

Original Article

**Evaluation of Analgesic, Antipyretic and Anti- Inflammatory Activities of
Ceratophyllum Demersum Linn. in Albino Rats.**S.S. Karale^{a,*}, S.A. Jadhav^b, N.B. Chougule^a, S.S. Awati^a, A.A. Patil^a^aK.L.E.S's College of Pharmacy, Belgaum, Karnataka, India, ^bNational College of Pharmacy, Shimoga, Karnataka, India.

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Abstract

The methanolic extract of whole plant of *Ceratophyllum demersum* Linn. at the doses of 250 and 500 mg/kg was screened for analgesic, antipyretic and anti-inflammatory activities. Acetic acid, Brewer's yeast and Carrageenan were used to induce algnesia, pyrexia and inflammation in albino rats of Wistar strain. The extract showed significant analgesic, antipyretic and anti-inflammatory activities. The presence of flavonoids in the methanolic extract may be contributed to its activities.

Keywords: *Ceratophyllum demersum* Linn., analgesic, antipyretic and anti-inflammatory.

1. Introduction

Traditional and folklore medicines play an important role in health services around the globe. About three quarters of the world population relies on plants and plant extracts for healthcare. India has an extensive forests cover, enriched with plant diversity. Several plants have been used in folklore medicine. The rational design of novel drugs from traditional medicine offers new prospects in modern healthcare [1]. *Ceratophyllum demersum* Linn. is an aquatic, rootless, perennial plant of Ceratophyllaceae family, about 8 inches to 3 feet long, densely leaved, green in colour. Leaves are long which are spreaded in water and forming a net-like, inter-jointly, gradually a dense coverage on water surface. The whole plant has been traditionally used in the treatment of ulcer, diarrhoea, dysentery, wounds, fever, burning sensation, haemorrhoids or piles, intrinsic haemorrhages, hyperdipsia, epistaxis, haematemesis [2,3]. From the existing information, it is evident that the plant may posses some important biological activities. No report on its biological activity has been found in the literature. The main objective of this study was to evaluate the analgesic, antipyretic and anti-inflammatory activities of the methanolic extract of *Ceratophyllum demersum* Linn. using popular preclinical screening models in laboratory animals.

2. Experimental Protocol**2.1. Plant Material**

The fresh whole plants of *Ceratophyllum demersum* were collected from Kolhapur district of Maharashtra state, India, in the month of June 2008.

The specimens were identified and authenticated by Dr. Harsha Hegde, research officer, Regional Medical Research Centre, Indian Council of Medical Research, Belgaum. A voucher specimen of the plant (RMRC-465) was deposited at the herbarium of Regional Medical Research Centre, Indian Council of Medical Research, Belgaum.

2.2. Preparation of Extract

The collected whole plants were washed under running tape water, dried under shade and coarse powdered in mechanical grinder. The dried powder (55 gm) was extracted in a Soxhlet extractor with methanol and a total of 50 cycles were run to obtain thick slurry. This slurry was then vacuum evaporated to yield solid extract. The dried extracts were stored in a well-closed, air tight and light resistant borosil glass container.

2.3. Preliminary Phytochemical Screening

In order to determine the presence of alkaloids, glycosides, flavonoids, tannins, steroids and saponins a preliminary phytochemical study with plant extracts was performed [4,5].

2.4. Preparation of Dose

For analgesic, antipyretic and anti-inflammatory activities, the methanolic extract was suspended in 1% w/v solution of Carboxyl methyl cellulose (CMC) in distilled water.

2.5. Animals Used

Female Swiss albino mice, weighing between 20-25 gm and Male Wistar albino rats, weighing 150-200 g were used for toxicity studies and analgesic, antipyretic, anti-inflammatory activities, respectively. Animals were acclimatized in the laboratory for 7 days before experimentation and housed in groups of four per cage at temperature 25 ± 1°C with 12:12 hours light: dark cycle was maintained. Animals

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were provided with standard rodent pellets diet (Gold Mohur food and feeds Ltd., Vikhroli (East), Mumbai) and water *ad libitum*. The experiment was carried out according to Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA No.25/1/99-AWD) and the study was approved by Institutional Animal Ethical Committee (IAEC).

2.6. Acute Toxicity Studies

The acute oral toxicity studies were performed according to OECD (Organization for Economic Control and Development) 423 guidelines on female Swiss albino mice by Acute Toxic Class Method [6]. Animals were fasted for 4 h with free access of water only. The methanolic extract was administered orally at a dose of 5 mg/kg initially and mortality if any was observed for 3 days. If mortality was observed in two out of three animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one animal out of three animals then the same dose was repeated again to confirm the toxic effect. If no mortality was observed, then higher (50, 300, 2000 and 5000 mg/kg) doses of extract were employed for further toxicity studies.

2.7. Drugs and Chemicals

Nimesulide (Nise, Dr. Reddy's Laboratory), Carrageenan (Sigma Lab.), Paracetamol (Calpol, Glaxo-Smithkline) and methanol (Sd fine-chem Ltd.) were used during the experimental protocol.

2.8. Analgesic Activity

Acetic acid induced writhing model was employed to evaluate the analgesic activity. Albino mice 20-25 gm body weights were divided into four groups of six animals each. First group of the animals received 1% CMC (10 ml/kg of b.w., p.o.) served as control, second group served as reference standard received Nimesulide (50 mg/kg of b.w., p.o) while third and fourth group received methanolic extract (250 and 500 mg/kg of b.w., p.o.), respectively. The vehicle, extract and standard drug administered orally 1 h. prior to the intraperitoneal administration of acetic acid injection (10 ml/kg of 0.6% v/v). The number of writhes induced in each mouse was observed for 10 min., period starting 10 min. after injection of acetic acid. The writhing effect indicated by the contraction of abdomen with simultaneous extension of hind limbs and trunk twist response [7,8]. The analgesic activity was expressed in term of percentage inhibition of writhes produced by acetic acid was calculated by using the formula,

$$\text{Percentage inhibition of writhes} = \frac{\text{Mean of Control} - \text{Mean of Test}}{\text{Mean of Control}} \times 100$$

2.9. Antipyretic Activity

The antipyretic activity was evaluated using Brewer's yeast (*Saccharomyces cerevisiae*) induced pyrexia method in Wistar rats. Before the experiment, the rats were maintained in separate cages with food and water *ad libitum* for 7 days and

the animals with approximately constant rectal temperature (37.5 – 38.4°) were selected for the study. Male Wistar albino rats weighing, 150-200 gm were divided into four groups of six animals each. First group of the animals received 1% CMC (10 ml/kg of b.w., p.o.) served as control, second group served as reference standard received Paracetamol (50 mg/kg of b.w., p.o) while third and fourth group received methanolic extract (250 and 500 mg/kg b.w., p.o.), respectively. Fever was induced by injecting 2 ml/kg of 20% aqueous suspension of Brewer's yeast in distilled water and 18 h after yeast injection the vehicle, extract and standard drug were administered. Rectal temperature was recorded by clinical thermometer at 0, 1, 2, 3 h after drug administration [9].

2.10. Anti-Inflammatory Activity

Anti-inflammatory activity of *C. demersum* was tested by using the carrageenan induced rat paw edema model. Male Wistar albino rats weighing, 150-200 gm were divided into four groups of six animals each. First group of the animals received 1% CMC (10 ml/kg of b.w., p.o.) served as control, second group served as reference standard received Nimesulide (50 mg/kg of b.w., p.o) while third and fourth group received methanolic extract (250 and 500 mg/kg b.w., p.o.), respectively. After 1 h of administration of vehicle, extract and standard drug, 0.1 ml of 1% w/v suspension of carrageenan was injected into the right hind paw to all four groups. The paw volumes were measured using plethysmometer (UGO Basile, Italy) every hour till 3 h after carrageenan injection, and mean increase in paw volume were calculated [1].

2.11. Statistical Analysis

The groups were compared using one-way analysis of variance (ANOVA) followed by Dunnett's test and $p < 0.05$ was considered as significant.

Results

The percentage yield of methanolic extract was found to be 15.90% w/w. It showed presence of glycosides, flavonoids, alkaloids, steroids and tannins. All the doses of (5, 50, 300, 2000 and 5000 mg/kg) of methanolic extract employed for acute oral toxicity studies were found to be non-toxic. It did not produce any mortality even at the highest dose (5000 mg/kg). Hence, 1/10th and 1/20th of the highest safer dose, i.e. 500 and 250 mg/kg body weight were selected for evaluation of analgesic, antipyretic and anti-inflammatory activities. The methanolic extract of *Ceratophyllum demersum* (MECD) at the dose of 250 and 500 mg/kg body weight showed significantly ($p < 0.01$) reduction in number of writhes and paw volume and at the dose 500 mg/kg body weight showed significantly ($p < 0.05$) reduction in pyrexia.

Table 1.Effect of methanolic extract of *C. demersum* on acetic acid-induced writhing in mice.

| Groups | Average no. of writhes | Percentage inhibition |
|----------|------------------------|-----------------------|
| Control | 36 ± 1.880 | - |
| Standard | 15.67 ± 0.989** | 56.47 |
| MECD250 | 25.33 ± 0.882** | 29.64 |
| MECD500 | 20.83 ± 0.910** | 42.14 |

Data are expressed as the mean ± S.E.M., n = 6 in each group. ** p < 0.01 when compared to control group (one way ANOVA followed by Dunnett's test).

Table 2.Effect of methanolic extract of *C. demersum* on carrageenan-induced rat paw edema.

| Groups | Mean paw volume at different time intervals (ml) | | | Percentage inhibition of edema volume | | |
|----------|--|-----------------|-----------------|---------------------------------------|-------|-------|
| | 1 h | 2 h | 3 h | 1 h | 2 h | 3 h |
| Control | 1.90 ± 0.058 | 2.017 ± 0.060 | 2.183 ± 0.048 | - | - | - |
| Standard | 1.017 ± 0.060** | 0.933 ± 0.067** | 0.817 ± 0.040** | 46.47 | 53.73 | 62.59 |
| MECD250 | 1.467 ± 0.049** | 1.383 ± 0.054** | 1.317 ± 0.040** | 22.79 | 31.43 | 39.67 |
| MECD500 | 1.083 ± 0.060** | 0.983 ± 0.070** | 0.883 ± 0.060** | 43 | 51.25 | 59.54 |

Data are expressed as the mean ± S.E.M., n = 6 in each group. ** p < 0.01 when compared to control group (one way ANOVA followed by Dunnett's test).

Table 3.Effect of methanolic extract of *C. demersum* on Brewer's yeast-induced pyrexia in rats.

| Groups | Mean rectal temperature (°C) | | | |
|----------|------------------------------|----------------|----------------|-----------------|
| | 0 h | 1 h | 2 h | 3 h |
| Control | 40.65 ± 0.175 | 40.42 ± 0.191 | 40.25 ± 0.177 | 40.12 ± 0.156 |
| Standard | 39.85 ± 0.315 | 39.60 ± 0.288* | 39.50 ± 0.246* | 39.39 ± 0.231** |
| MECD250 | 40.55 ± 0.161 | 40.38 ± 0.168 | 40.10 ± 0.132 | 40.01 ± 0.123 |
| MECD500 | 40.37 ± 0.112 | 40.27 ± 0.203 | 39.58 ± 0.114* | 39.49 ± 0.102* |

Data are expressed as the mean ± S.E.M., n = 6 in each group. * p < 0.05, ** p < 0.01 when compared to control group (one way ANOVA followed by Dunnett's test).

Discussion

The experimental findings from the acetic acid induced writhing model, carrageenan induced paw edema and Brewer's yeast induced pyrexia model showed that the methanolic extract of *C. demersum* reduced number of writhes, paw volume and pyrexia, respectively. The extract showed significant analgesic, anti-inflammatory and antipyretic activities. Flavonoids present in the extract are known to target prostaglandins which are involved in the late phase of acute inflammation, pyrexia and pain perception. Hence the presence of flavonoids in the methanolic extract of *C. demersum* may be contributed to its analgesic, antipyretic and anti-inflammatory activities.

Conclusion

In Conclusion, it could be suggested that, the crude methanolic extract of *C. demersum* possesses analgesic, antipyretic and anti-inflammatory activities. Further studies to isolate and reveal the active compound (s) contained in the crude extract of *C. demersum* and to establish the mechanism (s) of action are required.

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