Role of *Strychnos bicirohssa* Benth. on anti-inflammatory activity in experimental model by using Wistar rats

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**ABSTRACT**

The study was intended to evaluate the anti-inflammatory activity of ethanol extract of *Strychnos bicirohssa* (*Loganiaceae*) (EESB). The anti-inflammatory activity was carried out in different methods such as carrageenan induced, cotton pellet induced, croton oil induced oedema. The study was compared to a standard drug indomethacin (10 mg/kg). The results indicated that EESB is a bioactive agent having significant role in anti-inflammatory action by inhibition of the exudation, and leukocytes recruitment into the inflamed tissues.

**KEYWORDS**: Anti-inflammatory, ethanol extract, *Strychnos bicirohssa*

**INTRODUCTION**

Inflammation is a common underlying factor contributing to the exacerbation of a wide variety of disease such arthritis, asthma and cardiovascular disease which represent a series health problem. Both steroidal and non steroidal anti-inflammatory drugs currently used in the treatment of inflammatory diseases are known to have various side effects. Thus, the hunt for natural products with anti-inflammatory properties and
minimum side effects is still a challenge and targeting the discovery of new non steroidal anti-inflammatory drugs in extracts from plants is an area of rapid growth as the average age of global populations, and hence the need for drugs to treat age related inflammatory diseases (Carretero et al., 2008).

Herbal medicines are widely used in the world primarily in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. Ancient literature also mentions herbal medicines for various diseases, for which no scientific proof is available. One such plant, *S. bicirohssa* is selected with a view to prove its medicinal properties scientifically. The plant genus of *Strychnos* family are found throughout the tropics and sub tropics with nearly 20 species in India, of which *Strychnos* is renowned for the drug value of its alkaloid strychnine and brucine (Bhatnagar and Sastri, 1960). Leaves are 5-nerved, transverse nerves parallel and conspicuous. Corolla whitish <0.75 cm across, thin-shelled, soon deciduous (Mathew, 1983). Brucine is much less toxic and has no economic or commercial importance. Of the nonpoisonous plants, the clearing-nut tree, which is used for clearing muddy water, is important from the economic point of view. Strychnine showed remarkable negative chronotropic activity on frog isolated heart and guinea pig atria. It retained its activity in vivo also (open chest dog); strychnine (50 mg/Kg) when injected s.c. increased level of acetylcholine in spinal cord and sustained convulsions in frog for 4 hours (Panda, 2002).

**MATERIALS AND METHODS**

**Plant collection and authentication**

The plant *S. bicirohssa* was collected from Ariyamangalam and the surrounding area, Tiruchirappalli districts of Tamil Nadu, and authenticated by Botanical Survey of India, Coimbatore, India. Plant authentication was given by Botanical survey of India, Agriculture University, Coimbatore.
Preparation of extract

500 g of shade dried coarsely powdered leaves of *S. bicirrhosa* was extracted exhaustively for 72 hours in a distillation apparatus with the double quantity of ethanol, which was previously distilled off before extraction. The excess ethanol from the crude extract was distilled off under reduced pressure and the concentrated crude extract was stored in a desiccator for further analysis (Harborne, 1984, Kokate, 1994 and Wagner and Roth, 1999).

Experimental animals

The toxicity studies was performed in Swiss albino mice (25-30 g) and anti-inflammatory activity only in Wistar strain rats (180-200 g). The animals were purchased from Kings Institute, Guindy, Chennai. They were housed in large spacious polypropylene cages and supplied with pellet feed and water *ad libitum*. The animals were acclimatized for at least one week in lab condition before commencement of the experiment in standard laboratory conditions 12±1 h day and night rhythm, maintained at 25±2°C and 35-60 % humidity. The study was approved by the Institutional Animal Ethical Committee (IAEC) of Committee for the purpose of control and supervision of Experiment on Animals (CPCSEA).

Acute and subacute toxicity studies

The acute oral toxicity study was carried out in Swiss Albino mice as per OECD guidelines (OECD, 2000). The LD<sub>50</sub> cut-off dose was found to be in EESB 5000 mg/kg body weight. They did not show any sign of toxicity to animals.

Anti-inflammatory activity

The anti-inflammatory activity of EESB was assessed by carrageenan, cotton pellet, croton oil induced oedema models.
Carrageenan induced paw oedema

The anti-inflammatory activity was evaluated by the carrageenan induced paw oedema in rats (Winter and Porter, 1957 and Schapoval et al., 1994). Animals were anesthetized with sodium pentobarbital (40 mg/kg i.p.) and injected subplantarly into the right hind paw, with 0.1 ml carrageenan in isotonic saline (3.0 mg/ml). The animals were treated with EESB in saline and administered intraperitoneally 1 hour prior to the subplantar injection of 0.1 ml carrageenan (10 mg/ml). Oedema measurements were made using a modified digital plethysmometer (made in Tokyo, Japan) (Winder et al., 1957). The paw volume was measured at 1, 2, 3, 4 and 24 hour after carrageenan injection. The results were expressed as percentage inhibition in relation to the control group.

Cotton pellet induced granuloma

Cotton pellets weighing about 10±1 mg were autoclaved up to 20 minutes. Cotton pellets were aseptically implanted in the interscapular distance under the skin on the previously shaved back of the rats which were anesthetized with 25 mg/kg sodium pentobarbital intraperitoneally (Yakovleva et al., 1988). Inflammation induced animals were treated with EESB intraperitoneally groups up to 7 days. After 7 days the animals were sacrificed and the pellets together with the granuloma tissues were carefully removed, dried in an oven at 60 °C. The pellets were weighed both moist and dry. Mean weight of the granuloma tissue was recorded. The weight of the pellets taken out from drug administered rats was compared with the weight of the pellets taken out from the control group and indomethacin administered rats as reported by earlier workers (D’Arcy et al., 1960 and Cylgielman and Robinson, 1963).

Croton oil-induced ear inflammation

Croton oil irritant solution prepared was applied (0.1 ml) to the inner surface of the right ear of rats. The rats were sacrificed after 4 h and 7 mm punches were made in the ear using cork borer. Each ear disc was weighed and compared with control. EESB
was administered intraperitoneally 30 minutes before croton oil application. Mean weight of the ear granuloma tissue was recorded. The weight of the ear granuloma taken out from drug administered rats was compared with the weight of the ear granuloma taken out from the control group and indomethacin i.e., standard drug was administered to rats (Mascolo et al., 1987).

**Statistical analysis**

ANOVA followed by Student–Duncan-test was used to determine significant differences between groups at $P<0.01$ and $P<0.001$.

**RESULTS AND DISCUSSION**

In first method carrageenan is an early exudative phase of inflammatory pathology was reported by (Ozaki et al., 1990) that involved the action of vasoactive amines, such as histamine, serotonin, and kinins on vascular permeability (Whittle, 1964, Green, 1984 and Vinegar and Truax, 1987). Subcutaneous injection of carrageenan into the rat paw produces plasma extravasations and inflammation characterized by increased tissue water and plasma protein exudation with neutrophils extravasations and metabolism of arachidonic acid by both cyclooxygenase and lipoxygenase enzyme pathways (Gamache et al., 1986). It was observed by the increased paw volume under the plethysmometer in experimental rats in this study. (Winter and Porter, 1957) suggested that the early hyperemia of carrageenan-induced oedema results from the release of histamine and serotonin. Thus Carrageenan-induced paw oedema in rats appears to be a biphasic events and the early phase (2.5–3 h) of the inflammation is due to the release of vasoactive amines such as histamine and serotonin. In the later phase (4.5–6 h) is due to the activation of kinin-like substances such as prostaglandins, proteases and lysosome (Olajide et al., 2000). (Table 1) showed the anti-inflammatory effect of the EESB on carrageenan induced oedema in rats. The EESB was performed (51.73 and 59.64%) inhibition of contractions at doses of 100 and 300 mg/kg b. wt, i.p., respectively a
maximum inhibitory effect at 24\textsuperscript{th} hour. In the carrageenan induced oedema test (Table 1), inhibition occurred predominantly during the second phase of the response and thus, after i.p., administration, EESB caused a (46.61\%) and (59.64\%) response inhibition of the first and second phase respectively at dose level on 300 mg/kg b.wt/i.p., The first phase showed that the EESB reduced the inflammation at 3\textsuperscript{rd} hour by control the proliferation of histamine and serotonin and in second phase at 24\textsuperscript{th} hour by controlled the stimulation of kinin like substances.

In the second method known as the cotton pellet induced granuloma (Table 2), showed that the percentage activity (27.78 and 36.93\%) at doses of EESB 100 and 300 mg/kg b.wt., i.p., exhibited negligible inhibitory effect when compared to other methods used. In this cotton pellet induced granuloma method the tissues were recovered and it was reproduced after seven days by the EESB treatment. Leukocyte adhesion represents one of the first steps in the inflammatory response initiation and it is essential for accumulation of active immune cells at sites of inflammation. (Wagner and Roth, 1999). It is observed that the tissue was reduced (from 28.64 to 25.01 mg) by dose dependent manner. By which the EESB acting on the immune cells and controlled the release of immune cells and moderately decreased the vasoactive amines.

In third, croton oil induced method (Table 2), although a moderate significant effect was observed, the percentage inhibition (34.98 and 52.75\%) in the latency to healing stimuli was observed doses at 100 and 300 mg/kg b.wt., i.p., after 30 min of drug administration, respectively. EESB 300 mg/kg b.wt., i.p., showed maximum inhibitory effect. Ear disc weight was reduced (from 10.50 to 7.63 mg) at the doses 100 to 300 mg/kg doses. Interestingly, in these cases, the effect was long lasting. The increase in stimuli could be observed 30 min after drug administration, at the doses of 100, 300 mg/kg respectively. The method has certain advantages for natural product testing (Segura et al., 1998). So, the herbal treatment is very suitable to this ear oedema method.

As expected, indomethacin was very efficient during both the first (50.66\% inhibition) and second (74.19\% inhibition) phases and its effect was totally responses in
carrageenan method, (50.10% inhibition) in cotton pellet method, (65.75% inhibition) in croton oil induced method. But peripherally acting drugs such as aspirin, indomethacin and dexamethasone only inhibit the later phase (Rosland et al., 1990). Although the direct evidence of mechanism of action of extract is not clear. Flavonoids exhibit anti-inflammatory activity (Alcaraz, 1988) it will be useful to elucidate the phytochemicals from EESB in further analysis. Thus the oral and intraperitoneal administration of the drugs, this constitutes the activity of the herbal or standard marketed drugs, in a dose dependent manner.

CONCLUSION

EESB showed that the anti-inflammatory activity by inhibition of the exudation, and leukocytes recruitment. Statistical analysis showed that the oedema inhibitions of preparations containing extract is significantly different from the control group at all the concentrations tested and the activity is dose-dependent. From the above study it can be concluded that the ethanolic extracts of *S. bicirehssa* showed the anti-inflammatory activity and the inhibitory effect in a dose dependent manner. From the above study the plant, *Strychnos bicirehssa* is considered to be a best medico biological property among the normal plant flora.

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Table 1: Anti-inflammatory activity of EESB in Carrageenan induced oedema method paw volume (in ml) and percentage inhibition (in %)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Normal Paw Volume</th>
<th>0&lt;sup&gt;th&lt;/sup&gt; Hour</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; Hour</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; Hour</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; Hour</th>
<th>4&lt;sup&gt;th&lt;/sup&gt; Hour</th>
<th>24&lt;sup&gt;th&lt;/sup&gt; Hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>0.54±0.04</td>
<td>1.43±0.04</td>
<td>1.53±0.06</td>
<td>1.66±0.05</td>
<td>1.77±0.03</td>
<td>1.82±0.02</td>
<td>1.93±0.03</td>
</tr>
<tr>
<td>Indomethacin (10 mg/kg)</td>
<td>0.49±0.01</td>
<td>1.12±0.11</td>
<td>1.06±0.11</td>
<td>0.94±0.0&lt;sup&gt;a&lt;/sup&gt; (30.944)</td>
<td>0.87±0.04&lt;sup&gt;a&lt;/sup&gt; (50.66)</td>
<td>0.78±0.05&lt;sup&gt;a&lt;/sup&gt; (56.81)</td>
<td>0.49±0.01&lt;sup&gt;a&lt;/sup&gt; (74.19)</td>
</tr>
<tr>
<td>EESB 100 mg/kg</td>
<td>0.54±0.03</td>
<td>1.25±0.04</td>
<td>1.19±0.07&lt;sup&gt;b&lt;/sup&gt; (22.14)</td>
<td>1.11±0.09&lt;sup&gt;c&lt;/sup&gt; (32.77)</td>
<td>1.03±0.09&lt;sup&gt;c&lt;/sup&gt; (41.80)</td>
<td>0.95±0.06&lt;sup&gt;c&lt;/sup&gt; (46.38)</td>
<td>0.93±0.04&lt;sup&gt;c&lt;/sup&gt; (51.73)</td>
</tr>
<tr>
<td>EESB 300 mg/kg</td>
<td>0.52±0.03</td>
<td>1.23±0.05</td>
<td>1.16±0.05&lt;sup&gt;a&lt;/sup&gt; (24.42)</td>
<td>1.09±0.06&lt;sup&gt;b&lt;/sup&gt; (34.33)</td>
<td>0.94±0.09&lt;sup&gt;a&lt;/sup&gt; (46.61)</td>
<td>0.85±0.07&lt;sup&gt;b&lt;/sup&gt; (53.04)</td>
<td>0.78±0.05&lt;sup&gt;a&lt;/sup&gt; (59.64)</td>
</tr>
</tbody>
</table>

An hour after treatment, 0.1 ml of carrageenan was injected into the plantar side of hindpaw of rats and the paw volume was measured by plethysmometrically.

Values are mean ± SD (N=6), <sup>a</sup>P<0.05, <sup>b</sup>P<0.01, <sup>c</sup>P<0.001, with respect to control. Ns, P>0.05 (Anova followed by DMRT).
Table 2: Anti-inflammatory activity of EESB in Cotton pellet, Croton oil induced oedema method. Percentage inhibition (in %)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cotton pellet weight (in mg)</th>
<th>Ear Disc weight (in mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>39.66±1.84</td>
<td>16.15±1.26</td>
</tr>
<tr>
<td>Indomethacin (10 mg/kg)</td>
<td>19.79±2.01(^a)</td>
<td>5.53±0.89(^a)</td>
</tr>
<tr>
<td>EESB (100 mg/kg)</td>
<td>28.64±2.21(^c)</td>
<td>10.50±1.37(^c)</td>
</tr>
<tr>
<td>EESB (300 mg/kg)</td>
<td>25.01±1.60(^a)</td>
<td>7.63±0.94(^b)</td>
</tr>
</tbody>
</table>

Values are mean ± SD. (N=6), \(^a\)P<0.05, \(^b\)P<0.01, \(^c\)P<0.001, with respect to control. Ns, P>0.05 (Anova followed by New Duncan’s comparison test).

Figure 1: Comparative evaluation of Carrageenan, Cotton Pellet, Croton oil induced Oedema by EESB treatment

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