IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
March 2021 Vol.:20, Issue:4

Therapeutic Effect of *Calanthe triplicata* in Carrageenan-Induced Inflammatory Model in Rats

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Mythili Krishnamurthy*

Assistant professor, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research (Deemed to be university) Chennai, Tamil Nadu, India

Submitted: 09 February 2021
Revised: 28 February 2021
Accepted: 20 March 2021

Keywords: *Calanthe triplicata*, herbal extract, Acute oral toxicity, Anti-inflammatory.

ABSTRACT

Calanthe triplicata (Willemet) Ames is a species of orchid from the genus *Calanthe* and belongs to the family of Orchidaceae. The present study was undertaken on standardized ethyl acetate extract of *Calanthe triplicata* to explore its possible anti-inflammatory. Acute oral toxicity study was carried out for the safety profile of the herbal extracts as per OECD (Organisation for Economic Co-operation and Development)– 423 guidelines. Carrageenan induced inflammatory model in rats were used to explore anti-inflammatory activity. The findings of the results showed that the percentage of inhibition was enhanced. All these reports sustain the capability of *Calanthe triplicata* as a good source of anti-inflammatory agents.

HUMAN JOURNALS
www.ijppr.humanjournals.com

INTRODUCTION

Calanthe triplicata (Willemet) Ames belongs to the family of Orchidaceae. Its common name is Christmas Orchid and synonym is *C.veratrifolia.* It is a low growing evergreen terrestrial orchid, snow white flowers with yellow or red callus found in the hilly parts of south India.

The review of literature shows that the herbs are reported to be used in diseases of stomach and intestine; and the root is chewed along with betel nuts or other aromatic substances, in diarrhoea. Besides, their ornamental value, orchids are also known for their medicinal usage especially in the traditional system of treatments. *Calanthe* species is reported to be applied to painful joints.

The selected herbal extract was standardized by botanical, physical, chemical, toxicological and biological methods. The selected plant *Calanthe triplicata* was collected from Kolli hills, Tamil Nadu, India and authenticated by Siddha Central Research Institute, Arumbakkam, Chennai, Tamil Nadu, India.

Besides their ornamental value, *Calanthe* is used as medicine to cure various diseases such as diabetes, diarrhoea, dysentery, paralysis, convalescence, cuts & wounds, bronchitis, arthritis and rheumatism [1-3]. Based on the literature of *Calanthe* species, *Calanthe triplicata* was chosen for the research work.

MATERIALS AND METHODS

Calanthe triplicata (CT), *Calanthe triplicata* extract (CTE), Ethyl acetate extract (EAE), Methanol extract (ME), Carrageenan and diclofenac sodium.

Ethical Guideline

Name of the Ethics Committee that approved the study and all protocols:

Centre for Toxicology and Developmental Research (CEFT), Sri Ramachandra Institute of Higher Education and Research (Deemed to be University), Chennai, Tamil Nadu, India.

(a) Date of this approval: 27.04.2013

(b) Number of the certification or document which verified approval of the study: IAEC/XXXIII/SRU/252/2013

The selected plant *Calanthe triplicata* was extracted using ethyl acetate and methanol and the extracts were standardized and compared by carrying out the entire quality control test parameters for the quality assessment of crude plant material. The standardized, therapeutically valuable ethyl acetate extract*Calanthe triplicata* was used for further studies [4].

Acute oral toxicity study as per OECD- 423 guidelines

Young, healthy adult Sprague Dawley female rats weighing between 140- 180 g body weights housed in groups in a well-ventilated polypropylene cage. A 12-hrs light/12-hrs dark artificial photoperiod was maintained. Room temperature 22° C (± 3° C) and relative humidity 50–80 % kept in the room. Animals had free access to pelleted feed and reverse osmosis purified water *ad-libitum*[5].

Experimental design

Sprague Dawley female rats divided into two groups of three animals each.

Group I animals received control.

Group II animals received **t**est (2000 mg/kg body weight).

The drug was administered once orally via gastric intubation at a dose level of 2000 mg/kg body weight. Lethality and abnormal clinical signs viewed on the day of dosing and after that for 13 days. Bodyweight was recorded before dosing and after that once in a week till completion of the experiment. Gross pathological changes also monitored at the end of the experiment.

Anti-inflammatory activity by Carrageenan model

Experimental animals

Sprague Dawley rats weighing 200-250 gm were housed in groups in a well-ventilated polypropylene cage and were preserved at 25 ± 2º C in 12-hrs dark/12-hrs light cycles, with both standard pelleted diet and water *ad libitum* in accordance to the guidelines for laboratory animal facility. All the animals were acclimatized, at least for a period of 7 days, to the laboratory conditions before experimentation [6-9].

Experimental design

Sprague Dawley rats were divided into five groups of six rats each.

Group I animals received normal Control (vehicle).

Group II animals received positive Control (Carrageenan induced).

Group III animals received carrageenan and diclofenac Sodium (25 mg/kg b.wt, p.o/day).

Group IV animals received carrageenan and EAE (200 mg/kg b.wt, p.o/day).

Group V animals receivedcarrageenan and ME (200 mg/kg b.wt, p.o/day).

Induction of inflammation in rats

Paw edema was induced by injecting 0.1 ml of 1 % Carrageenan saline solution into the hind paw subplantar region of experimental animals except standard control.

One hour before the Carrageenan injection, vehicle, standard control (Diclofenac Sodium – 25 mg/kg), EAE and ME (200 mg/kg) were administered orally to the animals accordingly.

Paw volume measured at intervals of 0.5 hr, 1 hr, 2 hrs, 3 hrs and 5 hrs. Increase in paw volume recorded. Percentage inhibitions of paw edema for the standard, control and test groups calculated.

RESULTS AND DISCUSSION

The body weight of all the experimental animals were given in Table-1. The observed results are as following.

Table No. 1: Bodyweight of the experimental animals

Group	Treatment	Bodyweight (g)		
		Day 0	Day 7	Day 14
I	Step 1 (2000mg/kg b.wt.)	156.80±8.74	158.53±8.24	162.00±8.24
II	Step 2 (2000mg/kg b.wt.)	151.27±4.76	160.87±5.33	169.83±4.14

Values expressed in mean ± SEM; n=3

There was no treatment-related death, abnormal clinical signs or remarkable body weight changes observed in all the experimental animals. No gross pathological observation recorded in all the experimental animals and results are depicted in Table-2.

Table No. 2: Individual animals gross pathological observation

Group	Treatment	Animal ID	Organs	Observations
I	Step 1 - (2000mg/kg b.wt.)	H	Skin, eyes, brain, lungs, heart, liver, kidney, adrenals, spleen and sex glands.	No abnormality detected
		B		
		CL		
II	Step 2 - (2000mg/kg b.wt.)	H		
		B		
		CL		

From the above results, LD50 of the test drug was ascertained to be higher than 2000 mg/kg b.wt. Hence, the test drug falls in the "category-5 or unclassified" in accordance with the Globally Harmonised System of classification of chemicals. In the rat paw edema testing, inflammation was induced by carrageenan injection for 1 hr and then treated with extracts of ethyl acetate, methanol, standard and vehicle.

The acute toxicity results provide evidence that ethyl acetate extract of *C. triplicata* was safe up to a dose of 2000 mg/kg body weight. From the acute toxicity data, two different treatments 200 and 400 mg/kg (p.o.) were selected for *in- vivo* anti-inflammatory study.

The effect of ethyl acetate extract of *C. triplicata* in reducing acute inflammation was found to be near with a comparable dose of standard. Two hrs, after the treatment with ethyl acetate extract of *C. triplicata*, percentage inhibition was increased by above 50 % compared with the standard.

The results of paw edema and percentage inhibition of inflammation in rats were depicted in Table 3 and 4 respectively.

The results data indicate that the extract was rapidly absorbed and biologically available. After 5 hrs, inflamed paw sizes continued to shrink, showing that the treatment was pharmacologically active for a prolonged time.

All these information support the capability of *C. triplicata*, as it has a significantly enhanced anti-inflammatory activity.

Table No. 3: Effect of extracts of CTE against carrageenan-induced paw edema in rats

Time (min.)	Paw volume (ml)				
	Normal control	Positive control	Standard drug	Ethyl acetate Extract	Methanolic Extract
30	0.30 ± 0.04	0.58 ± 0.1*	0.40 ± 0.03*	0.45 ± 0.06*	0.53 ± 0.03*
60	0.28 ± 0.03	0.59 ± 0.08*	0.33 ± 0.05*	0.43 ± 0.03*	0.52 ± 0.03*
120	0.27 ± 0.02	0.60 ± 0.09*	0.32 ± 0.03*	0.41 ± 0.04*	0.51 ± 0.04*
180	0.27 ± 0.04	0.61 ± 0.06*	0.32 ± 0.03*	0.39 ± 0.02*	0.51 ± 0.03*
300	0.25 ± 0.03	0.63 ± 0.06*	0.31 ± 0.02*	0.38 ± 0.02*	0.51 ± 0.04*

Each point represents the Mean ± S.D of six observations.

* Statistical significant difference ($p<0.05$) compared to normal control

Table No. 4: Percentage Inhibition of CTE against carrageenan-induced paw edema in rats

Time (minutes)	Percentage Inhibition		
	Standard drug	Ethyl acetate	Methanol
30	31.33	21.71	9.00
60	44.51	27.15	12.20
120	46.39	31.33	14.48
180	47.83	36.62	16.87
300	51.13	39.71	19.34

The effect and percentage purity of extracts of CTE against carrageenan-induced paw edema in rats were shown in Figures 1 and 2 respectively.

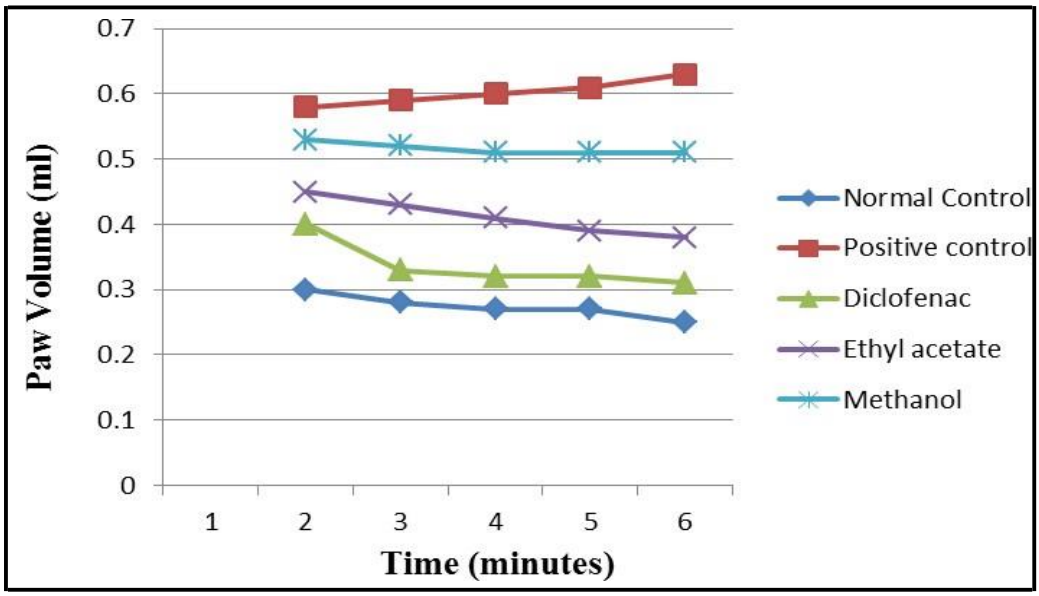

Figure No. 1: Effect of extracts of CTE against carrageenan-induced paw edema in rats

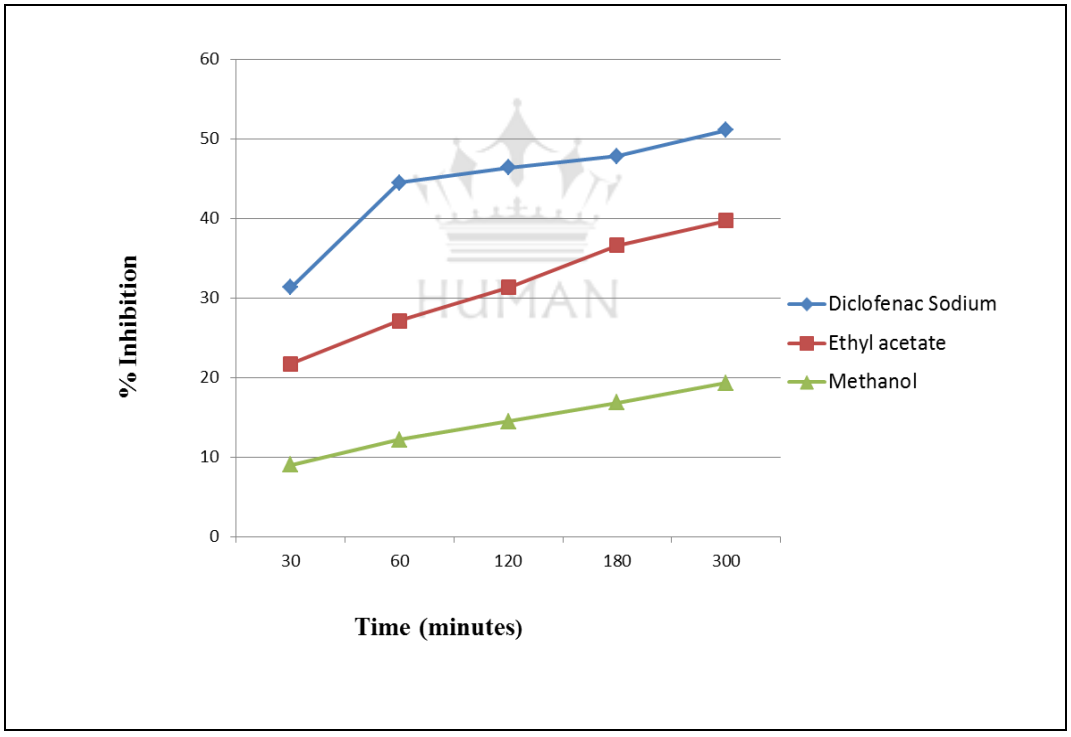

Figure No. 2: Percentage Inhibition of CTE against carrageenan-induced paw edema in rats

Statistical analysis

The collected data analyzed with SPSS 16.0 version. To illustrate about the data descriptive statistics mean and S. D used.

To locate the significance difference between the bivariate samples in paired groups (Paw volume in different time proportion) Wilcoxon signed rank test was used for independent groups (Normal control, positive control, standard and sample) Mann-Whitney U test was used.

For the multivariate analysis, the Kruskal-Wallis test was used. In all the above statistical tools, the probability value $P<0.05$ is considered as a significant level.

CONCLUSION

In the present study, the percentage inhibition of ethyl acetate extract of *Calanthe triplicata* against carrageenan-induced paw edema in Sprague Dawley female rats were performed. All the studies performed provide strong evidence for the use of the *Calanthe triplicata* as an anti-inflammatory and can be used as an alternative remedy for the management and treatment of inflammation.

ACKNOWLEDGEMENT

I thank the management of Sri Ramachandra Faculty of Pharmacy and Centre for Toxicology and Developmental Research, Sri Ramachandra Institute of Higher Education and Research (Deemed to be University) for providing all the necessary facilities to carry out this work.

REFERENCES

1. Henry A.N., *Calanthe triplicata,* Flora of Tamil Nadu, Botanical survey of India, Seidenfaden, Vol. III, 1989.
2. Ames, O., *Calanthe triplicata* (Willemet) Ames. Philipp J. Sci 1907; 2: 326.
3. Karthikeyan Mohanraj *et al.* IMPPAT: A curated database of Indian Medicinal Plants, Phytochemistry and Therapeutics.Sci Rep.2018;8:4329.
4. K Mythili *et al.* Quality Assessment of *Calanthe triplicata* by using various standardization parameters. Res & Rev in Pharma and Pharma Sci. 2014; 3 (3): 19-26.
5. OECD, Guidelines for the Testing of Chemicals/Section Health Effects Test No. 423: Acute Oral toxicity - Acute Toxic Class Method. Organization for Economic Cooperation and Development 2001: 1-14.
6. Ravi Kant Upadhyay. Anti-arthritic potential of plant natural products; its use in joint pain medications and anti-inflammatory drug formulations 2016. International Journal of Green Pharmacy 2016; 10(3):S120-S130.
7. Manjusha Choudhary *et al.* Medicinal plants with potential anti-arthritic activity, J Intercult Ethnopharmacol. 2015; 4(2): 147–179.
8. Alamgeer *et al.* Anti-arthritic activity of aqueous-methanolic extract and various fractions of *Berberis orthobotrys* Bien ex Aitch. BMC Complementary and Alternative Medicine 2017; 17: 371.
9. Zhang Q *et al.* Anti-arthritic activities of ethanol extracts of *Circaea mollis* Sieb. & Zucc. (Whole plant) in rodents. J Ethnopharmacol. 2018; 225: 359-366.
10. Murugananthan G. *et al.* Anti-Arthritic and Anti-Inflammatory Constituents from Medicinal Plants. J. Appl. Pharm. Sci. 2013; 3 (04): 161-164.

11. Gupta Sumeet *et al.* Anti Inflammatory and Anti Arthritic Activity of Different Milk Based Formulation of Curcumin in Rat Model. Current Drug Delivery 2018; 15 (2): 1-10.
12. Singh S *et al*. Anti-inflammatory and antiarthritic activity of UNIM-301 (a polyherbal unani formulation) in Wistar rats. Pharmacognosy Res. 2015; 7(2): 188-92.
13. Srinivasa Reddy Jitta *et al. Terminalia tomentosa* Bark Ameliorates Inflammation and Arthritis in Carrageenan Induced Inflammatory Model and Freund's Adjuvant-Induced Arthritis Model in Rats. Journal of Toxicology 2019: 11.