Anti-inflammatory and antipyretic activities of the ethanolic extract of *Shorea robusta* Gaertn. f. resin

T A Wani, H H Chandrashekara, D Kumar, R Prasad, K K Sardar, D Kumar* and S K Tandan

Division of Pharmacology and Toxicology, Indian Veterinary Research Institute, Izatnagar-243 122 (U.P), India

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*Shorea robusta* Gaertn. f. (Sal) is one of the most important traditional Indian medicinal plants. The resin of the plant has been used in the treatment of inflammation in folklore medicine. In the present study, ethanolic extract (70%) of *S. robusta* resin (SRE) was investigated for its anti-inflammatory and antipyretic activities. Acute inflammation was produced by carrageenan-induced hind paw edema and sub-acute by cotton pellet-induced granuloma in male Wistar rats. The antipyretic activity of SRE was studied using Brewer’s yeast-induced pyrexia in rats. The rats were divided into five groups with five animals in each group. Group I was treated with vehicle i.e. 1% v/v Tween-80 and served as control. Groups II to IV were treated with three different doses of SRE (30, 100 and 300 mg/kg orally). Group V was treated with standard drug etoricoxib (10 mg/kg orally). The anti-inflammatory activity of SRE was assessed by per cent reduction in edema volume of carrageenan-induced hind paw edema and by per cent decrease in granuloma formation in cotton pellet-induced granuloma test. SRE (100 and 300 mg/kg) produced a significant reduction in edema volume and decrease in granulation tissue formation in rats. Significant reduction in pyrexia was observed at all the dose levels of SRE i.e. 30, 100 and 300 mg/kg. The results of the present study demonstrated anti-inflammatory and antipyretic activities of *S. robusta* resin and supported its traditional therapeutic use in painful inflammatory conditions and fever.

Keywords: *Shorea robusta*, Resin, Inflammation, Carrageenan, Pyrexia

Inflammation is a complex biological response of vascular tissues to harmful stimuli including pathogens, irritants, or damaged cells. It is a protective attempt to remove the injurious stimuli, as well as to initiate the healing process for the tissue repair\(^1\). Inflammatory diseases are a major cause of morbidity throughout the world and have been called the ‘King of Human Miseries’\(^2\). Acute and chronic inflammatory diseases are still one of the most important health problems in the world\(^3\). Although several agents are known to treat inflammatory disorders, their prolonged use often leads to gastric intolerance, bone marrow depression, water and salt retention. Therefore, there is a need to develop new anti-inflammatory drugs with less side effects\(^4\).

*Shorea robusta* Gaertn. f. (Sal) belonging to the family Dipterocarpaceae (two-winged fruit) is most commonly distributed in Indonesia and is also found in Malaysia, the Philippines and certain parts of Northern and Eastern India. The various parts of plant are traditionally used in India for the treatment of diverse ailments. The leaves are used to treat wounds, ulcers, itching, leprosy, gonorrhoea, cough, earache, headache and as anthelmintic. In Unani system of medicine, the resin is used for treating menorrhagia, enlargement of the spleen and for relieving eye irritations. The powdered stem, bark or bark paste is applied to stop bleeding and promote healing of cuts among the tribal inhabitants of Southern Bihar and the Kondhs of Southwestern Odisha\(^5\). The resin obtained from the plant is considered as astringent and detergent and is used for fumigating the rooms of sick persons. The resin with honey or sugar is given in dysentery, bleeding piles and for weak digestion\(^6\). The bark decoction is used as drops in ear problems. Fruits of *S. robusta* have been used in diarrhoea\(^6\), while leaf extract has been found to possess significant anti-inflammatory activity\(^7\).

Although *S. robusta* resin is traditionally used in various diseases including inflammatory disorders, but to the best of our knowledge, there is no report available on the use of ethanolic extract of *S. robusta* (SRE) resin in inflammation and pyrexia. Thus, in the present study, we have evaluated the anti-inflammatory and antipyretic activities of SRE.

\(^{*}\)Author for correspondence:
E-mail: dineshks17@ivri.res.in
Fax: +91-581-2303284
Materials and Methods

Plant material and preparation of extract
Clean i.e. unadulterated and uncontaminated S. robusta resin was obtained from Odisha State of India, where the plant grows naturally. The resin was identified and authenticated for its cleanliness by the Departments of Botany and Chemistry, College of Basic Sciences and Humanities, Odisha University of Agriculture and Technology, Bhubaneswar, India. 250 g of resin powder was extracted with 1 L of 70% ethanol in a Soxhlet apparatus at 60-75°C. Extract was concentrated by evaporation. The yield of the extract was about 16.80%. SRE was suspended using 1% v/v Tween-80 for oral administration.

Phytochemical study
Qualitative analysis of SRE for the presence various phytochemicals, such as alkaloids, anthraquinones, flavonoids, saponins, tannins, sterols, reducing sugars, glycosides, resins and triterpenes was carried out as per the methods described earlier.

Experimental animals
Adult healthy male Wistar albino rats (150-250 g) were used in the present study. Animals were kept in clean polypropylene cages and maintained at room temperature (25 ± 1°C) on a balanced ration obtained from the Feed Technology Unit of the Institute. Fresh drinking water was offered to the animals daily ad libitum. The experiments were carried out in accordance with the guidelines of Animal Ethics Committee, IVRI, Izatnagar.

Carrageenan-induced hind paw edema in rats (acute inflammation)
This test was conducted as per the method described elsewhere. Adult male albino rats weighing 150-180 g were randomly divided into different groups containing five animals each and fasted for 16 h, but allowed fresh water ad libitum. The paw volume (0 h) was measured using plethysmometer (Ugo Basile, Italy). Group I or control received the vehicle. Groups II to IV received SRE in doses of 30, 100 and 300 mg/kg b.wt., respectively. Group V received the standard drug etoricoxib (10 mg/kg b.wt.). The paw volume (0 h) was measured using plethysmometer (Ugo Basile, Italy). Group I or control received the vehicle. Groups II to IV received SRE in doses of 30, 100 and 300 mg/kg b.wt., respectively. Group V received the standard drug etoricoxib (10 mg/kg b.wt.), while group I served as control group receiving comparable volume of vehicle.

Percent inhibition = \( \frac{V_c - V_t}{V_c} \times 100 \)

where Vc and Vt represent average paw volume in control and treated groups, respectively.

Cotton pellet-induced granuloma in rats (sub-acute inflammation)
The effect of extract was studied on proliferative phase of inflammation induced by cotton pellet in albino rats (150-180 g) as per the method described previously. The rats were randomly divided into five groups of five animals each. The animals were anaesthetized using ketamine hydrochloride (100 kg b.wt., i.p.) and xylazine (15 mg/kg b.wt., i.m.). Autoclaved cotton pellets weighing 35 ± 1 mg each were implanted (subcutaneously) one on each side in the flanks or axillae of rats. From the day of cotton pellet insertion, oral administration of the drugs was done for 7 consecutive days. Groups II, III and IV received SRE at the dose of 30, 100 and 300 mg/kg b.wt., respectively. Group V received the standard drug etoricoxib (10 mg/kg b.wt.), while group I served as control group receiving comparable volume of vehicle.

The rats were sacrificed on day 8 and the pellets covered with granulation tissue were dissected out and dried in hot air oven at 60°C for 48 h till constant weight was obtained. Per cent change in granuloma was compared with the untreated control group.

Antipyretic activity
The effect of SRE on pyrexia in male rats (150-180 g) was studied using the method described previously. The rectal temperature was recorded using a clinical thermometer, which was lubricated and inserted into the rectum approximately for 45 s. Fever was induced by 20 ml/kg of 20% aqueous suspension of Brewer’s yeast in normal saline, injected subcutaneously below the nape of neck. The animals were then fasted for the duration of the experiment (approx 24 h), but water was provided ad libitum.

After 18 h of yeast injection, rectal temperature was recorded to identify the pyretic animals and the rats showing an increase of 0.5 to 1.5°C were selected.
and divided into five groups of five animals each. Groups II to IV animals received the test extract of SRE 30, 100 and 300 mg/kg b. wt. orally. Group V received a standard drug treatment (etoricoxib @ 10 mg/kg orally). Group I or control received a proportionate volume of the vehicle (Tween-80) alone. At 1, 3 and 5 h after oral dosing (0 h), rectal temperature was recorded to determine whether the rise in temperature could be reversed.

**Statistical analysis**

All the data were analyzed by GraphPad Instat Software using one-way ANOVA with Bonferroni’s multiple comparison test and two-way ANOVA with Bonferroni’s multiple comparison test. A value of P<0.05 was considered to be statistically significant.

**Results**

**Phytochemical analysis**

The phytochemical analysis of the extract showed the presence of triterpenoids, sterols and resin.

**Anti-inflammatory activity**

*Carrageenan-induced hind paw edema*

The results of oral administration of SRE extract on carrageenin-induced edema are summarized in Fig. 1. SRE (100 mg/kg) produced significant (P<0.05) inhibition in edema volume by 54.34%, as compared to the control at 5 h of its administration. At 3 h and 5 h interval, SRE 300 mg/kg produced significant (P<0.05 and P<0.01, respectively) inhibition in edema volume by 36.84% and 56.52%, respectively. None of the doses of SRE showed significant inhibition in edema volume at 1 h of administration. SRE did not show any significant dose-dependent inhibition of edema volume i.e. results were insignificant when compared with SRE 30 mg/kg. Reference drug etoricoxib (10 mg/kg) at 3 and 5 h observation period on administration significantly (P<0.01 and P<0.05, respectively) inhibited the edema volume by 45.61 and 50.00%, respectively.

*Cotton pellet granuloma*

Figure 2 shows the results of orally administered SRE on cotton pellet granuloma test in rats. SRE significantly (P<0.001) decreased the granuloma weight at all the dose levels i.e. 30, 100 and 300 mg/kg by 16.28, 24.23 and 32.52%, respectively. SRE showed significant (P<0.001) dose-dependent decrease in the granuloma weight i.e. results were significant when compared with SRE 30 mg/kg. The standard drug etoricoxib (10 mg/kg) significantly (P<0.001) decreased the granuloma weight by 31.30%.

**Antipyretic activity**

The effect of oral administration of SRE on Brewer’s yeast-induced pyrexia in rats is summarized in Table 1. SRE significantly (P<0.001) reduced the

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, oral)</th>
<th>Rectal temp. (°F) (Mean ± S.E)</th>
<th>0 h</th>
<th>1 h</th>
<th>3 h</th>
<th>5 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td></td>
<td>103.38 ± 0.17</td>
<td>104.00 ± 0.27</td>
<td>103.24 ± 0.25</td>
<td>103.22 ± 0.31</td>
</tr>
<tr>
<td>SRE 30</td>
<td>30</td>
<td></td>
<td>102.90 ± 0.32</td>
<td>102.08 ± 0.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>101.62 ± 0.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>101.60 ± 0.30&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SRE 100</td>
<td>100</td>
<td></td>
<td>102.92 ± 0.19</td>
<td>101.88 ± 0.27&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>101.62 ± 0.18&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>101.64 ± 0.33&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>SRE 300</td>
<td>300</td>
<td></td>
<td>103.38 ± 0.28</td>
<td>101.90 ± 0.40&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>101.42 ± 0.41&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>101.60 ± 0.51&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Etoricoxib</td>
<td>10</td>
<td></td>
<td>103.28 ± 0.26</td>
<td>101.08 ± 0.21&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>100.30 ± 0.19&lt;sup&gt;ac, ba&lt;/sup&gt;</td>
<td>100.54 ± 0.30&lt;sup&gt;ac&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>P<0.01, <sup>ac</sup>P<0.001 (compared with control); <sup>ab</sup>P<0.05 (compared with SRE 30)
pyrexia at all the dose levels i.e. 30, 100 and 300 mg/kg at 1 h of administration. SRE 30, 100 and 300 mg/kg significantly (P<0.01) reduced the pyrexia at 3 and 5 h of administration. Similarly, SRE 300 mg/kg at 3 and 5 h of administration, significantly (P<0.001 and P<0.01, respectively) reduced the pyrexia. SRE did not show any significant dose-dependent reduction in pyrexia i.e. results were insignificant when compared with SRE 30 mg/kg. The reference drug etoricoxib (10 mg/kg) also produced significant (P<0.001) antipyretic effect at the same time.

Discussion

Inflammation is a complex and dynamic condition in which many changes take place at the site of inflammation as well as systemically. It involves a complex array of enzymes activation, release of mediators, extravasation of fluid, migration of cells, tissue breakdown and repair. It is known that the acute inflammatory response consists of three main vascular effects viz. vasodilatation and increased vascular flow, increased vascular permeability and leucocytes migration to the injured tissues. Anti-inflammatory effects can also be elicited by a variety of chemical agents and there is no remarkable correlation between their pharmacological activity and chemical structure. This coupled with the complexity of the inflammatory process makes the use of several different experimental models necessary when conducting pharmacological studies. Hence in this study, we used the following test models i.e. i) Acute inflammation: carrageenan-induced hind paw edema, and ii) sub-acute inflammation or proliferative phase of inflammation: cotton pellet-induced granuloma formation.

Carrageenan-induced paw edema is the standard experimental model of acute inflammation and carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs, as it is not known to be antigenic and devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility. Carrageenan-induced edema is a biphasic response, the first phase is mediated through the release of histamine, serotonin and kinins, whereas the second phase is related to the release of prostaglandins and mediated by bradykinin, leucotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages.

In the present study, 70% ethanolic extract of S. robusta resin was used. The 70% ethanol is one of the most preferred solvents for the extraction of crude plant material, as almost all the constituents in the plant material (polar and non-polar) are liable to get dissolved in 70% ethanol.

SRE at the dose of 100 mg/kg b.wt. significantly reduced the edema volume at 5 h, while 300 mg/kg b.wt. dose showed significant reduction in edema volume at 3 and 5 h interval. The results suggested that anti-edematous effects of SRE on carrageenan-induced paw edema might be related to inhibition of inflammatory mediators.

As evident from the results, the anti-inflammatory activity shown by SRE (100 and 300 mg/kg) in carrageenan-induced paw edema over a period of 5 h was quite similar to that exhibited by the group treated with standard etoricoxib. These results indicated that the extract acts on later phase of inflammation. Later phase activity might probably involve with arachidonic acid metabolites which produce an edematous response by mobilization of the neutrophils.

Sub-acute/chronic inflammation is a reaction arising when the acute response is insufficient to eliminate proinflammatory agents, which includes proliferation of fibroblasts and the infiltration of neutrophils and exudation. To assess the efficacy of SRE against sub-acute inflammation, cotton pellet granuloma test was employed. This model is an indication of proliferative phase of inflammation and it has been established that dry weight of pellet is well correlated with granulomatous tissue. Inflammation involves proliferation of macrophages, neutrophils, fibroblasts and multiplication of small blood vessels.
which are the basic sources of forming a highly vascularized reddish mass termed “granulation tissue”\textsuperscript{24}. SRE significantly reduced the granuloma weight at all the three dose levels i.e. 30, 100 and 300 mg/kg b.wt. when compared to control, with 16.28, 24.23 and 32.52\% inhibition, respectively. This could be related to the capability of the extract to act on proliferative events of granulation tissue formation. Reference drug etoricoxib also significantly reduced the granuloma weight with 31.30\% inhibition.

The prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) is believed to be the rate-limiting enzyme for prostaglandin (PGE\textsubscript{2}) synthesis\textsuperscript{25}. Thus, it might be plausible to conclude that the extract inhibited the synthesis of prostaglandins. However, it must be noted that several biochemical events occur, leading ultimately to the synthesis of PGE\textsubscript{2}. Fever is believed to result from a finely tuned, complex event which involves both the peripheral immune system and the brain through which a series of inflammatory and metabolic processes are regulated\textsuperscript{26,27}. It is established that there are two pathways, leading to the transcription and induction of cyclooxygenase (COX)-2, the rate-limiting enzyme for prostaglandin (PGE\textsubscript{2}) synthesis\textsuperscript{26}. Both pathways are activated by cytokines e.g. IL-1\textalpha, IL-6 and tumor necrosis factor (TNF) which trigger central mechanisms that act via the transcription factors, such as nuclear factor (NF)kB and signal transducer and activator of transcription (STAT-3)\textsuperscript{26}. It may, therefore, be worthwhile to investigate the exact point in the biochemical events, where the extract exerts its antipyretic effect. Based on the results obtained it can be concluded that the ethanolic extract of resin of \textit{S. robusta} possessed potential anti-inflammatory and antipyretic activities.

Our data demonstrated that the ethanolic extract of \textit{S. robusta} might be capable of effectively countering the inflammation and pyrexia due to its ability to significantly reduce edema volume in carrageenan-induced paw edema, significant decrease in granulation tissue formation, as revealed in cotton pellet-induced granuloma and significant reduction in Brewer’s yeast-induced pyrexia. However, it needs further evaluation in clinical settings before consideration for the treatment of inflammation and pyrexia.

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