In Vitro Anti-inflammatory Activity of Exacum wightianum Arn. (Gentianaceae)- An Endemic Medicinal Plant

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ABSTRACT

The anti-inflammatory activity of ethanol, methanol and chloroform extracts of Exacum wightianum, was evaluated by carrageenan induced rat paw oedema method. The dose effects of 200 mg/kg b.wt of the ethanolic, methanolic and chloroform extracts of E. wightianum was more active than 100 mg/kg b.wt. and found to be statistically significant. In acute inflammation as produced by carrageenan 72.30% and 86.89% protection was observed in methanol extract of E. wightianum. These extracts did not show any sign of toxicity up to a dose of 1000 mg/kg.bw in rats. The results suggested that the methanolic extract of E. wightianum has exhibited an effective anti-inflammatory activity mediated via either by inhibition of cyclooxygenase cascade and by blocking the release of vasoactive substances like histamine, serotonin and kinins. The results also justified the use of this plant in traditional Indian medicine in the treatment of inflammation.

Keywords: Exacum wightianum, Carrageenan induced paw oedema, Methanol, Ethanol and Chloroform extract.

INTRODUCTION

Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses (Kumar et al., 2004). Although it is a defense mechanism, the complex events and mediators involved the inflammatory reaction can induce, maintain or aggravate many diseases (Sosa et al., 2002). Currently used synthetic anti-inflammatory drugs are associated with some severe side effects. Therefore, the development of potent anti-inflammatory drugs with fewer side effects is necessary from medicinal plants origin.

MATERIALS AND METHODS

The whole plant unadulterated powdered materials of Exacum wightianum was successively extracted with ethanol, methanol and chloroform in a soxhlet apparatus and concentrated to dryness. These extracts were made free of any solvent by distillation. All the extracts were subjected for acute toxicity studies and LD50 doses were determined for the pharmacological activity. These extracts were used as an emulsion in 5% suspension with gum acacia and administered orally at the dose of 100and 200 mg/kg b.wt. The animals were grouped in cage in an air conditioned room at the temperature of 22±1°C with 12 hour light and dark cycle.
The animals were maintained with pellet diet and water *ad libitum*. They were further segregated in to various groups. This experiment was performed according to ethical guidelines for the investigation of experimental pain in conscious animals (659/02/a/CPCSEA). Stranded Intra Gastric Catheter tube (IGC) was used for oral drug administration.

**Carrageenan-induced paw oedema in albino rats (Winter and Poster, 1957)**

Animals were divided into 5 groups comprising five animals in each group. In all groups acute inflammation was produced by sub plantar injection of 0.1 ml freshly prepared 1% suspension of carrageenan in normal saline in the right hind paw of the rats and paw volume was measured plethysmometrically at 0 to 180 mins after carrageenan injection. All the animals were premedicated with indomethacin (10mg/kg b.w.t.) orally two hour before infection. Mean increase in paw volume was measured and percentage was calculated for all the extract. All the extracts were subjected for acute toxicity studies and 1/10th of the LD 50 dose was selected for pharmacological activity. Percentage inhibition of paw volume was calculated by the following formula

\[
\% \text{ inhibition} = \frac{V_c - V_t}{V_c} \times 100
\]

Where

- \( V_t \) means increase in paw volume in rats treated with test compounds.
- \( V_c \) means increase in paw volume in control group of rats.

**Statistical analysis**

The mean paw volume was expressed in terms of mean ± SEM and evaluated for statistical significance by ANNOVA followed by Dunnett’s test, \( P<0.05 \) was considered by statistically significant.

**RESULT**

*E. wightianum* was evaluated by carrageenan induced rat paw oedema method. The extracts were tested at two different dose levels. The dose effect of 200 mg/kg b.wt. of the ethanolic, methanolic and chloroform extracts of *E. wightianum* was more active than 100 mg/kg b.wt. Which were found to be statistically significant. In the present study 100mg/kg b.wt. and 200mg/kg, b.wt of ethanolic, methanolic and chloroform extracts of *E. wightianum* significantly reduced the carrageenan induced paw oedema inflammation as compared with that of the standard drug, indomethacin. This results indicated that the methanol extract with the dose of 200mg/kg,b.wt and the chloroform extract with the dose of 200 mg/kg b.wt showed a maximum anti-inflammatory activity as compared to the reference drug, indomethacin. The methanol extract with a dose of 200mg/kg b.wt produced 86.89% of inhibition and it is also high as compared to the reference drug. The ethanol extract with a dose of 200mg/kg b.wt produced 39.49% of inhibition and it was lower as compared to the reference drug. The chloroform extract with two different doses of 100mg/kg b.wt and 200mg/kg b.wt. showed only 55.98% and 61.71% inhibition respectively. It was lower as compared to the reference drug, whereas in case of ethanol, methanol and chloroform extracts of *E. wightianum* at the doses of 100 mg/kg b.wt. inhibited 14.68%, 72.30% and 61.71% respectively and it was higher inhibition per cent in case of methanol extract as compared to reference drug, Indomethacin which showed 58.14%. However, the ethanol and the methanol extract at the doses of 100mg/kg b.wt. showed 14.68% and 55.98% inhibition as compared to the control drug (Table 1 and Fig 1a, b and c).

**Table 1. Effect of Exacum wightianum extracts on the Percentage inhibition of Carrageenan induced paw oedema**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose mg/kg</th>
<th>0 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
<th>% Inhibition after 180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL (Group-I)</td>
<td>Normal saline</td>
<td>39.63±2.16</td>
<td>85.11±4.15</td>
<td>103.2±2.33</td>
<td>123.31±9.33</td>
<td>-</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>100 mg/kg</td>
<td>35.08±4.57</td>
<td>74.20±3.60</td>
<td>94.12±3.18</td>
<td>105.46±5.43</td>
<td>14.68 %</td>
</tr>
<tr>
<td>(Group-II)</td>
<td>200 mg/kg</td>
<td>22.11±2.18*</td>
<td>39.73±4.05*</td>
<td>63.35±4.18*</td>
<td>74.77±3.58*</td>
<td>39.49 %</td>
</tr>
<tr>
<td>Methanol extract (Group-III)</td>
<td>100 mg/kg</td>
<td>28.32±1.95</td>
<td>38.43±2.18*</td>
<td>31.26±3.93*</td>
<td>34.24±2.87*</td>
<td>72.30 %</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>200 mg/kg</td>
<td>27.02±1.84*</td>
<td>51.17±1.86**</td>
<td>17.57±4.18**</td>
<td>16.21±1.86**</td>
<td>86.89 %</td>
</tr>
<tr>
<td>(Group-IV)</td>
<td>100 mg/kg</td>
<td>31.37±1.98*</td>
<td>71.37±2.67*</td>
<td>54.14±1.69*</td>
<td>47.33±3.90*</td>
<td>55.98 %</td>
</tr>
<tr>
<td>Indomethacin (Group-V)</td>
<td>10 mg/kg</td>
<td>29.31±1.89</td>
<td>53.43±2.68*</td>
<td>54.76±1.98*</td>
<td>47.33±3.90*</td>
<td>61.71 %</td>
</tr>
</tbody>
</table>

Each Value is SEM ± 5 individual observations * P < 0.05; ** P<0.01 Compared paw oedema induced control vs drug treated rats

Group I : Control rats given normal saline orally by using an Intra Gastric Catheter tube (IGC).
Group II : Rats given ethanol EW extract at the dose of 100 and 200 mg/ Kg body weight by IGC
Group III : Rats given methanol EW extract at the dose of 100 and 200 mg/ Kg body weight by IGC
Group IV : Rats given chloroform EW extract at the dose of 100 and 200 mg/ Kg body weight by IGC
Group V : Rats given Indomethacin at the dose of 10 mg/ Kg body weight by IGC

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![Fig 1a: Effect of Exacum wightianum extracts on the Percentage inhibition of Carrageenan induced paw oedema](image-url)
The present results of carrageenan induced paw edema model indicated dose dependent anti-inflammatory activity.

The efficacy of various extracts of *E. wightianum* used as an efficient therapeutic agent in acute anti-inflammatory conditions. The results of the study supported the traditional use of this plant in some inflammation and painful conditions which confirm the presence of active chemical compounds related to these activities, phytochemicals such as flavonoids, terpenoids, steroids and phenolic compounds expressed their anti-inflammatory activity at least in part by modulation of pro-inflammatory gene expression such as cyclooxygenase-2, inducible nitric oxide synthase and several pivotal cytokines. These are considered to be reasonable candidates for new anti-inflammatory drugs (Kim et al., 2004). The plant *E. wightianum* also could have similar phytochemicals which support the Kim et al., (2004) study. Numerous plants constituents have demonstrated anti-inflammatory properties. For example, numerous flavonoids compounds (gospin, quereetin, gnaphalin) have been associated with anti-inflammatory and may have potential in the management of inflammatory disorders (Harbone and Baxter, 1993).

**CONCLUSION**

Indomethacin showed more or less uniform inhibition of edema in early intermediate and later phases. Methanolic extract showed also more or less significant inhibition of carrageenan induced paw edema in early phases while significant inhibition at later phases. Out of ethanol, methanol and Chloroform extracts from *E. wightianum*, methanol extract was more significant than the other extract in percentage inhibition of paw edema. Acute inflammation Induced by carrageenan results from cell damage, which provokes the production of endogenous mediators, such as, histamine, serotonin, prostaglandins, and bradykinin. It is well known that inhibition of edema induced by carrageenan in rats is one of the most suitable test procedures to screen anti-inflammatory agents as it closely resembles human arthritis. As some of the above extracts significantly inhibited this model of inflammation they can be thought to possess antiproliferative and antiarthritic activities similar to indomethacin, a cyclooxygenase inhibitor.

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**REFERENCES**


