Available online at www.elixirpublishers.com (Elixir International Journal)

Bio Technology

Elixir Bio Tech. 78 (2015) 29450-29452

ISSN: 2229-712X

Anti-inflammatory activity of Spathodea campanulata P. Beauv. Leaves against Carrageenan induced Paw Edema

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ARTICLE INFO

Article history:

Received: 31 July 2014;

Received in revised form:

19 December 2014;

Accepted: 29 December 2014;

Keywords

Carrageenan,

Spathodea campanulata,

Anti-inflammatory,

Methanol.

ABSTRACT–

The Present study, methanol and aqueous extracts of *Spathodea campanulata* leaves were tested for anti-inflammatory activity (paw edema induced by carrageenan) in rats. The methanolic extract in dose of 200 mg/kg bw showed the significant inhibition of paw edema at the end of 180 min was 0.431% followed by aqueous extract (0.593%). Among the two extracts tested, the methanolic extract showed remarkable activity at 200 mg/kg dose level whereas aqueous extract was not able to show such remarkable significant activities. The extract exhibited significant anti-inflammatory activity, which supports the traditional medicinal utilization of the plant.

Introduction

Inflammation is the reaction of living tissues to injury, infection or irritation. Lysosomal enzymes released during inflammation produce a variety of disorders which leads to the tissue injury by damaging the macromolecules and lipid peroxidation of membranes which are assumed to be responsible for certain pathological conditions as heart attacks, septic shocks and rheumatoid arthritis etc. A variety of chemical mediators or signaling molecules such as histamine, serotonin, leukotrienes, prostaglandins and oxygen derived free radicals (O2, OH, ONOO-) are produced by inflammatory and phagocytic cells predominantly in the sequences which participates in onset of inflammation (Vijayalakshmi *et al*., 1997; Safayhi and Sailer, 1997).

As presently available synthetic analgesic and anti-inflammatory drugs pose several health problems during their clinical use, search to develop new and more effective drugs with fewer side effects is necessary. Medicinal plants have a wide variety of chemicals from which novel anti-inflammatory agents can be discovered. Research on the biological activities of plants during the past two centuries has yielded compounds for the development of modern drugs (Arivazhagan *et al.*, 2000). The use of natural products is growing in the world especially in developing countries like India where over 75% of the population relies mainly on plants and plant extracts for healthcare.

Spathodea campanulata P. Beauv. is a species belonging to the Bignoniaceae family, native of equatorial Africa. It is commonly found and planted in the coffee estates of Munnar, South Tamilnadu and is denoted by the name Malaria Maram (tree). Its flowers and stem bark extracts have shown molluscicidal activity and also employed in diuretic, anti-inflammatory treatments. The leaves are used for kidney diseases, urethra inflame-mations and as an antidote against animal poisons. The stem bark preparations are used for enemas, fungus skin diseases, herpes, stomach aches and diarrhea (Mendes *et al*., 1986; Adriana *et al*., 2007). Hypoglycemic, anti-HIV and anti-malarial activities were also observed in the stem bark extracts (Makinde *et al*., 1988; Niyonzima *et al*, 1999). On the basis, the objective of the present investigation was to study the anti-inflammatory effects of leaves of methanol and aqueous extracts of *S.campanulata*.

Materials and methods

Collection of plants

Fresh aerial parts were collected randomly from the villages of Kovilpatti region, Thoothukudi District, Tamil Nadu from the natural stands. The plant was authenticated by Dr.V.Sampath Kumar, Scientist – C, Botanical Survey of India (Southern Circle), Coimbatore, Tamil Nadu and a voucher specimen was maintained at PG & Research Department of Botany, National College, Tiruchirapalli.

Preparation of extracts

Methanol

100 gm of dried plant powdered samples were extracted with 200 ml of methanol kept on a rotary shaker for 24 h. Thereafter, it was filtered and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fifth of the original volume. It was stored at 4^{o}C in airtight bottles for further studies

Aqueous

100 gm of dried powder were extracted in distilled water for 6 h at slow heat. Every 2 h it was filtered through 8 layers of muslin cloth and centrifuged at 5000 g for 15 min. The supernatant was collected. This procedure was repeated twice and after 6 h the supernatant was concentrated to make the final volume one-fifth of the original volume.

Acute toxicity study

Acute toxicity study was carried out according to the method described by Burger *et al*. (2005). No adverse effect or mortality was detected in albino rats up to 1200 mg/kg bw of methanol and aqueous extracts of *Spathodea campanulata* during 24 hr observation period basing on which the respective doses are selected for further study.

Determination of Anti-inflammatory potential

Extracts of leaves of Spathaodea campanulata were evaluated. Swiss albino rats (150 -180 g) were used taking into account

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international principles and local regulations concerning the care and use of laboratory animals. The animals had free access to a standard commercial diet and water *ad libitum* and were kept in rooms maintained at 22 ± 1ºC with a 12 h light/dark cycle. The *in vivo* anti-inflammatory activity was performed in Department of Pharmacology, RVS Pharmaceutical Sciences College, Coimbatore. The institutional animal ethical committee has approved the protocol of the study.

Carrageenan-induced edema in rats

For screening *in vivo* anti-inflammatory activity for each of the extracts, 4 Groups of five animals each were used.

Group I: Normal control (Carrageenan 1%w/v)

Group II: Positive control (indomethacin 10mg/kg, i.p.)

Group III: Rats received *S.campanulata* methanol extract (200mg/kg of bw)

Group IV: Rats received *S.campanulata* aqueous extract (200mg/kg of bw).

Paw swelling was induced by sub-plantar injection of 0.1 ml 1% sterile carrageenan in saline into the right hind paw. The crude extracts of plant at dose of 200 mg/kg were administered orally 60 minutes before carrageenan injection. The inflammation was quantified by measuring the volume displaced by the paw, using a plethysmometer at time 0, 30, 60, 120, 180 min after carrageenan injection. The difference between the left and the right paw volumes (indicating the degree of inflammation) was determined and the percent inhibition of edema was calculated in comparison to the control animals (Kulkarni, 2005). The percentage of inhibition of paw-edema is calculated by

$$\% \text{ inhibition of paw edema} = \frac{C - T}{C} \times 100$$

Where,

C = increase in paw volume of control group

T = increase in paw volume after administration of extracts

Results and Discussion

Medicinal plants will continue to provide a source for generating novel drug compounds. Plants may become the base for the development of a new medicine or they may be used as phyto medicine for the treatment of disease. The primary benefit of using plant-derived medicine is that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatments. Many plants have proved to successfully aid in various ailments leading to mass screening for their therapeutic components.

The present study of anti-inflammatory activity of ethanol extract of *spathodea campanulata* against carrageenan induced paw edema shows that the extracts have significant effect on inflammation and markedly reduced the swelling. Methanolic extract at a dose of 200mg/kg showed highly significant anti-inflammatory activity ($P<0.01$) as compared to control group at 30, 60, 120 and 180 min respectively. The aqueous extracts also showed significant activity ($P<0.01$) at 30, 60, 120 and 180 min (Table 1). The standard drug Indomethacin at a dose of 4mg/kg body weight inhibited the development of edema significantly from 30 min onwards. It showed maximum percentage reduction in paw edema at 180 min. Methanolic extract of *S.camapanulata* at the dose of 200mg/kg body weight showed percentage of inhibition of paw edema at 180 min 0.593%.

Carrageenan induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility (Winter *et al*., 1962). Intraperitoneal injection of carrageenan leads to inflammation of the peritoneum resulting from carrageenan induced release of interleukin-1 from macrophages in the carrageenan insulated tissue. Interleukin-1, a pro-inflammatory cytokine, induce accumulation of polymorpho nuclear cells by a variety of processes including adhesion and cell mobility (Meade *et al*., 1986).

Indomethacin is a cycloxygenase inhibitor, the ethanol extract has activity which is compareable to indomethacin and can be said to inhibit the cycloxygenase enzyme but lipoxygenase inhibitors also possess significant anti-inflammatory activity against carrageenan induced paw edema, so inhibition of carrageenan induced paw edema by the crude extract could also be due to its inhibitory activity on the lipoxygenase enzyme. The present experimental findings suggest that herbal product is a promising anti-inflammatory agent of plant origin in the treatment of inflammatory disorders and conditions. Hence, it is necessary to evaluate its anti-inflammatory activity on humans in clinical conditions. Further studies in future context on the basis of present studies are however needed to isolate the active, principle (s) responsible for anti-inflammatory potential.

Table 1. Anti-inflammatory activity of methanol and aqueous extracts of *Spathodea campanulata* (% of paw edema volume)

Group	Dose	Paw edema volume in ml as measured by mercury displacement at				
		0 min	30 min	60 min	120 min	180 min
I	0.1ml	0.531 ± 0.004	0.581 ± 0.007	0.71 ± 0.006	0.876 ± 0.002	1.2 ± 0.030
II	10 mg/kg	0.427 ± 0.005	0.459 ± 0.002	0.53 ± 0.009	0.523 ± 0.004	0.454 ± 0.002
III	200 mg/kg	0.432 ± 0.005	0.472 ± 0.002	0.51± 0.001*	0.583 ± 0.003*	0.431± 0.003*
IV	200 mg/kg	0.399 ± 0.003	0.452 ± 0.005	0.49± 0.003*	0.543 ± 0.002*	0.593± 0.003*

Group I: Normal control (Carrageenan 1%w/v), Group II: Positive control (indomethacin 10mg/kg, i.p.), Group III: Rats received *S.campanulata* methanol extract (200mg/kg of bw), Group IV: Rats received *S.campanulata* aqueous extract (200mg/kg of bw).

P values: * * P< 0.01; * P <0.05.

Values are expressed in mean ±SEM, n=5 animals in each group

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