ANTI-INFLAMMATORY ACTIVITY OF SARCOSTEMMA SECAMONE (L) BENNET WHOLE PLANT AGAINST CARRAGEENAN INDUCED PAW EDEMA

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ABSTRACT
This study was intended to evaluate the anti-inflammatory activity of the whole plant of Sarcostemma secamone. The anti-inflammatory activity study was carried out by using carrageenan induced paw edema. The ethanol extract of whole plant of Sarcostemma secamone was injected at different doses such as 150 and 300 mg/kg body weight and the study was compared with standard drug Indomethacin (10mg/kg). The extract exhibited significant anti-inflammatory activity, which supports the traditional medicinal utilization of the plant.

KEYWORDS: Sarcostemma secamone, Anti-inflammatory, Carrageenan.

INTRODUCTION
Sarcostemma secamone (L) Bennet, is an important medicinal plant belonging to the family Asclepiadaceae. It is used in the traditional systems of medicine for various ailments. The decoction of the plant is useful to gargle for throat and mouth infection. The latex is bitter and used as a vulnerary. Fresh roots are prescribed for jaundice (Chopra et al., 1956, Chopra et al., 1958, Anonymous, 1966; Nadkarni, 1982). The milky sap forms a wash for ulcers. In combination with turpentine, it is prescribed for itch (Kirtikar and Basu 1976). The plant is hot, bitter, tonic, expectorant, pungent, dry and indigestible causes flatulence, diuretic, laxative, aphrodisiac, anthelmintic, useful in leucoderma and bronchitis. The juice is used in gleet, gonorrhoea, pain in the muscles, cough and given to children as an astrigent (Poornima et al., 2009). Leaf powder stimulates arculatory system, increases secretion of urine and activates uterus (Prajapati et al., 2003). The fruit juice is used in gonorrhoea and pain in muscles (Kirtikar and Basu, 1976). The leaves, roots and latex of Sarcostemma secamone are employed in treating many diseases like mouth ulcer, sour throat, jaundice and ulcers (Khan, 2002, Satyavathi et al., 1987, Jain, 1991).

The attention of pharmacologist throughout the world has been focused on finding out safer and potent anti-inflammatory drug. The natural products today symbolize safety in contrast to the synthetic drugs that are regarded as unsafe to humans and environment. So, people are returning to the natural products with the hope of safety and security. However, so far there is no systematic study on anti-inflammatory activity has been reported in the literature. Hence the present study focuses on evaluating the anti-inflammatory activity of whole plant of Sarcostemma secamone.

To our knowledge more report on the effect of this plant on experimental information. This study was therefore undertaken to evaluate the effect of ethanol extract of the whole plant of Sarcostemma secamone on anti-inflammatory activity in carrageenan induced rat paw edema.

MATERIALS AND METHODS
The well grown and healthy whole plant of Sarcostemma secamone (L) Bennet were collected from natural forests of Western Ghats at Thannipari, Srivilliputhur, Virudhunagar District, Tamil Nadu.

PREPARATION OF PLANT EXTRACT FOR ANTI-INFLAMMATORY ACTIVITY
The dried whole plant materials of Sarcostemma secamone were powdered in a Wiley mill. Hundred grams of whole plant powder was packed in a Soxhlet apparatus and extracted with ethanol. The ethanol extract was concentrated in a rotary evaporator. The concentrated ethanol extract was used for anti-inflammatory activity.
ANIMALS
Adult Wistar Albino rats of either sex (150-200g) were used for the present investigation. Animals were housed under standard environmental conditions at temperature (25±2°C) and light and dark (12:12 h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water ad libitum.

ACUTE TOXICITY STUDY
Acute oral toxicity was performed by following OECD-423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study (OECD, 2002). The animals were kept fasting for overnight and provided only with water, after which the extracts were administrated orally at 5mg/kg body weight by gastric incubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administrated was assigned as toxic dose. If mortality was observed in one animal, then the same dose repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50,100 and 2000 mg/kg body weight.

ANTI-INFLAMMATORY ACTIVITY
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Albino rats of either sex weighing 150-200 grams were divided into four groups of six animals each. The dosage of the drugs administered to the different groups was as follows. Group I - Control (normal saline 0.5 ml/kg), Group II and III - Sarcostemma secamone (100 mg/kg and 200 mg/kg, p.o.), Group IV – Indomethacin (5 mg/kg, p.o). All the drugs were administered orally. Indomethacin served as the reference standard anti-inflammatory drug.

After one hour of the administration of the drugs, 0.1 ml of 1% W/V carrageenan solution in normal saline was injected into the sub plantar tissue of the left hind paw of the rat and the right hind paw was served as the control. The paw volume of the rats were measured in the digital plethysmograph (Ugo basile, Italy), at the end of 0 min., 60min., 120min., 180min., 240min., 360min., and 480min. The percentage increase in paw edema of the treated groups was compared with that of the control and the inhibitory effect of the drugs was studied. The relative potency of the drugs under investigation was calculated based upon the percentage inhibition of the inflammation. Percentage inhibition was calculated using the formula,

\[ \text{Percentage inhibition} = \left( \frac{V_c - V_t}{V_c} \right) \times 100 \]

Where, Vs the percentage represents the percentage difference in increased paw volume after the administration of test drugs to the rats and Vc represents difference of increased volume in the control groups.

STATISTICAL ANALYSIS
The data were analyzed using student’s t-test statistical methods. For the statistical tests a P values of less than 0.001, 0.01 and 0.05 was taken as significant.

RESULTS AND DISCUSSION
The present study of anti-inflammatory activity of ethanol extract of Sarcostemma secamone against carrageenan induced paw edema shows that the extracts have significant effect on inflammation and markedly reduced the swelling. The percentage reduction in the paw volume in the group of animals treated with Sarcostemma secamone extract 150mg was 71.98% and for the 300mg/kg was 77.95% at 3 hours. It shows that the plant extract have significant (P <0.01;P < 0.001) anti-inflammatory effect and the results were compared with indomethacin 10mg/kg and show percentage paw volume reduction of 76.87 %. (Table 1)

Carrageenan induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility (Winter et al.,1962). 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 prostaglandin and slow reacting substances which peek at 3h (Vinegar et al., 1969). It has been reported that the second phase of edema is sensitive to drugs like hydrocortisone, phenylbutazone and indomethacin.
Indomethacin is a cyclooxygenase inhibitor, the ethanol extract has activity which is comparable to indomethacin and can be said to inhibit the cyclooxygenase enzyme but lipoxygenase inhibitors also possess significant anti-inflammatory activity against carrageenan induced paw edema, so inhibition of carrageenan induced paw edema by the crude extract could also be due to its inhibitory activity on the lipoxygenase enzyme.

GC-MS analysis of *Sarcostemma secamone* whole plant revealed the presence of phytol, 9, 12-Octadecadienoic acid (Z, Z)-, phenyl methyl ester and 9-Octadecanoic acid (Z)-, phenyl methyl ester. These compounds may have a role in anti-inflammatory effects (Thangakrishnakumari *et al.*, 2012). In the present study, the anti-inflammatory activity of *Sarcostemma secamone* whole plant can be attributed to the above chemical constituents. Therefore, from the results of the present preliminary study, it can be concluded that *Sarcostemma secamone* possessed marked anti-inflammatory effect against its carrageenan induced paw edema. Further definitive studies are necessary to ascertain the mechanisms and constituents behind its anti-inflammatory action.

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**Table 1: Effect of *Sarcostemma secamone* whole plant extract on the percentage inhibition of Carrageenan induced paw edema.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Edema volume (ml)</th>
<th>% Inhibition after 180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Dose mg/kg</td>
<td>0 min</td>
</tr>
<tr>
<td>CONTROL (Group-I)</td>
<td>Normal saline</td>
<td>28.41±1.74</td>
</tr>
<tr>
<td><em>Sarcostemma secamone</em> extract (Group-II)</td>
<td>150</td>
<td>26.21±1.11</td>
</tr>
<tr>
<td><em>Sarcostemma secamone</em> extract (Group-III)</td>
<td>300</td>
<td>29.43±1.26</td>
</tr>
<tr>
<td>Indomethacin (Group-V)</td>
<td>10 mg</td>
<td>25.91±1.66</td>
</tr>
</tbody>
</table>

Each Value is SEM ± 5 individual observations * P < 0.05; ** P<0.01 *** P<0.001 Compared normal control vs edema. Control rats and drug treated rats.

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