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SCREENING OF ANTI-INFLAMMATORY ACTIVITY OF ACACIA TORTA (ROXB.) CRAIB USING COTTON PELLET INDUCED GRANULOMA

Girish N*, Anil Kumar KV, Beere Nagaraju

Department of Pharmacology, Visveswarapura Institute of Pharmaceutical Sciences, Bengaluru-560070, India.

ABSTRACT

Inflammation is part of self-limiting non-specific immune response, which occurs during bodily injury. In some disorders, the inflammatory process becomes continuous, leading to the development of chronic inflammatory diseases including cardiovascular diseases, diabetes, cancer etc. Several Indian tribes especially Gummanayakanapaly tribes in Karnataka used the stem bark extracts of *Acacia Torta (Roxb.) Craib* for treating inflammatory disorders. The objective of the present study was to evaluate the stem extracts of *Acacia Torta (Roxb.) Craib* were evaluated for anti-inflammatory activity in albino wistar rats using cotton pellet induced granuloma model and indomethacin as a reference drug. Ethanol and water were used to prepare stem extract by soxhlet extraction method. The extract was evaluated by using cotton pellet induced granuloma model in albino wistar rats at the dose of 100 mg/ kg/ p.o. and 400 mg/ kg/ p.o. and indomethacin at 10 mg/kg were used for the study. The hydro alcoholic extract of stem *Acacia Torta* was screened for cotton pellet induced granuloma in rats and the results are showed that hydro alcohol extract stem *Acacia Torta* exhibited 29.4% and 48.9% inhibition, ($P < 0.001$) of granuloma formation at the doses 100 and 400 mg/kg respectively, whereas indomethacin showed 52.01% when compared to vehicle control. To conclude, cotton pellet induced granuloma formation and adjuvant induced arthritis showed inhibition of prostaglandin synthesis might be the major mechanism by which the hydro-alcoholic extract of the stem *Acacia torta (Roxb.) Craib* exerts anti-inflammatory.

Key Words: *Acacia Torta (Roxb.) Craib*, Anti-inflammatory, Prostaglandin, Indomethacin.

INTRODUCTION

Most of the synthetic anti-inflammatory drugs are costly, and have adverse effect including gastrointestinal and respiratory irritation, nephrotoxicity, physical dependence and constipation in long-term use. Therefore, scientists are looking for cost-effective natural agents with low toxicity and better tolerance. The ethnomedicinal plants are considered as an important source of candidate therapeutics [1, 2] to combat long-term toxicity and escalating costs. Inflammation is a complex biological response of the damaged vascular tissues with protective attempt of healing, and classified as acute or chronic. The acute inflammation is the initial response of the body to the

harmful stimuli, when increased movement of plasma and granulocytes takes place from blood to the injured tissues [3], followed by a cascade of events involving the propagation and maturation of vascular and immune system, along with the cells of the injured tissues [4]. The affected cells are then activated to release several mediators including eicosanoids, cytokines, and chemokines to elicit the inflammatory response from acute to the chronic phase [1, 4].

In chronic (prolonged) inflammation, a progressive shift of injured cells occurred at site with simultaneous destruction and healing of the injured tissues, along with the release of cyclooxygenase (COX) mediated prostaglandins (PGs), leading to the pain, edema and fever. Thus, COX inhibitors are used as antiinflammatory drugs. However, many COX inhibitors have serious adverse effects [5] and conventional nonsteroidal anti-inflammatory drugs (NSAD) are unsuitable for the management of

Corresponding Author

Girish N

Email:- Girishn534@gmail.com

chronic and silent inflammations. Consequently there is a need to develop a new anti-inflammatory agent with minimum side effects. Search for safe and effective anti-inflammatory agents have been given priority in scientific research in herbal system of medicine.

Acacia torta (Roxb.) Craib, an important medicinal plant, is commonly known as Aadaari, Lataa Khadira has traditionally used in cough, bronchitis, dysentery, tubercular fistula, measles, inflammation, sepsis (skin diseases) and in emmenagogue [6]. The extract contains tannins, saponins, steroids, triterpenoids and alkaloids. In our laboratory, it was observed that crude extract of *Acacia Torta* (Roxb.) Craib exert anti-inflammatory activity in rats. Several plants of this genus have been used in folk medicine to treat stomach pain, cough, diarrhoea, piles, sore throat, as astringent, antipyretic, antimicrobial, antimalarial, antiviral, anti-oxidant, anti-hypertensive and as liver tonic [7]. *Acacia Torta* (Roxb.) Craib has been traditionally used in Maharashtra, Kerala, Karnataka and in Himalayas for the treatment of inflammation and rheumatism. However, systematic study of this plant has not been carried out for the anti-inflammatory activity. Therefore, present study was undertaken to evaluate the anti-inflammatory effect of stem of *Acacia Torta* (Roxb.) Craib stem extract in cotton pellet induced granuloma in rat model.

MATERIALS AND METHODS

The plant *Acacia torta* (Roxb.) Craib stem bark, were selected for the present study. The plant belonging to family Mimosaceae were collected from the Savandurga state forest of Karnataka. The plants were authenticated by Dr. V. Rama Rao, Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH, Govt. of India, G.C.P. Annexe, Ashoka Pillar, Jayanagar, Bengaluru. The air dried and powdered stem of *Acacia Torta* (Roxb.) Craib were successively extracted with alcohol and water in a Soxhlet apparatus.

Animals used

Albino rats of either sex weighing between (150-200 g) were procured from animal house of Visveswarapura Institute of Pharmaceutical Sciences Bengaluru for experimental purpose. The animals were acclimatized to laboratory conditions for 7 days. The animals were supplied with commercially available standard diet. Water was allowed *ad libitum* under hygienic conditions. The animals were grouped in cages in an air conditioned room at the temperature of $22 \pm 1^\circ\text{C}$ with 12 h light and dark cycle [8]. The ethical guidelines for the investigation of the animals used in experiment were followed in all the tests.

Acute oral toxicity studies

The acute toxicity of stem extracts of *Acacia torta* (Roxb.) Craib was determined by using female albino rats

of weight between (180-200) g, maintained under standard conditions. The animals were fasted for 12 hr prior to the experiments. Animals were administered with single dose of stem extracts of *Acacia torta* (Roxb.) Craib and observed for its mortality up to 48 hr study period (short term toxicity). Based on the short-term toxicity profile, the next dose was decided as per OECD guidelines No 423. From the LD50 dose 1/5th dose was selected and considered as high dose [9].

Experimental Design for evaluation of anti-inflammatory activity through cotton pellet induced granuloma method

Albino Wistar rats of either sex weighing 150–200 g were maintained in animal house and they were divided into 4 groups of 6 animals each. Prior to the experimentation they were acclimatized to housing conditions for at least one week period of time to adjust to the new environment providing with food and water *ad libitum*. The experimental protocol was for seven days [10, 11].

Group I-Control, animals were treated with 10% Tween-80 *p.o* for seven days.

Group II-Standard group, animals were treated with 10mg/kg body weight administered Indomethacin orally for seven days.

Group III-Animals were treated with 100mg/kg b. w. *p.o*. of hydro alcoholic extract of stem *Acacia torta*.

Group IV-Animals were treated with 400mg/kg b. w. *p. o*. of hydro alcoholic extract of stem *Acacia torta*.

Autoclaved cotton pellets (50 ± 1 mg) were surgically inserted in the lumbar region under ether anaesthesia. Different groups of the animal respectively were treated orally with vehicle (10% tween 80), indomethacin and hydro alcoholic extract of stem *Acacia torta* for seven days. On 8th day, rats were sacrificed under light ether anaesthesia, granuloma was surgically removed, dried at 60°C in an oven and weighed to determine the percentage inhibition of granuloma formation.

$$\% \text{ inhibition} = \frac{\text{Mean increase in paw volume in control group} - \text{Mean increase in paw volume in test group}}{\text{Mean increase in paw volume in control group}} \times 100$$

Preliminary phytochemical screening

It was observed from the preliminary phytochemical screening of the stem that Carbohydrates and Flavonoids were absent in the extract of stem *Acacia torta*. Moreover it was found that Saponins, steroids, triterpenes, alkaloids and tannins were present in the hydro alcoholic extract of stem *Acacia torta*.

Statistical analysis

Results were expressed as mean \pm SEM. Statistical significance was assessed using One-way and single measures of Analysis of variance (ANOVA) followed by Dunnett's comparison tests. *** $P < 0.001$ and ** $P < 0.01$ was considered as significant Vs control.

RESULTS

The acute toxicity studies reveal that the hydro alcoholic extract of stem *Acacia torta* (Roxb.) Craib were found to be non-toxic at dose of 2000 mg/kg and the extracts were found to be safe at dose of 2000 mg/kg. Hence 2000 mg/kg was the maximum tolerable dose considered for the hydro alcoholic extract. Thereby the

screening doses selected for systemic administration of test extracts for organ protective activities are mentioned below.

The hydro alcoholic extract of stem *Acacia torta* - 100 mg/kg (1/20th of the 2000 mg). The hydro alcoholic extract of stem *Acacia torta* - 400 mg/kg (1/5th of the 2000 mg).

The hydro alcoholic extract of stem *Acacia Torta* was screened for cotton pellet induced granuloma in rats and the results are shown in Table.1. The hydro alcohol extract stem *Acacia Torta* exhibited 29.4% and 48.9% inhibition, ($P < 0.001$) of granuloma formation at the doses 100 and 400 mg/kg respectively, whereas Indomethacin showed 52.01% when compared to vehicle control.

Table 1. Effect of hydro alcoholic extract of acacia torta (roxb.) craib stem on carrageenan induced paw edema

Treatment	Mean of dry cotton pellets (mg)	Percentage inhibition	Mean of wet cotton pellets (mg)	Percentage inhibition
Vehicle (10% Tween 80)	216.7 \pm 1.745		212.2 \pm 3.655	
Indomethacin (10mg/kg)	104 \pm 2.366	52.01 %	166.7 \pm 3.073	21.5 %
<i>Acacia Torta</i> (100mg/kg)	153 \pm 3.651	29.4 %	191.8 \pm 2.522	9.7 %
<i>Acacia Torta</i> (400mg/kg)	110.8 \pm 1.579	48.9 %	179.8 \pm 2.242	15.27 %

All values were expressed in Mean \pm S.E.M, N = 6

Fig 1. Effect of Acacia Torta extract on weight of dry cotton pellets

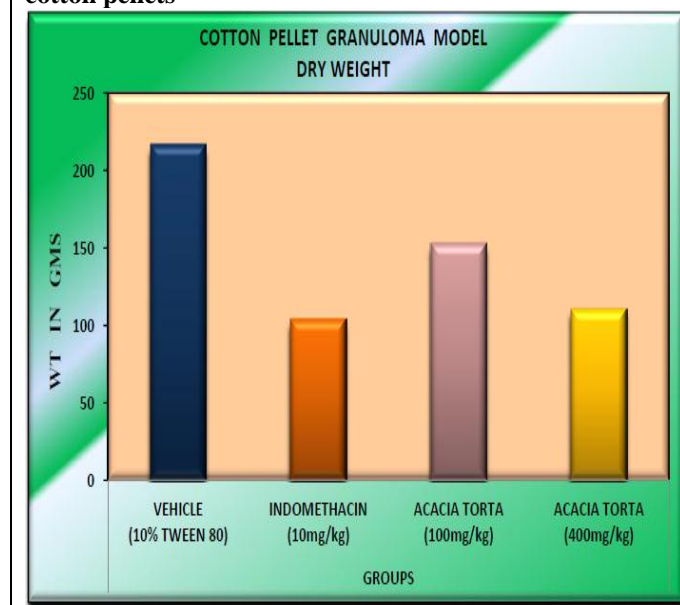
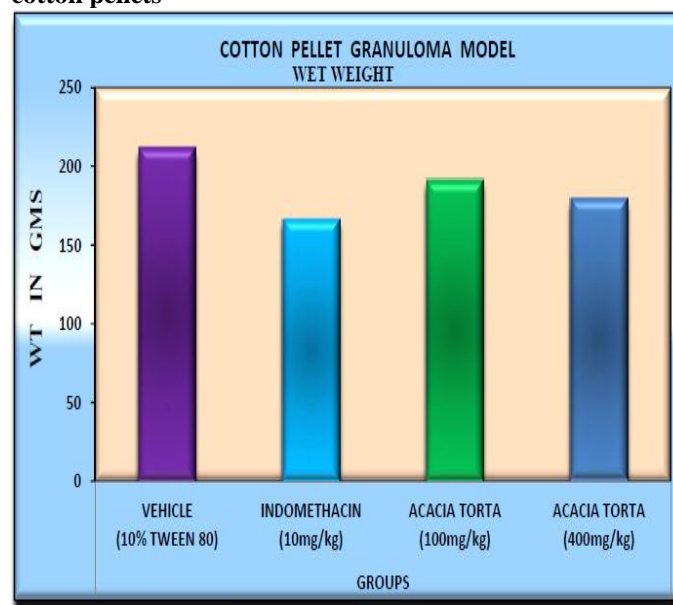


Fig 2. Effect of Acacia Torta extract on weight of wet cotton pellets



DISCUSSION

Natural products are a source for bioactive compounds and have potential for developing some novel therapeutic agents. *Acacia torta* (Roxb.) Craib possess many therapeutic activities. More recently there has been an interest in their analgesic and anti-inflammatory activities following reports about their ability to relieve pain and inflammation [12, 13].

The cotton-pellet granuloma is widely used to evaluate the transudative and proliferative components of the chronic inflammation. The moist weight of the pellets correlates with transudate, the dry weight of the pellet correlates with the amount of granulomatous tissues [14, 15]. Chronic inflammation occurs by means of the development of proliferate cells. These cells can be either spread or in granuloma form. Non-steroidal anti-inflammatory drugs decrease the size of granuloma which

results from cellular reaction by inhibiting granulocyte infiltration, preventing generation of collagen fibres and suppressing mucopolysaccharides [16, 17]. The hydro alcoholic extract of stem *Acacia Torta* showed significant anti-inflammatory activity in cotton pellet induced granuloma and thus found to be effective in chronic inflammatory conditions, which reflected its efficacy in inhibiting the increase in the number of fibroblasts, preventing angiogenesis and synthesis of collagen and mucopolysaccharides during granuloma tissue formation. The suppression of the T helper 1 in T-lymphocyte pathway, which releases inflammatory cytokines such as interleukin-12 and interferon- γ may also be responsible for this action. However mechanistic studies measuring specific cytokine levels may help elucidate this reasoning [18].

CONCLUSION

Thus, it is concluded that the hydro alcoholic extract of stem *Acacia torta* (Roxb.) Craib produced

significant anti-inflammatory activity against cotton pellet induced granuloma model. The results support the traditional use of this plant in some painful and inflammatory conditions and suggest the presence of biologically active components which may worth further investigation and elucidation.

CONFLICT OF INTEREST

None declared.

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ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee.

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