Evaluation of Analgesic, Antipyretic and Anti Inflammatory Activity of Different Fractions of Schima wallichii Barks

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ABSTRACT
Background: Presently medicinally important plants are the important safe therapeutic weapon to fight pain, pyrexia and inflammation in which Schima wallichii is showing interest for their reported biological activities in indigenous system of medicine. Objective: To evaluate the analgesic, antipyretic and anti-inflammatory activity of different fractions of Schima wallichii barks. Material and Methods: The analgesic activity of Schima wallichii fractions (F Nicholas, F Chlorella, and F Ethylacetate) was studied by using acetic acid induced anaesthesia and hot plate method in mice. The antipyretic activity of the fractions was studied in Brewer’s yeast induced pyrexia in rats. The anti-inflammatory activity was estimated volumetrically by measuring the mean increase in hind paw volume of rat using plethysmometer by inducing carrageenan. Results: F Chlorella and F Ethylacetate significantly decreased (p < 0.05 and 0.01, respectively) the writhing in mice where F Ethylacetate fraction persisted the action to increase the retention time in hot plate from 27 ± 1.99 to 65 ± 0.83% up to 120 min. The experimental results were comparable with the standard drug morphine sulphate. F Ethylacetate fraction reduced the swelling of hind paw in inflammatory rats from 1 h to 3 h of carrageenan injection because of decrease in percentage increase of paw volume. The antipyretic study revealed the bioequivalence of F Ethylacetate with paracetamol in respect of antipyretic activity at the time period of 15-30 h of drugs administration. Therefore, isolated flavonoid enriched Schima wallichii fraction F Ethylacetate exhibits anti-inflammatory, analgesic and antipyretic activities which may be due to its association with direct or indirect inhibition of prostaglandins because all inflammatory responses are due to increase in prostaglandin levels that results in pain, swelling and fever.

Key words: Schima wallichii, flavonoid, fractionation, antipyretic, analgesic, anti-inflammatory

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INTRODUCTION
Presently the drugs, used for the treatment of pain, pyrexia and inflammation, are synthetic having well known side effects and toxic effects. Moreover synthetic drugs are very expensive to develop since, for the successful introduction of a new product approximately 3000-4000 compounds are to be synthesized, screened and tested who’s cost of development ranges from 0.5 to 5 million dollars. Plants represent still a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs. It is therefore essential that efforts should be made to introduce new medicinal plants to develop safe and cheaper drugs. The lack of potent analgesic, antipyretic and anti-inflammatory drugs now actually in use prompted the present study, in which Schima wallichii had been selected for their reported biological activities in indigenous system of medicine. Schima wallichii (Terstroemiacæae) is a well known plant of Sikkim in the Himalayan region, India. The bark of this plant is traditionally used as antipyretic, antiseptic, anthelmintic, wound healing agent. In the present study the bark portion of the plant is selected and different isolated fractions were evaluated for analgesic, antipyretic and anti-inflammatory activity in animal models.

MATERIALS AND METHODS
Extraction and isolation of fractions: The authenticated barks of Schima wallichii were successively extracted by hydro alcohol (double distilled water: 99% absolute alcohol = 30: 70%) using soxhlet extraction apparatus. Then solvent was completely removed under reduced pressure and stored in a vacuum dessicator. The yields of the hydro alcoholic extraction were about 9.80%. The hydro alcoholic extract of Schima wallichii was used for isolation of F Nicholas, F Chlorella and F Ethylacetate fractions described earlier.

Experimental animals: Swiss albino mice (20-25 g) were used for analgesic activity and Wistar Rats (200±20 g) were used for antipyretic and anti-inflammatory studies. The animals were kept in...
polypropylene cages by maintaining balanced ration with free access of water and foods. Animals were grouped and housed in polyacrylic cages (38 x 23 x 10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2°C, relative humidity 55-65%, with dark/light cycle 12/12 h). They were allowed free access to standard dry pellet diet (Povimi Food Product Ltd. Mumbai India) and water ad libitum. The animals were acclimatized to laboratory conditions for 7 days before commencement of the experiment. All experimental methods described were reviewed and approved by the Institutional Animal Ethics Committee.

Toxicity study: Acute toxicity study of all the fractions was performed as per reported method⁶ in male Swiss albino mice. All the fractions are safe up to the dose of 2 g kg⁻¹ b.wt. p.o. in mice; therefore during this comparative study 100 mg kg⁻¹ b.wt. was selected for every animal models.

Analgesic activity: Hot plate method: The mice were divided into four groups (n = 6). Group I served as saline control. Group II to IV were treated with F₄₃, F₄₃, and F₄₃ respectively (100 mg kg⁻¹ b.wt. p.o) and Group V treated with morphine sulphate (5 mg kg⁻¹ s.c.). All of the mice were placed one by one on a hot plate⁶ maintained at 55 ± 1°C and the reaction latency (in seconds) for licking of hind paw or jumping noted. The cut off period was 15 seconds to avoid the tissue damage. All the fractions and morphine sulphate were treated 1 h before the mice were placed on a hot plate.

Acetic acid induced writhing test⁶: The mice were divided into four groups (n = 6). Group I received acetic acid (1% v/v, 10 mL kg⁻¹ b.wt., i.p) and writhing reflex was noted for the period of 15 minutes. Group II to IV received the fractions F₄₃, F₄₃, and F₄₃ respectively at the doses of 100 mg kg⁻¹ b.wt. p.o. and then acetic acid (1% v/v, 10 mL kg⁻¹ b.wt., i.p) was treated with these groups. Group V received reference drug aspirin (100 mg kg⁻¹ b.wt. p.o.) followed by acetic acid (1% v/v, 10 mL kg⁻¹ b.wt., i.p). All the fractions and aspirin were administered 1 h before administration of acetic acid (1% v/v, 10 mL kg⁻¹ b.wt., i.p) and writhing reflex was noted for the period of 15 min.

Antipyretic activity: Antipyretic activity was measured by Brewer’s yeast induced pyrexia in rats. Male wistar rats were fasted overnight with water ad libitum before the experiments. Pyrexia was induced by subcutaneously injecting 20% w/v Brewer’s yeast suspension (10 mL kg⁻¹) into the animals’ dorsum region. After the injection, the rectal temperatures of the animals were recorded by digital thermometer. The rats showed increases in temperature of at least 0.5°C were selected for the experiment. The fractions F₄₃, F₄₃, and F₄₃ were administered, respectively at the doses of 100 mg kg⁻¹ b.wt., p.o and the temperature was measured at 1, 2, 3 and 5 h after administration. Paracetamol (150 mg kg⁻¹ p.o.) was used as standard drug⁶.

Anti-inflammatory activity: Rats were randomly allocated into four groups (n = 6), each with six mice and treated as follows: First group served as normal control. Second, third and fourth group received three fractions F₄₃, F₄₃, and F₄₃ respectively (100 mg kg⁻¹ b.wt. p.o) daily for 3 consecutive days. Fourth group served as aspirin (100 mg kg⁻¹ b.wt., p.o) control. On day 3rd all the groups except normal control received 0.1 mL of carrageenan (1%) in the one hind paw of rats and after 60-120-180 min, the paw volume were measured by plethysmometer⁶.

Statistical analysis: Results are expressed as Mean ± SEM. Statistical significance (p) was calculated by using Graph Pad Prism version 4.03 and GraphPad Instat software (GraphPad Software Inc, San Diego, CA) and one-way ANOVA followed by Dunnett’s post hoc test of significance where p<0.05 and p<0.01 considered to be significant and highly significant, respectively.

RESULTS
Previously the analgesic activity of the Schima wallichii was reported⁸. Similarly in our study, only 13% decrease in acetic acid induced writhing was observed when mice treated with F₄₃ but in case of F₄₃ and F₄₃ there was a significant (p<0.05, and 0.01, respectively) decrease in writhing at a dose of 100 mg kg⁻¹. Interestingly more that 70% inhibition of writhing was observed in F₄₃ that was similar (p<0.01) with standard drug aspirin at dose of 100 mg kg⁻¹ (Table 1).

The F₄₃ exhibited 30% increase in retention time in hot plate in 150 min onward only, where as F₄₃ fraction persist the action to increase the retention time from 27 ± 1.09 to 65 ± 0.83% up to 150 min and this experimental result are comparable with standard drug morphine sulphate (10 mg kg⁻¹) (Table 1).

In first study, F₄₃, F₄₃, and F₄₃ and aspirin were administered 1 h before administration of acetic acid (1% v/v, 10 mL kg⁻¹ b.wt., i.p) and writhing reflex was noted for the period of 15 min. But in hot plate study all the fractions and morphine sulphate were treated 1 hr before the mice were placed on a hot plate. And the time of licking of hind paw or attempt of jumping were recorded.
Table 1: Comparative Analgesic study of the *Schima wallisii* fractions

<table>
<thead>
<tr>
<th>Groups</th>
<th>Acetoc acid induced writhing reflex</th>
<th>% Increase of retension time in Hot Plate Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>Control</td>
<td>54.33 ± 1.21</td>
<td>-</td>
</tr>
<tr>
<td>F<em>Fother</em></td>
<td>47.00 ± 0.88</td>
<td>3.57 ± 0.54</td>
</tr>
<tr>
<td>F<em>Chloroform</em></td>
<td>27.00 ± 0.89*</td>
<td>12.12 ± 0.40</td>
</tr>
<tr>
<td>F<em>Ethylacetate</em></td>
<td>16.18 ± 0.94*</td>
<td>77.71 ± 1.08</td>
</tr>
<tr>
<td>Aspirin</td>
<td>11.33 ± 1.36**</td>
<td>-</td>
</tr>
<tr>
<td>Morphine</td>
<td>31.70 ± 0.75</td>
<td>56.92 ± 0.51</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SEM (n = 6), where *p < 0.05 and **p < 0.01 when compared with control.

![Graph showing Effect of *Schima wallisii* fractions on carrageenan induced acute paw edema in rats](image1)

**Fig. 1:** Effect of *Schima wallisii* fractions on carrageenan induced acute paw inflammation in rats, after 3 days pretreatment of F*Fother*, F*Chloroform*, F*Ethylacetate* at 100 mg kg⁻¹ and aspirin 160 mg kg⁻¹, carrageenan (0.1 mL each, 1%) was injected in the hind paw of all groups of rats except normal. Paw volume was measured with a plethysmometer 1, 2 and 3 h after carrageenan injection and finally the % increase of paw volume was calculated. Values are represented as Mean ± SEM (n = 6).

In another study, aspirin significantly decreased carrageenan induced rat paw edema in an acute inflammation model. *Schima wallisii* fractions at a dose of 100 mg kg⁻¹ were used for the evaluation of its effect on acute inflammation. But only F*Ethylacetate* fraction reduced the swelling of hind paw in inflammatory rats from 1 to 3 h of carrageenan injection which was comparable to standard aspirin (Fig. 1). The percentage increase in rat paw volume was inversely proportional to anti-inflammatory activity of the drugs/fractions with compared to control.

![Graph showing Effect of *Schima wallisii* fractions on yeast-provoked elevated body temperature.](image2)

**Fig. 2:** Effect of *Schima wallisii* fractions on yeast-provoked elevated body temperature. Pyrexia was induced by subcutaneously injecting 20% w/v brewer's yeast suspension (10 mL kg⁻¹) into the animals' dorsal region. After the injection, the rectal temperatures of the animals were recorded by digital thermometer. Paracetamol (PCM) (150 mg kg⁻¹ p.o.) was used as standard drug. Values are expressed as Mean ± SEM (n = 6).

**DISCUSSION**

The acetic acid induced writhing test is normally used to evaluate the peripheral analgesic antinociceptive effect of drugs and chemicals as because acetic acid causes algnesia by the generation of endogenous substances like PGE2 and PGF2α in the peritoneal fluid, that have been reported to be responsible for peripheral pain sensation. Previously the analgesic activity of the hydro alcohol extract *Schima wallisii* was reported. In this study chloroform and ethyl acetate fractions (F*Chloroform* and F*Ethylacetate*) significantly reduce the acetic acid induced writhing in mice but the activity of F*Ethylacetate* is equivalent to standard drug aspirin (p < 0.01). Hot-plate tests have been claimed as models for studying the central analgesic properties of a given substance. Hydroalcoholic extract of *Schima wallisii* significantly attenuates the thermally induced algnesia by central effect reported previously. Similarly, in this study F*Ethylacetate* fraction persist central analgesic action to increase the retention time in hot plate from 27 ± 1.09-65 ± 0.83% up to 150 min and this experimental result conclude that flavonoid
enriched $F_{phyto}
$ fraction showed analgesic action both peripherally and centrally.

It is evident that carrageenan induced edema in rats is commonly used as an experimental model for inflammation and is believed to be biphasic: the first phase is attributed to the release of histamine, serotonin and kinin and the second phase is related to the release of prostaglandin and bradykinins. The release of these substances results in enhanced vascular permeability, thereby promoting accumulation of fluid in tissues that accounts for the edema. $S. wallaschi$ fraction $F_{phyto}$ at a dose of 100 mg kg$^{-1}$ reduces the paw volume up to 3 h of carrageenan injection. $S. wallaschi$ bark is a rich source of flavonoid compound reported earlier and flavonoids are known to inhibit the enzyme prostaglandin synthesis, therefore the anti-inflammatory activity of $F_{phyto}$ fraction is due to presence of flavonoid compound.

There was a 2°C increase in the body temperature after 5 h of Brewer's yeast (20% w/v) injection in rats but present results showed that the $F_{phyto}$ possesses a significant time dependent antipyretic effect in yeast provoked elevation of body temperature in rats and its effect is comparable to that of standard drug paracetamol. It has been observed that the isolation and purification of the constituents of an extract may lead to an increased/partial or total loss of specific bio-activity. Very often the chemical complexity of the crude or partially purified extract seems to be essential for the bioavailability of the active constituents. It has been observed that complexation with certain other clinically useful nutrients (like saponin, steroids etc.) substantially suppressed the bioavailability of such extracts and their individual constituents. In this experiment, isolated flavonoid enriched $S. wallaschi$ fraction $F_{phyto}$ exhibited anti-inflammatory, analgesic and antipyretic activities which may be due to its association with direct or indirect inhibition of prostaglandins as the inflammatory responses are due to increase in prostaglandin levels that results in pain, swelling and fever.

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REFERENCES