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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF HERBAL METHANOLIC FLORAL EXTRACT OF *CROSSANDRA INFUNDIBULIFORMIS* (LINN.) NEES

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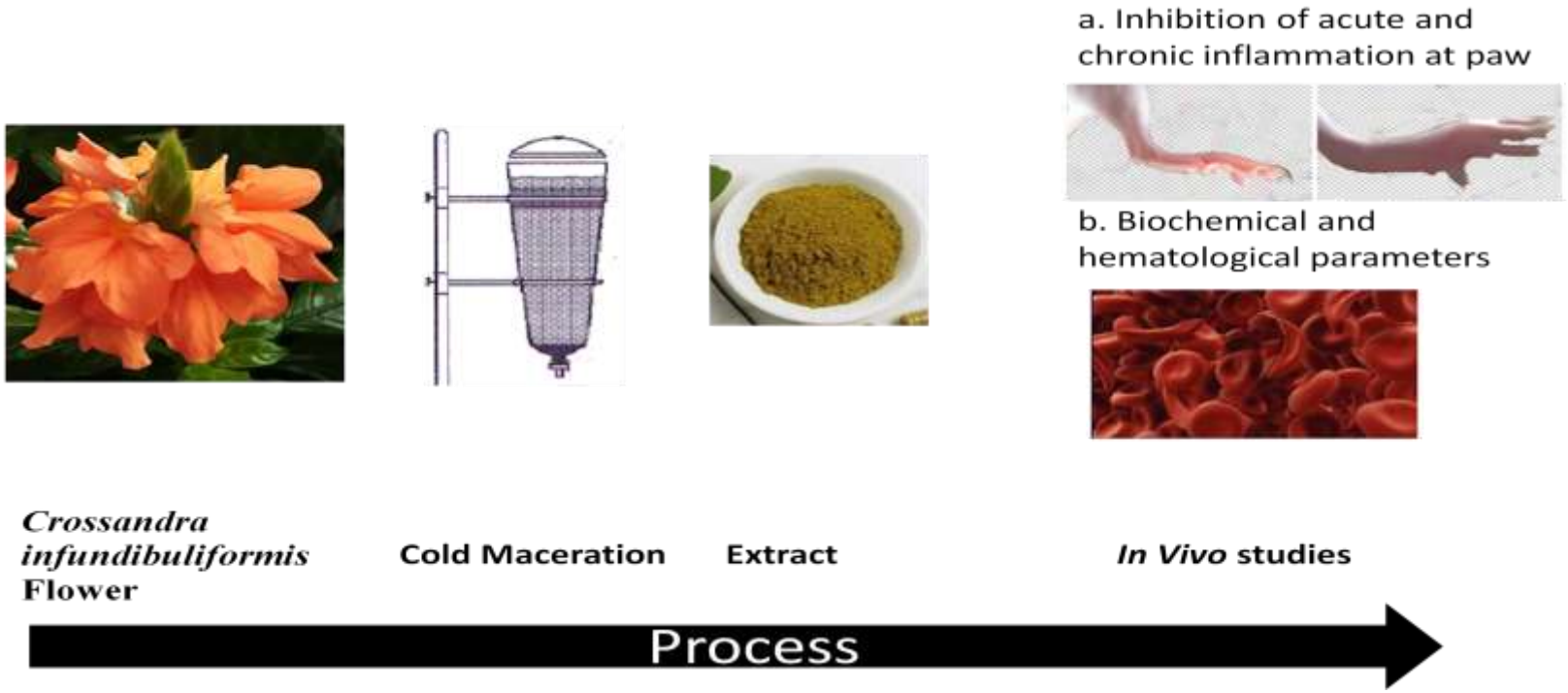
ABSTRACT

Crossandra infundibuliformis, a herbal source of drug utilized in many diseases, infections, injuries and other physiological situations. Natural source has been recognized as an origin for many classes of drugs, which further used as basic moiety for auxiliary development and has the advantage of being devoid of side effects in comparison to synthetic one. Methanolic extract of *Crossandra infundibuliformis* flower was evaluated for the acute and chronic inflammation study and its effects on levels of biochemicals and various hematological parameters were determined. Carrageenan induced paw edema and formaldehyde induced paw edema models were used for acute and chronic inflammation studies, respectively. Nitric oxide (NO) level, C-reactive protein (CRP) level, RBC, WBC & platelet count, hemoglobin (Hb) level, erythrocyte sedimentation rate (ESR) were also estimated. Extract showed significant anti-inflammatory activity, when observed at and after 2 hours of administration at all the given concentrations (75, 150 and 300 mg kg^{-1}). The results were further strengthened by outcomes of NO level, ESR value, CRP level, WBC, RBC, and platelets count. Extract at 300 mg kg^{-1} showed effect comparable to that of standard i.e. diclofenac sodium (10 mg kg^{-1}) in both acute and chronic study. Although the dose of the extract is much higher than the standard but it can be reduced by injecting the isolated, chemical entity responsible for this activity. Thus, methanolic extract of flower of *C. infundibuliformis* could further be studied for isolation and evaluation of novel chemical constituent.

Keywords: Anti-inflammatory, Extract, Flower, Hematological Parameters, Nitric Oxide, Paw Edema

Graphical Abstract



INTRODUCTION

There are various allopathic treatments available in inflammation, but they have one or the other limitation due to their side effects. Therapeutic agents like non steroidal anti-inflammatory drugs (NSAIDS) cause gastrointestinal **(Musumba *et al.*, 2012)** and cardiac toxic effect **(McKenna, 2011)** and steroidal anti-inflammatory drugs provoke the risk of infection **(Ruyssen-Witrand *et al.*, 2010),** diabetes **(Kim *et al.*, 2011)** and osteoporosis **(Lekamwasam *et al.*, 2012)** based on which discovery of natural anti-inflammatory drugs with least side effect is essential and this demand is mounting along with time. The recognized Indian systems of medicine are Ayurveda, Siddha and Unani, which comprises of herbs and minerals in the formulation **(Khan *et al.*, 2012)**. Not only medicinal plants but many plants of nutritional value have also been observed to be effective in diverse ailments e.g. *Tamarindus indica* **(Dhamija and Parle, 2013)**, *Sesamum indicum* **(Parle and Dhamija, 2013)** in depression, flowers of ornament e.g. saffron, violet **(Rop *et al.*, 2012)**, rose **(Choi and Hwang, 2003)** in antibacterial, antifungal activity etc.

One of the potential herbal source for curing many diseases, infections, injuries and other physiological situations, is *Crossandra infundibuliformis* (Firecracker flower), which is a species of flowering plant belonging to family Acanthaceae, native to Southern India. *C. infundibuliformis* was found to have flavonoids, steroids, tannins, volatile oils, alkaloids, glycosides, saponins, carbohydrates, proteins and amino acids which helps in reducing inflammation **(Madhumitha and Saral, 2011)**. Many extracts of plants showed their anti-inflammatory effect due to these contents e.g alkaloids **(Sharifi-Rad *et al.*, 2014)**, phenolic compounds **(Miles *et al.*, 2005)**, flavenoids **(Kang *et al.*, 2011)**, terpenoids **(Ku and Lin, 2013)**. In many circumstances flowers has been prescribed presently and traditionally, than other parts of the plant for treatment of particular disease or condition e.g. *Punica granetum* flower for diabetes **(Rop *et al.*, 2012)**.

On the basis of vast literature survey *C. infundibuliformis* flower methanolic extract was explored for acute and chronic anti-inflammatory property. To the best of our knowledge, there is no study reported in this direction.

MATERIALS AND METHODS

Materials

All the chemicals used were of analytical grade or higher grade and used as such

without any purification. Plant was procured from Vishakhapatnam (India) and was authenticated from National Institute of Science Communication and Information Resources, New Delhi, India (voucher no. NISCAIR/ RHMD/ Consult/ 2013/ 2299/ 79) and sample was stored in refrigeration for future use. Digital plethysmometer (Medicaid, Digital volume meter, India) was used to determine the volume of edema.

Experimental Animals

Wistar rats (150-200 g), of either sex were taken for study. They were procured from the Animal house of the PDM College of Pharmacy, after the approval of Institutional Animal Ethics Committee (IAEC) (protocol No. PDM/CPCSEA/RES/2013/05). Animals were housed according to standard protocols for animal rearing and handling.

Carrageenan-Induced Paw Edema Model: Acute Study

Anti-inflammatory activity was evaluated on the basis of inhibition of carrageenan induced hind paw edema as given in literature, (Kumar et al., 2013) with little modifications. In brief, rats were divided into 6 groups, with 6 animals in each group. A mark was made on the hind paw just below the tibio-torsal junction. The carrageenan solution (0.1 mL of 1% w/v) was injected into the sub plantar region of the left hind paw of rat for inflammation. The intraperitoneal and oral administration of the standard and test substance respectively, was done 30 minutes before the generation of inflammation. The relative increase in paw volume below the mark given at tibio-tarsal junction was measured in all groups at 1, 2, 3, 4 and 5 h after carrageenan injection and % paw edema was calculated (**Appendix 1**).

Formaldehyde-Induced Paw Edema: Chronic Study

Rats were injected with 0.1 mL of 2 % (v/v) of formaldehyde solution in the plantar region of the left paw on the first and third day of the test (Vasudevan et al., 2007). Drug treatment was given for 21 consecutive days, initializing from 0 day, i.e. day of formaldehyde injection. Each treatment was given per-orally once a day to individual group. Inflammation was measured (**Appendix 1**) on 1^{st}, 7^{th}, 14^{th} and 21^{st} day; 30 min after administration of the respective vehicle/drug treatment.

Biochemical and Hematological Parameters in Chronic Inflammation

After the completion of protocol of chronic inflammation study, the blood was collected from retro orbital plexus in the micro-centrifuge tube and centrifuged at 1000 x g for 15 min. Different reactions and procedures were carried out, as per given in the literature, to estimate nitric oxide (NO) level **(Yu *et al.*,**

2011), C-reactive protein level **(Tugirimana *et al*., 2011)**, RBC, WBC & platelet count **(Mariappan *et al*., 2011)**, hemoglobin (Hb) level, erythrocyte sedimentation rate **(Talwar *et al*., 2011)**.

RESULTS AND DISCUSSION

The extraction of the plant material was performed based on the literature (Bero et al., 2011) with little modifications (**Appendix 2**). The extract obtained showed percentage yield to be 6 %. Results showing evaluation of anti-inflammatory activity of *Crossandra infundibuliformis* methanolic flower extract in acute and chronic inflammation is shown in **Figure 1**. The extract was showing significant anti-inflammatory activity ($p<0.05$) in comparison to "control" i.e. average of group with carrageenan induced edema and no treatment/ injected vehicle in both acute and chronic inflammation with all the three concentrations/ doses i.e. 75, 150 and 300 mg kg^{-1} (assigned as CI 75, CI 150 and CI 300, respectively) on and after 2 hours. Parallel study with diclofenac sodium at 10 mg kg^{-1} dose ("standard/positive control") was also carried out for activity comparison. CI 300 was showing result equivalent to that of "standard". Inhibitory effect of the extract in carrageenan induced edema suggests its involvement in prostaglandin mediated pathway (Talwar et al., 2011). Formaldehyde inflammatory pathway inhibition represents the neutralization of active globulins i.e. action of NSAIDs (Vasudevan et al., 2007).

Change in plasma nitric oxide level and other blood parameters by *C. infundibuliformis* were recorded on 21^{st} day of chronic inflammation (**Table 1**). NO production is increased in inflammation and has proinflammatory and regulatory effects (Hamalainen et al., 2007). Some flavanoids reported to bring the NO level to normal (Kim et al., 2001) but precise mechanism of action is not known. Reduction in Hb count during inflammation may be attributed to the premature destruction or decreased response of bone marrow to erythropoietin. ESR is increased, which reveals the increased concentration of acute phase reactant proteins in plasma during inflammation (Talwar et al., 2011). NO level, ESR value, CRP level, WBCs count, platelets count, RBCs count in *C. infundibuliformis* treated groups (CI 75, CI 150 and CI 300) were significantly ($p<0.05$) decreased (and increased in case of Hb level) in comparison to the "control" group.

CONCLUSION

All the concentrations i.e. 75, 150 and 300 mg kg^{-1} were showing significant activity, even equivalent effect (CI 300) to that of positive control or standard i.e. diclofenac sodium (10 mg kg^{-1}). Biochemical and hematological

parameters were showing values closer to the normal after treatment with extract. In nutshell, the flower extract should be investigated further in expectation of obtaining novel, natural and effective chemical entity.

ACKNOWLEDGMENTS

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DECLARATION OF INTEREST

The authors report no declarations of interest.

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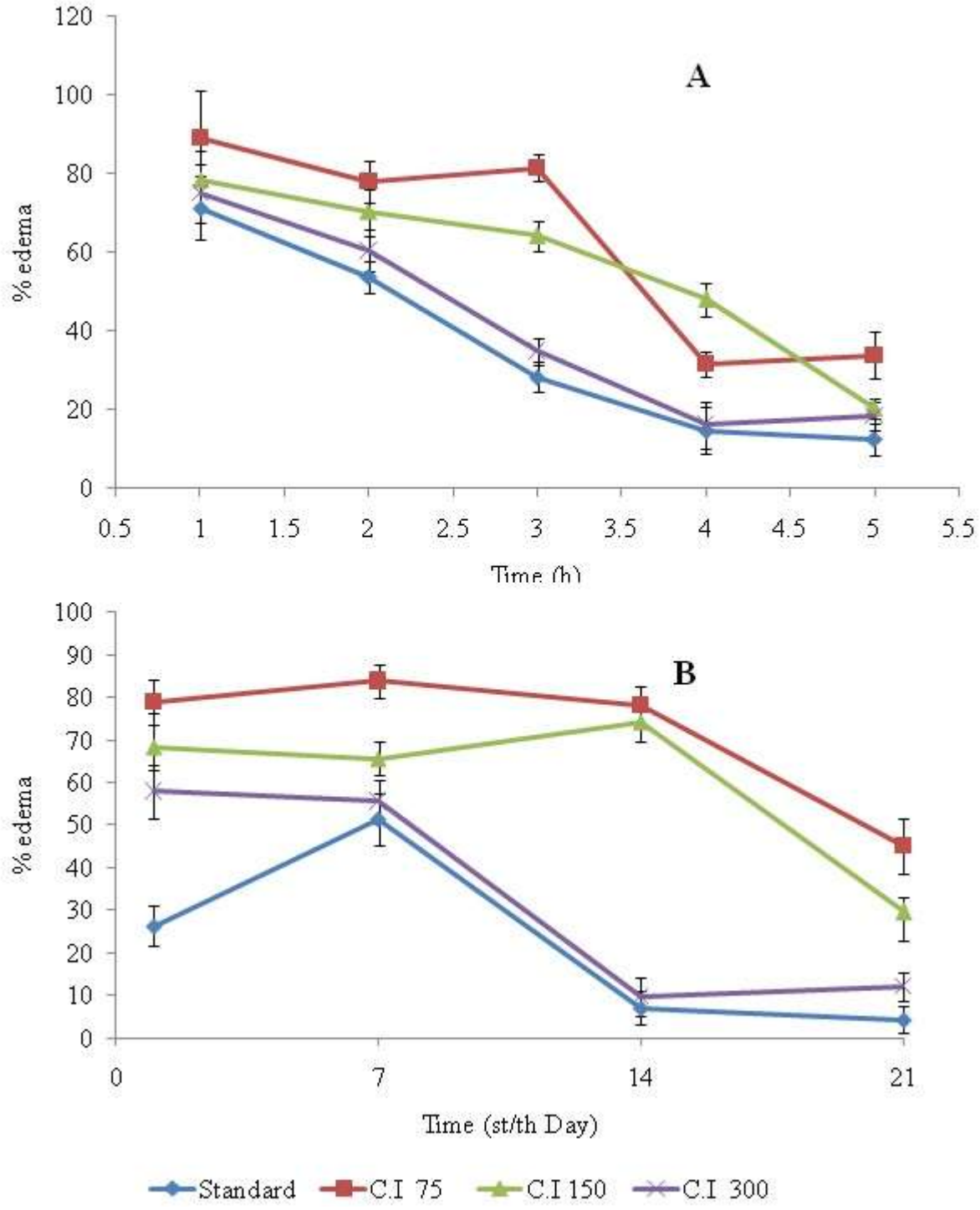

Figure 1: Effect of *C. infundibuliformis* on A. acute paw edema B. chronic paw edema Values are expressed as mean±SEM (n=6). "Standard" means treatment with diclofenac (10 mg/kg) 30 minutes prior to carrageenan induced inflammation, "CI 75" i.e. treatment with C.I extract (75 mg kg^{-1}) 30 minutes prior to carrageenan induced inflammation, "CI 150" i.e. treatment with C.I (150 mg kg^{-1}) 30 minutes prior to carrageenan induced inflammation and "CI 300" i.e. treatment with C.I (300 mg kg^{-1}) 30 minutes prior to carrageenan induced inflammation. % edema was calculated with taking edema of "control" i.e. inflammation but no treatment (equal volume of vehicle was given) as 100%. Data is analyzed by repeated measures two way ANOVA

Table 1: Effect of *C. infundibuliformis* on plasma NO level and various hematological parameters

S. No.	Group name	NO level (μmol L^{-1})	ESR (mm h^{-1})	CRP level (mg L^{-1})	WBC count (10^3 cells μL^{-1})	Platelets count (10^5 cells μL^{-1})	RBC count (10^6 cells μL^{-1})	Hb level (g %)
1.	Normal	20.83±1.579	3.82±0.14	4.20±0.12	3.12±0.15	6.83±0.14	5.32±0.19	13.6±0.26
2.	Control	51.00±0.966	11.45±0.13	7.40±0.23	6.2±0.12	9.63±0.13	3.92±0.06	7.7±0.43
3.	Standard	25.16±1.47	5.37±0.17	5.41±0.13	3.82±0.16	7.06±0.20	4.77±0.12	11.6±0.29
4.	CI 75	43.50±2.291	11.06±0.23	6.60±0.14	5.28±0.20	8.70±0.15	4.08±0.16	8.12±0.26
5.	CI 150	36.50±1.857	8.9±0.18	6.22±0.12	5.16±0.22	8.10±0.22	4.41±0.05	9.75±0.53
6.	CI 300	30.00±1.653	6.76±0.32	6.11±0.15	4.28±0.19	7.34±0.14	4.59±0.09	11.28±0.48

"Normal" means "control" means carrageenan induced inflammation but no treatment (equal volume of vehicle was given), "standard" means treatment with diclofenac (10 mg/kg) 30 minutes prior to carrageenan induced inflammation, "CI 75" i.e. treatment with C.I extract (75 mg/kg) 30 minutes prior to carrageenan induced inflammation, "CI 150" i.e. treatment with C.I (150 mg/kg) 30 minutes prior to carrageenan induced inflammation and "CI 300" i.e. treatment with C.I (300 mg/kg) 30 minutes prior to carrageenan induced inflammation. Each value is mean of 6 samples taken on 21st day

Supplementary material

Appendix 1: Inflammation measurement

Paw edema volume of all rats was measured by using digital plethysmometer before and after the treatment, while paw edema thickness or paw diameter was measured using vernier caliper.

The increase in paw volume or thickness was calculated according to the following formula:

$$\text{Paw volume/thickness change (V)} = \text{Final paw volume/thickness} - \text{Initial paw volume/thickness} \quad (1)$$

Initial and final paw volumes are the volumes obtained before and after injecting carrageenan.

Then, edema was expressed as the mean increase in paw volume relative to untreated animals. The percentage of edema was calculated by the equation

$$\% \text{ edema} = 100 \times (V_t/V_c) \quad (2)$$

where, V_c is the edema volume or thickness in the control group and V_t is the edema volume or thickness in tested group.

Appendix 2: Preparation of Extract

The extraction of the plant material was performed based on the literature **(Bero *et al.*, 2011)** with little modifications. The flowers of *C. infundibuliformis* were collected and healthy flowers were shade dried and then powdered using electric blender to get a coarse powder. The powdered material was extracted with methanol by cold maceration process. The extracts were prepared by

taking 30 g of dried flower powder in separate containers and to this 200 mL of solvent was added and kept for 24 h. The extracts were collected by filtration through 5 layers of muslin cloth. Then the collected filtrates were pooled, concentrated and dried at mild temperature.

Bero, J., Hannaert, V., Chataigne, G., Herent, M.-F., Quetin-Leclercq, J. *In vitro* antitrypanosomal and antileishmanial activity of plants used in Benin in traditional medicine and bio-guided fractionation of the most active extract. J. Ethnopharmacol. 2011; 137: 998-1002.