EVALUATION OF ANALGESIC, ANTI-INFLAMMATORY AND ANTIPYRETIC POTENTIAL OF METHANOL EXTRACT OF SWERTIA CORYMBOSA (GRISEB.) WIGHT EX C.B. CLARKE

G. MAHENDRAN*, V. NARMATHA BAI
Department of Botany, School of Life Sciences, Bharathiar University, Coimbatore 641046, Tamil Nadu, India.

Email: mahendran0007@gmail.com.

Received: 04 Feb 2013, Revised and Accepted: 25 Mar 2013

ABSTRACT
Objective: To evaluate the Analgesic, anti-inflammatory and antipyretic effects of methanolic extract of Swertia corymbosa, a folklores medicinal plant.

Methods: Methanol extract of Swertia corymbosa (100 and 200mg, body weight) was screened for analgesic (Eddy's hot plate and acetic acid induced writhing), anti-inflammatory (Carragenan induced paw edema) and yeast induced hyperthermic in rats.

Results: Treatment with methanol extract of S. corymbosa showed significant (P < 0.05 and P < 0.01) and dose dependant increase in paw licking time in Eddy's hot plate method. In writhing test, extract was significantly reduced the number of writhes. A dose dependant and significant inhibition of edema was observed in carragenan induced paw edema. Methanal extract at a dose of 200 mg/kg significantly reduction in elevated body temperature.

Conclusions: In vivo studies of S. corymbosa showed prominent antinociceptive, anti-inflammatory and antipyretic activities with ample safety profile and thus provided pharmacological base for the traditional uses of the plant in various painful conditions and pyrexia. Additional detail studies are required to ascertain its clinical application.

Keywords: Analgesic, Anti-inflammatory activity, Carragenan-induced paw edema, Methanol extract, Swertia corymbosa

INTRODUCTION
Inflammatory response is a complex process mediated by a variety of signaling molecules released by nerve endings, mast cells, platelets and leukocytes. Some of these molecules and their precursors (prostaglandins, nitric oxide, adenosine deaminase and myeloperoxidase) are used as markers of inflammation [1]. Study of inflammation with carragenan as a phlogistic agent has revealed increased vascular permeability, formation of exudate and a large number of cellular infiltrates a polymorphonuclear leukocyte [2].

Tumor necrosis factor alpha (TNF-α), an important inflammatory mediator, is a multifunctional cytokine that can regulate many cellular and biological processes, such as immune function, cell differentiation, proliferation, apoptosis, and energy metabolism. Furthermore, TNF-α can regulate the production of other pro-inflammatory cytokines, such as interleukin-6 (IL-6) and interleukin-1 (IL-1), to mediate and/or amplify their effects in peripheral organs [3]. During the acute inflammatory process, overproduction of TNF-α is crucial to the induction of inflammatory genes and the recruitment and activation of host immune cells [4, 5].

Non-steroidal anti-inflammatory drugs (NSAIDs) are used throughout the world for the treatment and management of inflammation, pain and fever. The use of NSAIDs, however, has not been therapeutically successful in all conditions of inflammation [6]. Moreover, adverse effects associated with NSAIDs can lead to ulcers and hemorrhage. As an alternative, plant based medicines are getting an increased therapeutics market share due to their mild action and fewer adverse effects. According to the World Health Organization nearly 80% of the world population prefers plant based drugs [7].

Swertia, an important genus of the family Gentianaceae, comprises about 250 species distributed in Euro-Asia, Africa and Madagascar and represented by 40 species in India [8]. Swertia species are widely used in Ayurvedic, Unani and Siddha systems of medicines [9]. In Chinese traditional medicine, Swertia are used for the treatment of hepatic, choleric, and inflammatory diseases [10]. Swertia corymbosa (Griseb.) Wight ex C.B. Clarke commonly known as Shirattakuhi by Irulars tribe. This plant has a long history of being used by Irulars and Palijan ethnic medical practitioners have been used for medicinally as diarrhea, fever, jaundice, diabetic, inflammation, anxiety, promote sleep, anti-epileptic, nervous disorders, antidote and as a stomach wash in cattle[10,11]. In order to understand the highly acclaimed properties of S. corymbosa and its usage in the traditional systems of medicine, we have attempted to evaluate its analgesic, anti-inflammatory and antipyretic potential through the use several animal models and this will provide insight information with reference to their medicinal value.

MATERIALS AND METHODS
Plant material
The fresh aerial parts of S. corymbosa were collected during the month of August, 2012 from the Vellingiri hills, Coimbatore, Tamil Nadu, India. The plant species was authenticated by Botanical Survey of India, southern circle, Coimbatore, and voucher specimen (BUBH6144) was deposited in the Department of Botany Herbarium, Bharathiar University, Coimbatore. Freshly collected plant materials were cleaned to remove adhering dust and then dried under shade. The dried samples were powdered in a Wily Mill to 60- mesh size and used for solvent extraction.

Preparation of the extract
Coarse powder from the shadow dried aerial parts was extracted in soxhlet extractor with methanol. The extracted solvent was then filtered and was concentrated by using rotary evaporator until the solvent was completely removed and a dark brownish viscous residue was obtained (yield: 30.11% w/w with respect to dried plant material).

Animals
Swiss albino mice weighing 20-30g and Wistar albino rats of 200-250 g were used for the pharmacological studies. They were housed in a clean polypropylene cage and maintained under standard laboratory conditions (temperature 25±5°C with dark/light cycle 12/ 12 h; 35-60 humidity). They were fed with standard pellet diet (VRK Nutritional solutions, Sangli, Maharashtra) and water ad libitum. The studies were carried out at Nandha College of Pharmacy and Research Institute, Perundurai, Tamil Nadu, India. The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethics Committee, and was cleared by same before beginning the experiment (688/02/C/CPSEA).
Acute toxicity

Acute oral toxicity study was performed according to acute toxic classical method on Swiss albino mice (n=6) of either sex selected by random sampling technique were used for acute toxicity study. The animals were kept fasting for overnight providing only water. Methanol extract of S. corymbosa (suspended in 0.5% carboxyl methyl cellulose) were administered orally at a dose of 5, initially to separate groups of mice and mortality was observed for 3 days. If mortality was observed in 4/6 or 6/6 animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one mouse out of six animals, then the same dose was repeated with higher doses such as 50, 300, 1000 and 2000 mg/kg. The general behaviors such as motor activity, tremors, convulsions, straub reaction, aggressiveness, piloerection, loss of lighting reflex, sedation, muscle relaxation, hypnosis, analgesia, ptosis, laceration, diarrhea and skin colour were observed for the first one hour and after 24 h of test drug administration.

Analgesic activity

Hot plate method

The hotplate method was used to measure response latencies according to the method described by MacDonald et al [12]. For the experiments, four groups (n=6) of Swiss albino mice (20–25g) were placed on a plate. Swiss albino mice (n=6) of either sex selected by random sampling technique were used for acute toxicity study. The animals were kept fasting for overnight providing only water. Methanol extract of S. corymbosa (suspended in 0.5% carboxyl methyl cellulose) were administered orally at a dose of 5, initially to separate groups of mice and mortality was observed for 3 days. If mortality was observed in 4/6 or 6/6 animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one mouse out of six animals, then the same dose was repeated with higher doses such as 50, 300, 1000 and 2000 mg/kg. The general behaviors such as motor activity, tremors, convulsions, straub reaction, aggressiveness, piloerection, loss of lighting reflex, sedation, muscle relaxation, hypnosis, analgesia, ptosis, laceration, diarrhea and skin colour were observed for the first one hour and after 24 h of test drug administration.

Anti-inflammatory activity

Carrageenan induced paw edema

For the experiment, the male wistar rats (120–150 g) were divided into four groups (n = 6). The animals were fasted overnight prior to the start of the experiment, and water ad libitum. The first group received distilled water (10 ml/kg, p.o.), while the second group was treated with indomethacin (25 mg/kg, p.o.). The third and the fourth groups were administered with the methanol extract of S. corymbosa (100 and 200 mg/kg, p.o. respectively). Acute inflammation was produced by the subplantar administration of 0.1 ml of 1% carrageenan (in 1% CMC w/v) in the right hind paw of the rats. The vehicle, extract and the standard drugs were administered 60 min prior to the injection of the phlogestic agent. The volumes of edema of the injected and the contralateral paws were measured at 1, 2, 3, 4, and 5 h after the induction of inflammation. The inflammation was measured by using an electronic vernier caliper (CD-6 CSX; Digimatic caliper, Mitutoyo, Japan) and calculates the percentage of paw edema inhibition [14].

Yeast –induced hyperpyrexia in rats

Before experimentation rectal temperature of rats were recorded by inserting a well lubricated bulb of a thermometer in the rectum. Hyperpyrexia was induced in rat by subcutaneous injection of 10ml/kg, b.w of a 15% aqueous suspension of brewer's yeast in the back below the nape of the rat. Pre-drug control temperatures were taken at 24 h after the yeast injection to determine the pyretic response of yeast. Methanol S. corymbosa extract (100 and 200mg/kg, b.w) and paracetamol (150mg/kg, b.w) served as the reference drug given orally 24 h after the yeast injection. The temperatures were recorded at 1-6 h after the drug treatment [15, 16].

RESULTS AND DISCUSSION

The methanol extract of S. corymbosa was evaluated for acute toxicity in mice. The extract did not alter the general behaviors and failed to produce any mortality even at the highest dose (2,000 mg/kg, p.o.) studied after 3 days and found to be safe. Based on acute toxicity, 2 doses i.e., 100 and 200 mg/kg were used for further pharmacological studies.

The analgesic activity of methanol extract of S. corymbosa assessed using hot plate test in swiss albino mice and the results are presented in Fig. 1.

![Fig. 1: Analgesic effect of methanol extract of S. corymbosa on heat stimulation response in the hot plate test.](image)

Values are the mean ± SD (n = 6). *P < 0.05, **P < 0.01 compared to control group control (vehicle) – distilled water.
In this analgesic testing model, pentazocine significantly prolonged the reaction time of animals with relatively extended duration of stimulation, confirming centrally mediated activity. Methanol extract of S. corymbosa showed significant analgesic activity at 100 and 200 mg/kg. Analgesic activity of the latter dose (200 mg/kg) was often higher over the positive drug pentazocine. At the dose level 200 mg/kg and 240 min reaction time, the analgesic activity (8.90 ± 0.40 s) of the test extract was higher than that of the standard drug pentazocine (8.10 ± 0.90 s). Thrombocytopenia and the values ± Sdins. Carrageenan induced paw edema formation due to carrageenan to an extent of 64.44 % (at 6h) at the dose of 25 mg/kg. The methanol extract of S. corymbosa significantly inhibited edema formation in rats (P < 0.01) in a dose dependent manner. The methanol extract at the dose of 200 mg/kg inhibited edema formation to the extent of 63.75% (at 6h) and the edema was found to be reduced to 3.15±0.24 mm. The presence of edema is one of the prime signs of inflammation [21]. It has been documented that carrageenan induced rat paw edema is suitable in vivo model to study anti-inflammatory drugs both steroidal and non-steroidal since it involves several mediators [22]. This method was chosen for the present study since edema induced by carrageenan is the most prominent acute experimental model in search for new anti-inflammatory drugs [21].

Carrageenan-induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic [23]. The early phase (1-2) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase (2.5-6) is due to the over production of prostaglandin and nitric oxide with peak at 3 h, produced by inducible isoforms of COX (COX-2) and nitric oxide synthase (iNOS) Panthong et al [24].

However, treatment with the methanol extract of S. corymbosa significantly reduced carrageenan on induced inflammation in both the phases (1-6 h) of the experiment.

### Table 1: Analgesic effect of methanol extract of S. corymbosa on acetic acid-induced writhing test.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Number of writhes (in 10 min)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 ml/kg</td>
<td>78.12 ± 2.08</td>
<td>---</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>25 mg/kg</td>
<td>21.94 ± 5.76**</td>
<td>71.91</td>
</tr>
<tr>
<td>Extract</td>
<td>100 mg/kg</td>
<td>34.65 ± 3.19*</td>
<td>55.64</td>
</tr>
<tr>
<td></td>
<td>200 mg/kg</td>
<td>19.25 ± 4.66**</td>
<td>75.01</td>
</tr>
</tbody>
</table>

The data represent the mean ± standard deviation (n = 6).

* **P < 0.05** **P < 0.01 compared to corresponding control. Control (vehicle): distilled water.

Peripheral analgesic activity was assessed by acetic acid-induced writhing test, which showed significant (P < 0.01) suppression of writhing (Table 1). Acetic acid is known to trigger the production of noxious substances within the peritoneum, which induces the writhing response [20]. The effect of the extracts against the noxious stimulus may be an indication that it depressed the production of irritants and thereby bringing a reduction in the number of writhes in animals. The writhing induced by chemical substances is due to sensitization of nociceptors by prostaglandins [14]. The abdominal constrictive response induced by acetic acid is a sensitive procedure to establish peripherally acting anti-nociceptives. The results indicate that the analgesic effect of methanol extract of S. corymbosa might be mediated by its peripheral effects by inhibiting the synthesis or action of prostaglandins.

The anti-inflammatory activity of S. corymbosa against acute pedal edema (induced by carrageenan) is shown in Table 2 and the results are comparable to that of the standard drug indomethacin, a potent inhibitor of the prostaglandins. Carrageenan induced paw edema remained even 6 h after its injection into the subplantar region of rat paw. Indomethacin as a reference standard drug inhibited the edema formation due to carrageenan to an extent of 64.44 % (at 6h) at the dose of 25 mg/kg. The methanol extract of S. corymbosa significantly inhibited edema formation in rats (P < 0.01) in a dose dependent manner. The methanol extract at the dose of 200 mg/kg inhibited edema formation to the extent of 63.75% (at 6h) and the edema was found to be reduced to 3.15±0.24 mm. The presence of edema is one of the prime signs of inflammation [21]. It has been documented that carrageenan induced rat paw edema is suitable in vivo model to study anti-inflammatory drugs both steroidal and non-steroidal since it involves several mediators [22]. This method was chosen for the present study since edema induced by carrageenan is the most prominent acute experimental model in search for new anti-inflammatory drugs [21].

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However, treatment with the methanol extract of S. corymbosa significantly reduced carrageenan on induced inflammation in both the phases (1-6 h) of the experiment.

### Table 2: Anti-inflammatory effect of S. corymbosa methanol extract on carrageenan induced paw edema in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg b. w.)</th>
<th>Swelling thickness (mm) ±SD (inhibition %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1h</td>
</tr>
<tr>
<td>Control</td>
<td>10 ml/kg</td>
<td>4.23±0.65</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>25 (22.69%)</td>
<td>3.27±0.15</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>100 (8.03%)</td>
<td>3.89±0.18</td>
</tr>
<tr>
<td></td>
<td>200 (26.24%)</td>
<td>3.12±0.41</td>
</tr>
</tbody>
</table>

The data represent the mean ± standard deviation (n = 6).

* **P < 0.05** **P < 0.01 compared to corresponding control. Control (vehicle): distilled water.

According to the result of our study, it may be concluded that the extract has a non-selective inhibiting effect on the release or actions of these mediators of inflammation. Based on this, it may be concluded that the suppression of the 1st phase may be due to inhibition of the release of early mediators, such as histamine and serotonin and the action in the 2nd phase may be explained...
by an inhibition of cyclooxygenase. The phytochemical investigation of genus Swertia revealed that xanthones, flavonoids, terpenoids, iridoids, secoiridoid glycosides and saponin [25]. Of these, xanthones are well known for their ability to inhibit pain perception. Xanthones also have anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation [26-30]. The synergistic effect of the anti-inflammatory in S. corymbosa may be responsible for the higher anti-inflammatory activity of this extract.

Subcutaneous injection of yeast suspension markedly elevated the rectal temperature after 24 h of administration. Treatment with the methanolic S. corymbosa aerial parts extract at the doses of 100 and 200 mg/kg significantly decreased the rectal temperature of the rats in a dose-dependent manner. The antipyretic effect stated as from the first and the effect was maintained for 4 h, after administration of the extract. The result obtained from both the standard (paracetamol) and methanolic extract treated rats were compared with that of control and a significant reduction in the yeast elevated rectal temperature was observed (Table 3).

Table 3: Effect of S. corymbosa methanol extract on Brewer’s yeast –induced pyrexia in rat model.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Rectal temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before treatment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24h</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>37.45±0.23</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>150</td>
<td>37.61±0.32</td>
</tr>
<tr>
<td>Methanol</td>
<td>100</td>
<td>37.11±0.12</td>
</tr>
<tr>
<td>extract</td>
<td>200</td>
<td>37.21±0.41</td>
</tr>
</tbody>
</table>

The results of the present study exhibited that methanol extract of S. corymbosa possesses analgesic, anti-inflammatory and antipyretic activities which may be mediated by the central and peripheral mechanisms. Further investigation on the isolation and characterization of individual compounds will be required to elucidate their different analgesic, anti-inflammatory and antipyretic mechanism and existence of possible synergism among the compounds.

REFERENCES


