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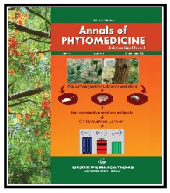

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Anti-inflammatory activity of leaf and leaf derived callus extracts of *Callicarpa tomentosa* L. : An endemic medicinal plant of Western Ghats, Karnataka, India on carrageenan induced rat paw edema

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Abstract

In the present investigation, anti-inflammatory activity of *Callicarpa tomentosa* L. leaf and leaf derived callus extracts were examined. This plant is used frequently in traditional medicine for the treatment of inflammatory and microbial diseases. In the current study, methanol extracts of the leaf and leaf derived callus were evaluated for the anti-inflammatory activity using carrageenan induced paw edema method by using diclofenac sodium as standard. Callus cultures were developed from the leaf explants on MS medium supplemented with 2,4-D (2 mg/l) and BAP (2 mg/l). The activity was carried out in three different dose concentrations (100, 200, 400 mg/kg body weight of the animal). Results revealed that the plant extracts showed significant anti-inflammatory activity with the percentage inhibition of 64.10% and 52.51% in case of leaf and leaf derived callus, respectively (at 400 mg/kg b.w). Results are promising and also ascertain that leaves of *C. tomentosa* have anti-inflammatory potential, compared to the standard. Therefore, there is a scope of using this as a potent anti-inflammatory drug.

1. Introduction

In the present world, 25% of the drugs are derived from plant sources and several others are synthetic analogues built on the prototype compounds isolated from plants (Yadav *et al.*, 2011). The attention of pharmacologists throughout the world is focused on finding a safe and potent anti-inflammatory drug. Today, the natural products symbolize safety in contrast to synthetic drugs that are regarded as unsafe to humans and environment (Malik *et al.*, 2020; Warrier, 2021). Medicinal plants are capable of synthesizing an overwhelming variety of low molecular weight compounds known as secondary metabolites usually with unique and complex structures (Tellez *et al.*, 2000). The Indian folklore medicine comprises numerous prescriptions for therapeutic purposes such as healing of wounds, inflammation, ulcers and snake bites (Jones *et al.*, 2007).

Inflammation, which is a pattern of response to injury, involves the accumulation of cells and exudates in irritated tissues, that allows the protection from further damage. Inflammation has been studied for thousands of years in an attempt to combat its various effects on the body (Yuan *et al.*, 2006). Although, it is a defence mechanism, the complex events and mediators involved the inflammatory reaction which can induce, maintain or aggravate many diseases. Therefore, the use of anti-inflammatory agents is helpful in the therapeutic treatment of several diseases (Sosa *et al.*, 2002).

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This type of research is of considerable importance in public health sector, since malnutrition (modern dietary habits) is linked to inflammation, ageing and other degenerative processes (Charami *et al.*,2008). Lamiaceae comprises approximately 236 genera, cosmopolitan in distribution (Li *et al.*, 2016). Plants of this family are widely used in traditional medicine due to their anti-inflammatory, antirheumatic, antiulcer, digestive and antimicrobial properties which are attributed to their phenolic and terpenoid content (Koleva *et al.*, 2003; Bhatt, 2019).

C. tomentosa is commonly known as velvety beauty berry belongs to the family Lamiaceae. It is endemic to Western-Ghats of Karnataka, India. This plant is widely used in ethnomedicine to cure various human diseases.The plant has been used for centuries in traditional medicine for prevention and treatment of wide range of health disorders such as inflammation and rheumatism (Mei *et al.*, 2011; Shrilakshmi *et al.*, 2021). Oral administration of root and bark decoction of the plant is used for the treatment of liver disorders (Lalawmpuii *et al.*, 2015). It is a useful medicinal plant for the treatment of various disorders like diarrhoea, dysentery, diabetes and fever. In Ayurvedic system of medicine, the plant is also known as Rishipatri and used for obstetric conditions. On the basis of the common uses of this plant in traditional folk medicine and its above reported activities, we have evaluated the anti-inflammatory potential of leaf and leaf derived callus extracts of *C. tomentosa*.

2. Materials and Methods

2.1 Collection of plant material

The fresh plant material was collected from Kodagu district, Karnataka and brought to the laboratory for further studies. The

material was identified by the plant taxonomist, department of studies in botany, University of Mysore. The collected plant material was washed thoroughly with running tap water and wiped with blotting paper. It was then shade dried in room temperature.

2.2 Preparation of media and callus induction

Defined medium for the growth of cell culture consists of inorganic salts, a carbon source, vitamins, growth regulators and some organic supplements.Various basal media like White medium, Nitsch and Nitsch medium, B5 medium and Gamborg medium (Khan *et al.*, 1988) have been employed, but most widely used culture medium is Murashige and Skoog (1962) medium (MS medium). Callus induction is significantly affected by the type of media used. MS medium supplemented with suitable concentration of growth regulators was prepared.Young and healthy leaf explants were washed under running tap water to remove the dirt and soil, then treated with Bavistin, followed by tween-20. Then, surface sterilized with 70% alcohol for 30 sec and 0.1% mercuric chloride for 2-3 min. They were further washed with sterile double distilled water. Under aseptic conditions, the explants were air dried and inoculated on MS medium supplemented with growth regulators [2,4-D (2 mg/l) and BAP (2 mg/l)].

2.3 Preparation of extracts

The dried plant (leaf and leaf derived callus) samples were ground well into a fine powder using blender and stored in air tight containers for further use. 30 g of each sample was extracted with 150 ml of methanol by Soxhlet extraction method. The methanol extracts were further evaporated to complete dryness and stored in airtight containers for further use.

2.4 Animals

Albino rats of either sex weighing 100-150 g were selected for the experiment with the permission of Institutional Animal Ethical Committee (No. UOM/IAEC/15/2021). They were housed in standard metal cages. The rats were allowed one-week acclimatization period before the experimental sessions. The rats were divided into 8 groups, each group containing 3 animals (Table 1).The animals were fasted for 12 h before the experiment. The experimental protocol was approved by the Institutional Animal Ethics Committee. The care of laboratory animals was taken as per the guidance of CPCSEA, Government of India.

2.5 Evaluation of anti-inflammatory activity using carrageenan paw edema method

The anti-inflammatory activity was carried out following the method of Winter *et al.* (1962).In brief, Group I animals served as negative control and received normal saline of 1 mg/kg concentration orally. The Group II animals served as positive control and were administered with standard drug diclofenac sodium at the dose concentration of 20 mg/kg body weight of the animal.The animals of the Groups III, IV, V were administered with the leaf extract of *C. tomentosa* in normal saline at the doses of 100, 200, 400 mg/kg b.w., respectively by peroral route. Similarly, animals of Groups VI, VII, VIII were administered with the same concentrations of leaf callus extract, respectively.

Table 1: Grouping of animals

Groups	Extract	Concentration (mg/kgb.w.)	Number of animals
I	Diclofenac sodium (+ control)	20	3
II	Saline (- control)	_	3
III	Methanol leaf extract	100	3
IV		200	3
V		400	3
VI	Methanol leaf callus extract	100	3
VII		200	3
VIII		400	3

Acute paw edema was induced by injecting 0.1 ml of 1% (w/v) carrageenan solution, prepared in normal saline in sub-plantar region of the left hind paw of the rat. The ankle joint of the rats was marked with permanent marker and the paw was dipped in mercury. The volume of the hind paw of the rats upto the ankle joint was measured plethysmographically by the mercury displace method. Measurements were taken at 1, 2, 3, 4 and 5 h after the administration of carrageenan. The change in the paw volume was determined by comparing it to the initial volume and the percentage inhibition was calculated for each group with the respective vehicle treated control. The percentage inhibition was calculated by the following formula:

$$\% \text{ inhibition} = (1 – Vt / Vc) \times 100$$

where Vt and Vc are the mean change in paw volume of treated and control rats, respectively.

3. Results

3.1 Callus induction

Callus cultures were developed from the leaf explants on MS medium supplemented with 2,4-D (2 mg/l) and BAP (2 mg/l). The callus obtained was soft creamish which later turned brownish. The callus obtained under *in vitro* conditions was further dried in the hot air oven at 60°C and stored in air tight containers.

3.2 Evaluation of anti-inflammatory activity

The methanol extracts of leaf and the leaf derived callus were used to study the anti-inflammatory activity. The results are represented as percentage inhibition of paw edema. The sub-plantar injection of carrageenan produced a local edema that increased progressively to reach its maximum in 3-5 h. Both the leaf and callus extracts showed significant anti-inflammatory activity. The extracts showed time dependent inhibitory activity over a period of 5 h.The extracts showed maximum inhibition at the concentration of 400 mg/kg during the 5th h of study. The methanol extract of the leaf showed a good inhibition of 64.10% compared to that of leaf callus which showed the inhibition of 52.51%. While, the standard drug diclofenac sodium exhibited the inhibition of 76.8% (Table 2).

At 100 mg/kg b.w. concentration (Figure 1), the leaf extract showed the percentage inhibition of 15.26, 16.13, 18.11, 19.82 and 22.11 (after 1, 2, 3, 4 and 5 h, respectively). While, the leaf derived callus extract exhibited the inhibition percentage of 11.11, 12.01, 14.64,

16.17, 18.19 (after 1, 2, 3, 4 and 5 h, respectively). At the concentration of 200 mg/kg b.w. (Figure 2), the leaf extract showed the percentage inhibition of 18.03, 21.84, 31.90,39.00, 50.30 (after 1, 2, 3, 4 and 5 h, respectively). While, the leaf callus extract exhibited the inhibition percentage of 14.37, 16.54, 22.33, 28.90, 39.45 (after 1, 2, 3, 4 and 5 h, respectively).

The extracts showed maximum percentage inhibition at 400 mg/kg b.w. concentration (Figure 3). After 1,2,3,4, and 5 h of study, the leaf extract exhibited the percentage inhibition of 22.87, 26.00, 37.32, 43.17 and 64.10, respectively, While, the leaf derived callus extract showed the percentage inhibition of 17.23, 20.87, 31.34, 39.00 and 52.51, respectively.

Table 2: Anti-inflammatory activity of methanol extracts of leaf and leaf callus of *C. tomentosa*

Extracts	Conc. (mg/kg b.w.)	Percentage inhibition				
		1 h	2 h	3 h	4 h	5 h
Leaf	100	15.26 ± 0.15^{d}	16.13 ± 0.12^{e}	18.11 ± 0.71^{e}	19.82 ± 0.34^{e}	22.11 ± 0.23^{e}
	200	18.03 ± 0.87^{c}	21.84 ± 0.31^{c}	31.91 ± 0.16^{c}	39.41 ± 0.75^{c}	50.31 ± 0.67^{c}
	400	22.87 ± 0.95^{b}	26.32 ± 0.24^{b}	37.23 ± 0.45^{b}	43.17 ± 0.17^{b}	64.31 ± 0.42^{b}
Leaf callus	100	11.11 ± 0.42^{f}	12.01 ± 0.17^{f}	14.64 ± 0.33^{f}	16.17 ± 0.12^{f}	18.19 ± 0.15^{f}
	200	14.37 ± 0.33^{e}	16.54 ± 0.41^{e}	22.33 ± 0.14^{d}	28.90 ± 0.61^{d}	39.45 ± 0.34^{d}
	400	17.23 ± 0.15^{d}	20.87 ± 031^{d}	31.34 ± 0.25^{c}	39.73 ± 0.54^{c}	52.51 ± 0.12^{c}
Standard	20	33.92 ± 1.04^{a}	42.33 ± 1.07^{a}	53.87 ± 0.97^{a}	64.18 ± 1.03^{a}	76.82 ± 1.02^{a}

Standard drug diclofenac sodium was used as reference at the dose of 20 mg/kg b.w. Mean ± SD, followed by the same superscripts are not statistically significant, when subjected to SPSS package ver. 14.0 Tukey's mean range test at 0.05 % level.

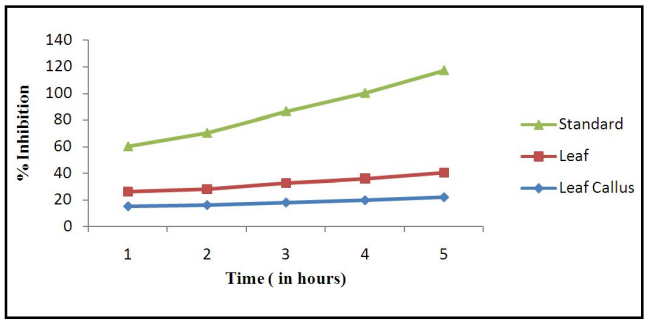

Figure 1: Anti-inflammatory activity of methanol extracts of leaf and leaf derived callus of *C. tomentosa* (at the dose of 100 mg/kg b.w.) with reference to standard.

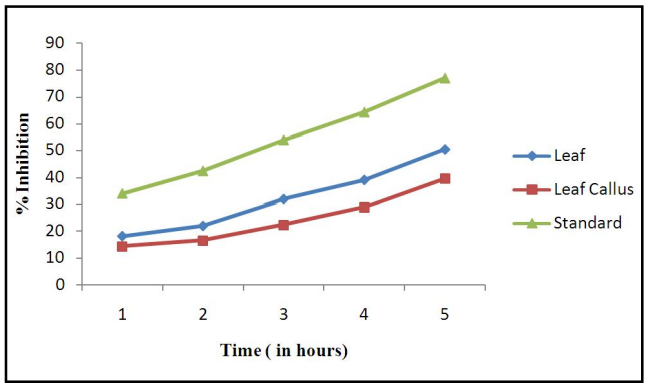

Figure 2: Anti-inflammatory activity of methanol extracts of leaf and leaf derived callus of *C. tomentosa* (at the dose of 200 mg/kg b.w.) with reference to standard.

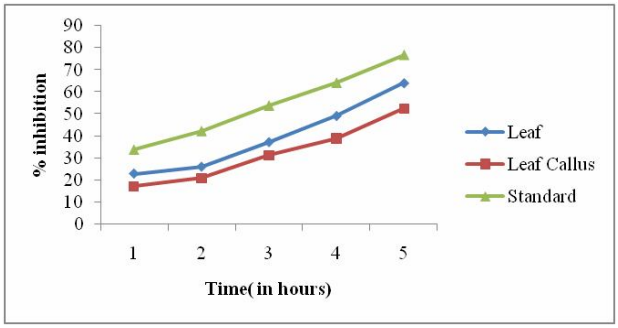

Figure 3. Anti-inflammatory activity of methanol extracts of leaf and leaf derived callusof *C. tomentosa* (at the dose of 400 mg/kg b.w.) with reference to standard.

4. Discussion

The present study was carried out to assess the validity of the plant *C. tomentosa* in the traditional medicine for the treatment of inflammatory disorders. Although, the anti-inflammatory effects of medicinal plants have been extensively studied, evaluation of these properties in rare and endemic species are scarcely reported. The evaluated plant extracts were effective in reducing the carrageenan-induced paw edema. This may support the popular use of plants in the treatment of inflammation (Meckes *et al.*, 2004). Both *in vivo* and *in vitro*, methods are available for the evaluation of anti-inflammatory agents. Among the *in vivo* methods, carrageenan induced rat paw edema assay is believed to be one of the most reliable and most widely used. Oral route of administration of drug is a common approach to administer the drug (Winter *et al.*, 1962).

Carrageenan is widely used to induce experimental inflammation for the screening of compounds possessing anti-inflammatory activity (Humbal *et al.*, 2019). This agent, when administered orally or injected locally into the rat paw, produces a severe inflammatory reaction (Yadav *et al.*, 2012).The development of edema induced by carrageenan corresponds to the events in acute phase of inflammation, mediated

by histamine, bradykinin and prostagladins produced under the effect of cyclooxygenase (Borgi *et al*., 2007).The edema developed in the rat paw after the injection of carrageenan is a biphasic event. The initial phase is attributed to the release of histamine and serotonin, followed by the release of prostaglandin like compound in the later phase (Nayak *et al*., 2010).

Callicarpa species are known to have therapeutic effects, hence, used in the formulation of herbal medicines. Several species of this genera have been evaluated for their bioactive potentialities including anti-oxidant and anti-inflammatory properties. *Callicarpa nudiflora* has been evaluated for its anti-inflammatory properties (Wu *et al*., 2020). Chemical profiling based on the identified components from *C. nudiflora*, a compound-target network for the anti-inflammatory effect was constructed. The anti-inflammatory activity fractions were isolated from *C. kwangtungensis* Chun. and were purified by column chromatography (Jia *et al*., 2012). Five new compounds were identified which showed significant *in vitro* anti-inflammatory activity. Aqueous as well as ethanolic extracts of leaves of *C. macrophylla* were evaluated for their anti-inflammatory activity using carrageenan paw edema method using diclofenac sodium as standard (Yadav *et al*., 2011). The ethanolic extract of *C. macrophylla* leaves showed better anti-inflammatory profile than the aqueous extract and could be the choice to be used as anti-inflammatory drug. The extracts of *C. japonica* has preventive potential for the development of allergic asthma. The plant exhibited the anti-inflammatory effects in lipopolysaccharide (LPS)-induced acute lung injury (ALI) animal models *via* regulation of inflammatory cytokines (Kim *et al*., 2019).

The protective effects of *C. japonica* has also been identified in cigarette smoke-induced pulmonary inflammation in mice (Lee *et al*., 2019). Studies have shown that two phenylpropanoids (forsythoside B and verbascoside) isolated from this plant exert anti-inflammatory properties under both *in vitro* and *in vivo* studies (Liu *et al*., 2019).

5. Conclusion

The findings presented here indicate that the methanol extracts of the leaf and leaf callus of *C. tomentosa* show a potent anti-inflammatory activity. Its effective in the inhibition of carrageenan induced paw edema in albino rats. The extracts showed time dependent inhibitory activity over a period of 5 h. The extracts were also tested at three different dose levels to know if they were dose dependent. At the different dose ranges used (100, 200, 400 mg/kg), there was a significant difference in their anti-inflammatory activity, hence, they were also found to be dose dependent.

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Conflicts of interest

The authors declare no conflicts of interest relevant to this article.

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