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In vitro anti-inflammatory activity and tissue culture studies on *Tinospora cordifolia*

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Abstract

The present study deals with the *in vitro* anti-inflammatory in methanolic extracts of *in vivo* (leaf and stem) and *in vitro* (callus) plant parts of *Tinospora cordifolia* (Acanthaceae) is a medicinal plant of immense therapeutic value. The present work is based on developing a protocol for the callus induction in *T. cordifolia* from stem and leaf explants. The highest rate of callus (70.82%) was observed on MS medium fortified with 1.5 mg/L 2, 4-D and 0.3 mg/L BAP. The methanolic extracts of all plant parts exhibited remarkable anti-inflammatory activity action. The maximum amount of membrane stabilization was found in stem callus (78.15% at a dose of 100µg/ml) as compare to other plant and callus parts tested. Therefore, our studies support the isolation and the use of active constituents from *in vivo* and *in vitro* plant parts of *T. cordifolia* in treating inflammations.

Keywords: *Tinospora cordifolia*, callus, *in vitro* anti-inflammatory, HRBC membrane stabilization.

Introduction

Inflammation is a pathophysiological response of living tissue to injury that leads to local accumulation of plasmatic fluid and blood cells. Although it is a defense mechanism that helps body to protect it-self against infection, burns, toxic chemicals, allergens or other noxious stimuli, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases (Sosa *et al*., 2002).

All the steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) which have been used since the introduction of acetyl salicylic acid are heterogeneous group of compounds which share many pharmacological properties and side effects, are probably polycompetent in that they are able to modulate more than one mediator or cellular event concerned with the inflammatory response (Whitehouse, 1974). For this reason, in recent time, more interest is shown in alternative and natural drugs for treatment of various diseases, but there is lack of scientific evidence (David *et al*., 2014).

The resurgence of public interest in plant based medicine coupled with rapid expansion of pharmaceutical industries have necessitated an increased demand for medicinal plants, leading to over-exploitation that threatens the survival of

many rare species. Also, many medicinal plant species are disappearing at an alarming rate due to rapid agricultural and urban development, uncontrolled deforestation and indiscriminate collection. Plant tissue culture technology holds great promise for micropropagation, conservation, and enhancement of the natural levels of valuable secondary plant products and to meet pharmaceutical demands and reduce the *in situ* harvesting of natural forest resources (Yogananth *et al*., 2012).

The herb *Tinospora cordifolia* (Menispermaceae) is commonly known as Guduchi in India. It has a long history of use in Ayurvedic medicine (the traditional medicine of India). Evidence hints that *Tinospora* may have anti-cancer (Singh *et al*., 2005; Singh *et al*., 2004), immune stimulating (Rawal *et al*., 2004), anti-diabetic (Stanely *et al*., 2003; Rathi *et al*., 2002), cholesterol-lowering (Stanely *et al*., 2003) and liver-protective (Bishayi *et al*., 2002) actions. Taking into consideration the medicinal value and utility, the present study has been initiated to evaluate the callus induction and *in vitro* anti -inflammatory activity in methanolic extracts of *in vivo* (leaf and stem) and *in vitro* (callus) plant parts of *Tinospora cordifolia*.

Materials and Methods

The explants of stem and leaf of *Tinospora cordifolia* were collected in Medavakkam, Chennai for callus induction. The explants were washed in the liquid detergent Tween 20 for 3 min and then rinsed in running tap water for 10 min. The cleaned explants were surface sterilized with aqueous 0.1% mercuric chloride solution for 8 min followed by 10 rinses in sterile distilled water with one minute intervals. After trimming the cut ends, surface sterilized explants were planted on MS medium supplemented with 2, 4-dichlorophenoxy acetic acid (2,4-D) (1.0, 1.25, 1.5 and 2.0 mg/l) along with Benzyl amino purine (BAP) and Kinetin (KIN) (0.30 mg/l) for callus induction. The cultures were incubated under cool fluorescent lights with 3000 lux for 16 h at a temperature of 25± 2ºC and 70±10 relative humidity. Each experiment had 10 replicates and repeated at least three times. Data were documented up to seven weeks of culture.

The fresh stem leaves, stem callus and leaf callus of *T. cordifolia* were shade dried and powdered mechanically and stored in an air tight container. The extraction was carried out by hot percolation method using Soxhlet apparatus. The solvent used was ethanol. About 40 gm of powder was extracted with 200 ml of ethanol. The extract was concentrated to dryness under controlled temperature 40-50°C. The extract was preserved in refrigerator till further use.

The human red blood cell (HRBC) membrane stabilization method (Gandhisan *et al*., 1991) was used for this study. The blood was collected from healthy human volunteer who was not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2% dextrose 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Drug was prepared by 4 gram of stem, leaves, stem callus and leaf callus were macerated with 10ml of hyposaline (0.36% NaCl) and extracts is centrifuged at 3000 rpm. Various concentrations of drugs were prepared (25, 50 and 100µg/ml) using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of HRBC suspension were added. Test solution was incubated at 37^0C for 30 min and centrifuged at 3,000 rpm for 20 min and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm8. Hydrocortisone was used as reference standard and a control was prepared omitting the extracts.

Results and Discussion

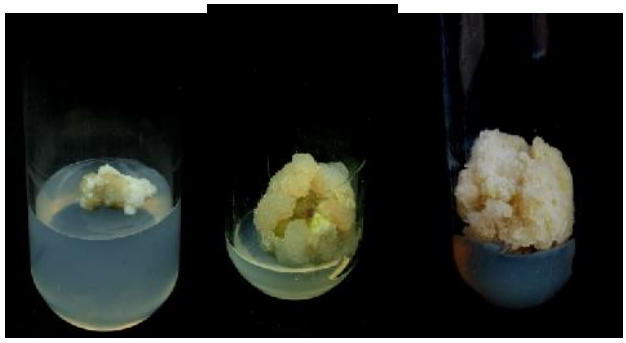
Stem and leaf pieces were used as a primary explants for callus induction. Callus initiation was observed from cut surface of leaves after 3 to 4 week of culture initiation. The leaf explants responded differently based on the concentrations of auxins and cytokinin present in the medium. In general, media containing high auxin and low cytokinin concentrations promote cell proliferation resulting in callus formation (Slater *et al*., 2003).Stem explants registered maximum amount of callusing (74.10 ± 0.55 %) was observed on the medium supplemented with combination of 2, 4-D and Kin (2.0 mg/l 2, 4-D,

and 0.3 mg/l KIN) after 3 weeks of culture initiation (Table 1 and Fig 1). Minimum response (41.83 + 0.32%) was obtained in leaf explants inoculated at 2,4-D 1.0 mg/l and BAP 0.30 mg/l combination (Table 2 and Fig 2). Nakano *et al*. (1994) found that NAA and 2,4-D alone could initiate callusing from stem, leaf and nodal segments but callus growth was slow. KIN along with auxins considerably enhanced callus growth. Similar to this result, the swelling of explants during the induction of callus was reported in the hypocotyl and leaf explants of *Meconopsis simpficifofia* (Sulaiman*et al*., 1991), nodal explants of *Dendrocalamus hamiltonii* Nees et Arn. Ex Munro (Godbole *et al*., 2002) and leaf explants of *Mentha piperita* (L.) (Sujana and Naidu, 2012).

The maximum growth rate in terms of fresh weight (3.83 ± 0.53 g) and dry weight (0.43 ± 0.06 g) was observed in the combination of 2, 4-D 1.5 mg/l and KIN 0.3 mg/l. Minimum growth rate 2.36 + 0.60 g fresh weight and 0.23 + 0.08 g dry weight was obtained in 1.0 mg/l 2, 4-D and 0.3 mg/l KIN combination. The induction of callus from other members of Menispermaceae *viz. Stephania cepharantha* Hayata (Suzuki *et al*., 1992) and *Coscinium fenestratum* (Nair *et al*., 1992) were reported when cultured on MS medium supplemented with 2, 4 – D + Kn/BAP. The callus obtained may be maintained on suitable culture condition either to regenerate the plantlets or to produce pharmaceutically important bioactive compounds.

Table 1. Effect of 2,4-D with kin and bap on callus induction, callus growth of young stem explants of *Tinospora cordifolia*

Hormones mg/l	Percentage of response	Fresh weight	Dry weight
2,4-D+KIN			
1.00 + 0.30	63.20± 1.21[a]	2.84±0.29[a]	0.27± 0.02[a]
1.25 + 0.30	66.20± 0.88[b]	3.32± 0.33[b]	0.33± 0.04[b]
1.50 + 0.30	72.06±0.67[d]	3.92± 0.50[d]	0.38± 0.01[e]
2.00 + 0.30	60.03 ± 0.78[e]	3.06± 0.18[c]	0.31± 0.02[d]
2,4-D+BAP			
1.00 + 0.30	62.10±1.05[a]	2.44 ±0.17[a]	0.23± 0.04[a]
1.25 + 0.30	69.06±0.67[c]	3.11±0.33[c]	0.27± 0.01[c]
1.50 + 0.30	71.53±0.48[c]	3.84±0.29[d]	0.31± 0.03[d]
2.00 + 0.30	74.10±0.55[d]	4.03± 0.15[e]	0.36± 0.02[e]



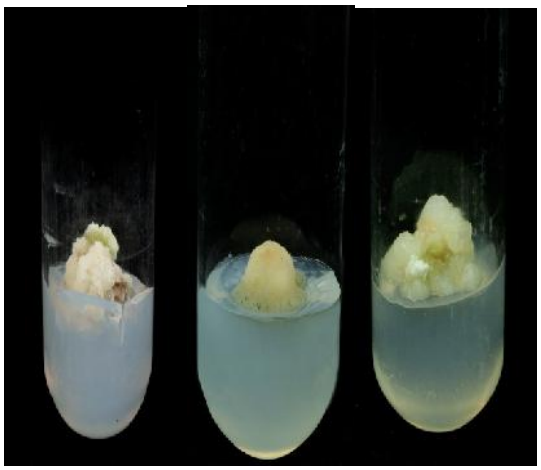
A) B) C)

Fig – 1: *In vitro* **callus induction from leaf explant of– Stage wise development in 2, 4-D + KIN concentration**

A) Callus induction in earlier stage**, B & C)** Matured callus

Table 2: Effect of 2,4-D with kin and bap on callus induction, callus growth of young leaf explants of *Tinospora cordifolia*

Hormones mg/l	Percentage of response	Fresh weight	Dry weight
2,4-D+KIN			
1.00 + 0.30	51.15 ± 0.83	2.93±0.53	0.30± 0.07
1.25 + 0.30	67.63 ± 0.65	3.80 ± 0.36	0.36 ± 0.02
1.50 + 0.30	70.82 ± 0.36	3.83 ± 0.53	0.43 ± 0.06
2.00 + 0.30	65.83 ± 0.368	3.10 ± 0.16	0.33 ± 0.01
2,4-D+BAP			
1.00 + 0.30	41.83 ± 0.32	2.36 ± 0.60	0.23 ± 0.08
1.25 + 0.30	64.25 ± 0.32	3.18 ± 1.93	0.30 ± 0.03
1.50 + 0.30	69.86 ± 0.53	3.66 ± 1.63	0.34 ± 0.03
2.00 + 0.30	58.30 ± 0.65	2.74± 0.83	0.24 ± 0.06



A) **B)** **C)**

Fig – 1: *In v itro* **callus induction from leaf explant of– Stage wise development in 2, 4-D + KIN concentration**

A) Callus induction in earlier stage,**B & C)** Matured callus

The extracts of the stem, leaves, stem callus and leaf callus of *T. cordifolia* were studied for *in vitro* anti-inflammatory activity by HRBC membrane stabilization method. Among all the extracts showed significant anti-inflammatory activity in a concentration dependent manner. Leaf callus extract at a concentration of 100µg/ml showed 78.15±1.13% protection of HRBC in hypotonic solution. All the results were compared with standard hydrocortisone which showed 80% protection (Table 2). The extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lyses of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane (Chou, 1997) and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release (Murugasan *et al*., 1981). Some of the NSAIDs are known to possess membrane stabilization properties which may contribute to the potency of their anti-inflammatory effect. Though the exact mechanism of the membrane stabilization by the extract is not known yet, hypotonicity-induced hemolysis may arise from

shrinkage of the cells due to osmotic loss of intracellular electrolyte and fluid components. The extract may inhibit the processes, which may stimulate or enhance the efflux of these intracellular components (Iwueke et al., 2006).

The present *in-vitro* study is a preliminary evaluation of anti-inflammatory activity of native and callus extracts of *Tinospora cordifolia* and demonstrated that folk medicine of these plants can be used to cure the inflammation. Further research work to analyze *in-vivo* anti-inflammatory activity of these two plants on animal models and to isolate the phytoconstituents responsible for anti-inflammatory activity are ongoing.

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