ISSN: 0976-4607
Research Article

International Journal of Innovative Pharmaceutical Research

Journal homepage: www.ijipr.com

The Analgesic and Anti-inflammatory Activities of the Leaf Extract of *Crossandra Infundibuliformis*

Jayaveera KN, Mallikarjuna M*, Venkateshwar Reddy A, Sailaja S and Yogananda Reddy K

*Department of Chemistry, JNTU Anantapur, Anantapur, Andhra Pradesh, India.

ABSTRACT

Inflammatory diseases are becoming common in aging society throughout the world. Recent studies indicate that the mediators and cellular effectors of inflammation are important constituents of the local environment of tumors (Mantovani A *et al*., 2008). Most of the available non-steroidal anti-inflammatory drugs are effective in inflammatory conditions, but are devoid of gastro protective property. The aim of the present study was to examine anti-inflammatory and analgesic activity of *Crossandra infundibuliformis*.

Key words: Non-steroidal anti-inflammatory drugs, Gastro protective property, *Crossandra infundibuliformis*, Anti-inflammatory, Analgesic activity.

INTRODUCTION

Inflammation, clinically, causes, as shown by 'Cornelius Celsus' of Rome 2000 years ago, *rubor (redness), calor (heat), dolor (pain)* of the affected region (Sujit K Chaudhary *et al*., 2001) and is a complex biological response of vascular tissues to harmful stimuli including pathogens, irritants or damaged cells (Denko C W *et al*., 1992). It is defensive mechanism of the body to remove the injurious stimuli as well as initiate the healing process for the tissue. Inflammation, however, if runs unchecked, leads to onset of diseases such as vasomotor rhinorrhoea, rheumatoid arthritis, and arthroscleroses (Henson P *et al*., 1989). It is believed that current drugs available such as Opioids and NSAIDs drugs are not useful in all cases of inflammatory disorders, because of their side effects, economy and potency (Ahmadiani A *et al*., 1998; Juvekar A R *et al*., 2009) as a result, a search for other alternatives is necessary. The use of plants to treat ailments is as old as antiquity. Records of humans using plants to treat diseases have been recorded as far back as 6000 to 4000 years when Ayurvedic physicians started treating tumors with extracts from Vinca- roseus (Ogunyemi AO *et al*., 1979; Usifoh C *et al*., 2009).

Taking into the account the most important analgesic prototypes (e.g. Salicylic acid and Morphine) were originally derived from the plant sources.

***Corrosponding author**

Mallikarjuna M
Email id: Jas_malli2000@yahoo.com

According to various medical literatures, several adverse reactions are known to be associated with the conventional non-steroidal anti-inflammatory drugs, thereby limiting the widespread application of these agents. The study of plant species traditionally used as pain killers should still be seen as fruitful research strategy in the search of new analgesic and anti-inflammatory drugs.

The plant *Crossandra infundibuliformis* commonly known as Kanakambaralu/ Firecracker flower belongs to family *Acanthaceae*. The plants are native to Southern India, Malaysia, Sri Lanka, Tropical Africa, and Madagascar where it grows as evergreen shrub. Crossandra infundibuliformis, an ornamental plant, is marketed as such. The species is often grown to beautify kitchen gardens as it is small in size and sports attractive flowers (Pandey BP *et al*., 1982; Kirtikar Basu, 2001). Thus, we are excited to learn from literature and also from folk (Labbie AR *et al*., 1995; Nareshkumar A *et al*., 2011; El-Hag AH *et al*., 2011; Patric RD *et* al., 2011; Mandal MD *et al*., 2011; Karthikai devi G, 2011; Prema B *et al*., 2011), the medicinal uses of Crossandra infundibuliformis. Flowers are grinded with pepper and the paste is applied for wounds (Sakthivel B *et al*., 2009) and the Capsules are aphrodisiac (Pullaiah T *et al*.,). The crushed plant is applied when cattle are bitten by a dog (Abhijit Dey JN *et al*., 2010) and leaves are used for cough and, cold (Madhava Chetty K *et al*., 2008). Leaf juice is applied on the surface of the thorn to expel it

from the foot by Malekudiya community (Kshirsagar RD *et al*., 2001).

In our continuing efforts at identifying medicinal plants with anti-inflammatory activity and establishing scientific evidence for activity, the acclaimed potency of the leaves of *Crossandra infundibuliformis* in inflammatory conditions stimulated our interest to screen the extract for effect on inflammation and analgesic activity. The present study was initiated with preliminary work relative to its phytochemical studies which are presented in this paper. Hence forth, anti-inflammatory, and analgesic activities of successive extracts of *Crossandra infundibuliformis* have been reported here.

MATERIALS AND METHODS

Plant material

Whole plant of *Crossandra infundibuliformis* was collected from Andhra Pradesh, India, and authentified by Asst. Prof. Dr. K. Madhava Chetty, Dept. of Botany S.V.University, Tirupathi, A. P.

Extraction

The Leaves were shade dried and powdered. The powder was passed through 40 mesh sieve, extracted with hexane (A), Dichloro methane (B), Acetone (C), Ethanol (D) by soxhlet and water (E). All the extracts of *Crossandra infundibuliformis* were evaporated and dried under reduced pressure.

Preliminary Phytochemical studies

Phytochemical screening of the prepared extracts was conducted with various qualitative tests to identify the presence of chemical constituents. To perform the tests the following chemicals and reagents were used: Carbohydrates with Molisch's test, Saponins with Foam test, Phytosterols with Liebermann-Burchard's test, Flavonoids with NaOH/HCl, Tannins and Phenolic, compounds with ferric chloride solution, Proteins and amino acids with Millon's test, Ninhydrin test, Biuret test, Gums with Molish reagents and concentrated sulfuric acid. Alkaloids were tested with Mayer's reagent, Hager's reagent and Dagendorff's reagent. These were identified by characteristic color changes using standard procedures (Purohit AP *et al*., 2004; Harborne JB *et al*., 1998; Suresh B *et al*., 2008; Amish AD *et al*.,2010).

From this study, Ethanolic extract of *Crossandra infundibuliformis* (EECI) was found to be containing most of the chemical constituents, hence it was chosen for further investigation.

Animals

Albino wistar rats of either sex weighing (200-250gms) were employed for study. They were housed in standard environmental conditions and fed with standard rodent diet with water *ad libitum*.

Acute toxicity study

An acute toxicity study was performed for *Crossandra infundibuliformis* to determine LD_{50} using different doses of the ethanolic extract (EECI) according to the standard method (Goush MN *et al*., 1984). It has been found that this extract is safe to use in animals even at a dose 3.2 gm/kg orally. Body weight before and after administration of the extract as per schedule was noted and any changes in skin, fur, eyes, mucous membranes, behavior pattern etc. were observed. No sign of tremors, convulsions, salivation, diarrhea and coma were seen.

Anti-inflammatory activity of EECI

The present anti-inflammatory activity was determined in albino rats of either sex according to the standard method (Winter CA *et al*., 1968). One hour after oral administration of the extract, edema was induced to all the groups by injecting 0.1 ml of 1% carrageenan in 0.9% w/v in saline (Turner. RA *et al*., 1965; Kulkarni SK *et al*., 2006) in the sub plantar region of left hind paw of rats. Animals are divided into four groups of six rats in each group.

All the doses were administered per orally according to the body weight of the animals. Paw edema volume was measured with plethysmometer just before 0 (before drug challenge) 30^{th}, 60^{th} and 90^{th} min after administration of drug. The percent inhibition of inflammation was calculated by using the formula.

Percentage of inhibition inflammation= (A-B/A) X100

Where, A and B denote mean increase in paw volume of control and drug treated animals respectively.

Analgesic activity of EECI

Acetic acid induced abdominal writhing test

The acetic acid induced abdominal writhing test was performed according to the standard method (Koester K *et al*., 1959) fasted normal mice divided into four groups of six mice in each group.

Animals were treated with scheduled doses, 60 min before acetic acid administration. The total number of writhings after intra peritoneal administration of 0.6% w/v acetic acid (0.1 ml for 10 gm) was recorded for 30 min, starting 5 min after the injection. Writhing response means contraction of the abdominal muscle together with stretching the hind limbs. The results of analgesic activity were expressed as the percentage reduction or inhibition of the abdominal writhings.

$$\text{Percentage inhibition} = \frac{(\text{Control average} - \text{test average})}{\text{Control average}} \times 100$$

Statistical analysis

Results were expressed as mean ± SEM, (n=6). Statistical analysis were performed with one way analysis of variance (ANOVA) followed by Student's t- test by using Graph Pad Software.

RESULTS

Phytochemical screening

Preliminary Phytochemical studies of ethanolic extract of *Crossandra infundibuliformis* (EECI) confirmed the presence of Alkaloids, Saponins, Flavonoids, Tannins, Carbohydrates, Gums, amino acids and Proteins (Table.1).

Acute toxicity study of EECI

Acute toxicity of ethanolic extract *Crossandra infundibuliformis* was shown in Table.2.

Anti-inflammatory activity of EECI

The percentage inhibition of edema of standard drug Diclofenac at dose 10 mg/kg at 30, 60, 90 min was found to be 50%, 61.54%, 87.60% respectively. The

percentage inhibition of edema at 100 mg/kg of EECI at 30, 60, 90 min was 32.50%, 41.02%, 52.63% and with 200 mg/kg of EECI at 30, 60, 90 min was 37.50%, 48.71%, 60.52% respectively. From these results it was found that the extracts have anti-inflammatory activity in dose dependent manner, but EECI at 200 mg/kg showed moderate anti-inflammatory activity compared to that of standard drug Diclofenac. The results were shown in Table.3.

Analgesic activity of EECI

Intraperitoneally injected acetic acid Produced abdominal contractions which were characterized by a stretching response. The percentage of inhibition of writhing of EECI at dose 100 and 200 mg/kg were found to be 45.44% and 57.80% respectively. The analgesic effect of EECI was comparable with that of standard drug Diclofenac at dose 200 mg/kg and the percentage of inhibition of writhing of Diclofenac was found to be 76.58%. The results were shown in Table.4.

Table.1 Preliminary Phytochemical screening of extracts

Type of Phytochemical constituents	A	B	C	D	E
Alkaloids	-	-	-	+	-
Carbohydrates	-	+	+	+	+
Flavonoids	+	+	+	+	-
Proteins & amino acids	-	-	-	+	-
Saponins	-	-	+	+	+
Tannins & Phenolic compounds	+	-	+	+	+
Gums	-	-	+	+	+
Phytosterols	+	-	+	+	+

+: Indicate presence of Phytochemical Constituents

-: Indicate absence of Phytochemical Constituents

A: Hexane, B: Dichloro methane, C: Acetone, D: Ethanol E: water

Table.2 Toxicity study

Treatment	Dose mg/kg	No. of animals	No. of Survival	No. of Death	Percentage of mortality	LD_{50}
Control	2%Tween80	6	6	0	0	
EECI	100	6	6	0	0	
	200	6	6	0	0	
	400	6	6	0	0	
	800	6	6	0	0	
	1600	6	6	0	0	
	3200	6	6	0	0	>3.2 g/kg body Wt.

Table.3 Anti-inflammatory effect of EECI carrageenan induced rat paw edema

Group	Treatment	Edema volume (ml)			
		0 h	30min	60min	90min
1	Control 2 ml of 2% Tween 80	0.42±0.13	0.40±0.1	0.39±0.12	0.38±0.14
2	Diclofenac 10 mg/kg	0.4±0.07	0.20±0.15* [50.00]	0.15±0.04 [61.54]	0.047±0.04* [87.60]
3	EECI 100 mg/kg	0.32±0.03	0.27±0.05** [32.50]	0.23±0.03** [41.02]	0.18±0.05** [52.63]
4	EECI 200 mg/kg	0.30±0.03	0.25 ±0.03** [37.50]	0.20±0.05** [48.71]	0.15±0.05** [60.52]

Each value represents the mean ± SEM (n=6). * P<0.001, ** P<0.005 when compared with control

Percentage inhibitions of the carrageenan-induced inflammation (oedema) are indicated in parenthesis

Table.4 Effect EECI by acetic acid induced writhing method in mice

Group	Treatment	No. of Writhing	% of Inhibition
1	Control 2ml of 1% NaCMC	65.18±3.44	-
2	Diclofenac 10 mg/kg	15.26±1.30	76.58
3	EECI 100mg/kg	35.56±1.58*	45.44
4	EECI 20mg/kg	27.50±2.20*	57.80

DISCUSSION

In carrageenan induced rat paw edema model, the anti-inflammatory effect of standard drug Diclofenac 10 mg/kg, EECI at two different doses 100 mg/kg and 200 mg/kg was observed up to 3 hrs after carrageenan challenge. Carrageenan is known inflammatory agent and causes increase in prostaglandins and bradykinin synthesis at various time intervals. Carrageenan induced paw edema has been reported to have more than one phase and the initial phase has been attributed to the release of histamine and serotonin, the maintenance of edema during the plateau phase is caused by kinin like substance and the second accelerating phase of swelling is due to prostaglandin like substances.

Administration of EECI at 100 and 200 mg showed decrease in paw edema volume for 0-30 min respectively. Interestingly the dose levels of extract exhibited similar pattern in reducing carrageenan induced paw edema from 30-90 min to the end of the experiment.

Acetic acid induced writhing in mice attributed visceral pain finds much attention of screening analgesic drugs. The crude extracts of the plant EECI showed significant analgesic action compared to the reference drug Diclofenac against acetic acid induced pain in mice at two dose levels i.e. 100 & 200 mg/kg b. wt.

Pain sensation in acetic acid induced writhing method is elicited by triggering localized inflammatory response resulting release of free arachidonic acid from tissue phospholipids via Cyclooxygenase (COX), and prostaglandin biosynthesis. In other words, the acetic acid induced writhing has been associated with increased level of PGE2 and PGF2á in peritoneal fluids as well as Lipoxygenase products. The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability. The acetic acid induced writhing method was found effective to evaluate peripherally active analgesics. The agent reducing the number of writhing will render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition. The significant pain reduction of the plant extracts might be due to the presence of analgesic principles acting with the prostaglandin pathways.

CONCLUSION

In conclusion, we can confirm that the ethanolic extracts of *Crossandra infundibuliformis* are endowed with Anti-inflammatory and Analgesic properties. However, further study is needed in order to understand the precise mechanism. In future experiments, studies with purified fractions of the extract can be conducted for further pharmacological and toxicological characterization, such as the research of the mechanisms involved in the Anti-inflammatory and analgesic effect.

REFERENCES

Abhijit Dey JN. Ethnoveterinary uses of Medicinal Plants by the Aboriginals of Purulia District, West Bengal, India. *International Journal of* Botany. 2010;6:433.

Ahmadiani A, et al. *J Ethanopharmacol*. 1998;61:229.

Amish AD, Kalidass C, Mohan VR. Pharmacognostical Investigation of *Hedyotis puberula* (G. Don) Arn. *Asian Journal of Exp. Biol, Sci*. 2010;1(2):373.

Denko CW. *A role of neuropeptides in inflammation. In: Whicher JT, Evans SW, editors. Biochemistry of Inflammation, London*: Kluwer Pub; 1992:177.

El-Hag AH, Habbal O, Hasson Al-Hashmi N, Al-Mahrooqi Z, et al. Antibacterial activity of Lawsonia inermis Linn (Henna) against Pseudomonas aeruginosa. *Asian Pac J Trop Biomed*; 2011;1(3):173.

Ghosh MN. Fundamental of experimental Pharmacology 2nd ed. Scientific book agency: Calcutta: India; 1984:53.

Harborne JB. *Phytochemical Methods,* 3rd edition, Springer (India) Private Limited, New Delhi, 1998.

Henson PM, Murphy RC. *Mediators of the inflammatory process.*6th ed. Amsterdam:Elsevier; 1989.

Juvekar AR, Gambhire MN, Wankhede SS. *Journal of Young Pharmacists*. 2009;1(3):220.

Karthikai devi G, Manivannan K, Anantharaman P, Balasubramanian T. Antimicrobial potential of selected brownseaweeds from vedalai costal waters, Gulf of Mannar. *Asian Pac JTrop Biomed*. 2011;1(2):114.

Kirtikar Basu. *Indian Medicinal Plant with illustration*. 2001;8:2604.

Koester K, Anderson M, Beer EJ. Acetic acid for analgesic screening. *Federal Proceedings*. 1959;18:412.

Kshirsagar RD, Singh NP. *Ancient Science of Life*. 2001;1:1.

Kulkarni SK. Hand book of experimental pharmacology. Vallabh Prakashan. New Delhi 3rd Ed; 2006:128.

Labbie AR, Guries RP, Ethnobotanical value and conservation of sacred groves of the Kpaa Mede in Soerra Leone; Econ *Bot*. 1995;49:297.

Madhava Chetty K, Sivaji K, Tulasi Rao K. *Flowering plants of Chittor district–Andhra Pradesh, India*; 1st Ed; 2008:255.

Mandal MD, Mandal S. Honey: its medicinal property and antibacterial activity. *Asian Pac J Trop Biomed*. 2011;1(2):154.

Mantovani A, Sica A, Allavena P. Cancer-related inflammation. *Nature*. 2008;454:436.

Nareshkumar A, Prabhu K, Murugan K, Ramasubramanian N, Bragedeeswaran S. Larvicidal and repellent potential of Moringa oleifera against malarial rector, Anopheles stephensi Liston (Insecta:Diptera: Culicidae). *Asian Pac J Trop Biomed*. 2011;1(2):124.

Ogunyemi AO. *The origin of herbal cure and its spread; proceedings of a Conference on African Medicinal Plants. Sofowora A. (Ed.) University Press, Ile-Ife*. 1979:20.

Pandey BP. *Taxonomy of angiosperms*. 4th ed. New Delhi: S. Chand and Company; 1982.

Patric RD, Johnoson M, Paul PK, Irudayaraj V. Phytochemicaland anti-bacterial activity of epidermal glands extract of Christellaparasitica (L.) H. Lev. *Asian Pac J Trop Biomed*. 2011;1(1):8.

Prema B, Yoga Latha L, Sasidharan S. Antimicrobial drug resistance of Staphylococcus aureus in dairy products. *Asian Pac J Trop Biomed*. 2011;1(2):130.

Pullaiah T. *Encyclopaedia of world medicinal plants*; 2, 662.

Purohit AP, Kokate CK, Gokhale SB. *Text book of Pharmacognosy*, 27th edition, Nirali Prakashan, Pune, India, 2004.

Sakthivel B, Rajendran K, Gayathri N, Shanmugam S, Ramar S. *Ethnobotanical Leaflets*. 2009;13:370.

Sujit K Chaudhary. *Quitessence Of Medical Pharmacology*,*New Central Book Agency (P) Ltd., Kolkata*,400 *Experimental Therapeutics*. 2001;166:96.

Suresh B, Ramesh M, Swamy MM, Mahadevan N, Sama V, Reddy YSR. Pharmacognostical Studies on *Dodonaea viscosa* leaves. *African Journal of Pharmacy and Pharmacology*. 2008;2(4):83.

Turner RA. Screening methods in pharmacology. *Academic Press Inc. London*; 1965:323.

Usifoh C, Okunrobo L, Bariweni M, Ching P. *Journal of Pharmacology*. 2009;7(1):27.

Winter CA, Risley EA, Nuss GW. Carrageenan induced oedema in hind paw of rats as an assay for anti-inflammatory drugs. *Proc. Soc. Exp. Biol. And Med*. 1962;111:544.