0164</td>
<td>0.334±0.0207</td>
<td>0.348±0.0164</td>
<td>0.354±0.02607*</td>
<td>0.332±0.01483**</td>
<td>0.306±0.01483**</td>
<td>0.27±0.0158**</td>
</tr>
</tbody>
</table>

Values are Mean ± SD, for 6 animals in each group. * P < 0.01; ** P < 0.05 when compared to control.

Table: 6, Effect of D. triangulare extract on formaline induced paw edema in Swiss albino.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial</th>
<th>0th day</th>
<th>1st day</th>
<th>2nd day</th>
<th>3rd day</th>
<th>4th day</th>
<th>5th day</th>
<th>6th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.20±0.0250</td>
<td>0.304±0.0134</td>
<td>0.336±0.1140</td>
<td>0.372±0.0113</td>
<td>0.392±0.0216</td>
<td>0.39±0.00707</td>
<td>0.38±0.0254</td>
<td>0.378±0.0178</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>0.20±0.1303</td>
<td>0.304±0.0134</td>
<td>0.336±0.0158</td>
<td>0.352±0.0228</td>
<td>0.372±0.0286</td>
<td>0.352±0.0189**</td>
<td>0.317±0.0170**</td>
<td>0.275±0.02081**</td>
</tr>
<tr>
<td>D. triangulare</td>
<td>0.21±0.0447</td>
<td>0.30±0.0141</td>
<td>0.334±0.0151</td>
<td>0.358±0.0148</td>
<td>0.356±0.0114*</td>
<td>0.374±0.0114</td>
<td>0.332±0.01483**</td>
<td>0.316±0.0070**</td>
</tr>
<tr>
<td>D. triangulare</td>
<td>0.206±0.2073*</td>
<td>0.298±0.0164</td>
<td>0.334±0.0207</td>
<td>0.348±0.0164</td>
<td>0.354±0.02607*</td>
<td>0.332±0.01483**</td>
<td>0.306±0.01483**</td>
<td>0.27±0.0158**</td>
</tr>
</tbody>
</table>

Values are Mean ± SD, for 6 animals in each group. * P < 0.01; ** P < 0.05 when compared to control.

53.99 % 0.25±0.221 (P < 0.01) in mice receiving diclofenac (10 mg/kg, i.p.) 1 hour before dextran (Table 3,4). D. triangulare given at two different doses (50mg/kg and 250mg/kg) 1 hour before dextran had a anti-oedema effect. D. triangulare produced significant and dose dependent decrease in paw edema, 50mg /kg lower dose (0.27±0.244) 30.84 % at higher dose (250 mg/kg) reduced the inflammatory effect of dextran by (0.235±0.274) 53.86 % at 3rd hour.in addition of d triangulare extract is anti-oedema effect.

Formaline induced paw edema

In the experiment designed to delineate the effect of the D triangulare extract on formalin induced oedema, the extract given at doses of 50 mg /kg and 250mg/kg showed marked anti-oedema effect at 6th day reducing oedema by 43.75% and 63.33%, respectively. (Tab.5,6)

**Discussion and Conclusion**

The anti-inflammatory activity was evaluated by both acute (carrageenan and dextran) and chronic (formalin) models in Swiss mice. The clinical treatment of inflammatory diseases is dependent on nonsteroidal or steroidal chemical therapeutics (Rainsford, 2007). However, long-term administration of NSAID may induce gastrointestinal ulcers, bleeding, and renal disorders (Robert, 1976; Peskar, 1977; Tapiero et al., 2002). Likewise, the use of steroidal antiinflammatory agents also causes multiple side effects (Schäcke et al., 2002; Reinke et al., 2002). Therefore, developing new agents with more powerful antiinflammatory activities and with lesser side effects will be of great interest. The carrageenan inflammatory model basically reflects the actions of prostaglandins.

Authors’ Contributions
Authors contributed equally to all aspects of the study.

Peer Review
Not commissioned; externally peer reviewed.

Conflicts of Interest
The authors declare that they have no competing interests.