Evaluation of Anti-Inflammatory Activity of Citrullus lanatus Seed Oil by *In-vivo* and *In-vitro* Models

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(Received: 14 August, 2012; Accepted: 27 August, 2012; Published: 29 August, 2012)

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**ABSTRACT**

The present study aimed for *in-vivo* and *in-vitro* evaluation of *Citrullus lanatus* seed oil for anti-inflammatory activity. *Citrullus lanatus* seed oil is extracted with n-hexane and tested for *in-vivo* and *in-vitro* anti-inflammatory activity. The oil was screened for *in-vivo* anti-inflammatory activity by carrageenan-induced paw edema in rat model and *In-vitro* anti-inflammatory activity by human red blood cell membrane stabilization method. The potency of the oil was compared with standard diclofenac (10 mg/kg). The oil showed significant reduction of edema in carrageenan induced rat paw edema model maximum at 3 hr (percentage reduction in paw volume 44.44%, 55.56% and 63.11% for CLSO(50 mg/kg), CLSO(100 mg/kg) and diclofenac(10 mg/kg) respectably and CLSO at concentration of 100, 250 and 500 mcg/ml showed 42.35%, 68.48% and 78.50% protection of HRBC in hypotonic solution respectably. All the results were compared with standard diclofenac at 50, 100 and 200 mcg/ml which showed 43.74%, 63.93% and 86.73% protection of HRBC in hypotonic solution respectably.

**Keywords:** *Citrullus lanatus*, anti-inflammatory activity, carrageenan-induced paw edema, human red blood cell membrane.

**INTRODUCTION**

Inflammation is a complex biological response of vascular tissue to harmful stimuli, pathogens, irritants characterized by redness, warmth, swelling and pain. Prolonged inflammation leads to the rheumatoid arthritis, atherosclerosis, hay fever, ischemic heart diseases etc and inflammation is a common manifestation of infectious diseases like leprosy, tuberculosis, syphilis, asthma, inflammatory bowel syndrome, nephritis, vascularitis, celiac diseases, auto-immune diseases etc.

Anti-inflammatory drugs like NSAIDs used to reduce the swelling and pain of inflammation. But these agents carry the risk of gastro-intestinal toxicity, cardiovascular and other toxicity for prolonged use. For these reason, there is a need for ant-inflammatory drugs having less severe side effects to use for chronic inflammatory disease as well. Therefore, in resent time, more interest is shown in alternative and natural drugs for treatment of various diseases, but there is lack of scientific evidence.

*Citrullus lanatus* of family Cucurbitaceae is commonly known as water melon and in local name Tarmuz (Hindi), Puchakaya (Telugu). The ripe fruits are edible and largely used for making confectionary. Its nutritive values are also useful to the human health. Fruit is used in cooling, strengthening, aphrodisiac, astringent to the bowels, indigestible, expectorant, diuretic, and stomachic, purifies the blood, allays thirst, cures biliousness, good for sore eyes, scabies and itches and as brain tonic to the brain. It also reported having analgesic and anti-inflammatory activity of roots and leaves, antimicrobial activity, laxative activity of fruit, anti-oxidant and antiulcerative activity.

**MATERIAL AND METHODS**

**Collection and extraction of oil**

The seeds of *Citrullus lanatus* of family Cucurbitaceae were collected from ripe fruits which were obtained from local fruit market, Kodad, Andhra Pradesh. The seeds collected from fruit and dried and extracted with n-hexane to obtain the oil. The percentage yield was 21.59 % w/w.

**Physiochemical studies of oil**
The oil obtained from watermelon seed were tested for qualitative tests for oragnoleptic characters, solubility, specific gravity, refractive index, saponification value, iodine value and chemical tests for oils.

**Drugs and Chemicals**

Diclofenac (Symed Pharm. Pvt. Ltd, Hyderabad) used as the standard anti-inflammatory drug. Carrageenan (Type-I, Sigma Chemicals) and all others laboratory reagents were obtained from the institute store and are analytical grade.

**Animals**

Rats of either sex weighing 150-200 g were used in experiment. Animals were obtained from Anurag Pharmacy College, Kodad. Animals were kept under standard conditions at 23-25°C 12 hr light/dark cycle and given standard pellet diet and water. Before performing the experiment the ethical clearance was obtained from institutional animal ethics committee (IEAC).

**Acute oral toxicity studies**

Acute oral toxicity study was carried out for n-hexane extracted *Citrullus lanatus* Seed oil (CLSO) using Acute Toxic Class Method as described in OECD (Organization of Economic Co-operation and Development) Guidelines No. 423. The *Citrullus lanatus* seed oil was safe up to a dose of 2,000 mg/kg body weight.

**Experimental design**

The animals were divided into four groups of six animals each as follows:

- **Group I**: Control: received 1 % aqueous solution of 2% Tween-80, p.o (1 hr before carragenan injection)
- **Group II**: Drug treated: received CLSO 50 mg/kg, p.o (1 hr before carragenan injection)
- **Group III**: Drug treated: received CLSO 100 mg/kg, p.o (1 hr before carragenan injection)
- **Group IV**: Drug treated: Diclofenac 10 mg/kg, p.o (1 hr before carragenan injection)

**In-Vivo anti-inflammatory activity**

**Carrageenan-induced paw edema in rats**

The acute hind paw edema in Wister rats (150-200 gm) was produced by Carrageenan-induced paw edema in rats 6. 0.1 ml of carrageenan (prepared as 1% w/v suspension in saline) locally injected into subplantar region of the left hind paw of rats. *Citrullus lanatus* seed oil (CLSO) (50 and 100 mg/kg, p.o. and diclofenac 10 mg/kg, p.o.) were given orally 1 hour prior of carragenan injection. Other group served as control in this experiment which got carragenan and vehicle. The rat paw volume up to the ankle joint was measured at 0 hr (30 min before caragennan injection), 1 hr, 2 hr, 3 hr and 4 hr after the injection of carrageenan using plethysmometer. Increase in the paw edema volume was considered as the difference between initial at 0 hr and of 1 hr, 2 hr, 3 hr or 4 hr. Percent inhibition of paw volume between treated and control groups were calculated as follows:

\[
\text{Percent inhibition} = \frac{V_c - V_t}{V_c} \times 100
\]

Where \(V_c\) and \(V_t\) represent the mean increase in paw volume in control and treated groups, respectively.

**Statistical Analysis**

The data were expressed as mean ± standard error mean (SEM). The data were analyzed by using Graph pad software Prism version 5 by one way analysis of variance (ANOVA). The test was followed by Dunnett’s ‘t’-test, p values less than 0.05 were considered as significance.

**RESULTS AND DISCUSSION**

**Preliminary Physicochemical Screening**

The CLSO was screened for various Physicochemical test as per the reported methods and found the oil as golden yellow colour, having pungent smell and soluble in organic solvent such as ethanol. It was found of Specific gravity 0.925 at 25°C, refractive index 1.46 at 25°C Saponification value 168.5, iodine value 121.3 and chemical tests confirms the presence of oil, terpenoids, and phenolic compounds.

**In-vivo anti-inflammatory activity of Citrullus lanatus seed oil (CLSO)**
The results of *in-vivo* anti-inflammatory activity of *Citrullus lanatus* seed oil (CLSO) on carragenan induced paw edema in rats were given in **Table 1**. Anti-inflammatory effect of *citrullus lanatus* seed oil was evaluated after sub plantar injection of carragenan in rats. Sub plantar injection of carragenan results in significant increased in paw edema after one hour in control rats as compared to normal rats which was subsequently increased up to 3 hrs. Rat treated with CLSO (50mg/kg and 100mg/kg) showed significant decrease in paw edema in paw edema on 1 hr, 2 hr, 3 hr and 4hr and was comparable with standard drug diclofenac (10 mg/kg). The difference in paw volume of treated group was most significant (p<0.001) at 3 hr.

**In-vitro Anti-Inflammatory of Citrullus lanatus seed oil (CLSO)**

The results of *in-vitro* anti-inflammatory activity of *Citrullus lanatus* seed oil (CLSO) on carragenan induced paw edema in rats were given in **Table 2**. *In-vitro* anti-inflammatory activity of CLSO was performed by using human red blood cell membrane stabilization method. CLSO showed significant anti-inflammatory activity in a concentration dependent manner. CLSO at concentration of 100, 250 and 500 mcg/ml showed 42.35%, 68.48% and 78.50% protection of HRBC in hypotonic solution respectively. All the results were compared with standard diclofenac at 50, 100 and 200 mcg/ml which showed 43.74%, 63.93% and 86.73% protection of HRBC in hypotonic solution respectively.

### Table 1: Effect of Citrullus lanatus Seed oil (CLSO) on carragenan induced paw edema in rats

<table>
<thead>
<tr>
<th>Treated groups</th>
<th>1 hr</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PV</td>
<td>% RPV</td>
<td>PV</td>
<td>% RPV</td>
</tr>
<tr>
<td>Control</td>
<td>0.300±0.02</td>
<td>-</td>
<td>0.366±0.03</td>
<td>-</td>
</tr>
<tr>
<td>CLSO 50mg</td>
<td>0.201 ±0.02 *</td>
<td>33.34</td>
<td>0.231±0.02*</td>
<td>36.88</td>
</tr>
<tr>
<td>CLSO 100 mg</td>
<td>0.21±0.01*</td>
<td>30.00</td>
<td>0.232±0.02*</td>
<td>36.61</td>
</tr>
<tr>
<td>Diclofenac 10 mg</td>
<td>0.166±0.02**</td>
<td>44.67</td>
<td>0.18±0.01**</td>
<td>50.81</td>
</tr>
</tbody>
</table>

Values are in Mean ± S.E.M (n=6); "- "Non Significant, *p<0.05, **p<0.01, ***p<0.001 when compared with Control using One way ANOVA followed by Dunnet’s “t” test, PV= Paw Volume, %RPV= Percentage Reduction in Paw Volume

### Table 2: In-Vitro anti-inflammatory activity of Citrullus lanatus Seed oil (CLSO) by HRBC method

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Conc. mcg/ml</th>
<th>Absorbance 560 nm</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>---</td>
<td>2.526</td>
<td>-</td>
</tr>
<tr>
<td>CLSO</td>
<td>100</td>
<td>1.456</td>
<td>42.35%</td>
</tr>
<tr>
<td>CLSO</td>
<td>250</td>
<td>0.796</td>
<td>68.48%</td>
</tr>
<tr>
<td>CLSO</td>
<td>500</td>
<td>0.543</td>
<td>78.50%</td>
</tr>
<tr>
<td>Diclofenec</td>
<td>50</td>
<td>1.421</td>
<td>43.74%</td>
</tr>
<tr>
<td>Diclofenec</td>
<td>100</td>
<td>0.911</td>
<td>63.93%</td>
</tr>
<tr>
<td>Diclofenec</td>
<td>200</td>
<td>0.335</td>
<td>86.73%</td>
</tr>
</tbody>
</table>
The edema and inflammation induced by Carrageenan is shown to be mediated by histamine and serotonin during first 1 h. After which increased vascular permeability is maintained by the release of kinins up to 2.30 h, followed by the release of kinins and finally through the release of bradykinin, prostaglandin and lysosomes from 2.30 to 6 h. The later phase is reported to be sensitive to most of the clinically effective anti-inflammatory agents. The mediators appear to be prostaglandins, the release of which is closely associated with migration of leucocytes into the inflamed site. The Carrageenan induced paw edema model in rats is known to be sensitive to cyclo-oxygenase (COX) inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents. Though CLSO (50 and 100 mg/kg, p.o.) significantly reduced the paw edema in rats but the effect was of less intensity, when compared with diclofenec (10 mg/kg, p.o).

The Citrullus lanatus seed oil exhibited membrane stabilization effect by inhibiting hypotonicity induced lyses of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release.

CONCLUSION
Citrullus lanatus seed oil possesses potent anti-inflammatory activity both in-vivo and in-vitro studies which is comparable to standard diclofenec. Since, serotonin, histamine and prostaglandins are the major mediators of inflammation, anti-inflammatory effect of Citrullus lanatus seed oil either due to inhibition of their synthesis or inhibition of prostaglandin synthesis at third stage of inflammation. Based on the present study, it can be concluded that Citrullus lanatus seed oil have potent anti-inflammatory activity and by further studies it can be possible to formulate natural anti-inflammatory drug of Citrullus lanatus.

Acknowledgements
This study was supported by giving permission to use instruments and ethical clearance from institute. Our special thanks to Dr. M. Chinna Eswaraih, Principal, Anurag Pharmacy College, Kodad, Andhra Pradesh, for his valuable suggestion and help.

REFERENCES
