



# Possible Effect of *Cinnamomum camphora* on Pain Amelioration and Pain Threshold in Mechanically Induced Pain in Female Wistar Rats

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## Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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## ABSTRACT

**Background:** Camphor, a long-standing chemical used in various home treatments, has been extensively studied for its antibacterial, antipruritic, and contraceptive properties, and is a key ingredient in topical home treatments. The study investigated the possible effect of *Cinnamomum camphora* essential oil on pain amelioration and pain threshold in mechanically induced pain in female Wistar rats.

**Method:** the study involves 25 adult Wistar rats, divided into five groups and each group was administered different milliliters of *Cinnamomum camphora*, except for group 1, pains were induced in the rats via experimental procedure; passive avoidance, paw withdrawer test, and tail clip test. Data obtained were analyzed using IBM SPSS version 23.

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**Results:** Group 2 showed a significant increase in avoidance time, a significant increase in pain threshold, and a significant decrease in pain sensitivity compared to the control group. The reverse was the case of group 4 when compared to the control group. Interestingly, the effects of *C. camphora* oil were more on the low-dose group than on the high-dose group.

**Conclusion:** These results suggest that *C. camphora* has an analgesic effect and is dose-dependent.

**Keywords:** Pain; pain threshold; *C. camphora*; pain amelioration; female.

## 1. INTRODUCTION

The discovery of novel pharmaceuticals is primarily reliant on medicinal plants. Camphor is well-known as a key component in topical home remedies for a variety of symptoms, and its use is widely accepted around the world due to its long history as an antipruritic, rubefacient, abortifacient, aphrodisiac, contraceptive, and lactation suppressant. Because of its long history of use, this drug has been the focus of various scientific examinations into its action and how it is metabolized in both human and animal bodies [1].

Camphor (*Cinnamomum camphora*) is a white, crystalline material with a strong odor and pungent taste obtained from the wood of camphor laurel (*Cinnamomum camphora*) and other laurel-related species. The camphor tree (*Cinnamomum camphora*) is a member of the Lauraceae family and is known to be native to China, India, Mongolia, Japan, and Taiwan. A variation of this aromatic evergreen tree is grown in the Southern United States, particularly in Florida [2,3].

Modern pharmaceutical technologies give a theoretical basis for the performance of *C. camphora*, which has long been utilized in traditional medicine [4]. According to current research, the pharmacological effects are primarily obtained from *C. camphora* essential oils. Because of technological advancements, essential oils may be isolated from various portions of *C. camphora*, and components of essential oils with pharmacological properties can be separated and purified. Essential oils are mostly derived through distillation. The tree's wood, twigs, and bark are steam-distilled, purified, and sublimated to produce camphor [2]. Camphor is readily absorbed via the skin and can be administered via injection, inhalation, or ingestion [5]. Camphor comes in various chemical types, each with a different essential oil content [6]. *Cinnamomum camphora* leaves include camphor as the major component, as well as cineol, linalool, eugenol, limonene,

safrole,  $\alpha$ -pinene,  $\beta$ -pinene,  $\beta$ -myrcene,  $\alpha$ -humulene,  $p$ -cymene, nerolidol, borneol, camphene, and other components [7].

Pain is a major worldwide health problem, and treatment is complex [8]. Despite current scientific advances in pain medicines, many pain problems still lack potent, safe, and effective medications [9]. Furthermore, several of the existing pain medications have negative side effects [10]. As a result, both the pharmaceutical business and academics continue to prioritize optimizing existing pain medications and developing new ones [11]. In recent years, there has been a growing interest in herbal medicines as potential therapeutic agents for pain and inflammation.

Among them, the species *cinnamomum camphora* (camphor) has a long tradition in classical medicine and it has been used around the globe for diverse purposes such as; analgesic, antiseptic, antispasmodic, antipruritic, anti-inflammatory, anti-infective, rubefacient, contraceptive, mild expectorant, nasal decongestant, cough suppressant [12], spasmodic effects of circulatory and respiratory diseases, treatment for muscle pain, inflammation, and rheumatism in the pharmaceutical sector [13], etc. While camphor has been shown to have various benefits, it can also be harmful in adults, causing congestion in the gastrointestinal tract, kidneys, and brain [13]. Symptoms of camphor poisoning in humans include nausea, vomiting, headache, disorientation, muscle excitability (producing tremors and twitching), convulsions, and delirium, depending on the dosage.

Camphor oil, extracted from the wood of the camphor tree, has been used for centuries as a topical analgesic. It has a cooling effect on the skin and can help alleviate minor aches and pains when applied externally [14]. A detailed assessment of the safety of ingesting camphor oil and its efficacy as a pain reliever is thus required and imperative [15].

This study aims to determine the possible effect of Cinnamomum on pain amelioration and pain threshold in mechanically induced pain in female Wistar rats.

## 2. MATERIALS AND METHODS

### 2.1 Animal Procurement and Acclimatization

25 healthy female albino wistar rats, weighing 128-193g, were used in the study. The animals were obtained from the animal house of the Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria. After identification, the animals were weighed and housed in a wire gauze cage and exposed to 12 hours of daylight and 12 hours of darkness for 7 days of acclimatization in an environment of normal room temperature. They were fed with standard rat diets (palletized poultry feeds) and distilled water throughout the study.

### 2.2 Cinnamomum camphora Procurement

*Cinnamomum camphora* 100% oil extract was procured from Jedwards International, Inc. China. With product ID; SKU: EHM1250.

### 2.3 Study Design

The study used an experimental design in which Wistar rats were randomly assigned to five groups. Group 1 was the control group, which consisted of five rats fed regular poultry feed and distilled water. There was no substance administered here. They were subjected to all levels of pain testing (passive avoidance test, analgesia meter, and tail clip) without receiving cinnamomum treatment. Group 2 comprised five rats who were also fed conventional poultry chow and purified water; 0.2ml of *Cinnamomum camphora* was given here. They were also subjected to all levels of pain testing (passive avoidance test, analgesia meter, and tail clip). Group 3 consisted of five rats fed standard poultry chow and distilled water, and 0.4ml of *cinnamomum camphora* was provided. They were also subjected to all levels of pain testing (passive avoidance, analgesia meter, and tail clip). Group 4 consisted of five rats who were fed standard poultry chow and distilled water, and 0.6 cc of cinnamomum caphora was provided. They were also subjected to all levels of pain testing (passive avoidance test, analgesic meter, and tail clip). Group 5 consisted of five rats who

were fed standard poultry feed and purified water and given 0.5ml of piroxicam. They were also subjected to all levels of pain testing (passive avoidance test, analgesic meter, and tail clip). The study lasted for four weeks, 7 days (1 week) for acclimatization, and three weeks for experimental processes.

### 2.4 Drug Administration

The rats were manually restrained by holding the loose skin on the back of their neck and raising it, thereby making the tented area of skin between the index finger and thumb bulge outward towards the holder, 1ml syringe containing the drug was injected into the tented skin of the rats and the content discharged into the system. Administration of the drug for each group commenced thirty minutes before the experimental tests were carried out.

### 2.5 Passive Avoidance Test

The passive avoidance test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior of rodents in response to mild stressors, that is novel environments and light. In this task, animals learn to avoid an environment in which they previously received a mild foot shock. In the step-through passive avoidance task, the animals are placed into an aversive brightly lit compartment and after stepping completely into the preferred dimly lit compartment a mild foot shock is delivered. The test apparatus consists of a small dark safe compartment (one-third) and a large illuminated aversive compartment (two-thirds). The latency to leave the dark compartment after receiving the shock, and also the time spent in the dark compartment after receiving the shock were recorded. The most consistent and useful measurement is the time spent in a dark box since this provides the most consistent dose-effect results in pharmacological evaluations

### 2.6 Paw Withdrawal Test

The analgesia-meter machine was used to determine the pain bearing and response of the rats. The test was carried out by applying force to the paw of the rat, the plinth increases at a constant rate, thereby enabling reproducible measurements to be made. The machine stops' running immediately after the pedal is released, at the point of paw withdrawal, recordings on the analgesia-meter were taken. After each test, the

slide is returned to its starting point by lifting and pushing to the left. The force is measured on the scale calibrated in 10-gram steps, by a pointer to the slide. This test was used to determine the anti-nociceptive activity of the drugs.

## 2.7 Tail clip

The tail clip is regarded as a stressful activating stimulus and can influence the rat's cognitive decisions and actions. The essence of the tail clip is to increase mechanical pressure on the tail of the rat throughout the experiment. The tail clip was padded with a soft material to avoid injury to the rat. The time spent for the rat to shout, bend its head towards its tail, and attempt to remove the clip was recorded.

## 2.8 Pain Induction

Pains were induced in the rats via the experimental test procedures.

## 2.9 Statistical Analysis

Data obtained were analyzed using the International Business Machine of Statistical Package for Social Science (IBM SPSS version 23) and results were presented as mean $\pm$ SEM. ANOVA was used as an inferential statistic. Thereafter, the post-hoc test of multiple comparisons was used to test the individual groups against each other. The confidence level was set at 95% and  $P < 0.05$  was considered significant.

## 3. RESULTS

Table 1 shows the result which revealed the mean value across the groups; at Week 1, Group 4 (0.6ml *Cinnamomum camphora*) showed a significant ( $p \leq 0.05$ ) decrease with a mean value of 70.20 when compared to the control group (group 1). At week 2, Group 2 (0.2ml *Cinnamomum camphora*) showed a significant ( $p \leq 0.05$ ) increase in time of avoidance when compared to a control group with a mean value of 300.0. Group 3(0.4ml *Cinnamomum camphora*) also showed a significant ( $p \leq 0.05$ ) increase with a mean value of 300.0. Group 4 (0.6ml *Cinnamomum camphora*) and 5 (piroxicam group) had no significant ( $p \leq 0.05$ ) difference in mean value when compared to the control group (group 1) At week 3, Group 2 (0.2ml *Cinnamomum camphora*) and 4 (0.6ml *Cinnamomum camphora*) had no significant ( $p \leq 0.05$ ) difference in

mean value when compared to control group (group 1) while Group 3(0.4ml *Cinnamomum camphora*) and 5 (piroxicam group) showed significant ( $p \leq 0.05$ ) increase in time of avoidance test when compared to control group with mean value of 300.0 and 300.0 respectively.

Table 2 shows task performance in the analgesymeter test. At week 1, Group 2 (0.2ml *Cinnamomum camphora*) group, group 3 (0.4ml *Cinnamomum camphora*) and group 5 (piroxicam group) showed no significant ( $p \leq 0.05$ ) difference in mean value when compared to control group (group 1). Group 4 (0.6ml *Cinnamomum camphora*) had a significant ( $p \leq 0.05$ ) decrease with a mean value of 6.16. At week 2, Group 2 (0.2ml *Cinnamomum camphora*) and 5 (piroxicam group) showed significant ( $p \leq 0.05$ ) increases when compared to a control group with mean values of 19.82 and 23.90 respectively. Group 3 (0.4ml *Cinnamomum camphora*) and 4 (0.6ml *Cinnamomum camphora*) had significant ( $p \leq 0.05$ ) decreases with mean values of 4.36 and 4.52 respectively. At week 3, Group 2 (0.2ml *Cinnamomum camphora*) group, group 3 (0.4ml *Cinnamomum camphora*) and group 5 (piroxicam group) showed no significant ( $p \leq 0.05$ ) difference in mean value. Group 4 (0.6ml *Cinnamomum camphora*) had a significant ( $p \leq 0.05$ ) decrease with a mean value of 4.52.

Table 3 shows the results obtained from the tail clip test. At week 1, there was no significant ( $p \leq 0.05$ ) difference in mean value in Group 2 (0.2ml *Cinnamomum camphora*) group, group 3 (0.4ml *Cinnamomum camphora*) group, group 4 (0.6ml *Cinnamomum camphora*) and group 5 (piroxicam group). At week 2, Group 2 (0.2ml *Cinnamomum camphora*) group, group 3 (0.4ml *Cinnamomum camphora*) and group 5 (piroxicam group) showed no significant ( $p \leq 0.05$ ) difference in mean value when compared to control group (group 1). Group 4 (0.6ml *Cinnamomum camphora*) had a significant ( $p \leq 0.05$ ) increase with a mean value of 92.4. At week 3, Group 2 (0.2ml *Cinnamomum camphora*) had a significant ( $p \leq 0.05$ ) decrease with a mean value of 13.0. Group 3 (0.4ml *Cinnamomum camphora*) and group 5 (piroxicam group) showed no significant ( $p \leq 0.05$ ) difference in mean value. Group 4 (0.6ml *Cinnamomum camphora*) had a significant ( $p \leq 0.05$ ) increase with a mean value of 85.0.

**Table 1. Assessment of degree of alertness and learning behaviour in the test and control groups using passive avoidance test**

Groups	Treatment	Week 1 Time (mean± SEM)	Week 2 Time (mean± SEM)	Week 3 Time (mean± SEM)
Group 1	Distilled water	120.60 ± 7.3	180.0 ± 73.5	132.40 ± 65.5
Group 2	0.2ml <i>Cinnamomum camphora</i>	197.0 ± 64.5	300.0* ± 0.0	240.0 ± 60.0
Group 3	0.4ml <i>Cinnamomum camphora</i>	187.20 ± 64.0	300.0* ± 0.0	300.0* ± 0.0
Group 4	0.6ml <i>Cinnamomum camphora</i>	70.20* ± 58.3	180.0 ± 73.5	160.60 ± 67.9
Group 5	0.5ml piroxicam	49.20 ± 18.6	180.0 ± 73.5	300.0* ± 0.0

Values are presented in mean ± SEM n=5, \* means values are statistically significant when compared to the control values (SEM= standard error mean)

**Table 2. Assessment of pain bearance threshold and response in the test and control groups using analgesymlmeter**

Groups	Treatment	Week 1 Gram (mean± SEM)	Week 2 Gram (means± SEM)	Week 3 Gram (mean± SEM)
Group 1	Distilled water	10.20 ± 1.8	10.64 ± 3.9	10.64 ± 3.9
Group 2	0.2ml <i>Cinnamomum camphora</i>	14.20 ± 4.5	19.82* ± 4.1	9.46 ± 4.2
Group 3	0.4ml <i>Cinnamomum camphora</i>	7.0 ± 1.1	4.36 *± 0.4	8.50 ± 2.0
Group 4	0.6ml <i>Cinnamomum camphora</i>	6.16 *± 1.5	4.52 *± 0.7	4.52* ± 0.9
Group 5	0.5ml piroxicam	9.60 ± 3.9	23.90* ± 1.1	11.12 ± 3.4

Values are presented in mean ± SEM n=5, \* means values are statistically significant when compared to the control values (p<0.05)

**Table 3. Assessment of pain sensitivity and response behaviour in the test and control Groups using tail CLIP procedure**

Groups	Treatment	Week 1 Time (mean ± SEM)	Week 2 Time (mean ± SEM)	Week 3 Time (mean ± SEM)
Group 1	Distilled water	15.80 ± 7.1	35.40 ± 21.9	35.40 ± 21.9
Group 2	0.2ml <i>Cinnamomum camphora</i>	28.0 ± 19.6	11.20 ± 4.9	13.0* ± 4.9
Group 3	0.4ml <i>Cinnamomum camphora</i>	21.80 ± 3.7	17.0 ± 4.3	14.0 ± 1.9
Group 4	0.6ml <i>Cinnamomum camphora</i>	16.60 ± 4.6	92.4* ± 53.3	85.0* ± 53.9
Group 5	0.5ml piroxicam	62.40 ± 27.2	10.40 ± 3.9	21.0 ± 10.7

Values are presented in mean ± SEM n=5, \* means values are statistically significant when compared to the control values

#### 4. DISCUSSION

The passive avoidance test. Groups 2, 3, and 5 were given 0.2ml *Cinnamomum camphora* essential oil, 0.4ml *Cinnamomum camphora* essential oil, and 0.5ml piroxicam, respectively, and showed a significant increase in avoidance time, while group 4 (0.6ml *Cinnamomum camphora* essential oil) showed a significant decrease in avoidance time when compared to the control group. This large increase in avoidance time is thought to be caused by the presence of 1,8-cineole. *C. camphor* has five chemotypes based on the primary components of its leaf oil: isoborneol, camphor, 1,8-cineole, linalool, and borneol [16,17,18,19,20,21].

Table 1's results support the Farivar et al. [22] study on the protective effect of 1,8-cineole on learning and memory impairment. 1,8-cineole, which is induced by cerebral hypoperfusion in male rats, is known to be a potent antioxidant. The study found that administering 1,8-cineole significantly strengthened passive avoidance memory (P<0.05). Their studies also showed that 1,8-cineole improves behavioral abnormalities following I/R-induced brain damage. Another study conducted by Moss and Oliver [23] examined the potential pharmacological correlations between absorbed 1,8-cineole following exposure to rosemary scent, cognitive performance, and mood.

Performance on cognitive tasks was found to be substantially related to the concentration of absorbed 1,8-cineole after exposure to rosemary scent, with larger concentrations resulting in better performance. Table 2 shows the assessment of pain-bearing threshold and response in the test and control groups using an analgesic meter. Groups 2 and 5 showed a significant increase in pain threshold, while groups 3 and 4 showed a significant decrease in pain threshold when compared to the control group. This considerable improvement in pain tolerance is thought to be caused by the presence of camphor, 1,8-cineole, linalool, and borneol. The pain threshold, also known as the pain threshold, is the point along a curve of increasing perception of a stimulus at which pain becomes noticeable (IASP. "IASP Pain Terminology" 2013). According to Xu et al. [19], camphor has an analgesic effect by activating and subsequently desensitizing transient receptor potential vanilloid-1 (TRPV1). The combination of TRPV1 desensitization and TRPA1 suppression provides a novel explanation for camphor's analgesic actions. Linalool has been demonstrated to reduce pain in models such as the acetic acid-induced writhing response, inflammatory pain, and the hot plate test in mice. The probable mechanism could be related to the inhibition of proinflammatory cytokines and the modulation of NMDA receptors. Topical use of borneol provided much more pain alleviation than placebo. Experiments in mice revealed that the TRPM8 channel could be the molecular target of borneol [24,25,26]. Zheng et al. [27] investigated the effects of 1,8-cineole on neuropathic pain mediated by the P2X2 receptor in the spinal cord dorsal horn and found that oral administration of 1,8-cineole inhibits over-expression of P2X2 receptor protein and mRNA in the spinal cord and dorsal horn in CCI rats.

Table 3 displays the assessment of pain sensitivity and response behavior in the test and control groups using the tail clip procedure. Group 2 (0.2ml *Cinnamomum camphora* essential oil) showed a significant decrease in pain sensitivity, while group 4 (0.6ml *Cinnamomum camphora* essential oil) showed a significant increase in pain sensitivity. This large decrease in pain sensitivity is thought to be caused by the presence of camphor, 1,8-cineole, linalool, and borneol, as observed in the research of Xu et al. [19]; Zheng et al. [27]; Fan et al. [24]; Peana et al. [25] and Wang et al. [26].

The significant decrease in avoidance time shown by group 4, a significant decrease in pain threshold shown by groups 3 and 4, and a significant increase in pain sensitivity shown by group 4 in Tables 1, 2, and 3 respectively is suspected to be a result of increased dose of *Cinnamomum camphora* essential oil.

## 5. CONCLUSION

The results obtained from the different test groups and control group using passive avoidance test, analgesymeter, and tail clip test showed a significant increase in avoidance time, a significant increase in pain threshold, and a significant decrease in pain sensitivity in group 2 which were administered with 0.2ml *Cinnamomum camphora* oil thereby indicating the analgesic effect of the essential oil. However, the reverse was found in group 4 which was administered with 0.6ml *Cinnamomum camphora* oil. Interestingly, the effects of *C. camphora* oil were more on the low-dose group than on the high-dose group. This study concludes that the analgesic effect of *Cinnamomum camphora* essential oil is due to the presence of camphor, 1,8-cineole, Linalool, and borneol in the oil and *Cinnamomum camphora* essential oil is dose-dependent i.e it was more effective in the low dose group than the high dose group.

## 6. RECOMMENDATION

It is therefore recommended that the analgesic use of *Cinnamomum camphora* oil should be done after the prescription of a safe dose by a health physician as it can be toxic when misused.

Further research should be carried out properly on *Cinnamomum camphora* to study the detailed mechanism of action in the body, the safe route of administration, and also the safe dose that can be taken to produce a positive effect.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

The study was approved by the University of Port Harcourt, Research and Ethical Committee and preserved by the author(s)

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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