EVALUATION OF ANTI-INFLAMMATORY AND ANTI-PYRETIC ACTIVITY OF ETHANOLIC EXTRACT OF CAPPARIS MOONII IN ALBINO RATS

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ABSTRACT
The current study is formulated to discover the mechanism of action and formulation of Capparis moonii against experimentally induced inflammation/paw oedema and pyrexia in rats. The effect of Ethanolic extract of Capparis moonii(fruit) was investigated in rats to evaluate the anti-inflammatory activity by using two models, i.e. Paw volume method by using Plethysmograph, the Vernier Calliper method and anti-pyretic activity by the model of Yeast induced pyrexia method. The parameters taken to assess anti-inflammatory activity were oedema, difference in paw volume of rat, amount of inflammation produced, percent oedema inhibition and yeast volume injected, difference in the rectal temperature, pyrexia. Indomethacin (25mg/kg) was used as positive control for both the activities. The results indicate that the Ethanolic extract of Capparis moonii (EECM) significantly (P <0.05) decreases the oedema, difference in paw volume of rat, amount of inflammation produced, percent oedema inhibition with respect to control and comparable with Indomethacin while ethanolic extract of Capparis moonii (EECM) significantly (P <0.05) decreases the difference in the rectal temperature, pyrexia.

KEYWORDS: Capparis moonii, inflammation, oedema, pyrexia, Plethysmograph, Vernier Calliper, anti-pyretic.

INTRODUCTION
Inflammation is identified to be a type of localized protective response to tissue damage and/or microbial invasion, which aids in isolation and destruction of the harmful agent, the tissue affected and to prepare the tissue for eventual process of repair and healing. Mostly an inflammatory reaction is short-lived and gives the desired protective response. But when there is excessive or prolonged inflammation, it can lead to extensive tissue damage, organ losing its function and mortality.[1]

The ancients described inflammation by five signs, namely redness (rubor), swelling (tumour), heat (calor), pain (dolor) and loss of function (functionlaesa). The increased redness is caused due to the additional passage of erythrocytes through the area, the swelling (oedema) is the result of increased passage of fluid from dilated and permeable blood vessels into the surrounding tissues or infiltration of cells into the damaged area, calor or increased temperature at that area is due to high rate of passing or erythrocytes and friction caused by them, pain is due to the direct effects of mediators, either from initial damage or that resulting from the inflammatory response and the stretching of sensory nerves due to oedema and the loss of function refers to either simple loss of mobility due to the oedema and pain, or to the replacement of functional cells with scar tissue. Previously, the standard treatments for such condition were using a non-steroidal anti-inflammatory drug (NSAID), such as aspirin, for pain relief and to use corticosteroids or even disease-modifying drugs to reduce other symptoms of the disease. Development of NSAIDs which shared the therapeutic action of aspirin but which did not cause the main side effect, namely gastric ulceration was carried out. This research led to the development of indomethacin, the fenamates, ibuprofen and many others. Most of these drugs had clinical utility they also eroded the gastric mucosa.[2]

Pyrexia or simply fever is a condition which is characterized by the event of raised temperature of the body which usually occurs in order to fight an infection or invasive condition. The most common causes are infectious viruses causing cold or flu. Other responsible conditions may be include hepatitis, sinusitis, urinary tract infections, dental swelling and drug reactions. In children its due to cold, flu, throat, UTI, or roseola. Less commonly, fever can be caused by extremely serious conditions such as meningitis, meningococcal disease etc. Chronic pyrexia may indicate hepatitis, tuberculosis,
lymphoma, and reactions to drugs (so-called drug fever).[3]

*Capparis moonii* Wight (belonging to family: Capparidaceae/Capparacea) commonly known as Large Caper, Rudanti in Sanskrit and Waghati in Marathi, is distributed in Maharashtra, Goa, Karnataka, Kerala and Tamil Nadu. Large Caper is the largest flower among all Caper flowers. The main active compounds present in the fruits of Rudanti are sitosterol, stachyhydrynin, rutin, Gallotannins (chebulinic acid derivatives).

Therapeutic Uses of Rudanti in Ayurveda are:[4]

- Rudanti nourishes each and every cell of the body (Rasyayani). It is useful in under nutrition and emaciating conditions (Shoshghani). It delays the signs of aging (JaraVinashnam) and is also useful in diseases which are having devastating effects on all the systems of the body (RajayakshaShaayasate).
- Rudanti has also been extensively used to get relief from asthma and cough by the people of India. Physical, chemical and physiological factors may lead to gastric ulceration in humans and experimental animals.

Reactive oxygen species (ROS) are reported in the pathophysiology of human diseases such as neurodegenerative inflammation, viral infections autoimmune GI inflammation and gastric ulcer.[6]

The results were comparable with the standard drug silymarin. (Prevention of carbon – tetra-chloride induced hepatotoxicity by the ethanolic extracts of Capparis moonii in rats (Pharma Biology, 42:286(2004) According to the literature survey there is no reported research on immunomodulatory activity so our main objective was to explore immunomodulatory activity.

**Actions**

- Fruit is used in purerpal sepsis and septic wounds, also for debility and cough.[5]
- EtOH (50%) extract of aerial parts is CNS depressant.
- Fruits contain L-stachyhydrine, rutin and beta-sitosterol.

**A. CNS depressant activity**

Fruit of Capparis moonii are used in the treatment of sepsis and septic wounds and the seeds are used for the treatment of cough. Ethanolic (50%) extract of aerial parts of Capparis moonii is CNS depressant.[6]

**B. Tuberculostatic Activity**

Capparis moonii fruit powder doesn’t have significant antituberculosis or bactericidal activity, whereas the seeds show slight activity. Capparis moonii fruit powder preparation exhibited anti-tuberculosis or bacteriostatic activity on Staphylococcus aureus (slight inhibitory effect) and marked effect on Shigella flexinerti.[6]

**C. Insulinomimetic Activity**

The two-new hydrolysable gallotannins, chebulinic acid derivatives obtained from the fruits of Capparis moonii, showed their significant effect on glucose uptake, IR-b phosphorylation, IRS-1 phosphorylation, GLUT4 and PI3-kinase mRNA expression in the L6 cells. The new compounds were isolated using bioassay guided fractionation technique and characterized using IR, MS, 1D and 2D NMR spectroscopic techniques. This is the first report of gallotannins from the fruits of Capparis moonii. Two new gallotannins were isolated and their antidiabetic activities were evaluated. They appeared to be primarily acting through stimulation of insulin signalling pathway, by major down signalling events on the insulin pathways (i.e., IR beta subunit and IRS-1 phosphorylation, PI3K and GLUT4 mRNA expression in L6 cells). The gallotannins may be regarded as potential candidates for development of new antidiabetic drugs.[6]

**D. Anti-hepatotoxicity**

The effect of the ethanol extract of C. moonii fruits was studied in carbon tetrachloride induced hepatotoxicity in rats. The hepatotoxicity was induced in rats with the administration of 1:1 (v/v) mixture of tetrachloride olive oil at the dose of 1ml/kg subcutaneously on day 7. The Ethanolic extract of C. moonii (200 mg/kg) and the standard drug Silymarin (25 mg/kg) were given orally from day 1 to day 9. The results were comparable with the standard drug Silymarin. The extract of C. moonii produced significant (p<0.001) lowering of the elevated serum glutamic oxalo acetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), (ALP) and a rise of depleted total protein when compared with the toxic control was reported.[6]

**MATERIAL AND METHODS**

**MATERIALS**

**Collection of plant material**

Fruits of Capparis moonii, were purchased from local suppliers in Jan. 2012 and authenticated from Agharkar Research Institute, Pune, India. The voucher specimen (No. F-176) was deposited in the herbarium of the Institute for future reference. The fruits were cut into small pieces and dried at controlled temperature 45°C and powdered.

**Preparation of extract**

The fruits were dried and powdered by grinding and sieved with a 44 # sieve. The powder was then kept in soxhlet apparatus with ethanol for 24 h. Later the extract was filtered and dried at 45°C (yield 7.46%). Extract was refrigerated at 4°C and used later.

**Chemicals**

**For Anti-Inflammatory Activity**

Ethanol (95%), Ethanolic extract of Capparis moonii (EECM), Indomethacin (25mg/kg of body weight of rats), Carrageenan (1% solution injected in plantar region of paw), carboxy methyl cellulose (CMC) were used in the study.
For Anti-Pyretic Activity
Ethanol extract of Capparis moonii (EECM), Indomethacin (25mg/kg of body weight of rats), Yeast solution containing dry yeast in a solution of Sodium Chloride (0.9% solution in water) at a temperature of 45-50°Celsius (110°F) injected subcutaneously, Carboxy methyl cellulose (CMC) were used in the study.

Animals
The study was conducted on Albino Wistar rats of 150-200 g and maintained under standard conditions (room temperature 24-27°C and humidity 60-65%) with 12 h light and dark cycle. The food in the form of dry pellets (Amrut Lab., Pune) and water were available ad libitum. Rats of either sex, were randomly allocated to groups of 6 animals each. The study was approved by the Institutional Animal Ethical Committee (IAEC) of Oriental College of Pharmacy, Sanpada, Navi Mumbai under the approval number OCP/IAEC/2015-2016/10.

Methods
For Anti-Inflammatory Activity
After Weighing and numbering the animals will be marked on both the hind paws (right and left) just beyond tibio-tarsal, so that every time the paw is dipped in the mercury column up to the fixed mark to ensure constant paw volume. Animals will be divided into 4 groups, each consisting of 6 animals. To first group distilled water will be given and to second group i.e. (standard group) 100 mg/kg body weight of aspirin aqueous solution will be orally administered. To 3 and 4 groups (test group) 100 and 200mg/kg of EECM drug will be orally administered respectively. Initial paw volume of each animal of control and drug treated animals will be compared and express as percent oedema inhibition by the drug.\(^9\)

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>DRUG</th>
<th>PAW MEASUREMENT AT DIFFERENT TIME INTERVALS (mm)</th>
<th>PERCENT INHIBITION OF OEDEMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP I (CONTROL-DISTILLED WATER)</td>
<td>0.5ML</td>
<td>3.20±0.02</td>
<td>3.25±0.03</td>
</tr>
<tr>
<td>GROUP II (STD-INDOMETHACIN)</td>
<td>25mg/kg</td>
<td>3.20±0.02</td>
<td>3.22±0.02</td>
</tr>
<tr>
<td>GROUP III - (EECM)</td>
<td>100mg/kg</td>
<td>3.20±0.02</td>
<td>3.50±0.02</td>
</tr>
<tr>
<td>GROUP IV - (EECM)</td>
<td>200mg/kg</td>
<td>3.20±0.02</td>
<td>3.24±0.04</td>
</tr>
</tbody>
</table>

Table 1: Rat Paw Measurement Table For Vernier Calliper Method.
Percent Difference in rectal temp. = \( \frac{\text{Final rectal temp.} - \text{Initial rectal temp.}}{\text{Initial rectal temp.}} \times 100 \)

Percent Inhibition of pyrexia = \( \left[ 1 - \frac{D}{S} \right] \) where,
\( D \) - Percent difference in rectal temp when drug is given.
\( S \) - Percent difference in rectal temp for control.

STATISTICAL ANALYSIS
The following values are expressed as mean± SEM (n=6). Significance of statistic data was evaluated by using GraphPad’s Prism 7.01 analysis software (grouped type of data where a single Y value was entered for each point using mean, SEM and n).

Table 2: Rat Paw Measurement Table For Plethysmometer Method.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>DRUG DOSE</th>
<th>INITIAL THICKNESS (mm of Hg)</th>
<th>FINAL THICKNESS IN TIME INTERVALS (mm of Hg)</th>
<th>PERCENT INHIBITION OF OEDEMA AT DIFFERENT TIME INTERVALS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30 min</td>
<td>60 min</td>
<td>120 min</td>
</tr>
<tr>
<td>GROUP I (CONTROL - DISTILLED WATER)</td>
<td>0.5ML</td>
<td>0.35±0.06</td>
<td>0.467±0.02</td>
<td>0.517±0.040</td>
</tr>
<tr>
<td>GROUP II (STD-INDOMETHACIN)</td>
<td>25mg/kg</td>
<td>0.217±0.017</td>
<td>0.283±0.037</td>
<td>0.217±0.037</td>
</tr>
<tr>
<td>GROUP III (EECM)</td>
<td>100mg/kg</td>
<td>0.250±0.021</td>
<td>0.300±0.043</td>
<td>0.250±0.049</td>
</tr>
<tr>
<td>GROUP IV (EECM)</td>
<td>200mg/kg</td>
<td>0.216±0.007</td>
<td>0.300±0.037</td>
<td>0.250±0.022</td>
</tr>
</tbody>
</table>

Table 3: Rectal Temp. Measurement Table For Yeast Induced Pyrexia Method.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>DRUG DOSE</th>
<th>INITIAL TEMPERATURE (°C)</th>
<th>FINAL TEMPERATURE IN TIME INTERVALS (°C)</th>
<th>PERCENT INHIBITION OF PYREXIA AT DIFFERENT TIME INTERVALS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>45min</td>
<td>90 min</td>
<td>150 min</td>
</tr>
<tr>
<td>GROUP I (CONTROL - DISTILLED WATER)</td>
<td>0.5ML</td>
<td>35.58±0.18</td>
<td>37.48±0.48</td>
<td>37.40±0.40</td>
</tr>
<tr>
<td>GROUP II (STD-INDOMETHACIN)</td>
<td>25mg/kg</td>
<td>35.65±0.15</td>
<td>37.33±0.35</td>
<td>36.9±0.50</td>
</tr>
<tr>
<td>GROUP III (EECM)</td>
<td>100mg/kg</td>
<td>35.15±0.10</td>
<td>37.5±0.22</td>
<td>37.3±0.20</td>
</tr>
<tr>
<td>GROUP IV (EECM)</td>
<td>200mg/kg</td>
<td>35.50±0.10</td>
<td>37.38±0.42</td>
<td>37.15±0.32</td>
</tr>
</tbody>
</table>

Figure 1: Anti-Inflammatory Action By Vernier Calliper Method.
DISCUSSION

Inflammation is characterized in phases, the first phase by vascular permeability, exudation of plasma, release of mediators; the second phase is due to migration of leucocytes and the final phase is by granuloma formation. This study is a subacute study using carrageenan as a phlogistic agent. The paw edema induced by carrageenan in rats is biphasic, the first phase (0-1 h) due to release of 5-HT, histamine, bradykinin from mast cells, plateau phase (2 h) maintained by kinins, second phase (3 h) produced by prostaglandins, protease and lysosomes.[11]

In this study ethanolic fruit extract of C. moonii showed significant (P < 0.01) reduction of paw edema at 100 and 200mg/kg in the comparison with control suggesting that the extract predominantly inhibited the release of prostaglandin like substances. The anti-inflammatory activity of ethanolic fruit extract of C. moonii may be attributed due to the phytochemical ingredients in it like flavonoids, 4-hydroxymellem, β sitosterol and vanillin. Flavonoids play a major role here as it not only inhibits prostaglandin biosynthesis, but inhibiting endoperoxidases but also enzymes like protein kinases and phosphodiesterases that are involved in the inflammation process.[12][13]

Brewer’s yeast here is an exogenous pyrogen (lipopolysaccharide that is the cell wall component of Gram-negative bacteria), which causes pathogenic fever by binding to lipopolysaccharide binding protein thus resulting in the release of cytokines like interleukin (IL-1, IL-6), tumor necrosis factor alpha (TNF-α) and ultimately prostaglandins. These pro-inflammatory mediators cross the blood brain barrier and act on the pre-optic/anterior hypothalamus triggering the release of prostaglandin E2 (PGE2) produced from (COX)-2 and thus elevating the body temperature. The anti-inflammatory activity of C. moonii is attributed to its cyclooxygenase inhibitory activity.[14] The phytochemical ingredients in the fruit extract such as phenolics, flavonoids, tannins, saponins, and terpenoids could be responsible for the antipyretic activity of this plant.[4]
RESULT
The given ethanolic extract of Capparis moonii (EECM) showed effect on Carrageenan induced inflammation / rat paw oedema with an action similar to that of the standard Indomethacin (25mg/kg of body weight) used. It was found that on giving 100mg/kg of EECM minor action was produced and on administration of double of original dose i.e. 200mg/kg of EECM results were comparatively better. The percentage of paw oedema inhibition shown by standard Indomethacin is 206.95% and in case of drug EECM in conc. 100 mg/kg and 200 mg/kg was 80.83% and 51.68% respectively at time 120 mins when performed by Plethysmometer method. When performed by Vernier calliper method the percent oedema inhibition shown by the standard Indomethacin is 67.1% and for drug given in conc. 100mg/kg and 200 mg/kg is 14.28% and 36.99% respectively for a period after 7 days.

For anti-pyretic activity done by Yeast induced pyrexia method, the pyrexia inhibition by the standard Indomethacin is 33.53% and drug EECM at conc. 100mg/kg and 200 mg/kg is 15.44% and 25.90% respectively at time 150 mins. Hence, the preliminary pharmacological screening of the extract showed significant dose dependent analysis. The inflammation reduction period was found to be 2 hrs. i.e after two hours of administration of oral dose of EECM there was substantial decrease in the oedema induced in rat paw. It was found that the effect of EECM on inhibiting inflammation was mostly effective in acute condition.

CONCLUSION
The Ethanol extract of Capparis moonii (EECM) was found in a dose dependent pattern, to have positive effect as an anti-inflammatory agent as well as an anti-pyretic agent at dose of 100mg/kg and comparatively better action at 200mg/kg conc. This makes it possible to utilize EECM as a potential treatment drug against inflammation and fever that too from a natural source. This also brings us to the conclusion that EECM proved to be effective in pre-clinical trials and hence this can further be followed with clinical trials.

ACKNOWLEDGEMENTS
We are grateful to our Principal Dr. (Mrs.) Sudha Rathod, mentors Dr. (Mrs.) Vanita Kanase and Mr. Imtiyaz Ansari for their guidance and support as well as to Pharmacology Dept., Oriental College of Pharmacy, Navi Mumbai.

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