Assessment of Antinociceptive and anti-inflammatory activities of *Galphimia glauca* stem methanol extract on noxious provocation induced pain and inflammation in *in-vivo* models

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ABSTRACT

This study assesses the antinociceptive and anti-inflammatory activities of *Galphimia glauca* stem methanol extract in mice and rats. The methanol extract of *Galphimia glauca* stem was prepared and evaluated for in-vivo antinociceptive effect and anti-inflammatory properties in Swiss albino mice and Wistar albino rats. The dose range of 100, 200 and 400 mg/kg b.w was administered orally for one day for assessing antinociceptive and seven days for anti-inflammatory activity respectively. Further experimental studies were conducted for determining the involvement of central and peripheral receptor actions in the antinociceptive activity of the extract by pre-challenging it with naloxone and acetic acid respectively. The in-vivo anti-inflammatory studies were conducted using carrageenan induced rat paw edema model and cotton pellet granuloma test. The LD₅₀ of the extract was found to be > 2000 mg/kg b.w. The stem methanol extract at 400 mg/kg dose exhibited significant (P ≤ 0.001) and dose-dependent antinociceptive and anti-inflammatory activity. It also exhibited central and peripheral antinociceptive actions when treated with naloxone and acetic acid respectively. The results revealed that the stem methanol extract has more potential in terms of antinociceptive and anti-inflammatory properties.

Keywords: Acetic Acid; Carrageenan; Diclofenac Sodium; *Galphimia glauca*.

INTRODUCTION

The importance of medicinal plants is recognized and documented well by research scholars since ancient history. Apart from the social benefits much attention has been given to the plants of medicinal significance. Most of the people in the developing nations believe in alternative system of medicine which uses medicinal plants for their primary health care. Due to the continuously increasing demand for medicinal plants, many herbal research institutes/centers across the globe are engaged in research, documentation and developing databases on the medicinal plants helping the scientific community. The plant *Galphimia glauca* (GG) is a native of Mexico, which has since been introduced in India.

*Galphimia glauca* is a dry habitat shrub which can be found distributed across South India belonging to the family of Malpighiaceae [1, 2]. It is commonly known as “*Calderonaamarilla*” and “*Florestrella*” in Spanish [3]. A tea made from the yellow leaves of the plant is used to relieve coronary pain as well as for soothing the nerves. The plant is used to bring down the fever, to help women in labor and it is also used as an emollient for injuries. The ethyl acetate extract of the plant was found to have wide usage in the treatment of asthma and allergies [2].
Hussein et al., 2013 reported the isolation of galloyl- and caffeoylqunic acids from *galphimia glauca* using pure zirconium silicate and bismuth citrate powders as sorbents inside micro spin columns [4]. Sharma et al reported the DNA Barcoding and metabolic profiling of the Mexican sedative and anxiolytic plant *Galphimia glauca* [5, 6]. Behavioral, Pharmaco-toxicological and genotoxicity tests were evaluated on standardized extracts of *Galphimia glauca* by Santamaria et al., 2007 [7].

Traditionally *Galphimia glauca* (GG) is used to relieve various forms of pain. There were no references reporting the use of GG in the treatment of pain and inflammation. Hence the present investigation is carried out to explore the beneficial effects of plant GG in assessing the pain and inflammation using *in-vivo* models.

**EXPERIMENTAL SECTION**

**Collection of Plant Material and its identification**

The shrub GG was cultivated in the lawn existing in the School of Pharmacy, Anurag Group of Institutions. GG stems were collected in the time of September, 2013. The plant was identified and authenticated by taxonomist, Dr. E. Narsimha Murthy, Satavahana University, Karimnagar, Telangana state, India. A voucher copy is stored with the reference number No.333, in the Department of Pharmacognosy and Phytochemistry, School of Pharmacy.

**Extraction**

GG stems were collected, dried in the shade and powdered coarsely. 100 g of stem powder was subjected to Soxhlet extraction using 500 ml of methanol. The extract was collected and then concentrated to dryness and stored. The yield obtained from GG stem methanol extract (GGSME), was 18 %.

**Animals**

Swiss albino mice of 6 to 8 weeks of age with 22.5 ± 2.5 g of either sex and Wistar albino rats of 12 to 14 weeks of age with 234 ± 24.8 g of either sex were used. Rodents were acclimatized for seven days to the laboratory conditions. The animals were retained in 12 hour light/dark cycles at 22 ± 2 °C with 60 to 70 % relative humidity. Entire pharmacological studies were carried out randomly using six animals of either sex in each group. The experimental protocol was approved by the Institutional Animal Ethics Committee of the institute (IAEC), School of Pharmacy, Anurag Group of Institutions (the approval number: I/IAEC/LCP/032/2013/SM-35).

**Chemicals and Drugs**

All the chemicals used were supplied from SD Fine Chemicals, India. Morphine was purchased from Troikaa Pharmaceuticals Inc. Gujarat, India. Carrageenan was procured from Sigma-Aldrich, USA. Diclofenac sodium and Naloxone drugs were collected from Novartis India Inc. and Samarth Pharma Inc. respectively as gift samples.

**Acute Toxicity Studies**

According to The Organization for Economic Co-operation and Development (OECD) guidelines, 423-2d, acute oral toxicity studies were conducted [8].

**Phytochemical Screening**

Phytochemical screening of the *G. glauca* stem methanol extract (GGSME) was carried out using standard procedures [9, 10].

**Antinociceptive activity**

**Thermal Stimulus Model**

The mice were placed on a hot plate (V. J Instruments, India) which was set at a fixed temperature of 55 ± 1°C. Mice were distributed randomly into VII groups (n = 6) and the pre-treatment response time (withdrawing their paws) for each mouse was recorded. One hour after oral and 30 minutes after intraperitoneal administration the post-treatment response was recorded with intervals of 30, 60 and 90 minutes respectively [11].

Group I received distilled water [10 ml/kg, body weight (b. w.), per oral (p. o.)].

Group II was treated with morphine [10 mg/kg, b.w., intraperitoneally (i. p)].

Group III-V was treated with GGSME [100, 200 and 400 mg/kg, b.w., respectively, (p.o)].

**Investigation of Opioid Receptors Involvement in the Anti-nociceptive activity of the Extract**

The GGSME with a dose of 400 mg/kg given orally was examined for this study. Specifically, two groups of mice (n = 6) “i.e.”; Group VI & Group VII were pre-challenged intraperitoneal with naloxone (5 mg/kg) 15 min prior to the administration of morphine (10 mg/kg; i.p.) and oral administration of GGSME (400 mg/kg) respectively. The
reaction time was recorded before and after the treatment as per the procedure mentioned in Thermal Stimulus Model [12, 13].

Chemical Stimulus Model

Formalin Test
Mice (s) used for this study were abstained from food overnight and were used for assessing the chemical stimulus induced pain (Formalin). Groups I to V were treated according to the procedure of the Thermal Stimulus Model. Group VI received diclofenac sodium (20 mg/kg; i.p.) which served as a standard drug. After thirty minutes of standard drug administration and 60 minutes after treatment with extracts, formalin (20 µl of 2.5% solution) was injected into the right hind paw of each animal subcutaneously. Individual animal was observed for pain responses in early (0-5 min) phase and in the late phase (15-30 min) respectively. The time (sec) spent for biting or licking the hind paw was observed and recorded [14, 15].

\[
\text{Inhibition (\%)} = \frac{\text{Reaction time [Control group] - Reaction time [Treated group]}}{\text{Reaction time [Control group]}} \times 100
\]

Writhing Test
The animals used for the study were grouped into VII groups (n = 6) and kept on a fast overnight. The group I received water (10 ml/kg), group II received Diclofenac sodium (20 mg/kg; i.p.), whereas group III to V received the GG extract treatment in accordance with the procedure of the Thermal Stimulus Model. After 30 min of drug/extract administration, all the animals were treated intraperitoneally with 0.7% acetic acid (10 ml/kg) and the numbers of writhing’s were recorded for a duration of 30 min [16, 17].

\[
\text{Inhibition (\%)} = \frac{\text{Number of writhes [Control group] - Number of writhes [Treated group]}}{\text{Number of writhes [Control group]}} \times 100
\]

Investigation of Peripheral Receptors Involvement in the Anti-nociceptive activity of the plant extract

Separately, two groups of mice (n = 6), Group VI and Group VII were pre-challenged intraperitoneally with naltrexone (5 mg/kg) 15 min prior to the i.p. administration of diclofenac sodium (20 mg/kg) and oral administration of GGSME (400 mg/kg) respectively. 30 min later, the animals were subjected to writhing test and the results were recorded [12].

Mechanical stimulus model
Haffner’s Tail Clip Method
The Wistar albino rats used in this study were screened initially for inducing pain at the root of the tail by applying a metal artery clip. Animals were grouped into V groups (n = 6) and pre-treatment response time for individual animal was recorded. Groups I to V were treated according to the procedure of the Thermal Stimulus Model.

After one hour of oral and 30 min of i.p. administration of the plant extracts and standard drug, the same procedure was used for recording the post-treatment response time [18].

\[
\text{Inhibition (\%)} = \frac{[\text{Post treatment latency} - \text{[Pre treatment latency]}]}{[\text{Cut off time} - \text{Pre treatment latency}]} \times 100
\]

Anti-inflammatory activity
Carrageenan Induced Paw Edema Model
The Carrageenan induced paw edema test was performed based on the method described by Laupattarakasem et al., 2006 [19, 20]. The rats used in this study were grouped into V groups (n = 6). The group I received water (10 ml/kg), Group II was treated with saline for initial seven days and with diclofenac sodium (20 mg/kg; i.p.) on the day of treatment. Groups III to V were treated with appropriate doses of stem methanol extract for seven consecutive days, as mentioned in the procedure of the Thermal Stimulus Model. The rats were kept on fast overnight and on the eighth day “i.e.”; one hour after the administration of diclofenac sodium and the stem methanol extract of varying doses, the rat paw edema was induced to all the groups by injecting carrageenan (0.1 ml, 1% w/v in saline) into a sub plantar region of right hind paw. The change in the paw volume was recorded immediately at different intervals of time (1st, 2nd, 3rd and 4th h) in both drug and extract treated groups before and after carrageenan challenge test using digital Plethysmometer (V. J Instruments, India).

\[
\text{Reduction in edema (\%)} = \frac{[\text{Mean edema in control group} - \text{Mean edema in drug treated group}]}{[\text{Mean edema in control group}]} \times 100
\]
Cotton Pellet Induced Granuloma experiment
The rats used in this study were grouped into V groups (n = 6) and were abstained from food overnight. Sterilized cotton pellets weighing about 20 mg each were used. The rats were anesthetized using urethane (1.5g/kg; i.p.) and the skin incision was made on the dorsal side of the rats and a cotton pellet was inserted subcutaneously, finally the incision was closed using surgical suture. Drug treatment started and continued for seven consecutive days. The group I received water (10 ml/kg), Group II was treated with diclofenac sodium (20 mg/kg; i.p.), whereas Groups III to V were treated with appropriate doses for seven days in accordance with the procedure of the Thermal Stimulus Model. Finally, on the eighth day, the animals were anesthetized and cotton pellets were removed out and foreign tissues were taken off and dried for about 24 h at 60° C and the dry weights were recorded. The transudative and granuloma weights were recorded and the percentage inhibition of granuloma tissue formation was determined applying the given formula [21].

\[
\text{Inhibition (\%)} = \frac{\text{Granuloma tissue weight [Control group]} - \text{Granuloma tissue weight [Treated group]}}{\text{Granuloma tissue weight [Control group]}} \times 100
\]

Statistical analysis
The results were reported as Mean ± S.E.M. Statistical analysis was performed with one-way analysis of variance (ANOVA), followed by Tukey’s multiple comparison test to calculate the significance of the results. All the statistical analysis was performed using Graph Pad Prism 5.0 software.

RESULTS AND DISCUSSION

Acute toxicity studies
The results showed no mortality/toxic symptoms in mice and rats treated with extract (2000 mg/kg) until the 14th day of the treatment period, according to the OECD 423-2d guidelines. Based on the results, we have selected 100, 200 and 400 mg/kg as low, moderate and high doses to assess the anti-nociceptive and anti-inflammatory studies.

Phytochemical Screening
In the preliminary Phytochemical Screening *G. glauca* stem methanol extract showed positive results for Carbohydrate, Proteins, Amino acids, Steroids, Terpenoids, Flavonoids, Tannins, Phenolic compounds and Saponins.

Antinociceptive activity

**Thermal Stimulus Model**
The GGSME increased the latency time in a dose dependent way. The onset of activity was seen at 30 min and it reached to a peak at 90 min. The activity was comparable (\(P \leq 0.001\)) with standard morphine (10 mg/kg). The results were tabulated in table I.

**Opioid Receptors Involvement in the Anti-nociceptive activity of the plant extract**
The central antinociceptive activity results of GGSME are depicted in table 1. The naloxone treated group (5 mg/kg) had significantly reversed the pain relieving property of GGSME at a dose of 400 mg/kg thus confirming the central actions of the extract.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>Reaction time after administering</th>
<th>Control/Standard/Extract (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Distilled water)</td>
<td>10 (ml/kg)</td>
<td>0 Min: 3.6 ± 0.08</td>
<td>3.3 ± 0.07</td>
</tr>
<tr>
<td>II (Morphine)</td>
<td>10</td>
<td>0 Min: 3.7 ± 0.1</td>
<td>7.9 ± 0.5⑨</td>
</tr>
<tr>
<td>III (GGSME)</td>
<td>100</td>
<td>0 Min: 3.7 ± 0.1</td>
<td>4.2 ± 0.1⑨</td>
</tr>
<tr>
<td>IV (GGSME)</td>
<td>200</td>
<td>0 Min: 3.9 ± 0.04</td>
<td>5.3 ± 0.7⑨</td>
</tr>
<tr>
<td>V (GGSME)</td>
<td>400</td>
<td>0 Min: 3.6 ± 0.09</td>
<td>7.0 ± 0.3⑨</td>
</tr>
<tr>
<td>VI (Naloxone + Morhine)</td>
<td>(5 + 10)</td>
<td>0 Min: 3.6 ± 0.08</td>
<td>4.0 ± 0.05⑨</td>
</tr>
<tr>
<td>VII (Naloxone + GGSME)</td>
<td>(5 + 400)</td>
<td>0 Min: 3.6 ± 0.01</td>
<td>4.1 ± 0.15⑨</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± S.E.M.; n = 6; the statistical significance done by ANOVA, followed by Tukey’s multiple comparison tests and is represented by a symbol.

\(⑨P \leq 0.001\) indicates comparison with group I.

\(⑨P \leq 0.001\) indicates comparison with group II.

\(⑨P \leq 0.001\) indicates the dose dependent activity on comparison of the high dose with respective low dose of the extracts.
Formalin Test
The GGSME (400 mg/kg) had showed 84.98 %, 97.30 % inhibition of pain in the early and late phase respectively. The results are represented in table 2.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>Paw licking time (s)</th>
<th>Early phase (0-5 min) Inhibition (%)</th>
<th>Late phase (15-30 min) Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Distilled water)</td>
<td>10 (ml/kg)</td>
<td>178.7 ± 5.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>II (Morphine)</td>
<td>10</td>
<td>40.6 ± 1.3&lt;sup&gt;a&lt;/sup&gt; 77.28</td>
<td>4.6 ± 0.5&lt;sup&gt;b&lt;/sup&gt; 96.12</td>
<td></td>
</tr>
<tr>
<td>III (GGSME )</td>
<td>100</td>
<td>83.5 ± 2.1&lt;sup&gt;c&lt;/sup&gt; 53.27</td>
<td>38.3 ± 1.2&lt;sup&gt;ab&lt;/sup&gt; 67.7</td>
<td></td>
</tr>
<tr>
<td>IV (GGSME )</td>
<td>200</td>
<td>52 ± 1.7&lt;sup&gt;de&lt;/sup&gt; 70.52</td>
<td>22 ± 0.5&lt;sup&gt;e&lt;/sup&gt; 81.48</td>
<td></td>
</tr>
<tr>
<td>V (GGSME )</td>
<td>400</td>
<td>26.83 ± 1&lt;sup&gt;ef&lt;/sup&gt; 84.98</td>
<td>3.2 ± 0.5&lt;sup&gt;ef&lt;/sup&gt; 97.30</td>
<td></td>
</tr>
<tr>
<td>VI (Diclofenac sodium)</td>
<td>20</td>
<td>91 ± 1.7&lt;sup&gt;a&lt;/sup&gt; 49.07</td>
<td>14 ± 0.5&lt;sup&gt;a&lt;/sup&gt; 88.21</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM.; n = 6; the statistical significance done by ANOVA, followed by Tukey’s multiple comparison tests and is represented by a symbol.

<sup>a</sup>P ≤ 0.001 indicates comparison with group I.
<sup>b</sup>P ≤0.001 indicates comparison with group II.
<sup>c</sup>P ≤0.01 indicates comparison with group II.
<sup>d</sup>P ≤0.001 indicates comparison with group III.
<sup>e</sup>P ≤0.01 indicates comparison with group III.
<sup>f</sup>P ≤0.001 indicates the dose dependent activity on comparison of the high dose with respective low dose of the extracts.

Writhing Test
The GGSME (400 mg/kg) exhibited significant (81.32 % inhibition) antinociceptive activity in a dose dependent manner when compared to that of diclofenac sodium (20 mg/kg; i.p.) which showed 74.97 % inhibition. The results are reported in Fig.1.

Peripheral Receptors Involvement in the Anti-nociceptive activity of the plant Extract
The drug naloxone (5 mg/kg) has shown no effect on the abdominal constriction in mice which were treated with GGSME (400 mg/kg) and diclofenac sodium (20 mg/kg). The results revealed that the activity was a result of the activation of peripheral receptors. GGSME (100, 200 mg/kg) results are not mentioned here. The above results are shown in Fig.1.

[Fig1. Antinociceptive effects of G. glauca stem methanol extract (GGSME) on Acetic acid induced pain in mice (Writhing test) a) Number of writhings; b) Writhings inhibition (%)]

Haffner’s Tail Clip Test
The GGSME showed the maximum effect with 83.48 %, 66.9 % and 40 % inhibition of pain at high, medium and low doses respectively. The results are tabulated in table 3.
Table 3: Antinociceptive effects of *G. glauca* stem methanol extract on Tail Clip Induced Pain in rats (Haffner’s Tail Clip Test)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>Pre-treatment reaction latency (s)</th>
<th>Post-treatment reaction latency (s)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Distilled water)</td>
<td>10 (ml/kg)</td>
<td>1.50 ± 0.02</td>
<td>1.54 ± 0.02</td>
<td>85.51</td>
</tr>
<tr>
<td>II (Morphine)</td>
<td>10</td>
<td>1.68 ± 0.2</td>
<td>11.6 ± 0.3</td>
<td>66.9</td>
</tr>
<tr>
<td>III (GGSME)</td>
<td>100</td>
<td>1.92 ± 0.1</td>
<td>3.2 ± 0.2</td>
<td>40</td>
</tr>
<tr>
<td>IV (GGSME)</td>
<td>200</td>
<td>1.72 ± 0.02</td>
<td>5.2 ± 0.1</td>
<td>66.9</td>
</tr>
<tr>
<td>V (GGSME)</td>
<td>400</td>
<td>1.80 ± 0.1</td>
<td>10.9 ± 0.1</td>
<td>83.48</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM.; n = 6; the statistical significance done by ANOVA, followed by Tukey’s multiple comparison tests and is represented by a symbol.

\*P ≤ 0.001 indicates comparison with group I.

\*P ≤ 0.001 indicates comparison with group II.

\*P ≤ 0.001 indicates the dose dependent activity on comparison of the high dose with respective low dose of the extracts.

### Anti-inflammatory activity

#### Carrageenan Induced Paw Edema Model

The obtained experimental results are reported in table 4. GGSME at 400 mg/kg dose showed 76.54 % and 88.60 % inhibition of paw edema at 3rd and 4th h, respectively, when compared with standard diclofenac sodium at respective time points.

Table 4: Anti-inflammatory effect of *G. glauca* plant extracts on Carrageenan Induced paw edema test in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>Changes in paw edema volume after administration of Control/Standard/Extracts (h)</th>
<th>IPE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1h IPE (%) 2h IPE (%) 3h IPE (%) 4h IPE (%)</td>
<td></td>
</tr>
<tr>
<td>I (Distilled water)</td>
<td>10 (ml/kg)</td>
<td>1.16 ± 0.02 0.002 1.32 ± 0.002 1.62 ± 0.09 1.93 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>II (Diclofenac sodium)</td>
<td>20</td>
<td>0.72 ± 0.02 0.002 0.01 0.02 0.02 0.02 0.02</td>
<td>69.75 32.0 0.01 83.41</td>
</tr>
<tr>
<td>III (GGSME)</td>
<td>100</td>
<td>0.99 ± 0.03 10.60 0.01 0.02 0.02 0.02 0.02</td>
<td>41.97 88.4 0.04 54.40</td>
</tr>
<tr>
<td>IV (GGSME)</td>
<td>200</td>
<td>0.91 ± 0.02 0.00 23.52 0.82 0.01 0.02 0.02</td>
<td>53.70 66.0 0.00 65.80</td>
</tr>
<tr>
<td>V (GGSME)</td>
<td>400</td>
<td>0.68 ± 0.002 0.002 0.03 0.05 0.05 0.05 0.05</td>
<td>76.54 0.01 88.60</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM.; n = 6; the statistical significance done by ANOVA, followed by Tukey’s multiple comparison tests and is represented by a symbol.

\*P ≤ 0.001, indicates comparison with group I.

\*P ≤ 0.001 indicates comparison with group II.

\*P ≤ 0.001 indicates the dose dependent activity on comparison of the high dose with respective low dose of the extracts.

\*IPE (%) indicates percentage inhibition of paw edema.

#### Cotton Pellet Induced Granuloma test

The results are reported in Table 5. The GGSME (400 mg/kg) showed an 84 mg reduction in transudative weight of cotton pellets and 80.95 % inhibition of granuloma formation when compared with diclofenac sodium, which showed a 98 mg reduction in transudative weight and 76.19 % inhibition of granuloma formation.

Table 5: Anti-inflammatory effects of *G. glauca* stem methanol extract on Cotton Pellet Induced Granuloma Test in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>Granuloma wet weight (mg)</th>
<th>Granuloma dry weight (mg)</th>
<th>Transudative weight (mg)</th>
<th>Granuloma weight (mg/mg cotton)</th>
<th>Inhibition of granuloma (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Distilled water)</td>
<td>10 (ml/kg)</td>
<td>189 ± 2.54 62 ± 2.5 127 ± 1.5 2.1 ± 0.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>II (Diclofenac sodium)</td>
<td>20</td>
<td>128 ± 2.90 30 ± 1.5 98 ± 2.0 0.5 ± 0.1</td>
<td>76.19</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>III (GGSME)</td>
<td>100</td>
<td>157 ± 3.0 38 ± 1.1 119 ± 1.6 0.9 ± 0.1</td>
<td>57.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IV (GGSME)</td>
<td>200</td>
<td>134 ± 3.8 33 ± 1.9 101 ± 3.2 0.6 ± 0.06</td>
<td>69.04</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>V (GGSME)</td>
<td>400</td>
<td>112 ± 2.21 28 ± 1.0 84 ± 2.5 0.4 ± 0.04</td>
<td>80.95</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM.; n = 6; the statistical significance done by ANOVA, followed by Tukey’s multiple comparison tests and is represented by a symbol.

\*P ≤ 0.001 indicates comparison with group I.

\*P ≤ 0.001 indicates comparison with group II.

\*P ≤ 0.001 indicates the dose dependent activity on comparison of the high dose with respective low dose of the extracts.

The methanol extract of *Galphimia glauca* stem was investigated for assessing in vivo antinociceptive and anti-inflammatory properties. The hot plate and tail clip methods were used for confirming the central effects, while the formalin and writhing’s tests were conducted for the peripheral effects. The GGSME was administered orally with 400 mg/kg dose showed significant and dose dependent antinociceptive activity in both models of pain (central and
peripheral). Further the central opioid receptors involvement is confirmed by the reversed pain relieving property through the administration of naloxone.

The opioids bind to specific receptors in the CNS and other tissues. The important classes of opioid receptors include mu receptors (\(\mu_1, \mu_2\)), kappa receptors (\(\kappa_1, \kappa_2\)) and delta receptors (\(\delta_1, \delta_2\)). Opioid receptors are G-protein coupled receptors and cause a decrease of adenyl cyclase activity leading to reduced formation of the cAMP. They mediate two types of actions. A presynaptic action results in closure of Ca\(^{+}\) channels, while the post synaptic activity results in opening of K\(^{-}\) channels leading to reduced neuronal excitability. The above results suggest the central analgesic actions of the plant extract was perhaps mediated by the inhibition of opioid receptors. Similar kind of results was earlier reported by [22].

Morphine inhibits both the phases of formalin induced pain, whereas diclofenac inhibits only late inflammatory phase. In formalin test the GGSME showed significant dose dependent action in both phases of pain. The acetic acid induced, caused writhings which is used for screening peripheral antinociceptive activity.

The drugs which were used act through inhibition of COX enzyme in the peripheral tissues by blocking the synthesis and or release of inflammatory mediators like Cell derived mediators like [Vaso active amines (Histamine, 5HT and Neuropeptide)], Eicosanoids (PGD\(_2\), PGE\(_2\), PGF\(_2\)-\(\alpha\), PGI\(_2\) and TXA\(_2\)), Lysosomal components (Granules of neutrophils, granules of monocytes and tissue macrophages), Platelet activating factor, Cytokines (IL-1 and IL-6 are active in acute inflammation, while IL-12 and IL-17 are active in chronic inflammation) and free radicals. [23]. GGSME lessened the number of writhings in mice in a dose dependent way to that of diclofenac sodium. The above results revealed significant central and peripheral actions of GGSME acting at both the phases in the formalin test and in acetic acid induced pain.

Carrageenan induced paw edema model is applied to study acute inflammation processes. The paw edema that was induced is biphasic; the initial phase involves the release of histamine, kinins and serotonin while the late phase is mediated by prostaglandins. The GGSME at higher dose exhibited a significant dose dependent activity suggesting its action to be similar to that of NSAIDS [24].

Usually cytokinins are of two types, proinflammatory and anti-inflammatory. Among the proinflammatory cytokinins TNF (tumor necrosis factor), interleukin-1 (IL-1) and interleukin-6 (IL-6) have been most widely studied with respect to metabolic regulation after inflammation. TNF stimulates the release of IL-1, and both the TNF and IL-1 stimulate the release of IL-6. Other cytokinins involved in inflammation include interleukin-4 (IL-4), interleukin -7 (IL-7), interleukin -8 (IL-8).

In chronic inflammation, there is a macrophage stimulation by IL-1\(\alpha\), IL-1\(\beta\), IL-2 and TNF-\(\alpha\) and proliferation of macrophages by M-CSF (Macrophage colony stimulating factor), proliferation of fibroblasts, and multiplication of small blood vessel. In chronic inflammation, there is a proliferation of macrophages, fibroblasts, and multiplication of small blood vessel. The anti-inflammatory drugs act by altering the endogenous factors involved in the migration of substances to the site of inflammation or by inhibiting the release of inflammatory mediators TNF-\(\alpha\), IFN-\(\gamma\), IL-1, IL-2 [25]. In cotton pellet induced granuloma test, the GGSME treated rats exhibited potential inhibitory effects in transudative and proliferative phases of inflammation. The activity may be due to the above said mechanisms acting on inflammatory mediators.

The Preliminary Phytochemical Screening results of G. glauca stem methanol extract showed the presence of Secondary metabolites like Flavonoids, Tannins, Phenolic compounds, Steroids, Terpenoids, and Saponins. One or a combination of the above plant constituents may be responsible for the anti-nociceptive and anti-inflammatory properties of GGSME in the present study.

**CONCLUSION**

The results conclude that the GGSME has potential antinociceptive activity involving both central and peripheral mechanisms as well as potential anti-inflammatory properties. Further studies are planned to explore the phytochemical profile using HPLC/HPTLC. The bioactivity studies on different fractions of the GGSME have to be performed to identify the probable active compound (s).

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