

## ANTI-INFLAMMATORY EFFECT OF *CARALLUMA EDULIS* AGAINST ACUTE AND CHRONIC INFLAMMATION

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### ABSTRACT

*Caralluma edulis* (Edgew.) Benth. ex Hk.f. (Asclepiadaceae) is traditionally used for the management of rheumatism. The current study aimed to assess the anti-inflammatory effect of *Caralluma edulis*, to explain its medicinal use in inflammatory ailments. *Caralluma edulis* crude extract (Ce.Cr) was investigated in acute (carrageenan and xylene mediated) and chronic (formalin evoked) models of inflammation in mice. Ce.Cr which shows the presence of alkaloids, glycosides, flavonoids, saponins, phenols, terpenes and tannins caused dose-dependent (100, 300 and 1000 mg/kg) reduction of the carrageenan, xylene and formalin-induced inflammation ( $P < 0.05$ ,  $P < 0.001$  vs. saline group). At the lower dose of 30 mg/kg it was effective in inhibiting the xylene mediated ear edema. No mortality observed with Ce.Cr upto 5 g/kg. This study reveals that *Caralluma edulis* possess therapeutic ability to reduce inflammation, hence proving its potential to be used as an alternative medicine in the management of inflammatory disorders and also validate its folkloric use in treatment of rheumatism.

**Key words:** Asclepiadaceae, anti-inflammatory, *Caralluma edulis*, formalin, carrageenan.

### INTRODUCTION

Inflammation is the complex immunological response against the invading irritant, pathogen, injury and trauma (Ma *et al.*, 2013). The inflammatory signs involve pain, swelling, redness, edema, loss of function associated with inflammatory mediators release such as nitric oxide, prostaglandins, interleukins and tumour necrosis factor-alpha (Posadas *et al.*, 2000; Zhang *et al.*, 2011). Inflammation leads to arthritis, cardiovascular complications or even cancer, if left untreated. It is the need of time to develop novel therapeutic agents which are safe, less prone to undesirable effects and are more efficacious (Burke *et al.*, 2006). Medicinal plants used in ethnomedicine worldwide for treatment of various ailments, including inflammation.

*Caralluma edulis* (Edgew.) Benth. ex Hook.f. commonly known as Choung belongs to family Asclepiadaceae (Ahmad *et al.*, 2009). Traditionally *Caralluma edulis* is used for the treatment of parasitic infections, Alzheimer disease, rheumatism, hypertension, gastric problems, diabetes and leprosy. Genus *Caralluma* enriched in pregnane glycosides, megastigmane glycosides, flavone and esters (Adnan *et al.*, 2014). *Caralluma edulis* has been reported to possess antidiabetic (Wadood *et al.*, 1989) and anti-oxidant (Ansari *et al.*, 2005) activities. In the present investigation, the crude extract of *Caralluma edulis* was screened for anti-inflammatory activity using different (acute and chronic) models of inflammation, with aim to rationalize its folklore use in rheumatism.

### MATERIALS AND METHODS

**Plant material collection and extraction:** The fresh whole plant of *Caralluma edulis* was purchased from the local market of Islamabad in May, 2015. It was identified from Dr. Mushtaq Ahmad taxonomist at Department of Plant Sciences, Quaid-e-Azam University, Islamabad. The sample specimen bearing voucher # 422 was deposited to the herbarium of same Department. Plant material was thoroughly washed, dried at room temperature and then coarsely ground. The powder material (3 kg) was extracted with 80% ethanol for seven days with mixing at regular intervals. The macerated plant material was filtered (Williamson *et al.*, 1998). The filtrate concentrated on the rotary evaporator, to obtain the semi-solid paste under reduced pressure i.e. *Caralluma edulis* crude extract (Ce.Cr).

**Chemicals:** Carrageenan was purchase from SigmaChemicals Co, StLouis, MO-USA and diclofenac sodium from Olive Laboratories National Industrial Zone Rawat, Islamabad respectively. Ethanol, formalin (37% v/v), and xylene were obtained from Merck, Darmstadt, Germany.

**Animals:** All the experiments performed on Balb-C mice (25-35 g), divided in different groups, 5 in each group. Animals were kept at the animal house of Riphah Institute of Pharmaceutical Sciences where controlled environmental conditions were maintained with 23-25°C, standard diet and tap-water *ad libitum*. All tests and experiments conducted in this study were in accordance

with the guidelines of Laboratory Animal Resources Institute, Commission on Life Sciences University, National Research Council (1996) and prior approval was granted by Ethics Committee of Riphah Institute of Pharmaceutical Sciences, Riphah International University (Ref.#: REC/RIPS/2015/001).

**Phytochemical analysis:** The *Caralluma edulis* crude extract was tested, for detection of various chemical constituents according to established protocols (Harborne, 1984; Evans, 1996). Plant material when treated with sodium hydroxide, yellow color appearance indicates the positive result for flavonoids and tannins were positive, when cream yellow color was produced with lead acetate. Blue color formation on the treatment of plant with ninhydrin reagent indicates the presence of proteins. Steroids were detected using chloroform and sulphuric acid, appearance of red coloration shows positive result. Dragendroff's reagent was used for detection of alkaloids, formation of red precipitates is considered positive. Saponins were positive, when froth formation of diluted sample occurs, on vigorous shaking. When extract treated with ferric chloride, appearance of black coloration indicates the presence of phenols. Glycosides were detected using keller-killiani test, brown ring formation at the interface was considered positive. Carbohydrates were detected using Molisch's test, violet ring formation indicates the presence of reducing sugars.

**Carrageenan-induced hind paw edema:** Overnight fasted mice were used in the study. The paw volume displacement was measured using Plethysmometer, UgoBasile, Italy before administration of any drug (Padilha *et al.*, 2010). Mice with similar paw volume displacement were placed in one group (n=5, each group). Inflammation was induced by carrageenan sub-plantar injection (0.1 mL of 1% solution in Normal saline). 30 minutes prior to carrageenan injection, the group 1<sup>st</sup> was treated with Normal saline in 10 mL/kg dose, served as control. The second, third and fourth group were treated with Ce.Cr (100, 300 and 1000 mg/kg) ip and fifth group was treated with diclofenac sodium (20 mg/kg) ip. The paw volume displacements were measured at one hour interval upto 5 hour after the carrageenan injection (Ray *et al.*, 2015).

**Xylene-induced ear edema:** Group-I treated with Normal saline in 10 mL/kg dose, served as negative control. Group II–V with Ce.Cr (30, 100, 300 and 1000 mg/kg) i.p and Group VI with Diclofenac sodium (20 mg/kg). Half an hour, after administration of extract and standard drug, inflammation was induced by the application of 30  $\mu$ L xylene to the both surface of right ear (Atta and Alkofahi, 1998). Two hour after the application of xylene, mice were sacrificed by cervical dislocation and both ears were excised. Circular sections were cut and weighed. The ear weight changes produced

by irritant was expressed as the difference of ear weight of the right and left ear (treated vs. untreated) and % inhibition was calculated as follows:

$$\% \text{ Inhibition} = [(W_c - W_t) / W_c] \times 100$$

Where,  $W_c$  = Ear weight difference of control,  $W_t$  = Ear weight difference of treated groups

**Formalin-induced arthritic inflammation:** The chronic anti-inflammatory activity was determined by formaldehyde-induced arthritic inflammation (Cho *et al.*, 2011). On the 1<sup>st</sup> day and 3<sup>rd</sup> day 0.1 mL of formalin (2% formaldehyde) was injected in the left hind-paw. Treatment with drug and extract started on the same day and continues for 10 days. Group-I was administered saline (10 mL/kg), served as negative control. Group II-IV with Ce.Cr (100, 300 and 1000 mg/kg) i.p and Group-V with diclofenac sodium (20 mg/kg). The changes in paw sizes were measured on daily basis and continue up to 10 days (Hosseinzadeh *et al.*, 2000).

**Acute toxicity test:** Mice divided in three groups. Saline (10 mL/kg) was injected to 1<sup>st</sup> group, served as negative control. Plant extract in increasing doses were administered (10 mL/kg volume) to 2<sup>nd</sup> and 3<sup>rd</sup> group. The mice were given standard diet and tap-water *ad libitum* and observed for 24 hours. The no. of deaths counted after 24 hours (Sanmugapriya and Venkataraman, 2006).

**Statistical analysis:** Data represented as Mean  $\pm$  SEM (standard error of mean). The results were analyzed using one-way ANOVA with Post-hoc Tukey test,  $P < 0.05$  considered as different significantly. The bar-graphs were analyzed using the Graph Pad program (GraphPAD, SanDiego, CA-USA).

## RESULTS

**Phytochemical screening:** Ce.Cr reveals presence of saponins, alkaloids, tannin, phenol, glycosides, terpenoids and flavonoids.

**Effect on Carrageenan-induced hind paw edema:** Ce.Cr in dose-dependent manner (100-1000 mg/kg) reduced carrageenan-induced paw edema. The animals of saline control group at 0, 1, 2, 3, 4 and 5<sup>th</sup> hour were having paw volume displacement values of  $0.218 \pm 0.009$ ,  $0.344 \pm 0.006$ ,  $0.35 \pm 0.008$ ,  $0.414 \pm 0.007$ ,  $0.376 \pm 0.002$  and  $0.358 \pm 0.002$  mL respectively. The paw volume displacement values of Ce.Cr (100 mg/kg) treated group at 0, 1, 2, 3, 4 and 5<sup>th</sup> hour were  $0.220 \pm 0.004$ ,  $0.338 \pm 0.006$ ,  $0.344 \pm 0.005$ ,  $0.33 \pm 0.004$ ,  $0.31 \pm 0.006$  and  $0.302 \pm 0.006$  mL ( $P < 0.001$  vs. saline-control group) respectively. The paw volume displacement values of Ce.Cr (300 mg/kg) treated group at 0, 1, 2, 3, 4 and 5<sup>th</sup> hour were  $0.224 \pm 0.005$ ,  $0.328 \pm 0.006$ ,  $0.326 \pm 0.008$ ,  $0.302 \pm 0.008$ ,  $0.294 \pm 0.005$  and  $0.286 \pm 0.006$

mL ( $P < 0.001$  vs. saline-control group) respectively. The paw volume displacement values of Ce.Cr (1000 mg/Kg) treated group at 0, 1, 2, 3, 4 and 5<sup>th</sup> hour were  $0.232 \pm 0.004$ ,  $0.316 \pm 0.007$  ( $P < 0.05$  vs. saline-control group),  $0.304 \pm 0.005$ ,  $0.288 \pm 0.006$ ,  $0.262 \pm 0.006$  and  $0.256 \pm 0.007$  mL ( $P < 0.001$  vs. saline-control group) respectively. The paw volume displacement of diclofenac sodium (20 mg/kg) treated group at 0, 1, 2, 3, 4 and 5<sup>th</sup> hour were  $0.176 \pm 0.005$ ,  $0.286 \pm 0.003$ ,  $0.24 \pm 0.004$ ,  $0.234 \pm 0.005$ ,  $0.206 \pm 0.004$  and  $0.202 \pm 0.004$  mL ( $P < 0.001$  vs. saline-control group) respectively as shown (Fig. 1).

**Effect on Xylene-mediated ear edema:** Ce.Cr dose-dependently (30-1000 mg/kg) caused marked reduction of xylene-induced ear edema. The control saline group has ear weight difference of  $0.053 \text{ g} \pm 0.002$ . The ear weight differences for Ce.Cr 30, 100, 300 and 1000 mg/kg treated groups were  $0.038 \text{ g} \pm 0.003$  ( $P < 0.05$  vs. saline group),  $0.03 \text{ g} \pm 0.002$ ,  $0.016 \text{ g} \pm 0.003$ ,  $0.013 \text{ g} \pm 0.003$  respectively ( $P < 0.001$  vs. saline group), causing 28.3, 43.39, 69.81 and 75.47% inhibition of ear edema. Diclofenac sodium (20 mg/kg) reduced the ear weight to  $0.017 \text{ g} \pm 0.004$  ( $P < 0.001$  vs. saline group), resulting in 67.92% inhibition (Table 1).

**Effect on Formalin-induced inflammation:** Ce.Cr (100-1000 mg/kg) reduced the inflammation induced by formalin. The animals of control saline group at 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10<sup>th</sup> day were having paw volume displacement values of  $0.514 \pm 0.007$ ,  $0.522 \pm 0.006$ ,  $0.572 \pm 0.008$ ,  $0.566 \pm 0.009$ ,  $0.558 \pm 0.005$ ,  $0.556 \pm$

$0.006$ ,  $0.526 \pm 0.007$ ,  $0.510 \pm 0.008$ ,  $0.508 \pm 0.008$  and  $0.496 \pm 0.007$  mL respectively. The paw volume displacement values of Ce.Cr (100 mg/kg) treated group at 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10<sup>th</sup> day were  $0.512 \pm 0.004$ ,  $0.510 \pm 0.005$ ,  $0.506 \pm 0.005$ ,  $0.490 \pm 0.005$ ,  $0.484 \pm 0.005$ ,  $0.478 \pm 0.004$ ,  $0.470 \pm 0.003$ ,  $0.462 \pm 0.005$ ,  $0.458 \pm 0.004$  and  $0.448 \pm 0.006$  mL ( $P < 0.001$  vs. saline group) respectively. The paw volume displacement values of Ce.Cr (300 mg/kg) treated animals at 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10<sup>th</sup> day were  $0.492 \pm 0.004$ ,  $0.476 \pm 0.005$ ,  $0.466 \pm 0.002$ ,  $0.462 \pm 0.005$ ,  $0.456 \pm 0.004$ ,  $0.438 \pm 0.006$ ,  $0.430 \pm 0.004$ ,  $0.422 \pm 0.006$ ,  $0.418 \pm 0.004$  and  $0.408 \pm 0.004$  mL ( $P < 0.001$  vs. saline group) respectively. The paw volume displacement values of Ce.Cr (1000 mg/kg) treated group at 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10<sup>th</sup> day were  $0.488 \pm 0.005$  ( $P < 0.05$  vs. saline group),  $0.468 \pm 0.006$ ,  $0.462 \pm 0.004$ ,  $0.456 \pm 0.004$ ,  $0.430 \pm 0.008$ ,  $0.408 \pm 0.007$ ,  $0.404 \pm 0.008$ ,  $0.398 \pm 0.006$ ,  $0.390 \pm 0.004$  and  $0.394 \pm 0.005$  mL ( $P < 0.001$  vs. saline group) respectively. The paw volume displacement values of diclofenac sodium (20 mg/kg) treated group at 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10<sup>th</sup> day were  $0.478 \pm 0.006$ ,  $0.462 \pm 0.005$ ,  $0.454 \pm 0.002$ ,  $0.442 \pm 0.005$ ,  $0.410 \pm 0.008$ ,  $0.404 \pm 0.008$ ,  $0.396 \pm 0.007$ ,  $0.394 \pm 0.007$ ,  $0.388 \pm 0.006$  and  $0.386 \pm 0.002$  mL ( $P < 0.001$  vs. saline group) respectively (Fig. 2).

**Acute toxicity test:** Mice were administered Ce.Cr in 3 and 5 g/kg dose and mortality was observed up to 24 hour. No mortality observed up to 5 g/kg dose.

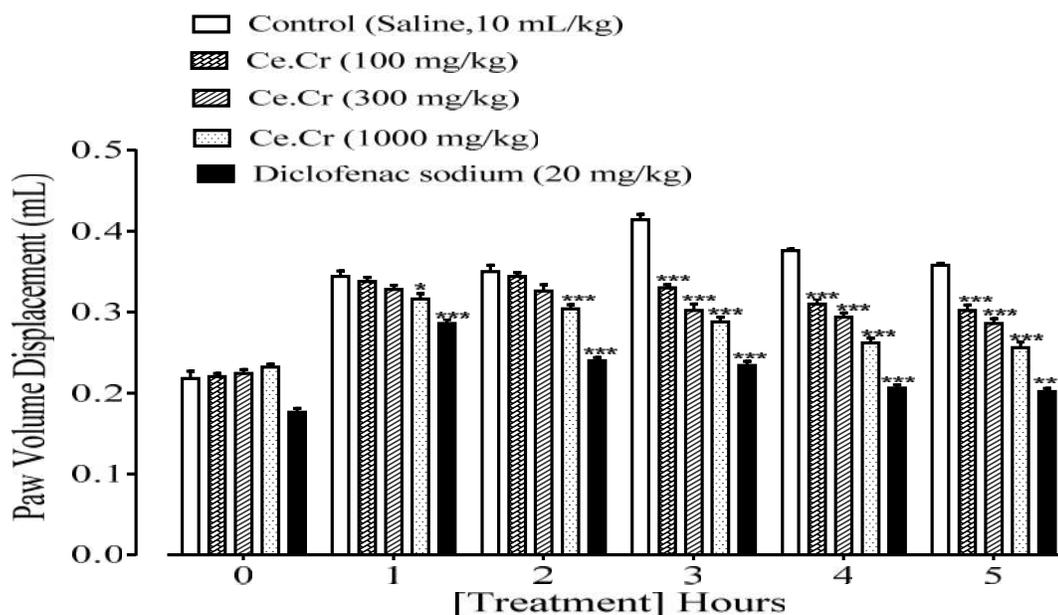
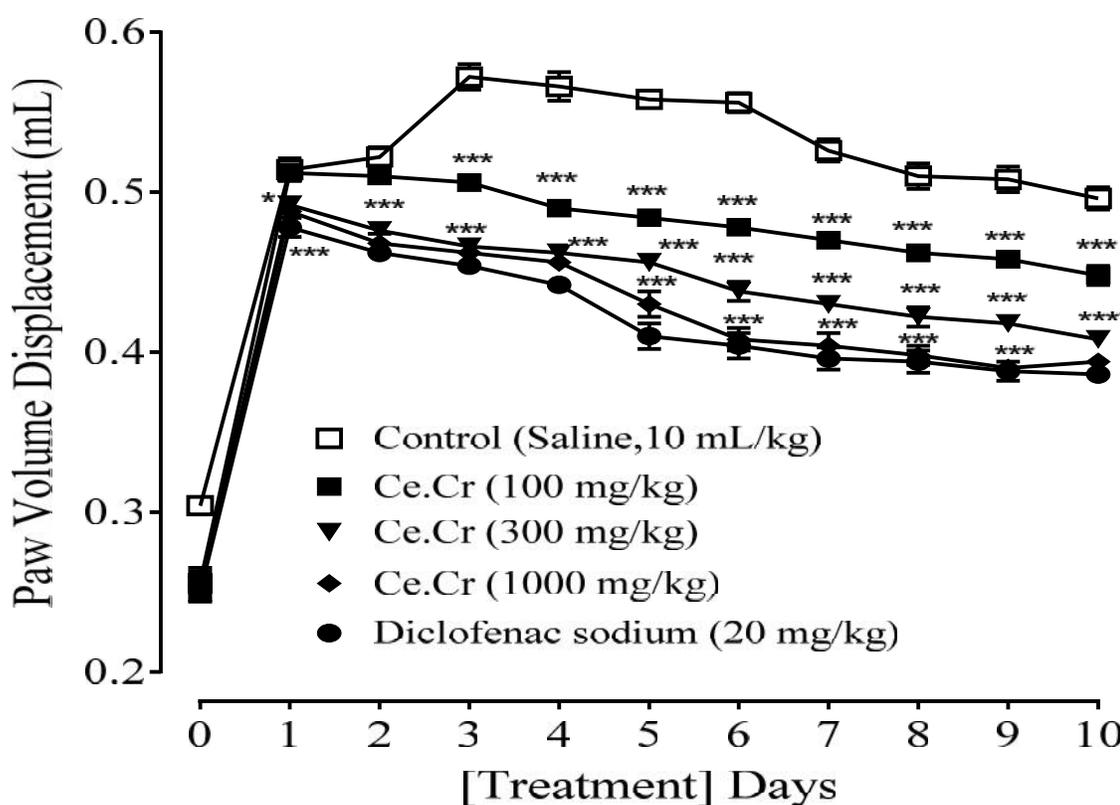


Fig. 1. Effect of *Caralluma edulis* crude extract (Ce.Cr) and diclofenac sodium on carrageenan-induced paw edema in mice. Values shown are mean  $\pm$  SEM,  $n=5$ . \* $P < 0.05$ , \*\*\* $P < 0.001$  vs. saline group, one-way analysis of variance with post-hoc Tukey test.

**Table 1.** Effect of *Caralluma edulis* crude extract (Ce.Cr) and diclofenac sodium on xylene-induced ear edema in mice.

Treatment	Weight of right ear (g)	Weight of left ear (g)	Difference (g)	Inhibition (%)
Control (Saline, 10 mL/kg)	0.093 ± 0.001	0.040 ± 0.002	0.053 ± 0.002	-
Ce.Cr (30 mg/kg)	0.090 ± 0.003	0.052 ± 0.003	0.038 ± 0.003*	28.30
Ce.Cr (100 mg/kg)	0.100 ± 0.005	0.070 ± 0.006	0.030 ± 0.002***	43.39
Ce.Cr (300 mg/kg)	0.082 ± 0.008	0.066 ± 0.007	0.016 ± 0.003***	69.81
Ce.Cr (1000 mg/kg)	0.077 ± 0.006	0.064 ± 0.006	0.013 ± 0.003***	75.47
Diclofenac sodium (20 mg/kg)	0.080 ± 0.006	0.063 ± 0.004	0.017 ± 0.004***	67.92

Values represented as mean ± SEM (n=5). \*P < 0.05, \*\*\*P < 0.001 vs. saline group, One-way analysis of variance with post-hoc Tukey test.



**Fig. 2.** Effect of *Caralluma edulis* crude extract (Ce.Cr) and diclofenac sodium on formalin-induced arthritis in hind paw of mice. Values shown are mean ± standard error of mean, n=5. \*P < 0.05, \*\*\*P < 0.001 vs. saline group, one-way analysis of variance with post-hoc Tukey test.

## DISCUSSION

In view of medicinal use of *Caralluma edulis* in inflammatory ailments such as rheumatism, its extract was evaluated for possible anti-inflammatory action against acute (carrageenan and xylene mediated) and chronic (formalin evoked) models of inflammation, to rationalize aforementioned ethnomedicinal use of the plant. Carrageenan-induced edema is a well-established

in-vivo animal model to ascertain the anti-inflammatory effect (Mossa *et al.*, 1995; Bukhari *et al.*, 2007). It's non-antigenic and did not produce any systemic effect (Bukhari *et al.*, 2016). Carrageenan induces inflammation through release of histamine, serotonin (early phase), prostaglandins and bradykinin (later phase) in different phases (Burch and DeHaas, 1990). It is reported that agents which produce reduction in carrageenan-induced edema, caused the inhibition of prostaglandins synthesis

via inhibiting the cyclo-oxygenase (COX) enzyme (Skoutakis *et al.*, 1988; Selvam and Jachak, 2004). Ce.Cr in dose-dependent fashion caused inhibition of carrageenan-induced paw edema, like that exhibited by Diclofenac sodium-standard nonsteroidal anti-inflammatory drug (NSAID). The NSAID's decrease swelling, inflammation, and pain by inhibiting prostaglandins synthesis through COX enzyme inhibition in the arachadonic acid pathway (Grosser *et al.*, 2011). The plant crude extract was more effective in inhibiting later phase (from 2-5 hours) of carrageenan-induced inflammation. Based on these findings, it can be ascribed that the anti-inflammatory effect of *Caralluma edulis* might be possibly occurred via inhibition of prostaglandin synthesis inhibitory mechanism.

When tested against xylene-induced inflammation, Ce.Cr reduced ear edema induced by xylene, even at lower tested dose of 30 mg/kg and at 1000 mg/kg it was found more effective than diclofenac sodium. Xylene induces inflammation through release of various inflammatory mediators, such as kinins, histamines and fibrinolysin, which further causes vasodilation and increase vascular permeability (Li *et al.*, 2011). The effectiveness of the plant extract against xylene-induced inflammation is attributed possibly to phospholipase A2 inhibition, involved in inflammation pathophysiology due to xylene (Sofidiya *et al.*, 2014).

*Caralluma edulis* was further investigated against formalin-induced chronic inflammation. It is a simple model to screen anti-inflammatory and anti-arthritic effect, because of its resemblance to human arthritis (Igbe *et al.*, 2010). Two sub-aponeurotic injections of formalin were applied to induce inflammation, characterized by increased paw thickness and volume as chronic inflammation responses (Cho *et al.*, 2011). These markers were used for testing anti-inflammatory activity. Ce.Cr, like diclofenac sodium was found significantly effective in reducing the formalin-induced cell damage.

The observed anti-inflammatory activity of *Caralluma edulis* may be due to the presence of phenols and flavonoids, as phyto-chemicals of such classes were known for the anti-inflammatory potential (Orhan *et al.*, 2007), though the importance of other constituent present in this plant cannot be ruled out.

In toxicity analysis, the Ce.Cr was safe up to 5 g/kg, which is in line with therapeutic use of *Caralluma edulis*.

**Conclusions:** The results from study reveals that *Caralluma edulis* possess the anti-inflammatory activity against carrageenan-induced hind paw edema, reduce xylene-mediated ear edema and effective in formalin-evoked chronic inflammation, and thus validate its medicinal use in rheumatism. Further advanced molecular studies are required to explain the active principles responsible for anti-inflammatory effect and to

explore the pharmacodynamics basis of the pharmacological action.

**Acknowledgements:** The authors are thankful to Riphah Academy of Research and Education (RARE), Islamic International Medical College Trust, Riphah International University for financial support of the study.

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