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Experimental evaluation of anti-inflammatory activity of *Cinnamomum zeylanicum* oil in male wistar rats

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Abstract

The present study was planned to evaluate *in vivo* anti-inflammatory activity of cinnamon oil (*Cinnamomum zeylanicum*) following single dose oral administration (50, 100 and 200 mg/kg) in male wistar rats. The *in-vivo* anti-inflammatory assay of cinnamon oil was carried out using carrageenan induced paw edema model in rats. Indomethacin was administered @ 10 mg/kg in standard drug control rats. Rats of control groups were kept untreated and other groups were treated with cinnamon oil @ 50, 100 and 200 mg/kg b.wt., respectively. The anti-inflammatory effect of cinnamon oil was highest at 3h (30.58%) at the dose of 200 mg/kg. It was lower than anti-inflammatory effect of standard drug indomethacin at 3h (42.99%). Cinnamon oil showed dose dependent anti-inflammatory activity in wistar rats.

Keywords: *Cinnamomum zeylanicum*, cinnamon oil, anti-inflammatory, paw edema

1. Introduction

Cinnamon is a common spice that has been used with different food items for several centuries by different cultures around the world. It is obtained from different parts of a tropical evergreen tree. According to Barceloux, (2009) the genus *Cinnamomum* (Lauraceae) includes more than 250 aromatic evergreen trees and shrubs of up to 10–20 m high, primarily distributed in Southeast Asia, China, and Australia. Sri Lanka is the major world exporter of cinnamon essential oil [1]. Investigations conducted at the beginning of the 1980s have shown that this genus has a center of diversity in south India [2]. According to a summary report on the essential oil of cinnamon bark by the Committee for veterinary medicinal products, the cinnamon bark essential oil mainly contains cinnamaldehyde (55%–76%), eugenol (5%–18%) and saffrole (up to 2%) [3].

A very detailed botanical characterization of different species of the genus *Cinnamomum* has been written by Ravindran *et al.* (2003) [2]. According to them there are mainly four types of cinnamon:

1. True cinnamon, *Cinnamomum zeylanicum* or *Cinnamomum verum* J. Presl
2. Cassia cinnamon, *Cinnamomum aromaticum* Nees
3. Vietnamese cinnamon, *Cinnamomum loureiroi*.
4. Indonesian cinnamon, *Cinnamomum burmannii*.

Cinnamon is used as a spice and flavoring material. Cinnamon and cassia are believed to have a broad spectrum of medicinal and pharmacological applications. Pharmacological evaluation of *cinnamomum zeylanicum* for anti-inflammatory activity in rat paw edema model [4]. Anti-inflammatory activity of an essential oil recipe consisting of the supercritical fluid CO₂ extract of cinnamon *in vivo* by inflammatory model of dimethyl benzene (DMB)- induced ear vasodilation in mice [5]. Recent pharmacological studies have shown that besides its role as a spice, cinnamon can be used as a hypoglycemic and cholesterol-lowering [6], wound pro-healing [7], and anti-inflammatory compound [8]. Gunawardena *et al.* (2015) reported anti-inflammatory activity of cinnamon (*C. zeylanicum*) extract and identification of E-cinnamaldehyde and o-methoxy cinnamaldehyde as the most potent bioactive compounds [9].

2. Materials and Methods**2.1 Experimental animals**

The study was conducted on adult healthy male wistar rats. Twenty five male rats (370 to 420 g) of 8-10 weeks of age were procured from Cadila Healthcare Ltd. (R & D Centre), Ahmedabad, Gujarat. The experimental protocol was approved by Institutional Animal Ethics

Committee (Project No. IAEC/280/VPT/2018) at College of Veterinary Science and Animal Husbandry, Anand, Gujarat and protocols were followed according to the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA). The animals were housed in standard polypropylene cages and maintained under controlled room temperature ($22 \pm 2^{\circ}\text{C}$) and humidity ($55 \pm 5\%$) with 12 h light and 12 h dark cycle. All the rats were fed normal pellet diet and deionized water was provided *ad libitum* throughout the course of the experiment. All the rats were kept under acclimatization for 5 days prior to grouping and initiation of experiment. Rats were kept under constant observation during entire period of study. All necessary managemental procedures were adopted to keep the rats free from stress.

2.2 Drugs and chemicals

Carrageenan (Non-gelling, mixture of λ & κ carrageenan) was purchased from Sigma-Aldrich, India. Indomethacin was purchased from local medical store of Anand district (Gujarat). Cinnamon essential oil was purchased from Sigma-Aldrich, India.

2.3 Preparation of carrageenan and indomethacin solution

For the preparation of 10% w/v carrageenan suspension, 0.5 gm carrageenan was weighed using digital analytical weighing balance which was dissolved in 5 ml of normal saline. For the preparation of Indomethacin suspension each 25mg capsule was dissolved in 5 ml of distilled water so each ml contains 5 mg/ml.

2.4 Induction of paw edema in rats

The *in-vivo* anti-inflammatory assay of cinnamon oil (*Cinnamomum zeylanicum*) was carried out using rat paw edema method as described by Winter *et al.*, (1962) [10]. All rats were injected subcutaneously with 0.1 ml of 10% w/v carrageenan suspension (0.1 ml of a 1% suspension in 10% saline) in the sub-planter region of the left hind limb as a local acute edema inducer after 30 minutes of oral administration of cinnamon oil as well as indomethacin.

2.5 Experimental design

The present study was conducted on 25 male rats were dividing them in 5 various groups, having 5 rats in each

group. Control rats were kept untreated. Standard control rats were treated with Indomethacin (10 mg/kg, orally). Five rats in each group were treated with cinnamon oil at the dose of 50, 100 and 200 mg/kg b.wt. orally, respectively.

2.6 Measuring of paw edema volume

Edema was expressed as the increase in paw volume (ml). The paw volume was measured up to the tibiotarsal articulation. Volume of edematous paw was measured by using plethysmometer (PLM-01 plus, Orchid Scientific Instrument, India) at 0 h (before treatment), 1, 2, 3, 4, 6 and 24 hours after treatments.

2.7 Percent inhibition of inflammation

Percent inhibition of paw edema volume in wistar rats was calculated.

$$(\%) \text{ Inhibition} = \frac{\text{Mean paw volume (control)} - \text{Mean paw volume (treated)}}{\text{Mean paw volume (control)}}$$

2.8 Statistical analysis

All the data have been presented as mean \pm SE. Statistical comparisons of the results were made by one way analysis of variance (ANOVA) using software SPSS (Version 25). Significant differences ($p < 0.05$) between different experimental groups were determined by Duncan's test.

3. Results

The present study was conducted to evaluate *in-vivo* anti-inflammatory activity of cinnamon oil @ 50, 100 and 200 mg/kg b.wt. in wistar rats. The result of anti-inflammatory effect was presented as change in paw volume (Table 1 and Figure 1) and percentage inhibition (Table 2 and Figure 2) in wistar rats. The results revealed that the cinnamon oil showed anti-inflammatory effect with varying magnitudes at various doses in male wistar rats. The anti-inflammatory effect of indomethacin was highest at 3h (41.75%) as compared to cinnamon oil treated rats. The anti-inflammatory effect of cinnamon oil was highest at 3h (30.15%) at the dose rate of 200 mg/kg. At 3h all doses gave higher anti-inflammatory effect. Cinnamon oil showed dose dependent anti-inflammatory activity in male rats

Table 1: Effect of oral administration cinnamon oil on carrageenan-induced rat paw edema (ml) in male wistar rats (Mean \pm SE, n=5)

Group	0h	1h	2h	3h	4h	6h	24h
Control	0.72 \pm 0.02	0.95 \pm 0.01 ^c	1.14 \pm 0.01 ^c	1.79 \pm 0.03 ^c	1.72 \pm 0.02 ^d	1.52 \pm 0.02 ^c	0.91 \pm 0.02 ^c
Indo	0.71 \pm 0.02	0.82 \pm 0.02 ^a	0.88 \pm 0.03 ^a	1.04 \pm 0.03 ^a	1.15 \pm 0.02 ^a	1.16 \pm 0.01 ^a	0.81 \pm 0.04 ^a
CO-50	0.71 \pm 0.02	0.9 \pm 0.01 ^b	1.04 \pm 0.04 ^b	1.39 \pm 0.02 ^b	1.41 \pm 0.01 ^c	1.16 \pm 0.02 ^b	0.9 \pm 0.02 ^{bc}
CO-100	0.73 \pm 0.02	0.88 \pm 0.03 ^{ab}	1.02 \pm 0.03 ^b	1.28 \pm 0.06 ^b	1.32 \pm 0.01 ^{bc}	1.16 \pm 0.04 ^b	0.87 \pm 0.02 ^{abc}
CO-200	0.72 \pm 0.01	0.86 \pm 0.01 ^{ab}	0.97 \pm 0.02 ^b	1.25 \pm 0.09 ^b	1.25 \pm 0.07 ^{ab}	1.16 \pm 0.03 ^a	0.83 \pm 0.01 ^{ab}

Mean value with dissimilar superscript in a column vary significantly at $p < 0.05$

Indo = Indomethacin @ 10 mg/kg b. wt. in wistar rats

CO-50 = Cinnamon oil @ 50mg/kg b. wt. in wistar rats

CO-100 = Cinnamon oil @ 100mg/kg b. wt. in wistar rats

CO-200 = Cinnamon oil @ 200mg/kg b. wt. in wistar rats

Table 2: Percent inhibition of paw edema in wistar male rats treated with cinnamon oil

Group	1h	2h	3h	4h	6h	24h
Indo	13.84	23.04	41.75	33.06	24.09	11.87
CO-50	5.84	8.81	21.92	17.91	13.38	1.70
CO-100	8.32	10.47	28.19	23.14	15.94	4.67
CO-200	9.44	14.94	30.15	27.35	22.50	9.40

Indo = Indomethacin @ 10 mg/kg b. wt. in wistar rats

CO-50 = Cinnamon oil @ 50mg/kg b. wt. in wistar rats

CO-100 = Cinnamon oil @ 100mg/kg b. wt. in wistar rats

CO-200 = Cinnamon oil @ 200mg/kg b. wt. in wistar rats

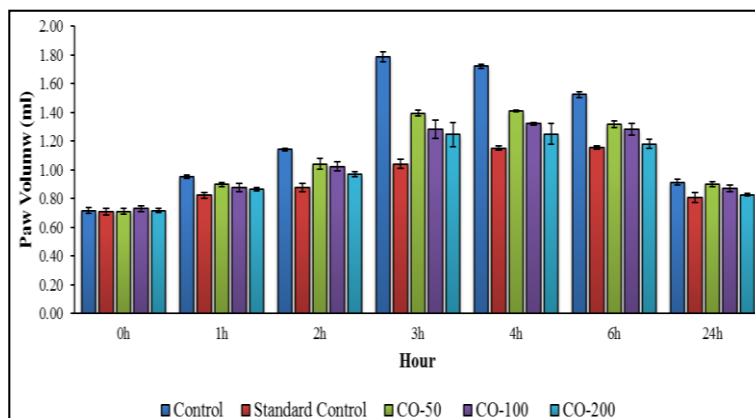


Fig 1: Effect of oral administration of cinnamon oil on carrageenan-induced rat paw edema (ml) in male wistar rats (Mean \pm SE, n=5)

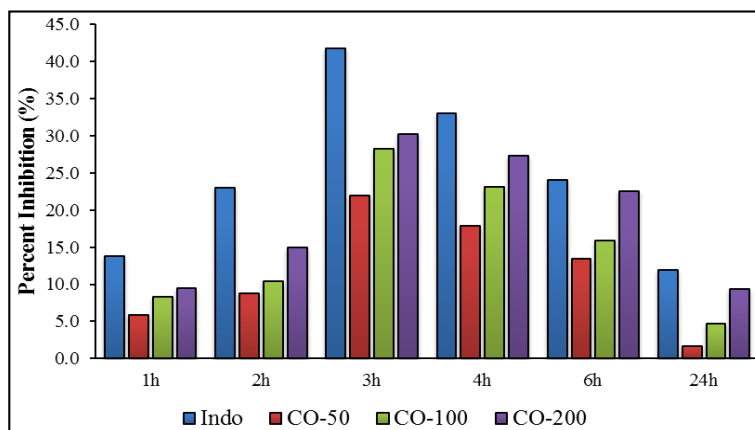


Fig 2: Percent inhibition of paw edema in male wistar rats treated with cinnamon oil

4. Discussion

In the present study, the significant decrease in paw edema volume was observed in carrageenan induced wistar rats treated with indomethacin (10 mg/kg) and cinnamon oil @ 50, 100 and 200mg/kg b.wt. treated rats. Cinnamon oil showed anti-inflammatory activity at all 3 doses in male wistar rats. Similar result was found by Maridass and Ghanthikumar (2008) [11]. They evaluated anti-inflammatory activity of ethanol extracts of *Cinnamomum keralaense* in rat paw edema model. The percentage inhibition of inflammation at 50, 100, 200 and 400mg/kg/ day was 7.17%, 38.01%, 45.17%, and 68.84% respectively, at 6h post-carrageenan administration. Pande *et al.* (2009) also reported similar result, the control group paw oedema volume was 0.62 ± 0.05 ml at 1h, 0.68 ± 0.05 ml at 2h, 0.74 ± 0.05 ml at 3h, 0.69 ± 0.05 ml at 4h, 0.67 ± 0.06 ml at 5h [4]. The reference (Diclofenac sodium) group paw oedema volume was 0.24 ± 0.03 ml at 1h, 0.19 ± 0.01 ml at 2h, 0.14 ± 0.02 ml at 3h, 0.21 ± 0.02 ml at 4h and 0.23 ± 0.02 ml at 5h. The *Cinnamomum zeylanicum* group paw oedema volume was 0.42 ± 0.04 ml at 1h, 0.38 ± 0.03 ml at 2h, 0.27 ± 0.03 ml at 3h, 0.41 ± 0.03 ml at 4h, 0.26 ± 0.04 ml at 5h at the dose rate of 250 mg/kg. The alcoholic extract of *Cinnamomum zeylanicum* was found to possess good anti-inflammatory activity [4]. Gambhire *et al.* (2009) also reported anti-inflammatory activity of aqueous extract of *Cinnamomum tamala* given at the doses of 100, 200 and 400 mg/kg in rat using paw edema model. *C. tamala* extract at the doses of 100 and 200 mg/kg moderately inhibited paw edema 25.65 and 31.57% respectively, whereas at the dose of 400 mg/kg and indomethacin at the dose of 10mg/kg significantly ($p < 0.05$) inhibited paw edema (54.4 and 62.5% respectively) at the end of 4h after carrageenan injection [12]. Azab *et al.* (2017) reported significant reduction

in paw edema to 39.8, 47.65 and 55.6% at 3 h following treatment by *Cinnamomum glanduliferum* oil at doses of 250, 500 and 1000 mg/kg, respectively [13].

5. Conclusions

The present study revealed that oral administration of cinnamon oil showed dose dependent anti-inflammatory activity in male wistar rats. The highest anti-inflammatory activity was observed at 3 hour post oral administration of cinnamon oil in male wistar rats.

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7. Conflict of interest

The authors declare that they have no conflict of interest.

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