

ANTI-INFLAMMATORY, ANALGESIC AND ANTIPYRETIC ACTIVITY OF METHANOLIC EXTRACT OF CINNAMOMUM TAMALA (NEES) IN EXPERIMENTAL ANIMAL MODELS

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Received for publication: June 8, 2012; Accepted: August 10, 2012.

Abstract: This study was aimed to evaluate the anti-inflammatory, analgesic and antipyretic activity of methanolic extract of *Cinnamomum tamala* leaves. **Materials and Methods:** Anti-inflammatory activity was studied in Swiss albino mice using carrageenan. Analgesic activity was evaluated in mice by using hot plate method, acetic acid-induced writhing movement and tail flick test. Antipyretic activity was evaluated by using brewer's yeast. **Result:** The study showed that *C. tamala* extract was having anti-inflammatory activity at the different test doses. The analgesic activity was also found to be significant ($p < 0.05$) and dose dependent prolongation of response latency in the hot plate test. Acetic acid induced writhing assay showed the decreased number of stretching episodes in the extract treated groups. The antipyretic assay revealed the temperature-decreasing pattern significantly ($p < 0.01$) when compared with control. **Conclusions:** These results demonstrated that *Cinnamomum tamala* has potential health benefits as it can produce significant anti-inflammatory, analgesic and antipyretic activities.

Keywords: Anti-inflammatory, analgesic, antipyretic, *Cinnamomum tamala*

INTRODUCTION

Medicinal plants, which form the backbone of traditional medicine, have in the last few decades been the subject for very intense pharmacological studies; this has been brought about by acknowledgement of the value of medicinal plants as potential sources of new compounds of therapeutics value and as sources of lead compounds in the drug development. *Cinnamomum tamala* (Nees) is one of the main species of Lauraceae family, found throughout India especially in altitudes. The plant is a moderate sized evergreen tree 7.5 m in height with dark brown or blackish rough bark and pinkish or reddish brown blaze. Leaves are simple, opposite, ovate-oblong, acoriaceous, glabrous and 3-nerved from base. Flowers are pale yellowish in axillary and terminal and fruits are ovoid, fleshy, black drupe, supported by enlarged perianth tube. This is widely used as anthelmintic, diuretic, stimulant carminative and tonic in the traditional medicine system. The present study is aimed to evaluate the anti-inflammatory, analgesic and anti-pyretic activities of *Cinnamomum tamala* leaves in the *in vivo* model system.

MATERIALS AND METHODS

Extraction: *Cinnamomum tamala* leaves were shade dried, powdered and extracted using soxhlet extraction apparatus by the 12hrs continuous hot methanol. Solvent free extract was prepared using rotovac and dissolved in 2.5% DMSO and 2.5% Tween 20.

Animals: Swiss albino mice (20-25 g) were used for the present study. They were purchased from Small Animal Breeding Station, Veterinary College, Mannuthy, Thrissur and housed in well-ventilated cages under controlled condition of light and humidity and provided with normal mouse chow and water *ad libitum*. All the animal experiments were done as per the instructions prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and implemented through the Institutional Animal Ethics Committee (No. 612/02/A/CPCSEA).

Chemicals and Reagents: Carrageenan was purchased from Sigma Aldrich, USA. Methanol and DMSO, Tween 20 and other chemicals were analytical grade procured from Merck, India.

Carrageenan-induced Acute Inflammatory model: Swiss albino mice were divided into four groups comprising of six animals in each group. Group I with Carrageenan alone was kept as control and Group II was treated with standard drug Ibuprofen (10mg/kg). Group III and IV were treated with different concentrations of *C. tamala* extract (250 and 500 mg/kg b.wt) orally. Carrageenan 0.1ml (1% in 0.9% saline) was administered into the plantar surface of right hind paw of animals. Before injection of carrageenan, the average volume of the right hind paw of each mouse was measured and 3 hours after the injection of carrageenan was also measured.

The edema was measured as an increase in the volume of paw, and from this the percentage of inhibition for each group was obtained as per the standard protocol^{2,3}.

Analgesic activity in Hot plate model: In this study animals were individually placed on a hot plate maintained at constant temperature ($55^{\circ}\text{C} \pm 0.2$) and the reaction of animals, such as paw licking or jump response was taken as the end point as per Eddy and Leimbach method⁴.

Acetic acid induced writhing: The animals were divided into four groups of six animals each as mentioned above. The animals were fasted for 16 hours. Two different doses of test extracts (250 and 500mg/kg b.wt) were administered 45 minutes before acetic acid (0.2ml of 3%) injection. After the acetic acid injection intraperitoneally, the contraction of abdominal muscles together with stretching of the hind limbs was cumulatively counted over a period of 30 minutes⁵.

Tail-flick test: The tail of the mice was placed on the analgesiometer and time taken by the animal to withdraw (flick) its tail from the source of heat was taken as the reaction time. Butrophan was used as standard drug. A reaction period of 10 seconds is considered as maximum analgesia and tail is removed from source of heat to avoid tissue damage^{6,7}.

Antipyretic activity: Pyrexia was induced in fasted animal by subcutaneous injection of a 20% aqueous suspension of Brewer's yeast in normal saline at rate of 1ml/kg b.wt. Rise in body temperature was noted 18 hrs after yeast injection and it was noted at an interval of 1 hour for 3 hours^{8,9}.

RESULTS

Anti-inflammatory Activity: The methanolic extract of *Cinnamomum tamala* was found to have anti-inflammatory activity in the carrageenan induced paw edema in Wistar albino rats. The percentage of inhibition of edema formation was 66.75% and 73.71% at 250 and 500 mg/kg b.wt dosage respectively. The efficacy of the extract was found to be dose dependent (Figure.1).

Analgesic activity of extract: The time course of antinociception produced by *Cinnamomum tamala* extract was evaluated and presented in Figure 2. Oral administration of *C. tamala* extract rendered significant ($p < 0.05$) analgesic activity and showed dose dependent prolongation of response latency in the hot plate method.

Acetic acid writhing method: In the acetic acid induced writhing experiment, the number of stretching episodes during 30 minutes after extract

administration was found to be 31.33, 27.50, 18.66 in the group of Indomethacin, *C. tamala* 250 and 500 mg/kg b.wt respectively as shown in Table.1.

Tail flick response method: Tail flick response method showed that the activity of test extract was having the peak analgesic activity at 180 minutes after oral administration of extract. The analgesic activity of extract was moderate when compared with Butrophan and it was dose dependent and significant ($P < 0.05$) when compared with control (Table.2).

Antipyretic Activity: The antipyretic activity was analyzed by observing the rectal temperature of pyrexia induced experimental animals at one –hour interval for total of three hours, after giving the oral administration of test extract (Table.3). The temperature decreasing pattern was observed significantly ($p < 0.01$) when compared with control.

Figure.1: Anti-inflammatory activity of *Cinnamomum tamala* in carrageenan induced paw edema

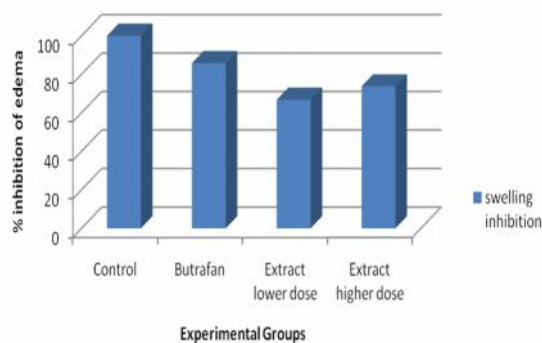


Figure.2: Effect of *Cinnamomum tamala* extract on thermic stimulus-induced pain (hot plate test) in mice.

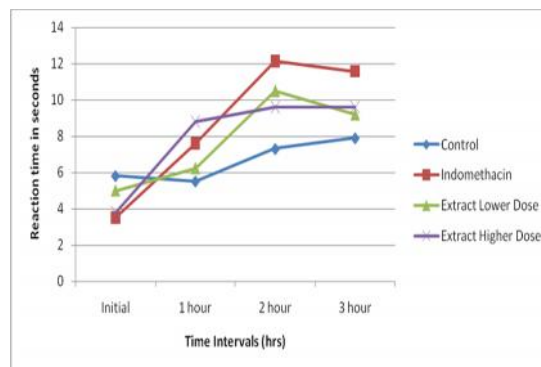


Table.1: Analgesic effect of *Cinnamomum tamala* extract on acetic acid induced writhing in mice

Group	Treatment	No. of mice protected from writhing	No. of stretching episodes during 30 min after extract administration
I	Control	0 (6)	67.6 ± 3.42
II	Indomethacin	0 (6)	31.33 ± 2.94**
III	<i>C. tamala</i> Lower dose (250 mg/kg b.wt)	0 (6)	27.50 ± 2.80*
IV	<i>C. tamala</i> Higher dose (500 mg/kg b.wt)	0 (6)	18.66 ± 3.12*

Values are Mean ± SEM, n=6. *P<0.05 ** p<0.01 when compared with control

Table.2: Effect of *Cinnamomum tamala* extract on latency of tail-flick response in rat.

Group	Treatment	Reaction Time (in seconds)			
		Recording at various time intervals (minutes after drug administration)			
		Initial	60 min	120 min	180 min
I	Control	15.88 ± 0.525	13.76 ± 0.77	16.11 ± 0.98	14.95 ± 2.50
II	Butraphan	19.88 ± 1.98	27.43 ± 4.80	33.30 ± 1.63**	34.31 ± 2.32**
III	<i>C. tamala</i> Lower dose (250 mg/kg b.wt)	13.33 ± 1.02	15.58 ± 0.79	20.65 ± 2.36**	22.65 ± 3.63**
IV	<i>C. tamala</i> Higher dose (500 mg/kg b.wt)	12.80 ± 1.35	15.60 ± 3.14	17.12 ± 2.27*	27.33 ± 4.19*

Values are Mean ± SEM, n=6. *P<0.05 ** p<0.01 when compared with control

Table.3: Effect of *Cinnamomum tamala* extract on Brewer's Yeast induced pyrexia in mice.

Group	Treatment	Rectal Temperature (in °C at time h)				
		-16a	0 b	1h	2h	3h
I	Control	36.5 ± 0.19	38.9 ± 0.22	38.30 ± 0.91	38.55 ± 0.53	38.43 ± 0.16
II	Paracetamol	36.11 ± 0.09	38.14 ± 0.13	38.45 ± 0.43*	36.57 ± 0.72*	36.60 ± 0.40**
III	<i>C. tamala</i> Lower dose (250 mg/kg b.wt)	36.12 ± 0.20	38.4 ± 0.35	37.83 ± 0.15*	37.74 ± 0.23*	37.16 ± 0.13*
IV	<i>C. tamala</i> Higher dose (500 mg/kg b.wt)	36.8 ± 0.26	37.88 ± 0.35	37.81 ± 0.41*	37.63 ± 0.22**	36.22 ± 0.17*

Values are Mean ± SEM, n=6. *P<0.05 **p<0.01 when compared with control a-Temperature just before yeast injection

b- Temperature just before drug administration

DISCUSSION

The present study showed the anti-inflammatory activity of methanolic extract of *C. tamala* leaves in the carrageenan induced model system. Carrageenan is known for its classic biphasic effect, first phase

mediated by release of histamine and serotonin during the first hour and release of kinins up to 2.5 hour, while the second phase is mediated by release of prostaglandin, proteases and superoxide radicals from 2.5 to 6 hours¹⁰. The development of non-steroidal anti-inflammatory agents in recent years has contributed a lot to overcome various medical problems like arthritis and also helped in understanding the time mechanism of inflammation. Extensive research has revealed that most chronic illness, including cancer, diabetes, and cardiovascular and pulmonary diseases, are mediated through chronic inflammation¹¹. Thus suppressing chronic inflammation has the potential to delay, prevent and even treat various chronic diseases including cancer. The analgesic activity of *C. tamala* extract was studied for central (narcotic) and peripheral (non-narcotic) activities. The analgesic activity of test extract against acute inflammatory pain was better as compared to Indomethacin. Aspirin and Indomethacin offer relief from inflammatory pain by suppressing the formation of pain substance in the peripheral tissues, where prostaglandin and bradykinin were suggested to play an important role in the pain process¹². The analgesic effects of acetic acid are produced due to liberation of mediators such as histamine, serotonin, bradykinin, cytokines and eicosanoids¹³. These factors promote increase of vascular permeability as well as reduce the threshold of nociception and stimulate the nervous terminal of nociceptive fibers¹⁴. Therefore it is likely that *C. tamala* extract might suppress the formation of these substances and thus exerts its analgesic activity in acetic acid induced writhing test. In the present study, *C. tamala* extract significantly increased the reaction time in hot plate test, suggesting central analgesic activity. It is well known that most of the anti-inflammatory, analgesic drugs possess antipyretic activity. In general, non-steroidal anti-inflammatory drugs produce their antipyretic activity through inhibition of prostaglandin synthetase within the hypothalamus. So it is inferred that the antipyretic activity of the extract may be due to the presence of non-steroidal compounds.

ACKNOWLEDGEMENT

Authors are thankful to the Director General, CCRAS, New Delhi for his support and encouragement and all the staff members of NRIP, Cheruthuruthy for their extended cooperation.

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**Source of support: Nil,
Conflict of interest: None Declared**