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RESEARCH ARTICLE

ANTI-INFLAMMATORY ACTIVITY OF *CYATHEA NILGIRENSIS* HOLTUM, AGAINST CARRAGEENAN INDUCED PAW EDEMA

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INTRODUCTION

A retrospection of the healing power of plants and a return to natural remedies are absolute need of our time. The medicinal plants assume a tremendous importance, especially at a time when the world is showing a resurgence of interest in the healing properties. The world is endowed with a rich wealth of medicinal plants. Herbs have always been the principal form of medicine in India and presently they are becoming popular throughout the developed world as people strive to stay healthy in the face of chronic stress and pollution and to treat illness with medicines that work in concert with the body's own defenses. Medicinal plants are economically important as the herbal medicines are regaining importance day by day and because of the fact that it has less or no side effects. The efficacy of Indian systems of medicine captured the interest of the elite and modern lines. Screening of natural sources such as plant extracts has led to the discovery of many clinically useful drugs that play important roles in the treatment of human diseases (Kumar *et al.*, 2009). Many medicinal plants are used in developing countries for the management of pain and inflammatory conditions. Scientific proof of the folkloric claims of these medicinal plants through research will provide the basis for the conservation of tropical and Western Ghats medicinal recourses. These could provide useful compounds in

ABSTRACT

The plant extracts of *Cyathea nilgirensis* were evaluated for anti-inflammatory activity in mice using the carrageenan induced-paw edema test. The study has shown that the ethanol and chloroform extracts of plant *C. nilgirensis* possess a significant anti-edematogenic effect on paw edema induced by carrageenan. Both the extracts of *C. nilgirensis* showed significant ($p < 0.001$) decrease in edema with a percentage of inhibition ranging from value 13.71 ± 1.25 to 7.15 ± 1.52 at 100mg/kg in chloroform extract and ethanol extract showed values ranging from 13.86 ± 2.12 to 6.7 ± 2.64 at 100mg /kg. These extracts have shown better results compared to standard drug diclofenac sodium. This study revealed the promising anti-inflammatory activity of *C. nilgirensis* which has been carried out scientifically for the first time. The evaluation could be an important finding globally, as inflammation has become a common disease. Hence the results of this study indicates that *C. nilgirensis* can be effective remedy in acute anti-inflammatory disorders.

drug development and testing processes (Musa *et al.*, 2009). Most of the local or indigenous people are not well known about the uses of Pteridophytes as source of medicine as they have been ignored. There is no much information available in the literature about the medicinal importance except a few studies (Caius, 1935; Benjamin, 2007). Therefore the study of *Cyathea nilgerinsis* was under taken in order to ascertain the medicinal properties of the plant.

Description of the plant

Cyathea nilgirensis Holtum, a southern Indian endemic tree fern (Fraser – Jenkins 2008) was seen growing in the deep shade of the swampy forest of Uttar Kannada. The plant belongs to the family Cyatheaceae. It is endemic to South India and 13 cm in diameter, it is unbranched; bearing crown of fronds at the apex and scales densely covering the younger fronds. Stipes up to 40 cm long swollen at base bearing small hairs. Lamina bipinnate, oblong, lanceolate, pinnae about 12 pairs, alternate, distinct petiolate, pale green below, dark green above, texture herbaceous. Sori situated in the vein forks of the lower half of the segments, exindusiate, paraphyses intermingled with sporangia, spores trilete. Grows as terrestrial species along shaded stream banks (Manickam & Irudayaraj 1992).

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MATERIALS AND METHODS

Collection and identification of plant material

Cyathea nilgirensis Holttum is an endemic tree fern was collected from Kathalekan (kathale-dark, Kan-sacred forest) relic forest in Uttar Kannada district of Central Western Ghats in Karnataka. The herbarium is archived at the JCB herbarium of Centre for Ecological Sciences, Indian Institute of Science, Bangalore. The allotted numbers of herbarium specimens are HJCB 189 (a), HJCB189 (b).

Preparation of crude extract

The collected plant material of *Cyathea nilgirensis* was shade dried, powdered. The powdered plants were sequentially extracted with petroleum ether, chloroform and ethanol according to increasing polarities, starting with the least polar solvent using the soxhlet apparatus. The Chronological extraction procedure was adopted based on the fact that different polarity of solvents facilitates the removal of desirable compounds soluble in particular solvents (Bazkinal *et al*, 2002). The extracts were air dried using the rotary evaporator.

In vivo Anti-inflammatory activity

Hind paw edema method: The animals used in this study were male and female mice weighing between 25-30gms. Animals were maintained at $22 \pm 2^\circ\text{C}$ with a 12 h light and dark cycle, fed on standard rodent chow with free access to water. All animal studies conducted were approved by the Institutional Animal Ethics Committee, DFRL Mysore, as stated by prescribed guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (DFRL/ IAEC/01/2015 dated 27/04/2015).

Preparation of plant extracts doses: *Cyathea nilgirensis* extracts were prepared in 1% DMSO (Dimethyl sulphoxide) as a suspension and administered to the respective doses. The doses of plant extracts and experiment design are given below.

Carrageenan induced paw edema model

Group-I: Vehicle control received 1% DMSO (dose: 10mL/kg).

Group-II: Animals treated with 1% carrageenan (dose: 0.1mL).

Group III: Animals treated with Diclofenac sodium (dose: 50mg/kg).

Group IV: Animals treated with chloroform extract of *Cyathea nilgirensis* (dose: 50 mg/kg).

Group V: Animals treated with chloroform extract of *Cyathea nilgirensis* (dose: 100mg/kg).

Group VI: Animals treated with ethanol extract of *Cyathea nilgirensis* (dose: 50mg/kg).

Group VII: Animals treated with ethanol extract of *Cyathea nilgirensis* (dose: 100mg/kg).

Protocol

Carrageenan induced paw edema is a classical model for determination of acute phase inflammation. The mice paw edema was provoked by sub planter injection 0.1 mL of 1% w/v of carrageenan in 0.9% saline in right hind paw. The hind paw volume was measured by dipping the foot in digital plethysmometer up to the lateral malleolus (Winter *et al*, 1962). The displacement of sodium chloride solution was measured by the plethysmometer. The initial paw volume considered as 0 h reading was measured and recorded. The drug or test substances like 1% DMSO (vehicle control), Diclofenac Sodium, and various extract doses were administered orally 60 min before administration of carrageenan. The hind Paw volume was measured at 1 hour interval up to 4th hour of the experiment. The difference between paw volumes at various time intervals indicated the edema volume due to inflammation. The percentage inhibition produced by the drug and extracts was calculated by following formula,

Percentage inhibition of paw edema (%) = (Control - Treated) / Control X 100

RESULTS AND DISCUSSIONS

The plant extracts of *Cyathea nilgirensis* were evaluated for anti-inflammatory activity in mice using the carrageenan induced-paw edema test. According to (Vineagar *et al*, 1987), the development of paw edema is derived from the release of cytoplasmic enzymes and serotonin from mast cells and the increase of prostaglandin in the inflamed area. The macrophages in carrageenan-insulted dermal tissue release interleukin-1 causing accumulation of polymorphic nuclear cells (PMNs) in the inflamed area, the activated PMNs then release lysosomal enzymes and active oxygen, which induces paw swelling. Carrageenan induced mice paw edema is a suitable test for evaluating anti-inflammatory activities of natural products (Panthong *et al*, 2003).

The results of anti-inflammatory activity of *Cyathea nilgirensis* of chloroform and ethanol extracts is shown in the Table. The effect of the extracts (50 and 100mg/kg) and the reference drug on Carrageenan induced paw edema were most pronounced at 3h after carrageenan injection, while the 100mg/kg dose of the ethanol and chloroform extracts showed their highest activity at 4h, indicating that the extracts may be more potent than the reference drug. Both the extracts of *C. nilgirensis* showed significant (p 0.001) decrease in edema with a percentage of inhibition ranging from value 13.71 ± 1.25 to 7.15 ± 1.52 at 100mg/kg in chloroform extract and where as ethanol extract showed values ranging from 13.86 ± 2.12 to 6.7 ± 2.64 at 100mg/kg. Development of edema induced by carrageenan is commonly correlated with early exudative stage of inflammation. This study has shown that the ethanol and chloroform extracts of plant *C. nilgirensis* possess significant anti-edematogenic effect on paw edema induced by carrageenan. These extracts exhibited better results compared to standard drug diclofenac sodium.

In *Cyathea nilgirensis* the preliminary phytochemical studies have revealed the presence of carbohydrates, alkaloids and flavonoids. The alkaloids and flavonoids might be responsible in part for the observed anti-inflammatory effect of *Cyathea nilgirensis* (Sahaya Mary et al, 2015).

To conclude, this study revealed the promising anti-inflammatory activity of *C. nilgirensis* which has been carried out scientifically for the first time. The evaluation could be an important finding globally, as inflammation has become a common disease. Hence the results of this study are an indication that *C. nilgirensis* can be effective in acute anti-inflammatory disorders.

Table Anti-inflammatory activity of Crude Extracts of ethanol and chloroform of plant *Cyathea nilgirensis* measuring Paw volume.

| Treatment Group | % inhibition of paw edema | | | |
|---|---------------------------|---------------------------|---------------------------|---------------------------|
| | 1 h | 2 h | 3 h | 4 h |
| Group I (Control) | 0.00 | 0.00 | 0.00 | 0.00 |
| Group II (Control Treated with Carrageenan at 1%) | 15.69 ± 1.92 | 20.96 ± 1.20 | 26.46 ± 1.80 | 37.30 ± 2.45 |
| Group III | 16.83 ± 1.67 ^b | 13.55 ± 2.10 ^b | 9.68 ± 2.50 ^c | 12.83 ± 2.06 ^c |
| Group IV | 13.25 ± 1.86 ^b | 14.06 ± 1.16 ^c | 14.55 ± 1.63 ^c | 11.80 ± 2.20 ^c |
| Group V | 13.71 ± 1.25 ^a | 11.51 ± 1.76 ^c | 9.05 ± 0.96 ^c | 7.15 ± 1.52 ^c |
| Group VI | 13.53 ± 1.04 | 11.91 ± 1.68 ^c | 17.43 ± 1.16 ^c | 13.88 ± 2.63 ^c |
| Group VII | 13.86 ± 2.12 ^b | 11.3 ± 2.01 ^c | 11.2 ± 3.43 ^c | 6.7 ± 2.64 ^c |

Percentage inhibition of paw edema values are presented in parentheses. Values are expressed as mean ± SEM ; n=6, p 0.05 Vehicle control Vs carrageenan a= p 0.05, b=p 0.01, c= p 0.001, Carrageenan Vs drug/test substance.

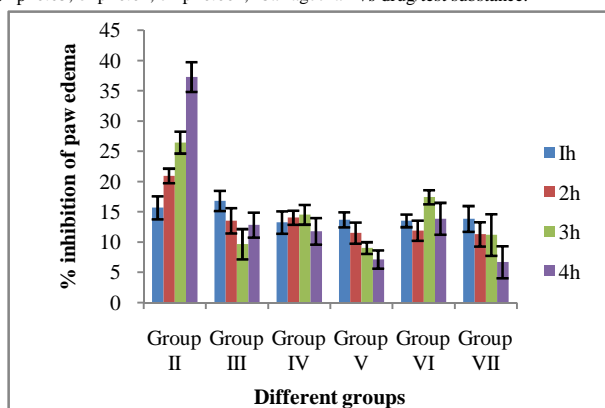


Fig Anti-inflammatory activity of *Cyathea nilgirensis*

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