



***In vivo* anti-inflammatory activity of methanolic extract of *Hydrocotyle conferta* Wight (Apiaceae)**

R. Nantha Kumar*, K. Arumugasamy, M.R. Udhayasankar and H. Abdul Kaffoor

PG and Research Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore-641 029, Tamil Nadu, India.

*For correspondence E-mail: nanthabio89@gmail.com

Abstract

The present study was investigated on the anti-inflammatory activity of methanolic whole plant extract of *Hydrocotyle conferta* carrageenan induced paw edema rats model, The percent inhibition with indomethacin and *H. conferta* methanolic extract in the carrageenan induced paw edema at the dose level 300mg/kg were 87.14% and it confirmed anti-inflammatory activity.

Introduction

Inflammation is a complex process, which is frequently associated with pain and involves occurrence of increase in vascular permeability, protein denaturation and membrane alteration. When cells in the body are damaged by microbes, physical or chemical agents, the injury is in the form stress. Inflammation is a defensive response that is characterized by redness, pain, heat, and swelling and loss of function in that area. Since inflammation is one of the body's nonspecific internal systems of defense, the response of a tissue to an accidental cut is similar to the response that results from other types of tissue damage, caused by burns due to heat, radiation, bacterial or viral invasion (Gerard *et al.*, 1993). The medicinal plants are most important sources of medicines. India is well known for the availability of medicinal plants in the different bioclimatic zones for anti-inflammatory diseases (Hemamalini *et al.*, 2010).

Apiaceae represents one of the best-known plant families, widely distributed in temperate climate regions where they are often used as spices, vegetables, or drugs owing to the presence of useful secondary metabolites (Heywood, 1971; Kubeczka, 1982). *Hydrocotyle conferta* Wight. (Apiaceae) is an endangered species found in Southern Western Ghats, India.

Materials and methods

Collection of plant material

H. conferta plant was collected from Kodanadu, the Nilgiris, the Western Ghats, Southern India. The identification of the plant was also confirmed by Dr. M. Murugasen, Scientist B, Taxonomist, Botanical Survey of India, Eastern region, Shillong.

Preparation of the extract

All freshly collected plant parts of *H. conferta* then were packed in the ice box (or) in a polycover and brought to lab there material was made into powder with help of small mechanical device and passed through 250mm sieve and kept under whether condition or in a deep freezer.

100 g of powder was packed in a thimble as part in the extractor unit of Soxhlet apparatus 250 ml of methanol was added in the rotted bottomed flasks which a part of the apparatus and it was run for at least 10-15 hrs. the methanol extract was removed from the flask and it was concentrated under reduced pressure vacuum pressure and residue was kept in the refrigerator at 4 °C for further study.

Experimental animals

The male albino rats were obtained from the animal house, Agricultural University, Trissur, Kerala. Feed (standard pellet diet) and water were supplied *ad libitum*. All rats were maintained at a temperature of 20-24°C, relative humidity of 50-70%, and a 12 h light/dark cycle. Rats were put into four groups of five in the

same cage for 1 week before treatment. The experimental protocol has been approved by the Institution Animal Ethics committee and by the Regulatory body of the government (659/02/a/CPCSEA).

Acute toxicity study

For the pharmacological study, residue was suspended in double distilled water which contains carboxy methyl cellulose (1% w/v, CMC). The acute toxicity was determined for the methanolic extract of *H. conferta* on wister albino rats by fixed dose method. 100-1000 mg/kg b.wt. of extract of *H. conferta* was administered by oral route to rats and mortality was observed for 3 days.

In vivo anti-inflammatory activity

Carrageenan-induced paw oedema in albino rats

All wister albino rats were divided into 5 groups constituter five rats in each group. In all groups acute inflammation was produced by sub plantar injection of 0.1 ml freshly prepared 1% suspension of carrageenan in normal saline in the right hind paw of the rats and paw volume was measured plethysommetrically at 0 to 180 mins after carrageenan injection. All the animals were premedicated with indomethacin (10mg/kg b.wt.) orally two hour before infection. Mean increase in paw volume was measured and percentage was calculated for all the samples extract was subjected for acute toxicity studies and 1/10th of the LD 50 dose was selected for pharmacological activity (Winter and Poster, 1957). Percentage inhibition of paw volume was calculated by the following formula

$$\text{Inhibition (\%)} = \frac{V_c - V_t}{V_c} \times 100$$

Where

V_t- means increase in paw volume in rats treated with test compounds

V_c- means increase in paw volume in control group of rats.

Results and Discussion

The anti -inflammatory activity of methanolic extracts of *H. conferta* were revealed by the carrageenan induced rat paw oedema method. The methanolic extracts were tested at 150 and 300 mg/kg b.wt., dose levels. The present study elucidates 150 and 300 mg/kg b.wt., methanolic extracts of *H. conferta* significantly reduced the carageenan induced paw oedema inflammation as compared with that of the standard drug, indomethacin. This result indicated that the methanolic extract dose of 300 mg/kg.b.wt showed a maximum anti-inflammatory activity (87.14%) as compared to the reference drug, indomethacin. The methanolic extract of plant it's with a dose of 150 mg/kg b.wt., produced 74.82% of inhibition(Table 1 and fig 1).

The present study showed that the rats treated with methanolic extract of *H. conferta* have exhibited a significant anti-inflammatory activity with an inhibition of the paw edema compared to the control. Carrageenan induced inflammation is a useful model for the estimation of orally active anti-inflammatory effects (Ratheesh and Helen, 2007). The test model basically reflects the action of prostaglandins involved in the inflammation process induced by carrageenan (Mujumdar and Misar, 2004). (Neha Mohan *et al.*,2013) Evaluation of anti-inflammatory activity in ethanolic extract of *Coriandrum sativum* using carrageenan induced paw oedema in albino rats. (Danya, 2017) have investigated for antiinflammatory activity by carrageenan induced paw oedema method by methanolic extract of *C. sarkariae* the methanolic extract at the dose showed a maximum anti-inflammatory activity compared to the standard drug.

Conclusion

In conclusion the present study showed that the methanolic extracts of *H. conferta* possessed potent anti-inflammatory properties than the whole plant extract. This study gives an idea that the compound of plant

H. conferta can be used as a lead compound for designing a potent anti-inflammatory drug which can be used to cure inflammation.

Table 1. *In vivo* anti-inflammatory activity of methanol extract of *H. conferta* on carrageenan induced hind paw oedema in rats.

| Treatment Groups | Dose mg kg ⁻¹ | Paw thickness (mm) | | | | % Inhibition |
|------------------|--------------------------|--------------------|------------|------------|-------------|--------------|
| | | 0 min | 60 min | 120 min | 180 min | |
| Group I | Normal saline | 69.71±1.30 | 86.74±3.58 | 92.71±3.54 | 127.31±9.33 | -- |
| Group II | 150 mg kg ⁻¹ | 31.37±1.98 | 74.77±3.58 | 54.76±1.98 | 34.24±2.87 | 74.82 |
| Group III | 300 mg kg ⁻¹ | 26.78±1.93 | 51.84±1.07 | 34.22±1.28 | 20.60±1.81 | 87.14 |
| Group IV | 10 mg kg ⁻¹ | 29.13±1.62 | 42.64±1.54 | 32.27±1.22 | 16.34±0.50 | 89.74 |

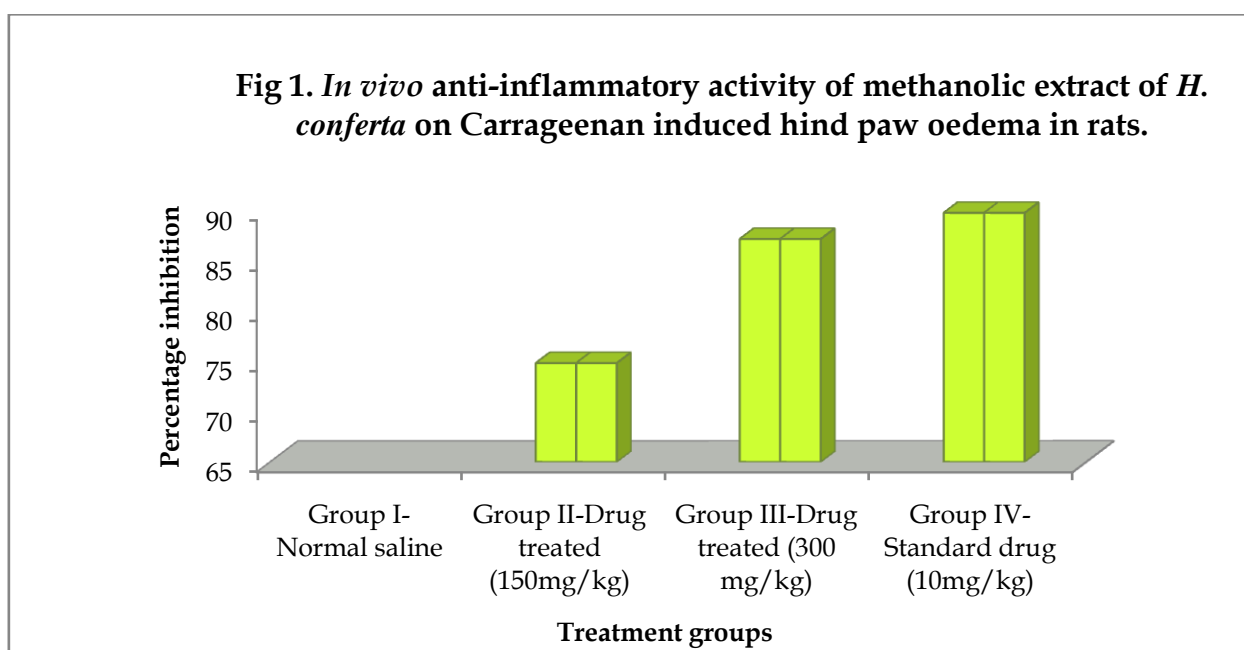
*Value is SEM ± 5 individual observations * P < 0.05; ** P<0.01 Compared paw oedema induced control vs drug treated rats.

Group I: Control rats given normal saline

Group II: Rats given methanolic extract of *H. conferta* at the dose of 150 mg/ Kg b.wt.

Group III: Rats given methanolic extract of *H. conferta* at the dose of 300 mg/ Kg b.wt.

Group IV: Rats given Indomethacin at the dose of 10 mg/ Kg b.wt.



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