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Evaluation of the antiinflammatory activity of field grown plant and Callus Extracts of *Tinospora cordifolia* (Willd.) Hook.F.Thoms under *in vitro* conditions.

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ABSTRACT

The investigation is aimed to carry out the anti inflammatory activity of ethanol extracts of field grown leaf and leaf derived callus of Tinospora cordifolia (Willd.) Hook.F.Thoms (Menispermaceae).Callus was initiated from the leaf explants of Tinospora cordifolia cultured on MS medium supplemented with auxins and cytokinins alone and in combinations. In the case of leaf derived callus, maximum biomass was recorded on medium containing 2,4-D, NAA and BAP combination. Leaf and leaf derived callus extracts were screened for active principles. Berberine an isoquinoline alkaloid is present in Tinospora cordifolia. Berberine content of callus was improved by imposing abiotic stress on their growth. Salt stress (high conc. of NaCl) showed more response compared to other stresses such as light stress (darkness for 16 h) and temperature stress. Ethanol extracts of leaf derived callus grown under salt stress and field grown leaf were used to analyze their anti inflammatory potential under in vitro conditions. It has been observed that the callus extract of leaves show more berberine content and revealed better anti inflammatory activity as compared to in vivo plant leaf extract.

Keywords: Tinospora cordifolia, callus culture, anti inflammatory activity, MS medium, carrageenan, isoquinoline alkaloid, berberine.

I. INTRODUCTION

Medicinal plants, which form the backbone of traditional medicine, have in the last few decades been the subject of very intense pharmacological studies. Approximately 80% of the world total population depends exclusively on plants for their health and healing. Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants as anti inflammatory agents. Plant derived drugs serve as a prototype to develop more effective and less toxic medicines [8]. Over 50% of all modern clinical drugs are of natural product origin [13] and natural products play an important role in drug development programs in the pharmaceutical industry [1]. Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, phenols, saponins, sterols etc. There is a growing attention in correlating the phytochemicals of a medicinal plant with its pharmacological activity [11].

II.MATERIALS AND METHODS

A.Collection of plants

The young leaves and stem of *Tinospora cordifolia* were collected from 3-5 months old healthy plant growing in the Medicinal plant garden of J.J. College, Pudukkottai, Tamil Nadu, India, that receives a mean annual rainfall ranging from 100 to 120 cm with an average temperature of 38˚C. The pH of the garden soil was 7. The plant leaves and stem were washed thoroughly with tap water followed by sterile distilled water and shade dried at room temperature for 10-15 days.

B. Media Preparation,Callus Initiation and its Proliferation:

MS media [9] supplemented with auxins *viz*., 2,4-D, NAA, BAP and Cytokinins *viz*., BAP and Kn alone at different concentrations was used for callus induction. The cultures were maintained in a culture room at 16/8 h light/dark conditions by using cool white fluorescent tubes (40 µM m^{-2} s^{-1}) with 55-60% relative humidity. Each treatment had 10-25 replicates and was repeated thrice.

C. Collection and storage of field grown plant and callus materials

The plant parts were collected from 3-5 months old mature plants and washed with water and then chopped into small fragments. The materials were then shade dried at ambient temperature (32°C) for 4 -5 days and the drying operation was carried out under controlled conditions to avoid chemical changes. The dried samples were crushed into fine powder using an electronic blender. The powdered samples were stored in polythene containers at room temperature. The callus material obtained from various explants were collected at the end of four week and dried in hot air oven at 50°C for 48 hours. Then the dried material was powdered using mortar and pestle and the powdered samples were stored in polythene containers at room temperature**.**

D. Preparation of extracts

The organic constituents from dried plant (Leaf & Stem) material were obtained by continuously extracting the powdered material in soxhlet apparatus with ethanol: water (4:1) as organic solvent for 24 hours at 55^{oC} until complete exhaustion of the material. After completion of extraction, the extracts were passed through Whatman No.1 filter paper and the filtrate was concentrated in vacuum rotary evaporator at 60^{oC} in order to reduce the volume. The paste like extracts were stored in labeled screw capped bottles and kept in refrigerator at 4^{oC} [10]. Likewise extracts were also prepared using the solvent Chloroform: water (4:1) and the aqueous extract was prepared using distilled water and extracted at 100 ^{oC}.

E. Pharmacological studies

In the present investigation pharmacological study (Anti-inflammatory activity) was performed with an animal model to find out the efficacy of ethanolic extracts of leaf and leaf derived callus of *Tinospora cordifolia* (Grown under NaCl stress conditions).

a. Animal study

Albino rats (180 – 230 g) 4 -6 weeks old were obtained from Periyar college of Pharmaceutical Sciences for girls, Tiruchirappalli, Tamil Nadu, India and were kept in standard plastic animal cages in 4 groups of six animals each with 12 h of light and dark cycle in the institutional animal house. The animals were fed with standard rodent diet and provided water *ad libitum*. After one week of acclimatization the animals were used for further experiments. Approval from the institutional animal ethical committee for the usage of animals in the experiments was obtained as per the Indian CPCSEA guidelines.

b.Anti-inflammatory activity : Carrageenan induced hind paw edema

Anti Inflammatory activity of extracts of *Tinospora cordifolia* and their callus was determined by treating the animals having carrageenan induced hind paw edema. Albino rats of either sex weighing 150-200 gms were divided into four groups of six animals each. The dosage of the drugs administered to different groups was as follows. Group I served as Control, Group 2 and 3 received ethanol extracts of leaf and leaf derived callus of *Tinospora cordifolia* (100 mg/kg)

respectively and Group 4 was treated with Indomethacin (10 mg/kg) which served as the standard reference drug. All the drugs were administered orally. All the above drugs were given to rats orally four hours before the commencements of the study. At the time of study a booster dose of each drug was given and 30 minutes after the administration of the booster dose 0.1 ml of 1% w/v carrageenan [Carrageenan is a mixture of polysaccharide composed of sulphated galactose units and is derived from Irish Sea moss *Chondrus crispus*] solution in normal saline was injected into the sub plantar tissue of the left hind paw of the rat to induce edema and right hind paw served as the control. The quantum of the swelling was measured by determining the volume of water [14] or mercury [15] displaced in the plethysmograph. The volume displaced in the plethysmograph was measured at the end of 0 min., 60 min., 120 min., 180 min, 240 min., 360 min and 480 min. The per cent increase in paw edema of the treated group was compared with that of the control and the inhibitory effect of the drugs were studied. The relative potency of the extracts under investigations was calculated based upon the percentage inhibition of the inflammation.

$$\% \text{ Inhibition of inflammation} = \frac{(\text{Volume of control} - \text{Volume of treated}) \times 100}{\text{Volume of control}}$$

III.RESULTS AND DISCUSSION

In the present study ethanol extracts of leaf and leaf derived callus extracts of *Tinospora cordifolia* were taken to find out their anti inflammatory effects on carrageenan induced hind paw edema in albino rats. Paw volume of carrageenan treated animals (Group I) were increased with increasing time (Table). Rise in paw volume was retarded in group II (carrageenan + *T. cordifolia* leaf extract 100mg/kg) animals. The reduction in paw volume was significant after 3 hours of treatment.The percent inhibition of inflammation in group II was 34.49 while group III has showed 39.78 percent inhibition of inflammation in paw volume. The percent inhibition of inflammation in group IV treated with standard drug Indomethacin (10mg/kg) was 57.06. Among the tested extracts, leaf derived callus extracts of *T. cordifolia* showed the maximum percent inhibition (39.78) which was significant at $p<0.001$ than the control by student's t test.

Anti-inflammatory activity of leaf and leaf derived callus extracts of Tinospora cordifolia against carrageenan induced paw edema in albino rats

Treatment	% increase in paw volume Mean ± S.E (n=6)					% inhibition in paw volume
	Post insult time of assay in minutes					
	0	60	120	180	240	
Group I	28.72±1.83	83.75±7.33	99.90±4.7	113.42±7.9	119.73±4.32	---
Group II	31.3±2.8	51.7±4.3	63.5±5.3	74.3*±5.7	79.0±7.7*	34.49
Group III	24.7±2.3	45.9±3.7	57.3±4.5	68.3±3.9**	71.3±6.9*	39.78
Group IV	27.8±1.72	35.8±2.63	39.7±3.11	48.7±3.99**	49.72±4.42**	57.06

* $P < 0.01$ Vs Control
** $P < 0.001$ Vs Control by Students 't' test
[Group I - Control (carrageenan 0.5 ml/kg), Group II - *T. cordifolia* leaf extract (100 mg/kg), Group III – *T. cordifolia* leaf derived callus extract (100 mg/kg), Group IV- Indomethacin (10mg/kg)].

The results presented here may help to establish the scientific basis for utilization of *Tinospora cordifolia* for the treatment of pain and inflammation in folk medicine. Carrageeenan

injection into the rat paw provokes a local, acute inflammatory reaction that is a suitable criterion for evaluation of anti-inflammatory agents [15]. Induction of acute inflammation in control rats resulted in a prominent increase in paw thickness. Given table shows the results of anti-inflammatory activity. These results indicate that ethanol extracts of *Tinospora cordifolia* leaf (100 mg/kg) possessed significant ($p<0.01$ vs control) anti-inflammatory activity with a percent inhibition of 34.49 at 180 and 240 minutes of edema induction. Under the same experimental conditions, anti-inflammatory activity of leaf derived callus extracts of *T. cordifolia* (100 mg/kg) showed 39.78% inhibition in paw volume at 180 min of carrageeenan administration as 68.3% compared to control and 71.3% ($p<0.01$ vs control) at 240 min of carrageeenan administration.

The extracts showed good anti-inflammatory activity against acute inflammation. The inhibition by extracts was maximum after 3 h of administration of phlogistic agent. The effect of indomethacin (10 mg/kg) was comparable to that of leaf derived callus extract of *T. cordifolia* (100 mg/kg) after 4 h. Inflammation induced by carrageenan involves three distinct phases of the release of the mediator, including serotonin and histamine in the first phase (0-2 h), kinins in the second phase (3 h) and prostaglandin in the third phase (>4 h). In the first phase increase in vascular permeability occurs. Infiltration of leukocytes occurs in second phase and granuloma formation in third phase [12].

Inflammation is generally considered as an essentially protective response to tissue injury caused by noxious, physical, chemical (or) microbiological stimulus. It is a complicated process involving various mediators, such as prostaglandins, leukotriens and platelet activity factor [5]. The major macrophage derived inflammatory mediators such as pro inflammatory cytokines, tumour necrosis factor – α (TNF – α) and the reactive free radical nitric oxide (NO) synthesized by inducible NO synthase (iNOs), contribute to the development of inflammatory diseases [4] Exudates formation and leukocyte infiltration are important components of inflammation [6].

The investigation is based on the need for newer anti-inflammatory agents from natural source with potent activity and lesser side effects as substitutes for chemical therapeutic. Anti-inflammatory activity of *T. cordifolia* can be attributed to its berberine content because berberine has a variety of pharmacological effect including inhibition of 12-0-Tetradecamylphorbol β-acetate induced mouse ear edema [16]. Berberine was compared with Indomethacin in inhibiting PGE_2 concentration and carrageenan induced flush cavity in rats. This PGE_2 inhibition is corrected with reduced Cox-2 protein synthesis, but not by enzymatic activity [2]. Berberine also inhibits cyclooxygenase – 2 transcriptional activity [3] and Lipoxyzenase activity [7] which are involved in inflammation reaction. Similar findings have been reported by other researchers [17] .The anti-inflammatory effect of callus can be improved by dark stress, salt stress and precursor feeding by enhancing the secondary metabolite production.

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