ANTI-INFLAMMATORY ACTIVITY OF CUCUMIS SATIVUS SEED IN CARRAGEENAN AND XYLENE INDUCED EDEMA MODEL USING ALBINO WISTAR RATS

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ABSTRACT
The present study was conducted to study the anti inflammatory activity of Cucumis sativus seed in Carrageenan-induced paw edema model and xylene induced ear edema model using Albino Wistar rats. A significant inhibition of carrageenan induced rat paw edema comparable to that produced by indomethacin, the standard anti inflammatory drug was obtained with all the two doses of the acetone extract, tested in the present study. The results from present study indicate the efficacy of the acetone extract as a therapeutic agent in acute as well as chronic inflammatory conditions. Thus it could be concluded that Cucumis sativus seed extracts possess significant anti-inflammatory properties.

Key words: Anti inflammatory activity, Cucumis sativus seed, Acetone extract, Indomethacin.

INTRODUCTION
Inflammation is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. Inflammation is not a synonym for infection, because infection is caused by an exogenous pathogen, while inflammation is the response of the organism to the pathogen. In the absence of inflammation, wounds and infections would never heal and progressive destruction of the tissue would compromise the survival of the organism. However, inflammation which runs unchecked can also lead to a host of diseases, such as hay fever, atherosclerosis, and rheumatoid arthritis. It is for this reason that inflammation is normally tightly regulated by the body.

Inflammation can be classified as acute and chronic. Acute inflammation is a short-term process which is characterized by the classic signs of inflammation - swelling, redness, pain, heat, and loss of function - due to the infiltration of the tissues by plasma and leukocytes. It occurs as long as the injurious stimulus is present and ceases once the stimulus has been removed, broken down, or walled off by scarring (fibrosis). The process of acute inflammation is initiated by the blood vessels local to the injured tissue, which alter to allow the exudation of plasma proteins and leukocytes into the surrounding tissue. The increased flow of fluid into the tissue causes the characteristic swelling associated with inflammation (Deraedt R et al., 1980) and the increased blood flow to the area causes the reddened color and increased heat. The blood vessels also alter to permit the extravasation of leukocytes through the
endothelium and basement membrane constituting the blood vessel. Once in the tissue, the cells migrate along a chemotactic gradient to reach the site of injury, where they can attempt to remove the stimulus and repair the tissue.

Chronic inflammation is a pathological condition characterized by concurrent active inflammation, tissue destruction, and attempts at repair. Chronic inflammation is not characterized by the classic signs of acute inflammation listed above. Instead, chronically inflamed tissue is characterized by the infiltration of mononuclear immune cells (monocytes, macrophages, lymphocytes, and plasma cells), tissue destruction, and attempts at healing, which include angiogenesis and fibrosis. Endogenous causes include persistent acute inflammation. Exogenous causes are varied and include bacterial infection, especially by Mycobacterium tuberculosis, prolonged exposure to chemical agents such as silica, or autoimmune reactions such as rheumatoid arthritis. This aim of the study is to investigate in vivo anti-inflammatory potential of extracts. Therefore an effort has been made to corroborate and establish scientific evidence for its ethno botanical uses and producing cost effective remedies.

MATERIALS AND METHODS

Plant material

The seeds of the plant Curcuma sativus were collected from Annavasal, Thiruvurur Dist, Tamilnadu. Further identification has also been done.

Preparation of extract

The seeds collected was washed with distilled water to remove impurities and dried in shade. The dried seed was extracted with acetone in a soxhlet apparatus. The solvent was completely removed under reduced pressure and oil was obtained (yield 12%). The extract obtained is emulsified in order to enhance bioavailability.

Chemical and reagents

All reagents used in the study were of high purity. All chemicals such as Carrageenan, Indomethacin formalin was purchased from Sigma Aldrich Chemical (Malaysia). Acacia (SD Fine Chemicals Ltd.) were used as emulsifying agent.

Mechanism of Action of Indomethacin

Indomethacin is a Non- Steroidal Anti-Inflammatory drug commonly used as anti-inflammatory, analgesic and anti-pyretic agents. Indomethacin is a non-selective inhibitor of cyclooxygenase (COX) 1 and 2, enzymes that participate in prostaglandin synthesis from arachidonic acid. Prostaglandins (PG) are hormone-like molecules normally found in the body, where they have a wide variety of effects, some of which lead to pain, fever, and inflammation (Hart F, 1963).

Mechanism of Action of Carrageenan induced inflammation

Carrageenan induced inflammation is related to neutrophil and the production of neutrophil derived free radicals, as well as to the release of other neutrophil derived mediators. The inhibition of carrageenan induced inflammation in rats is an established model for evaluating anti inflammatory drugs (Headley PM, 1985; Wolfe MN, 1999) which has been used frequently to assess anti inflammatory effect of natural remedies. The development of carrageenan induced edema consist two phase system; the first phase occurs within one hour of carrageenan inflammation and is attributed to the release of cytoplasmic enzymes serotonin, from the mast cells. The second phase is mediated by an increased release of prostaglandins in the inflammatory area and continue between the two phases is provided by kinins (Phelan K, 2003).

Animals

Experimental animals

Experiments were carried out on healthy adult male Albino wistar rats weighing 180 ± 20 grams. Animals were housed in polypropylene cages with stainless steel grill top at 25 ± 2°C with 12:12 hours light and dark cycle was followed. They were fed a standard diet of pellets and tapped water ad libitum. Rats were routinely acclimatized to laboratory conditions for 7 days prior to experiments after acclimation, the animals will be subjected to a gross observation, to ensure that the selected animals are in good state of health.

Fasting

The animals were fasted prior to dosing by withholding food overnight. Fasted body weight of rats was determined and the dose was calculated according to their body weight.

Experimental Procedure:Carrageenan-induced paw edema in rats

The rats were divided into 4 groups (n = 3). The different groups were treated with acetone extract of Cucumis sativa (200 and 400 mg/kg, p.o.), indomethacin (10 mg/kg, p.o.) and control vehicle per oral and the paw volume were measured at 30, 60, 120, and 180 min after carrageenan administration. Basal volume measurement was carried out using a plethysmometer (Model 7150, UGO Basile, Italy). The animals were pretreated with the extract 1 h before the administration of carrageenan.

Acute inflammation was produced by the subplanter administration of 0.1 ml of (1%, w/v) carrageenan in normal saline in the right paw of the rats. The ratio of the anti-inflammatory effect of Cucumis sativa oil was calculated by the following equation:

Anti-inflammatory activity (%) = (1 - D / C) x 100
Where, D represents the percentage difference in paw volume after *Cucumis sativa* oil was administered to the rats, and C represents the percentage difference of volume in the control groups (Suleyman *et al.*, 1999).

**Xylene induced ear edema**

Albino rats were divided into four groups of three animals each. Animals were treated orally with the extract (200 and 400 mg/kg), dexamethasone (1 mg/kg) and distilled water (3 ml/kg). Thirty minutes later, edema was induced in each group by applying a drop of xylene to the inner surface of the right ear. After 15 min, the animals were sacrificed under ether anesthesia and both ears cut off, sized and weighed. The anti-inflammatory activity was expressed as the percentage inhibition (Dobois *et al.*, 2004) of edema in the treated mice in comparison with the control rats.

**Statistical analysis**

The values of each experimental group were expressed as mean ± SEM and compared with the control group followed by student's *t* test.

**Xylene induced ear edema**

The xylene ear edema model shows the evaluation of anti-inflammatory steroids and is less sensitive to non-steroidal anti-inflammatory agents. Severe vasodilation, edema changes of skin and infiltration of inflammatory cells are detected as signs of acute inflammation after topical application of xylene. In the present study, the increases in ear weight were inhibited in a dose related manner by the extract. The results of extract are shown in Table 2.

There is significant in reducing the ear edema formation through this plant extract and with the dose of 400 mg/kg shows the significant value of less than 0.01. Comparable to standard drug, the plant extract showed good activity.

**Table 1. Anti-inflammatory activity of acetone extract of *Cucumis sativus* seed oil (ACS) on carrageenan-induced paw edema method**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, po)</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1% acacia</td>
<td>0.23±0.12</td>
<td>0.36±0.56</td>
<td>0.66±0.32</td>
<td>0.89±0.26</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>0.15±0.41</td>
<td>0.19±0.23</td>
<td>0.28±0.68</td>
<td>0.26±0.14</td>
</tr>
<tr>
<td>Test I</td>
<td>200</td>
<td>0.21±0.54</td>
<td>0.29±0.16</td>
<td>0.45±0.68</td>
<td>0.50±0.46</td>
</tr>
<tr>
<td>Test II</td>
<td>400</td>
<td>0.19±0.62</td>
<td>0.28±0.14</td>
<td>0.39±0.35</td>
<td>0.42±0.43</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n = 3). P value <0.05. The values inside parenthesis indicates the percentage inhibition of paw edema.

**Table 2. Anti-inflammatory activity of the extract and Dexamethasone (reference drug) on xylene induced ear edema in Wistar rats.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg)</th>
<th>Weight of right ear (mg)</th>
<th>Weight of left ear (mg)</th>
<th>Difference (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3ml/kg</td>
<td>23.70 ±1.08</td>
<td>14.92 ±1.17</td>
<td>8.78</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>1</td>
<td>23.99 ±0.64</td>
<td>20.89 ±0.57</td>
<td>3.10*</td>
</tr>
<tr>
<td>ACS</td>
<td>200</td>
<td>22.68 ±0.51</td>
<td>18.30 ±1.20</td>
<td>4.38*</td>
</tr>
<tr>
<td>ACS</td>
<td>400</td>
<td>23.91 ±0.44</td>
<td>16.12 ±0.63</td>
<td>3.89**</td>
</tr>
</tbody>
</table>

Data are the mean ± SEM values for three mice in each group. *p < 0.05, **p < 0.01 as compared to the control.

**Figure 1.** Group of animals (1, 2, 3, and 4) versus weight of the ear (mg)
RESULTS AND DISCUSSION
The effect of acetone extract of *Cucumis sativus* seed on carrageenan-induced paw edema in rats is shown in Table 1 and chart-1. The results obtained indicate that the acetone extract has significant anti-inflammatory activity in rats. The acetone extract of *Cucumis sativus* seed reduces the paw edema induced by carrageenan by 43.82% and 52.80% on oral administration of 200 and 400 mg/kg respectively after 3 hour of carrageenan induction, as compared to the control group. Indomethacin at 10 mg/kg inhibited the edema volume by 70.78%.

DISCUSSION
In the present investigation, acetone extract of *Cucumis sativus* seed was studied for its Anti-inflammatory activity using acute animal model. Due to the increasing frequency of intake of NSAID’s and their reported common side effects, there is need to focus on the scientific exploration of herbal drugs having fewer side effects. So, there is a continuous search for indigenous drugs, which can provide relief to inflammation and pain. The traditional medical practitioners use this plant to cure inflammation and pain (Govindarajan R et al., 2007). To give a scientific validation for this plant, an attempt was made to study the anti-inflammatory activity.

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 CA, 1962). Carrageenan-induced edema is a biphasic response. The first phase occurs within one hour of carrageenan administration and is mediated through the release of cytoplasmic enzymes, histamine, and serotonin from the mast cells. Plateau phase is maintained by kinin like substances. The second phase (>1.0 h) is related to the release of prostaglandin and slow reacting substances which peak at 3 hrs. The knowledge of these mediators involved in different phases is important for interpreting mode of drug action.

Xylene is known to cause severe vasodilation and edematous changes of skin as signs of acute inflammation (Kim HD et al., 2007). The increased thickness of ear tissues is caused by these histopathological changes. In the present investigation, the plant extract significantly inhibited the xylene – induced increases in ear weight in a dose related manner. This inhibition capacity of the plant extract can be regarded as the evidence of anti-inflammatory efficacy through reducing vasodilation ad so that improving edematous condition.

According to the results of the present investigation, it can be concluded that the seeds of the plant *Cucumis sativus* has anti-inflammatory effect that support to the traditional use of this plant for the treatment of related diseases. This study also suggests for the further detail investigation of mechanisms of the pharmacological (Kirkova M, 1992) effects and also to isolate the active compound(s) responsible for those properties.

Declaration of interest
The authors report no conflicts of interest.

REFERENCES