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## RESEARCH ARTICLE

### EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF *CITRULLUS COLOCYNTHIS*

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#### ABSTRACT

*Citrullus colocynthis* had been widely used for its reported biological activities in indigenous system of medicine. The present investigation was carried out to find the effect of different phlogistic agents-induced paw edema and carrageenan-induced air-pouch inflammation in rats. Methanol extract of *Citrullus colocynthis* significantly inhibited carrageenan, serotonin and prostaglandin E1-induced paw edema. Maximum inhibition was observed in prostaglandin E1-induced paw edema. In carrageenan air-pouch model, methanol extract of *Citrullus colocynthis* significantly reduced the volume of exudate and migration of neutrophils and monocytes. The extract significantly decreased formation of granuloma tissue in chronic inflammation model. Hence, present investigation established some pharmacological evidences to support the *Citrullus colocynthis* is used as anti-inflammatory agent.

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#### INTRODUCTION

Inflammation is a local response of living mammalian tissues to injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agent. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Edema formation, leukocyte infiltration and granuloma formation represent such components of inflammation (Mitchell *et al.*, 2000). *Citrullus colocynthis* (L.) Schrad is a fruit commonly known as bitter apple or bitter cucumber, found in Sudan, Iran and India and in the deserts (Trease and Evans, 1970). The dried pulp of *Citrullus colocynthis* has been used for constipation, edema, bacterial infections, cancer and diabetes (Al-Ghaithi and El-Ridi, 2004). Recently, the antioxidant effects and the effect of the aqueous extract of the pulp on kidney and liver functions were reported (Al-Hader *et al.*, 1994). Nevertheless, to date, the scientific scrutiny of *Citrullus colocynthis*, is insufficiently documented and warrants systematic analysis. In particular, the acute effect of aqueous extract of the leaf in vivo remains untested. The anti diabetic effects of leaf of *Citrullus colocynthis* was reported (Gurudeeban, 2008). In the present study, we have evaluated the effect of CLEt on acute inflammation using different inflammatory mediators-induced paw

edema and subacute inflammation (leukocyte infiltration and exudation) using carrageenan air-pouch model in rats.

#### MATERIALS AND METHODS

##### Plant material

Fresh *Citrullus colocynthis* leaves were collected from the Gulf of Mannar Biosphere, (Tamilnadu) India. The specimen was certified by Botanical Survey of India (BSI) Coimbatore, and documented in the Herbaria of C.A.S. in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu, and India. Mature leaves were separated manually from the aerial part of the plant. Then, the leaves was dried and minced with a grinder into a powder in preparation for extraction. The experimental chemicals were purchased from Sigma Chemicals, Mumbai.

##### Preparation of extract

The leaves were shade dried and subjected to size reduction to get a coarse powder. The powdered material was subjected to successive extraction in a Soxhlet apparatus, using methanol (75%) as solvent at 50°C. The extract was then evaporated on a rotary evaporator.

##### Experimental animals

Male albino Wistar rats (150 to 200 g) bred in the Central Animal House, Rajah Muthiah Medical College, Annamalai University, was used in this study. The animals were fed on a pellet diet (Hindustan Lever, India) and water *ad libitum*. The animals were maintained in their respective groups for 60 days. All studies were conducted in accordance with the National Institute of

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Health's Guide for the Care and Use of Laboratory Animals [NIH,1985], and the study was approved by the Institutional Ethical Committee of Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalai Nagar, Tamilnadu, India.

#### Phlogistic agents-induced paw edema

The rats were divided into different groups of six each. Acute inflammation was induced by intraplantar administration of 0.1 ml of carrageenan (1%) and chemical mediators *viz* ; serotonin (0.1%), and prostaglandin E<sub>1</sub> (0.0001%). Rats were treated with CLEt (250 mg/kg, p.o.), 1 h before administration of phlogistic agents. The paw volume was measured prior to injection of phlogistic agent (0 h) and then at predetermined interval for each agent. For carrageenan, bradykinin and prostaglandin E<sub>1</sub>, the interval was 3 h, whereas, for serotonin was 1 h and 2 h, respectively. Paw volume was measured using digital Plethysmometer (UGO BASIL, Italy). Change in the paw volume was measured and antiinflammatory activity was calculated by using the formula: % Inhibition of inflammation =  $1 - (Vt/Vc) \times 100$ , where, VT represents the change in the paw volume in CLEt treated group and VC represents the change in the paw volume in the corresponding vehicle treated control group.

#### Carrageenan air-pouch model

The rats were divided into three groups (n=6). Air-pouch was produced according to the method described by (Salvemini *et al*, 1995). Briefly, rats were anesthetized and air cavities were produced by subcutaneous injection of 20 ml of sterile air into the intrascapular area of the back (0 day). An additional 10 ml of air was injected into the cavity every 3 dose (3rd and 6th day) to keep the space open. On the 7th day, 2 ml of 1% solution of carrageenan dissolved in saline was injected directly into the pouch to induce an inflammatory response. The rats were orally pre-treated with CLEt or indomethacin 2 h prior to the injection of carrageenan into the air-pouch. The second dose of treatment was repeated after 24 h of the first treatment. Forty-eight hours after carrageenan injection, the rats were anesthetized with ether and the pouch was carefully opened by a small incision. The volume of exudate was collected and measured. An aliquot of the exudate was used for quantification of leukocyte concentration using a hemocytometer and differential cell count was performed using a manual cell counter after staining with Wright's stain. The results were expressed as the total number of neutrophils and monocytes.

#### Statistical analyses:

All data were expressed as mean  $\pm$  SEM. The statistical analyses were performed using Student's 't' test ( $p < 0.05$  was considered as significant).

## RESULTS

CLEt significantly ( $p < 0.05$ ) inhibited carrageenan, serotonin and prostaglandin E<sub>1</sub> -induced paw edema. Maximum inhibition (48%) was found in prostaglandin E<sub>1</sub> -induced paw edema, whereas, it was 35% in carrageenan induced paw edema and 30.28% inhibition, respectively relative to vehicle treated control group. In the carrageenan air-pouch model CLEt and indomethacin significantly ( $p < 0.05$ ) reduced carrageenan-induced

exudate volume and infiltration of neutrophils and monocytes into the air-pouch compared to vehicle treated control group (Table 2). Inflammation has different phases; the first phase is caused by an increase in vascular permeability, the second one by infiltration of leukocytes and the third one by granuloma formation. In the present study, we have examined the effects of CLEt on two phases of inflammation. In the preliminary experiment, the different doses of extract (100, 250, 500 mg/kg, p.o.) were tested in carrageenan-induced acute inflammation in rats (results not shown). We observed that CLEt at the doses of 250 and 500 mg/kg significantly inhibited carrageenan-induced paw edema. Based on these observations and the previous report (Raphael, 2000), we selected 250 mg/kg, p.o. dose for further studies. In the present study, CLEt significantly inhibited the paw edema induced by prostaglandin E<sub>1</sub> (48.56%) and serotonin (30.28%). Further, except prostaglandin E<sub>1</sub> -induced edema, the magnitudes of these inhibitions were less than that observed with carrageenan-induced edema (35.26%). It has been reported that these inflammatory mediators are released endogenously and contribute to the various phases of paw edema (Smith, 1974). These observations suggest that probably, CLEt shows antiinflammatory effect not only by blocking the effects of serotonin, and prostaglandin E<sub>1</sub> on vascular membrane but also by inhibiting the release of these mediators. Since maximum antiinflammatory effect was observed in prostaglandin E<sub>1</sub> -induced paw edema, our results favor the notion that CLEt may contain active constituent that block prostaglandin E<sub>1</sub> effects. In the current study, CLEt significantly reduced the neutrophil and monocyte infiltration and volume of exudate in carrageenan-induced air-pouch inflammation. These results indicate that CLEt may alter the action of endogenous factors that are involved in neutrophil and monocyte migration to the site of inflammation. However, CLEt was less potent than indomethacin.

## DISCUSSION

In the present study, anti inflammatory activity of methanolic extract of *Citrullus colocynthis* leaves were evaluated. Anti-inflammatory activity was tested by different *in vivo* screening models, represents different phases of inflammation. Carrageenan induced edema has been commonly used as an experimental animal model for acute inflammation study and is believed to be biphasic. The early phase of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. Histamine, serotonin, bradykinin and prostaglandins are established mediators of acute phase of inflammation causing increase in vascular permeability and vasodilatation (Smith, 1974). The later phase is sustained by prostaglandins release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostoglandins produced by tissue macrophages (Whittle, 1964).

The anti-inflammatory activity of CLEt extract found may be due to the presence of therapeutically active flavonoids such as apigenin, quercetin, naringenin and luteolin (Khare, 2009). Flavonoids are known to prevent the synthesis of prostaglandins and have therapeutic application on inflammation (Havsteen, 2002).

**Table 1. Effect of *Citrullus colocynthis* on paw edema induced on different phlogistic agents**

Treatment	Change in paw volume (ml)	% Inhibition of inflammation
Carrageenan (0.1 ml of 1% solution) + Vehicle	2.56 ± 0.15	-
Carrageenan (0.1 ml of 1% solution) + CLEt	1.82 ± 2.12	35.26
Serotonin (0.1 ml of 1% solution) + Vehicle	3.56 ± 0.13	-
Serotonin (0.1 ml of 1% solution) + CLEt	2.34 ± 0.23	30.28
Prostaglandin E1 (0.1 ml of 1% solution) + Vehicle	2.41 ± 0.18	-
Prostaglandin E1 (0.1 ml of 1% solution) + CLEt	1.26 ± 0.42	48.56

**Table 2. Effect of *Citrullus colocynthis* on leukocyte infiltration and exudates volume in Carrageenan-induced air pouch inflammation**

Treatment	Dose mg/kg	Exudate volume (ml)	Neutrophils ( $\times 10^6$ cells)	Monocytes ( $\times 10^6$ cells)
Control	0	3.56 ± 0.12	255.60 ± 5.65	86.12 ± 6.35
CLEt	250	1.3 ± 0.056	124.35 ± 6.24	56.12 ± 2.14
Indomethacin	10	0.86 ± 0.07	76.82 ± 1.24	39.76 ± 3.25

## CONCLUSION

The data obtained from the present study indicated that several factors may contribute to the anti-inflammatory action of CLEt. It showed inhibitory effect on different phlogistic agents-induced paw edema, carrageenan-induced leukocyte infiltration and exudate formation, thus exhibiting anti-inflammatory effect against acute and sub acute phases of inflammation.

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