Anti-Inflammatory activity of *Morinda tinctoria* on Carrageenan induced Rat Paw Edema

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Research Article

Abstract

*Morinda tinctoria* (Rubiaceae) is commonly found in the regions of India, Burma, Sri Lanka, Cambodia and Indonesia. This plant is used in the treatment of asthma and bronchitis. Ethanolic extract of whole plant of *M. tinctoria* was screened for anti-inflammatory activity in carrageenan induced rat paw edema at the dose level 100, 200 and 400mg/kg was compared with the standard drug, Diclofenac sodium. The extract produced significant and dose dependent inhibition of carrageenan induced significant (p<0.05) when compared to control. Preliminary phytochemical analysis of *M. tinctoria* indicated the presence of PO, GSH, Vitamin E, Vitamin C, Calcium and magnesium. Further studies may reveal the exact mechanism of action and phytochemical constituents responsible for anti-inflammatory activity.

Keywords: Anti-inflammatory, Carrageenan, Paw edema, Morinda tinctoria

Introduction

The genus *Morinda*, belonging to the family Rubiaceae, is indigenous to tropical countries and is considered an important traditional folk medicine. Many species of *Morinda* are available in India of which *Morinda tinctoria* predominantly grows as a weed tree in vacant agricultural land and especially on uncultivated lands and along the boundaries of the cultivated field. Ancient writings reveal that *Morinda* has long been cultivated in different parts of Tamil Nadu state in India. In particular, the species *Morinda citrifolia* is reported to have a broad range of therapeutic and nutritional values [1]. There is great demand for its fruit juice in alternative medicine for different kinds of illnesses such as arthritis, diabetes, muscle aches, menstrual difficulties, heart disease, cancers, gastric ulcer, blood vessel problems, and drug addiction [2]. Although the South Indian ancestor realized the therapeutic value of *M. tinctoria* and used it in the traditional Indian medicinal systems like siddha, lack of proper documentation resulted in loss of that knowledge.

Previously wound healing property and anticonvulsant activity has been reported in this plant [3 & 4]. Inflammation and pain are local response of living mammalian tissue to injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agent. There are various components to an inflammatory reaction that can contribute to the associate symptoms and tissue injury. Edema formation, leukocyte infiltration and granuloma formation represents such components of inflammation [1]. Edema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability or the mediators that increase blood flow [5]. Carrageenan – induced paw edema is widely used for determining the acute phase of inflammation. Histamine 5-hytrax tryptamine and bradykinin are the first detectable Mediators in the early phase of carrageenan induced inflammation [6] whereas prostaglandins are detectable in the late phase of inflammation [7]. It is believed that current analgesia inducing drugs such as opiates and non-steroidal inflammatory drugs are not useful in all cases, because of their side for other effects and potency [8]. The present study was initiated with the aim of investigating the medicinal and therapeutic properties of *M. tinctoria* by evaluate the anti-inflammatory potentials of the methanolic extract.
Materials and Methods

Plant material

Raw leaves were collected from a *M. tinctoria* tree located in Thanjavur, Tamilnadu India and identified, verified at Botanical survey of India, Coimbatore. Voucher specimens are being deposited in the department of Botany, A.V.V.M. Sri Pushpam College (Autonomous), Poondi (Var tinctoria–NHSK/ 1001-1010) for future references.

Preparation of Extract

The fresh leaves were washed with tap water, rinsed with distilled water, and air-dried. They were ground to a powder using a mortar and pestle and stored at 20°C until use. The dried powder material (5000) of the leaves of *M. tinctoria* was extracted with 2000 ml of ethanol in an extract apparatus. The menthols extract was then distilled evaporated and dried in vacuum. The resulted extract yield was 7.45%.

Phyto chemical profile

The phytochemical investigation showed the presence of the following, constituents, the presence of Malondialdehyde (LPO) [9], Glutathione (10), Vitamin C [11], α - Tocopherol (Vitamin – E) [12] Serum total protein [13], Calcium [14] and Magnesium [15].

Animals

Swiss albino mice of either sex weighting 20 – 25g or albino wistar rats of either sex weighting 180 – 300g were used for study the were maintained under standard environmental conditions and were *jel* with standard pellet diet with water and libitum.

Determination of Anti-inflammatory activity

*M. tinctoria* was evaluated for anti-inflammatory activity by carrageenan induced rat paw edema method [16 & 17]. Male wistar rats (150 – 200g) were randomly distributed in for group of five animals each. The first group served as a control. Second group served as the standard (diclofenac sodium 5 ml) while the third and fourth group received 200g and 400g of *M. tinctoria*. One hour after the administration of the test drugs 0.1ml of 1% w/v suspension was injected in to the sub plantar region of the hind paw to the all four groups.

The paw volumes were measured using plethysmometer. Every hour noted paw values. The parentage inhibition was calculated by using the formula [18].

\[
\% \text{Inhibition} = \frac{V_c - V_t}{V_c} \times 100
\]

Where \(V_c\) = edema volume of control

\(V_t\) = edema volume of test

Statistical analysis

The results were expressed as mean ± S.E.M. statistical analysis was carried out by using ANOVA followed by Dunnet's multiple comparison tests graph pad PRISM software version 4.03 (2005). \(p < 0.5\) was considered significant.

Results and Discussion

The methanol extract of *M. tinctoria* was found to be non-toxic up to the dose of 2000 mg/kg and did not cause any death of the tested animals. The phytochemical tests with the methanol extract of *M. tinctoria* indicated the presence of Malondialdehyde (LPO), Glutathione (GSH), Vitamin E, C, Calcium, Magnesium, Total protein, albumin, antibody and WBC (neutrophils).

The paw value was observed in the 4 groups of rats in the time interval of 1st, 2nd, 3rd and 5th period. In the control group 3rd hr sample had maximum volume of paw value than others. In the drug treated, paw value was decreased than control. Whereas root treated group had higher percentage than leaf treated group (Fig 1). Similarly results were observed (19)

The level of LPO and GSH were analyzed in both (normal and experimental) conditions. The level analyzed LPO was increased in control than other groups. GSH level was decreased in control than other groups. Similarly, [20] reported that the level of LPO increase was due to carrageenan damage on membrane lipids and also it may causes nitrate production. However synthetic drug and plant drug treated groups had minimized the production of LPO than control group. Similarly the level of GSH was decreased in control than other groups. (Fig 2)
**Fig. 1** The level of LPO and GSH in Carragennan induced paw edema

**Fig. 2** The level Vitamin E and Vitamin C in Carragennan induced paw edema
Fig. 3 The level of Calcium and Magnesium in Carragennan induced paw edema

Fig. 4 The level of Total protein and Albumin in Carragennan induced paw edema
The despit level of Vitamin E and Vitamin C in normal and experimental animals. The level of Vitamin E, Vitamin C decreased in control than others. However vitamin E is an important chain breaking antioxidant [21] and protects cellular membrane against lipid per oxidations [22], as well as Vitamin C is also important water soluble antioxidant in biological system and essential micro nutrient required for normal metabolic functioning of the body [23]. Vitamin C needs to maintain the level of Vitamin -B at optimum concentration. The reduced level of vitamin C and vitamin E in control group in direct that utilization of these of scavenging of free radicals which are produced from carrageenan induction. Administration of Diclofenac sodium, plant drug (leaf, root) was significantly increased the level of Vitamin c and Vitamin E in carrageenan injected groups. (Fig 3)

Shows the level of calcium and Magnesium in normal, carrageenan and drug treated animal groups. The level of serum calcium was increased and magnesium levels were decreased in control than other groups. The progression of bone destruction appeared in rheumatoid arthritis is due to increased free radical activity [24]. Carrageenan induced paw edema was taken as prototype of exclusive phase of attributable to the release histamine, serotonin and kin in the first hour after injection of carrageenan. A more pronounced second phase is reeled to the release of prostaglandins like sub stannous in 2-3 hrs. So the level of calcium was increased and the magnesium level was decreased in control group, utilization of migration for complement pashvay may leads to declined level.

The level of total protein and albumin were analyzed in normal and experimental condition (Fig 4). These levels were decreased in control than other groups. The site specific oxidative damage of some of the susceptible amino acids of protein is now recorded as the major causes of metabolic dysfunction during pathogenesis [25]. Hence, decline in total protein content can be demand as useful index of the severity of cellular dysfunction in diseases. Albumin level also declined in inflammatory conditions. But plant treated and drug treated groups got near normal level by its anti-inflammatory activity.

The inflammatory antibody IgE level and WBC level were increased in control group than other groups (Fig 5). The rapid extracellular mediators those are stored in the cells cytoplasmic granules and synthesis and secretion of leukotrienes and prostaglandins [26]. The functions of mast cells can be manipulated for therapeutic ends by regulating their function with appropriate drugs. In this direction the Moringa tinctoria is an attractive candidate in this present study.

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References


