

Assessment of acute anti-inflammatory effect of *Carissa carandas* leaf extracts in Wistar Albino Rats

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ABSTRACT

Carissa carandas (Apocynaceae), commonly known as *Karamcha* in Bengali is a woody, climbing shrub indigenous to India and grown in South-East Asian countries. The present study assessed two defatted successive solvent (ethyl acetate and methanol) extracts of *C. carandas* leaf for their acute anti-inflammatory potential by carrageenan induced hind paw oedema in Wistar albino rats. Both the test extracts exhibited significant and comparable anti-inflammatory activity. The methanol extract was found to be a bit more active than the ethyl acetate extract. The present preliminary study demonstrated promising acute anti-inflammatory activity of *C. carandas* leaf in Wistar rats.

Keywords: Anti-inflammatory, *Carissa carandas*, oedema, leaf, carrageenan.

INTRODUCTION

Inflammation is a patho-physiological response to injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed functions. Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. It is the body response to inactivate or destroy the invading organisms, to remove the irritants and set the stage for tissue repair. It is triggered by the release of chemical mediators from injured tissue and migrating cells. [1] The most commonly used drug for management of inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs), which have several adverse effects especially gastric irritation leading to formation of gastric ulcers. [2, 3]

Natural products have contributed significantly towards the development of modern medicine. Recently traditional medicine worldwide is being re-evaluated by extensive research on different plant species and their active therapeutic principles. The

rich wealth of plant kingdom can represent a novel source of newer compounds with significant anti-inflammatory activities. [4] The major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects and low cost.

Carissa carandas L. (Apocynaceae), commonly known as *Karamcha* in Bengali is a woody, climbing, flowering shrub indigenous to India and common throughout much of India, Burma and Malacca and dry areas of Ceylon. It is often grown in Thailand, Cambodia, South Vietnam and in East African countries. [5] It is commercially cultivated in India for its consumable and palatable fruits commonly used as a condiment in Indian pickles and spices. Several phytochemical studies were reported on this plant. [6] However, pharmacological studies on this plant are comparatively scanty. Therefore, in the present investigation we attempted the acute anti-inflammatory assessment of defatted ethyl acetate and methanol extracts from *C. carandas* leaf grown in India.

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MATERIALS AND METHODS

Plant material: The mature leaves of *Carissa carandas* L. (Apocynaceae), were collected during November 2013 from Kalyani, Nadia, West Bengal, India. The plant material was taxonomically identified

at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India. The voucher specimen (CNH/18/2014/Tech.II/74) was maintained in our research laboratory for future reference. The plant material (leaves) was shade-dried with occasional shifting and then powdered with mechanical grinder, passing through sieve no. 40, and stored in an air-tight container.

Preparation of plant extracts: The dried powdered material was defatted with benzene. The defatted powdered material thus obtained was further successively extracted with ethyl acetate and methanol for 72 h in a percolator. The solvent was distilled off in reduced pressure and resulting semisolid mass was vacuum dried using rotary flash evaporator to yield a solid residue and the percentage extractive values were accordingly 2.43% w/w and 14.1% w/w respectively. The preliminary phytochemical analysis was performed for all three extracts to identify the phytoconstituents present in the extracts. [7]

Drugs and chemicals: Indomethacin and λ -carrageenan were from Sigma-Aldrich Chemical Corp. (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade obtained commercially.

Experimental animals: Adult Wistar rats of Wistar strain weighing 200 ± 20 g were procured from registered breeders (Reeta Ghosh & Co., Kolkata, India) and maintained under standard laboratory conditions (temperature $25 \pm 2^\circ\text{C}$ with dark and light cycle 14/10 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The mice were acclimatized to laboratory condition for 10 days before commencement of the experiment. All experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee (Reg. no. 367001/C/ CPCSEA).

Anti-inflammatory evaluation: Carrageenan-induced rat paw oedema:

The overnight fasted rats were divided into four groups ($n = 6$). The first group (which served as

control) received normal saline (5 ml/kg body wt., p.o.). The second and third groups received the ethyl acetate and methanol extracts at the doses of 150 mg/kg b.w., p.o. respectively. The fourth group (which served as reference) received indomethacin (10 mg/kg body wt., p.o.). After 30 mins, acute inflammation was produced by the subplantar administration of 0.1 ml of 1 % (w/v) of freshly prepared suspension of λ -carrageenan in the right hind paw of each rat. The paw volume was measured at 0 h and after 1 h intervals up to 4 h after carrageenan challenge by using a plethysmometer (Ugo Basile, Italy). The difference between the two readings was taken as the volume of oedema and the percentage of inhibition was calculated with respect to control and expressed the percentage of protection by using the following formula: [8]

$(\text{Control mean} - \text{Treated mean} / \text{Control mean}) \times 100$ %.

Statistical analysis: The data are represented as mean \pm standard error of mean (SEM). Degree of significance was assessed by Student's 't' test. *P* values less than 0.001 were considered as statistically significant.

RESULTS AND DISCUSSION

Preliminary phytochemical studies showed the presence of triterpenoids and steroids in the benzene and ethyl acetate extracts; and triterpenoids, alkaloids, phenolic compounds, glycosides and carbohydrates in the methanol extract.

The present study establishes the significant acute anti-inflammatory activity of *C. carandas* leaf extracts in experimentally induced acute inflammation in Wistar rats. The inflammatory response can be readily produced in the form paw oedema with the help of irritants or phlogistic agents. Such agents like carrageenan, formalin, bradykinin, histamine, serotonin etc when injected into the dorsum of the foot of the rats they produce acute paw oedema within a few minutes of injection. Carrageenan induced rat paw oedema has been most commonly used as an

ideal experimental animal model for acute inflammation. [9, 10]

Carrageenan-induced acute inflammatory oedema is generally believed to be a biphasic response. The early phase (1-2 h) of the carrageenan model is mainly mediated by histamine and serotonin (5-HT). The late phase (2-4 h) is mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages [11]. In the present study, both the test extracts produced significant inhibition of carrageenan induced rat paw oedema after a period of 4 h (Table 1). This indicates the two test extracts were active in both the early and late phases of carrageenan induced acute hind paw inflammation in rats. The methanol extract was found to be slightly more active than the ethyl acetate extract.

Preliminary phytochemical studies revealed presence of phenolic compounds in methanol extract. Polyphenolic compounds are putative natural

products that are known to several important biological activities including anti-inflammatory properties. [12, 13] The polyphenols content may be responsible for its higher anti-inflammatory action against carrageenan-induced acute inflammation in albino rats.

Based on the results obtained from the present preliminary study, it can be concluded that both the defatted ethyl acetate and methanol extracts of *Carissa carandas* leaf possessed comparably effective acute anti-inflammatory actions in Wistar albino rats. Further studies are presently necessary to confirm the identity of the bioactive principles responsible for these actions.

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Table 1: Effect of *Carissa carandas* leaf extracts on carrageenan induced rat paw oedema.

| Treatments | 1 h | 2 h | 3 h | 4 h | % Inhibition |
|-----------------------------------|------------|------------|------------|------------|--------------|
| Normal control | 0.73±0.08 | 1.40±0.57 | 1.80±0.57 | 1.66±0.08 | - |
| Indomethacin (10 mg/kg) | 0.20±0.05* | 0.50±0.05* | 0.36±0.03* | 0.23±0.03* | 86.14 |
| Ethyl acetate extract (150 mg/kg) | 0.33±0.06* | 0.54±0.09* | 0.42±0.02* | 0.29±0.06* | 82.53 |
| MeOH extract (150 mg/kg) | 0.28±0.04* | 0.44±0.03* | 0.33±0.05* | 0.26±0.07* | 84.34 |

Values are mean ± SEM (n = 6). *p < 0.001 when compared with normal control.

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