ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACTS OF LEAVES OF CAPPARIS SEPIARIA LINN. IN WISTAR STRAIN RATS

RAJESH, P., SELVAMANI, P., LATHA, S., SARASWATHY, A. AND RAJESH KANNAN, V.

Abstract: The study was intended to evaluate the anti-inflammatory activity of ethanolic extract of leaves of Capparis sepiaria (Capparidaceae) (EECS) in Wistar rats. Phytochemical analysis was carried out by using standard methodology. The plant has alkaloids, amino acids, glycosides, reducing sugars, saponins, starch, steroids, tannins and terpenoids. The anti-inflammatory activity was carried out in different methods such as carrageenan, cotton pellet and croton oil induced oedema. Different doses (100, 200 and 300 mg/kg/i.p.) of EECS were injected to rats and the results were compared with standard drug indomethacin (10 mg/kg). Paw volume was measured using digital plethysmometer. The C. sepiaria extract showed the maximum inhibitory activity at 300 mg/kg/i.p. in a dose dependent manner. These inhibitions were statistically significant (p<0.01-0.001). These results indicate that C. sepiaria extract is a bioactive agent and having significant results in anti-inflammatory action by inhibition of the exudation, and leukocytes recruitment into the inflamed tissues.

Key words: Capparis sepiaria; Anti-inflammatory.

INTRODUCTION

Inflammation is a common underlying factor contributing exacerbation of a wide variety of disease states including arthritis, asthma and cardiovascular diseases. Both steroidal and non steroidal anti-inflammatory drugs, currently used in the treatment of inflammatory diseases, are known to have various side effects. Thus, the hunt for natural products with anti-inflammatory properties and minimum side effects is still a challenge and targeting the discovery of new non steroidal anti-inflammatory drugs [1] from plants.

Herbal medicines are being used by about 80% of the world population mainly, in the developing countries for primary health care. Ancient literature also mentions herbal medicines for various diseases, for which no scientific proof is available. One such plant, C. sepiaria, is selected with a view to prove its medicinal properties scientifically. Among Bhil, Dhanka, Dubada and Nayaka tribes of Khedbrahma region in north Gujarat, the roots are crushed along with ginger and asafetida and the paste is applied externally to cure mumps. Among Agari, Bhil, Dhodia, Dubla, Khakari, Koli and Raikare tribes of Dahanu forest division in Maharashtra, the decoction of bark and root powder is given in dropsy and gout reported by Jain et al. [2]. Thus the shrub is the source of the multiple drugs that has beneficial effects, particularly in skin troubles was reported by Prajapati and Kumar [3]. The leaves, flowers, fruits and roots are extensively used for treating cold, cough, whooping-cough, asthma, antispasmodic and antipyretic and antiseptic properties. The juice of inner bark of the root is used for scabies and eczema [4]. Leaves contain α- and β-sterol, taraxasterol, erythrodial, betulin and two terpenoid alcohols. The root contains indole...
glucosinolates, glucobrassicin and 4-methoxy-
glucobrassicin [2]. *C. sepiaria* is very rich in alkaloids,
glycosides, carbohydrates, terpenes and sterols [5].
A complex alkaloid was isolated from methanol 
extract of *C. sepiaria* and the root of *C. sepiaria*
that showed analgesic [6] and antipyretic [7] 
properties.

Limitations in the conventional medical management
of inflammation indicate a real need for safe and
effective treatment of inflammatory patients. Herbal 
medicines may provide a solution to this problem with
comparatively less side effects. Therefore, the 
objective of present study is to evaluate the anti-
inflammatory activity of ethanolic extract of leaves 
of *C. sepiaria* Linn.

**MATERIALS AND METHODS**

**Plant Collection and Identification:** The plant *C.
sepiaria* was collected from Mathur and the 
surrounding area, Tiruchirappalli districts of Tamil 
Nadu, and authenticated by Botanical Survey of India,
Coimbatore, India.

**Phytochemical screening:** The methods of 
Harborne [8], Trease and Evans [9], Ikhiri et al. [10] 
and Dahou et al. [11] were used to screen the 
chemical constituents the EECS. The presence of 
alkaloid (Dragendorff reagent and Mayer’s reagent),
flavonoids (Shinoda test), steroids (Liberman 
Burchard test) and terpenes (Vanillin–sulfuric acid 
reagent) were assessed. The dry ethanolic extracts 
of *C. sepiaria* were separately tested for the 
presence of alkaloids, amino acids, glycosides, 
proteins, saponins, starch, tannins and terpenoids.

**Preparation of extract:** 500 g of shade dried 
coarsely powdered leaves of *C. sepiaria* was 
extracted exhaustively for 72 hours in a soxhlet 
apparatus with ethanol, which was previously distilled 
off before extraction. The excess ethanol from the 
crude extract was distilled off under reduced pressure 
and the concentrated crude extract was stored in a 
desiccator for further analysis as reported by Harb-
orne [8], Kokate [12] and Wagner and Roth. [13].

**Acute and subacute toxicity studies:** The acute 
oral toxicity study was carried out in Swiss Albino 
mice as per OECD guidelines [14]. The LD<sub>50</sub> cut-
off dose was found to be in EECS 5000 mg/kg bw. They 
did not show any sign of toxicity to animals.

**Experimental animals:** The toxicity studies was 
performed in Swiss albino mice (25-30 g) and anti-
inflammatory activity only in Wistar strain rats (180-
200 g). The animals were purchased from Kings 
Institute, Guindy, Chennai. They were housed in large 
spacious polypropylene cages and supplied with pellet 
feed and water *ad libitum*. The animals were 
acclimatized for at least one week in lab condition 
before commencement of the experiment in standard 
laboratory conditions 12 ± 1 h day and night rhythm, 
maintained at 25 ± 2°C and 35-60 % humidity. The 
study was approved by the Institutional Animal Ethical 
Committee (IAEC) of Committee for the purpose of 
control and supervision of Experiment on Animals 
(CPCSEA).

**Anti-inflammatory activity:** The anti-inflammatory 
activity of EECS was assessed by carrageenan, 
cotton pellet, croton oil induced ear oedema models.

**Carrageenan induced paw oedema:** The anti-
inflammatory activity was evaluated by the 
carrageenan induced paw oedema in rats [15,16]. 
Animals were anesthetized with sodium pentobarbital 
(40 mg/kg i.p.) and injected subplantarly into the right 
hind paw, with 0.1 ml carrageenan in isotonic saline 
(3.0 mg/ml). The animals were treated with EECS 
in saline and administered intraperitoneally 1 hour 
prior to the subplantar injection of 0.1 ml carrageenan 
(10 mg/ml). Oedema measurements were made using 
a modified digital plethysmometer was reported by 
Winder et al. [17] before injecting carrageenan and 
1, 2, 3, 4 and 24 hour after carrageenan injection. 
The results were expressed as percentage inhibition 
in relation to the control group.

**Cotton pellet induced granuloma:** Cotton pellets 
weighing about 10 ± 1 mg were autoclaved up to 20 
minutes. Cotton pellets were aseptically implanted in 
the interscapular distance under the skin on the 
previously shaved back of the rats which were 
anesthetized with 25 mg/kg sodium pentobarbital 
intraperitoneally [18]. Inflammation induced animals 
were treated with EECS intraperitoneally groups up 
to 7 days. After 7 days the animals were sacrificed 
and the pellets together with the granuloma tissues 
were carefully removed, dried in an oven at 60 °C. 
The pellets were weighed both moist and dry. Mean 
weight of the granuloma tissue was recorded. The 
weight of the pellets taken out from drug administered 
rats was compared with the weight of the pellets 
taken out from the control group and indomethacin
administered rats was reported by earlier workers [19-21]

**Croton oil-induced ear inflammation:** Croton oil irritant solution prepared was applied (0.1 ml) to the inner surface of the right ear of rats. The rats were sacrificed after 4 h and 7 mm punches were made in the ear using cork borer. Each ear disc was weighed and compared with control. Extract or vehicle was administered intraperitoneally 30 minutes before croton oil application. Mean weight of the ear granuloma tissue was recorded. The weight of the ear granuloma taken out from drug administered rats was compared with the weight of the ear granuloma taken out from the control group and indomethacin i.e., standard drug was administered to rats reported by Brooks et al. [22]

**Statistical analysis:** ANOVA followed the Student–Duncan-test was used to determine significant differences between groups and P<0.05, P<0.01 and P< 0.001 was considered significant.

**RESULTS**

(Table 1) shows the anti-inflammatory effect of the EECS on carrageenan induced oedema in rats. The extracts caused (53.36, 61.13 and 62.69%) inhibition of contractions at doses of 100, 200 and 300 mg/kg b. wt, i.p., respectively a maximum inhibitory effect at 24th hour. In the carrageenan induced oedema test (Table 1), inhibition occurred predominantly during the second phase of the response and thus, after i.p., administration, in which, EECS caused a (62.69%) response inhibition of the second phase at dose level on 300 mg/kg b.wt/i.p., In the second method known as the cotton pellet induced granuloma (Table 2), shows the percentage activity (30.48, 37.36 and 44.12%) at doses of EECS 100, 200 and 300 mg/kg b.wt., i.p., this is exhibit negligible inhibitory effect when compared to other methods used. It is observed that the cotton pellet granuloma tissues were reduced (from 27.57, 24.84 to 22.16 mg). In third, croton oil induced method (Table 3), although a significant effect was observed, the percentage inhibition (37.02, 43.59 and 50.09%) in the latency to healing stimuli were observed at doses of 100, 200 and 300 mg/kg b.wt., i.p., after 30 min of drug administration, respectively. EECS 300 mg/kg b.wt., i.p., showed maximum inhibitory effect.

Ear disc weight was reduced (from 10.17, 9.11 to 8.06 mg) at the doses 100, 200 to 300 mg/kg doses. Interestingly, in these cases, the effect was long lasting. The increase in thermal stimuli could be observed 30 min after drug administration, at the doses of 100, 200 and 300 mg/kg respectively. As expected, indomethacin was very efficient during

**Table 1:** Anti-inflammatory activity of crude ethanol extracts of *C. sepiaria* in carrageenan induced oedema method paw volume in ml (mean value ± S.D) and percentage (in %)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Normal Paw Volume</th>
<th>0th Hour</th>
<th>1st Hour</th>
<th>2nd Hour</th>
<th>3rd Hour</th>
<th>4th Hour</th>
<th>24th Hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>0.54 ±0.04</td>
<td>1.43 ± 0.04</td>
<td>1.53 ± 0.06</td>
<td>1.66 ± 0.05</td>
<td>1.72 ±0.03</td>
<td>1.82 ± 0.02</td>
<td>1.93 ± 0.03</td>
</tr>
<tr>
<td>Indomethacin (10 mg/kg)</td>
<td>0.49 ±0.01</td>
<td>1.12 ± 0.11</td>
<td>1.06 ± 0.11</td>
<td>0.94 ± 0.06</td>
<td>0.87 ±0.04</td>
<td>0.78 ± 0.05</td>
<td>0.49 ±0.01</td>
</tr>
<tr>
<td>EECS (100 mg/kg)</td>
<td>0.51±0.01</td>
<td>1.37 ± 0.02</td>
<td>1.32±0.03</td>
<td>1.26 ±0.02</td>
<td>1.20 ±0.03</td>
<td>1.07 ± 0.08</td>
<td>0.90 ± 0.05</td>
</tr>
<tr>
<td>EECS (200 mg/kg)</td>
<td>0.56±0.04</td>
<td>1.26 ± 0.04</td>
<td>1.19±0.04</td>
<td>1.05 ±0.02</td>
<td>0.91 ±0.04</td>
<td>0.82 ± 0.05</td>
<td>0.75 ± 0.07</td>
</tr>
<tr>
<td>EECS (300 mg/kg)</td>
<td>0.54±0.03</td>
<td>1.22 ± 0.02</td>
<td>1.11±0.01</td>
<td>0.98 ±0.01</td>
<td>0.89 ±0.02</td>
<td>0.81 ± 0.04</td>
<td>0.72 ± 0.03</td>
</tr>
</tbody>
</table>

**Table 2:** Anti-inflammatory activity of crude ethanol extract of *C. sepiaria* in cotton pellet induced granuloma method

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cotton pellet weight (Mean value ± S.D)</th>
<th>Percentage Inhibition (in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>39.66±1.84</td>
<td>-</td>
</tr>
<tr>
<td>Indomethacin (10 g/kg)</td>
<td>19.79±2.01</td>
<td>50.10</td>
</tr>
<tr>
<td>EECS (100 mg/kg)</td>
<td>27.57±1.69</td>
<td>30.48</td>
</tr>
<tr>
<td>EECS (200 mg/kg)</td>
<td>24.84±1.35</td>
<td>37.36</td>
</tr>
<tr>
<td>EECS (300 mg/kg)</td>
<td>22.16±1.09</td>
<td>44.12</td>
</tr>
</tbody>
</table>

**Table 3:** Anti-inflammatory activity of crude ethanol extract of *C. berryi* in croton oil induced oedema method

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ear weight (in mg) (Mean value ± S.D)</th>
<th>Percentage Inhibition (in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.15 ± 1.20</td>
<td>-</td>
</tr>
<tr>
<td>Indomethacin (10 mg/kg)</td>
<td>5.53 ± 0.89</td>
<td>65.75</td>
</tr>
<tr>
<td>EECS (100 mg/kg)</td>
<td>10.17 ± 1.14</td>
<td>37.02</td>
</tr>
<tr>
<td>EECS (200 mg/kg)</td>
<td>9.11 ± 1.25</td>
<td>43.59</td>
</tr>
<tr>
<td>EECS (300 mg/kg)</td>
<td>8.06 ± 1.11</td>
<td>50.09</td>
</tr>
</tbody>
</table>
Figs. 1 and 2: Anti-inflammatory activity of crude ethanol extracts of *C. sepiaria* in carrageenan induced oedema method

Figure 3 and 4: Anti-inflammatory activity of crude ethanol ether extracts of *C. sepiaria* in cotton pellet induced granuloma method
both the first (50.84% inhibition) and second (74.61% inhibition) phases and its effect was totally responses in carrageenan method, (50.10% inhibition) in cotton pellet method, (65.75% inhibition) in croton oil method. Thus the oral and intraperitoneal administration of the drugs, this constitutes the activity of the herbal or standard marketed drugs, in a dose dependent manner.

**DISCUSSION**

Carrageenan early is an exudative phase of inflammatory pathology was reported by Ozaki et al. [23] that involved the action of vasoactive amines, such as histamine, serotonin, and kinins on vascular permeability [24-26]. Subcutaneous injection of carrageenan into the rat paw produces plasma extravasations and inflammation characterized by increased tissue water and plasma protein exudation with neutrophils extravasations and metabolism of arachidonic acid by both cyclooxygenase and lipoxygenase enzyme pathways [27]. It was observed by the increased paw volume under the plethysmometer in experimental rats in this study. Winter and Porter [15] suggested that the early hyperemia of carrageenan-induced oedema results from the release of histamine and serotonin. Thus Carrageenan-induced paw oedema in rats appears to be a biphasic events and the early phase (2.5–3 h) of the inflammation is due to the release of vasoactive amines such as histamine and serotonin. In In the later phase (4.5–6 h) is due to the activation of kinin-like substances such as prostaglandins, proteases and lysosome [28]. The first phase showed that the EECS reduced the inflammation by control the proliferation of the histamine and serotonin and in second phase controlled the stimulation of kinin like substances. Wagner and Roth [13] reported that leukocyte adhesion represents one of the first steps in the inflammatory response initiation and it is essential for accumulation of active immune cells at sites of inflammation. Although direct evidence of the mechanism of action of extract is not clear. Flavonoids exhibit anti-inflammatory activity was reported [29]. According to this, flavonoids presented in leaves extract activate the immune cells to control the inflamed area.

In the second method, EECS reduced the cotton pellet weight by three different doses in a dose dependent manner. In the third method ear oedema the most important mediators are involved as prostaglandins,
histamine and serotonin, whereas the lypoxygenase pathway has no important role [30]. The method has certain advantages for natural product testing [31]. But peripherally acting drugs such as aspirin, indomethacin and dexamethasone only inhibit the later phase [32]. So, the herbal treatment is very suitable to this ear oedema method. Also the EECS showed that the anti-inflammatory activity by reduced ear disc weight. Statistical analysis showed that the oedema inhibitions of preparations containing extract is significantly different from the control group at all the concentrations tested and the activity is dose-dependent. From the above study we can be concluded that the ethanolic extracts of C. sepiaria shows the anti-inflammatory activity and the inhibitory effect is dose dependent.

ACKNOWLEDGEMENTS

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REFERENCES