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Determination of *in-vivo* anti-inflammatory potential of *Cinnamomum zeylanicum* oil in female wistar rats

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Abstract
Objective: The aim of the present study was to evaluate *in vivo* anti-inflammatory activity of cinnamon oil (*Cinnamomum zeylanicum*) following single dose oral administration (50, 100 and 200 mg/kg) in female wistar rats.
Methods: Carrageenan induced paw edema model was used for the *in-vivo* anti-inflammatory activity of cinnamon oil in female wistar rats. As a standard drug control indomethacin was administered at the dose rate of 10 mg/kg female wistar rats. Rats of control groups were kept untreated. Other three groups were treated with cinnamon oil at the dose rate of 50, 100 and 200 mg/kg b. wt., respectively.
Results: Cinnamon oil showed dose dependent anti-inflammatory effect at various doses in female wistar rats. The anti-inflammatory effect of cinnamon oil was highest at 3h (30.58%) at the dose of 200mg/kg. It was lower than anti-inflammatory effect of standard drug indomethacin at 3h (42.99%).
Conclusion: The highest anti-inflammatory activity was observed at 3-hour post oral administration of cinnamon oil @ 50, 10 and 200 mg/kg b. wt. in female wistar rats.

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

1. Introduction
Essential oils (volatile or ethereal oils) are aromatic oily liquids obtained from various plant materials (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). The term 'essential oil' is derived from the name coined by Paracelsus von Hohenheim in the 16th century. He named the effective component of a drug Quinta essential. These oils can be obtained by expression, fermentation, enfleurage or extraction but the method of steam distillation is most commonly used for commercial production of essential oils. As estimated 3000 essential oils are known, of which about 300 are commercially important, destined chiefly for the flavors and fragrances market [1]. Researchers from all over the world are trying to characterize a range of biological properties of essential oils which includes antimicrobial, antiviral, ant mutagenic, anticancer, antioxidant, antiinflammatory, immunomodulatory and antiprotozoal activities [2].
Cinnamon oil is extracted from *Cinnamomum zeylanicum*, also called Cinnamon in English, Dalchini in Hindi, Taj in Guajarati (Indian spices board). *C. zeylanicum*, the source of cinnamon bark and leaf oil, is an indigenous tree of Sri Lanka, although most oil now comes from cultivated areas. Smaller areas of wild trees are also found in the south-western parts of India [3]. The genus *Cinnamomum* comprises of about 250 species, of which 20 occur in India [4]. 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%) [5].
Cinnamon is used as a spice and flavoring material. Cinnamon and cassia are believed to have a broad spectrum of medicinal and pharmacological applications. Several studies have reported the anti-inflammatory activity of cinnamon and its essential oils [6]. Recent pharmacological studies have shown that besides its role as a spice, cinnamon can be used as a hypoglycemic and cholesterol-lowering [7], wound pro-healing [8], and anti-inflammatory compound [9]. Anti-inflammatory activity of cinnamon (*C. zeylanicum*) extract and identification of E-cinnamaldehyde and o-methoxy cinnamaldehyde as the most potent bioactive compounds [10].

2. Materials and Methods
2.1 Experimental animals
The study was conducted on adult healthy female wistar rats. Twenty five female rats (220 to260 g) of 8-10 weeks of age were procured from Cadila Health Care 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). All the rats were housed in polypropylene cages at Laboratory Animal House Facility in an environmentally controlled room with 22 ± 3 ºC temperature and 30-70% relative humidity. Light/dark cycles of 12/12 hours were provided throughout the study period. Rats were provided with standard pellet diet. Deionized water was provided *ad libitum* throughout the course of the experiment. All necessary manage mental procedures were adopted to keep the rats free from stress.

2.2 Drugs and chemicals

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

2.3 Preparation of carrageenan and indomethacin solution

For the preparation of 10% w/v carrageenan suspension, 0.5 gm carrageenan was weigh 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

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) [11]**.** 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 female rats were dividing them in 5 various groups, having 5 rats in each group. Control rats were given ad libitum drinking water. Rats of standard control group were treated orally with indomethacin at the dose rate of 10 mg/kg b. wt. as a reference drug in female rats, respectively. Cinnamon oil was given orally to female rats at three different dose rate 50, 100 and 200 mg/kg b. wt., respectively.

2.6 Measuring of paw edema volume & Percent inhibition of inflammation

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 Scintific Instrument, India) at 0 h (before treatment), 1, 2, 3, 4, 6 and 24 hours after treatments. Percent inhibition of paw edema volume in wistar rats was calculated.

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

2.7 Statistical analysis

All the data have been presented as mean ± SE. Statistical comparisons of the results were made using 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 female wistar rats. The anti-inflammatory effect of indomethacin was highest at 3h (42.99%) in female as compared to other doses of cinnamon oil. The anti-inflammatory effect of cinnamon oil was highest at 3h (30.58%) in female at the dose rate of 200 mg/kg. In the present study cinnamon oil showed dose dependent anti-inflammatory activity in both male and female rats. At 3h all doses gave higher anti-inflammatory effect.

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

Group	0h	1h	2h	3h	4h	6h	24h
Control	0.53 ± 0.02	0.73 ± 0.02^{c}	1.01 ± 0.03^{c}	1.46 ± 0.05^{c}	1.4 ± 0.06^{c}	1.19 ± 0.04^{b}	0.74 ± 0.02
Indo (F)	0.53 ± 0.02	0.63 ± 0.01^{a}	0.84 ± 0.03^{a}	0.83 ± 0.03^{a}	0.95 ± 0.03^{a}	0.90 ± 0.02^{a}	0.66 ± 0.04
CO-50 (F)	0.53 ± 0.02	0.68 ± 0.01^{b}	0.94 ± 0.02^{b}	1.14 ± 0.04^{b}	1.16 ± 0.02^{b}	1.07 ± 0.07^{ab}	0.72 ± 0.03
CO-100 (F)	0.53 ± 0.01	0.68 ± 0.02^{b}	0.9 ± 0.01^{ab}	1.07 ± 0.12^{b}	1.08 ± 0.05^{ab}	1.02 ± 0.08^{a}	0.71 ± 0.03
CO-200 (F)	0.53 ± 0.01	0.66 ± 0.01^{ab}	0.87 ± 0.02^{ab}	1.01 ± 0.03^{ab}	1.02 ± 0.08^{ab}	0.93 ± 0.05^{a}	0.68 ± 0.04

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 female rats treated with cinnamon oil

Group	1h	2h	3h	4h	6h	24h
Indo	13.78	16.67	42.99	31.41	24.00	11.05
CO-50	6.60	7.19	21.71	16.35	10.38	3.23
CO-100	7.72	10.64	27.60	22.06	15.45	4.59
CO-200	9.88	13.66	30.58	27.18	21.91	8.69

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

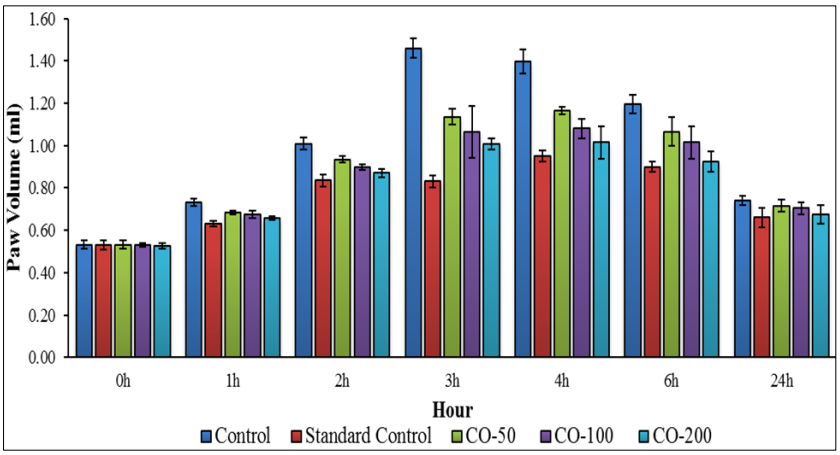

Fig 1: Effect of oral administration of cinnamon oil on carrageenan-induced rat paw edema (Ml) in female wistar rats (Mean ± SE, n=5)

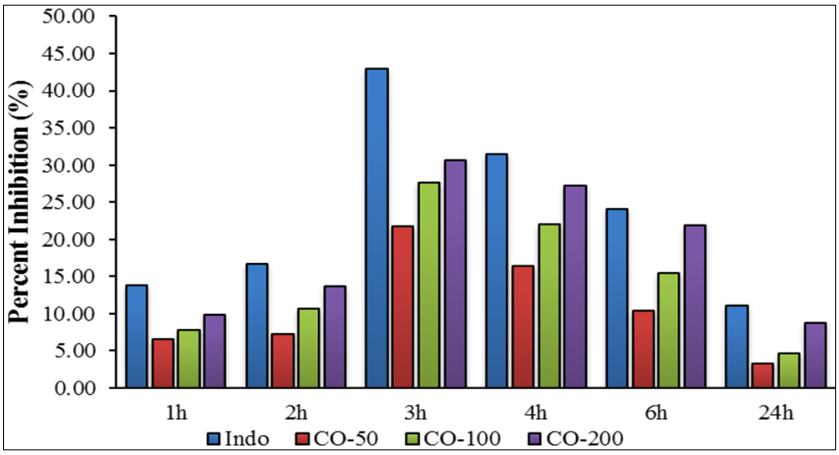

Fig 2: Percent inhibition of paw edema in wistar female rats treated with cinnamon oil

4. Discussion

In our 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 female wistar rats. Similar result was found by Maridass and Ghanthi kumar (2008) [12]. 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) [13] 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 and 0.67 ± 0.06 ml at 5h [13]. 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 at 4h, 0.23 ± 0.02 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 at 4h, 0.26 ± 0.04 at 5h at the dose rate of 250 mg/kg. The alcoholic extract of *Cinnamomum zeylanicum* was found to possess good anti -inflammatory activity [13]. Gambhire *et al.* (2009) [14] also reported anti-inflammatory activity of aqueous extract of *Cinnamomum tamala* given at the doses of 100, 200 and 400 mg/kg in rats 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 [14]. Azab *et al.* (2017) [15] 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 [15].

5. Conclusions

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

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