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# Evaluation of Analgesic Activity of Hydroalcoholic Extract of *Cinnamomum zeylanicum* Bark in Albino Rats

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## ABSTRACT

*The hydroalcoholic extract of Cinnamomum zeylanicum (HAECZ) bark was evaluated for its analgesic activity in albino rats. The HAECZ bark was evaluated for its toxicity study and priliminary phytochemical analysis using standard methods. HAECZ bark was evaluated for its analgesic activity at a dose of 100, 200 and 400 mg/kg, p.o. by using Tail immersion and Hot plate method in Wister albino rats. HAECZ bark showed no toxicity up to 1000 mg/kg body weight and the preliminary phytochemical study indicates presence of carbohydrates, terpenoids, tannins, saponins, flavonoid, glycosides and steroids. The extract showed significant and dose dependent analgesic activity in tail immersion test whereas in hot plate test it showed significant activity at 100 and 400 mg/kg body weight respectively. HAECZ bark showed analgesic activity in albino rats and was comparable with pentazocin.*

**Keywords:** *Cinnamomum zeylanicum*, Analgesic activity, Tail immersion test, Hot plate test.

## INTRODUCTION

Analgesics are most commonly known as painkillers used to reduce the feeling of pain without loss of consciousness. Analgesia is an unpleasant sensation which may be either acute or chronic, usually evoked, by external stimuli and is a consequence of complex processes of the peripheral and central nervous system [1]. The entire analgesic agent produces their therapeutic effects by inhibiting various prostaglandins substances involved in development of pain. The common mechanism of analgesic is

inhibition of cyclooxygenase (COX) enzyme(s) which leads to a decrease in the synthesis of various prostaglandins and thromboxanes [2]. Drugs used to relieve pain are broadly divided into two groups i.e., opioid and nonopioid drugs. The introduction of these drugs has revolutionized the treatment of pain [1]. Various types of plants used in herbalism and some of these plants have medicinal activities. These medicinal plants are rich resources of phytoconstituents which can be used as a source of lead compound for the treatment of various diseases [3]. According to the World Health Organization (WHO), traditional medicines has established and proved to have preventive, curative and rehabilitative roles. From the literature review it was observed that various herbal drugs like *Sida acuta*, *Stylosanthes fruticosa*, *Toona ciliata*, *Bougainvilla spectabilis*, *Ficus glomerata*, *Polyalthia longifolia* and *Toona ciliata* have analgesic activity [4]. The word Cinnamon comes from the Greek word Kinnamon [5] which is a very good spice cultivated in tropical area like Sri Lanka and South east of India. It obtained from dried inner bark of the tree *Cinnamomum zeylanicum* belongs to the family Laureaceae. The bark of cinnamon mainly contains essential oil, cinnamal dehyde, euginol, camphene, cinnamyl acetate and cinnamyl alcohol, which is the main active phytoconstituent of this drug [6]. It shows number of pharmacological effects like anti-inflammatory, anti-oxidant, anti-microbial, antidiabetic, and memory enhancing activity [6]. In ayurvedic system of medicines it is used in preparations like flue preventive, indigestion, flatulence, mouth washes and as per the unani system, the bark is used for gastroenterological problems. Though inflammation is generally coexist with analgesia and from the literature survey it was observed that it is having anti-inflammatory activity Hence, our aim of present study was to evaluate analgesic activity of hydro-alcoholic extract of *Cinnamomum zeylanicum* (HAECZ) bark in wistar albino rats by using two commonly used animal models i.e., Tail immersion test and hot plate test.

## MATERIALS AND METHODS

Dried barks of the *Cinnamomum zeylanicum* were collected from the local market of Berhampur, Odisha on 2$^{nd}$ June 2015. Then it was coarsely powdered using hand grinding machine and were passed through sieve no 60 and stored in air tight containers before extraction.

***Preparation of hydroalcoholic extract***

About 100 g of coarsely powdered bark was taken in a beaker and macerated with 50 ml of equal proportion of solvents containing methanol and water 1:1 for 24 hrs. After 24 hrs the macerated powder was extracted using soxhlet apparatus with 250 ml of solvent containing equal proportion of water and methanol i.e., 1:1, which was maintained at a temperature of 60°C. The powder was extracted up to 4-6 hrs until color of the extract becomes faded. Then the extract was evaporated to dryness in water bath with continuous stirring. The concentrated exact was stored in the decicator till further study.

***Animals***

Wister albino rats weighing 120-140 g of either sex were maintained under controlled condition of light and dark (12 hr each) and a temperature 25 ± 1°C in the animal house of Roland Institute of Pharmaceutical Sciences, Berhampur. The animals were acclimatized for one week prior to actual experiment. All the Pharmacological activities were carried out after obtaining the approval from the Institutional Animal Ethical Committee of Roland Institute of Pharmaceutical Sciences, Berhampur.

***Acute toxicity***

OECD guide line 423 was followed for the acute oral toxicity study. Two animals of either sex were selected randomly for the oral toxicity study. A single dose of hydroalcoholic extract of *Cinnamomum zeylanicum* (HAECZ) bark starting at a dose of 200 mg/kg and progressively increasing to 400 mg/kg, 600 mg/kg, 800 mg/kg and 1000 mg/kg body weight was administered orally to each group of animals. Animals were closely observed for the behavioral changes, locomotion, muscle spasm, loss of righting reflex, tremor, convulsion and mortality for 24 hrs and further observed on $14^{th}$ days for occurrence of toxic symptoms and mortality [7].

***Preliminary phytochemicals testing***

One gram of HAECZ was dissolved in 100 ml of distilled water to obtain a stock solution of concentration 1% (w/v) and was subjected to various phytochemicals testing like carbohydrate, protein, terpenoids, tannins, saponins, flavonoids, alkaloids, glycosides and steroids using standard procedure [8].

***Evaluation of analgesic activity***

**Tail immersion test**

The withdrawal of tail completely from the hot water is called pain reaction time (PRT) if the PRT increases with administration of test substance are considered as analgesic activity [9]. Rats were randomly divided into five groups of six animals each (n=6), food was withdrawn for 12 hrs but not with water. After 12 hrs group-I received distill water p.o., group-II was given pentazocin 20 mg/kg, i.p., group-III, IV and V were administered orally with 100, 200 and 400 mg/kg of HAECZ respectively. After 30 min of pentazocin and 1hr of extract administration, about 3-5 cm of the tail of each rat was dipped into a beaker containing warm water maintained at a temperature of 50 ± 10°C and the time taken for the rat to flick the tail completely from the hot water was recorded for all the rats using a stop watch.

**Hot plate test**

Hot plate induced pain in rat is used to evaluate central analgesic activity of the test substance [10,11]. Adult rats of either sex were randomly divided into five groups containing six animals each (n=6), Food was withdrawn for 12 hrs but not with water. The pre-drug reaction time (PRT) was assessed by placing each rat upon a heated metal plate (Hot plate) maintained at a temperature of about 55 ± 1°C within a restraining cylinder. The PRT for each rat was determined using a stop watch to measure the time taken to produce response like licking of paw and jumping response. A cut off time was put at 20 seconds, this served as control reaction time for the prevention of tissue damage. After the basal reaction time Group-I received distill water, p.o., group-II was given pentazocin 20 mg/kg, i.p and group III, IV and V was given 100, 200 and 400 mg/kg of HAECZ bark p.o. respectively. The PRT for each rat was again determined after the treatment.

***Statistical analysis***

Values are expressed as mean ± SEM. Statistical analysis was performed using ANOVA followed by Dunnett's post hoc test using graph pad prism. P<0.05 was considered as significant.

## RESULTS

***Acute toxicity study***

In acute toxicity study the HAECZ did not show any mortality up to 1000 mg/kg body weight. There were no change in behavior, locomotion, muscle spasm, loss of righting reflex, tremor and convulsion was observed in 24 hours. Further there was no mortality observed after 14 days.

***Preliminary phytochemical evaluation***

The phytochemical test of HAECZ bark contents various phytocostituents is shown in Table 1.

**Table 1:** Preliminary Phytochemical Evaluation

| Phyto-constituents | Results |
|---|---|
| Carbohydrates | + |
| Proteins | - |
| Terpenoids | + |
| Tannins | + |
| Saponins | + |
| Flavonoids | + |
| Alkaloids | + |
| Glycosides | - |
| Steroids | + |
| Note: + = Present,-= Absent | |

***Analgesic activity***

**Tail immersion test in albino rats**

Complete withdrawal of the tail from the warm water was taken as the parameter for the evaluation of analgesic activity. There was no significant difference in reaction time observed in control group at different time interval signifies no analgesic activity, further there was no significant difference in reaction time between the treatment group with control group at basal time implies that all the animal have same basal reaction time. Pentazocin at a dose of 20 mg/kg showed significant ($p$<0.01) difference in

reaction time compared to control group at 15, 30 and 60 min respectively (Table 2). The HAECZ at a dose of 200 and 400 mg/kg showed significant difference ($p$<0.05) and ($p$<0.01) respectively at 30 min time interval compared to control group. Whereas at 60 min the extract at a dose of 200 and 400 mg/kg body weight showed significant ($p$<0.01) difference compared to control group. Hence from the above study it was found that HAECZ bark have significant analgesic activity (Table 2).

**Table 2:** Reaction time of hydro alcoholic extract of *Cinnamomum zeylanicum* (HAECZ) bark and pentazocin using tail immersion test in albino rats.

| Groups | Treatment | Dose(mg/kg) | Reaction Time (Sec) Mean ± SEM | | | |
|---|---|---|---|---|---|---|
| | | | Basal | 15 min | 30 min | 60 min |
| I | Control | --- | 4.6 ± 0.49 | 5.6 ± 0.49 | 5 ± 0.36 | 4.6 ± 0.66 |
| II | Pentazocine | 20 | 4.8 ± 0.30 | 7.6 ± 0.21** | 10.6 ± 0.33** | 10.6 ± 0.33** |
| III | HAECZ | 100 | 4 ± 0.36 | 4.6 ± 0.33 | 5 ± 0.36 | 5.3 ± 0.21 |
| IV | HAECZ | 200 | 3.6 ± 0.21 | 4.6 ± 0.21 | 6.3 ± 0.21* | 6.6 ± 0.21** |
| V | HAECZ | 400 | 3.6 ± 0.21 | 5.6 ± 0.33 | 9.5 ± 0.42** | 9.6 ± 0.33** |
| **Note:** Each values were represented as Mean ± SEM, ( n=6); * p<0.05, ** p<0.01 as compared to control group | | | | | | |

**Hot plate test in albino rats**

Hot plate test is popularly used for the evaluation of analgesic activity. The reaction time was taken as the parameter for the evaluation of analgesic activity. The control group showed no significant difference in reaction time at different time interval. The treatment groups also showed no significant difference in reaction time compared to control group at basal point. Pentazocin 20 mg/kg showed a significant difference ($p$<0.01) compared to control group at 15, 30 and 60 min respectively (Table 3). HAECZ 100 and 400 mg/kg showed significant difference ($p$<0.01) at 15, 30 and 60 min interval compared to control group (Table 3). Hence we found that HAECZ bark have analgesic activity which is shown in Table 3.

**Table 3:** Reaction time of hydro alcoholic extract of *Cinnamomum zeylanicum* (HAECZ) bark and pentazocin using hot plate model in albino rat.

| Groups | Extract/drug | Dose(mg/kg) | Reaction Time (Sec) Mean ± SEM | | | |
|---|---|---|---|---|---|---|
| | | | Basal | 15 min | 30 min | 60 min |
| I | Control | --- | 4 ± 0.33 | 4.3 ± 0.1 | 4.6 ± 0.19 | 4.6 ± 0.30 |
| II | Pentazocine | 20 | 5 ± 0.36 | 7.3 ± 0.33** | 10 ± 0.36** | 10.6 ± 0.42** |
| III | HAECZ | 100 | 5.3 ± 0.33 | 6 ± 0.36** | 7 ± 0.36** | 7.3 ± 0.21** |
| IV | HAECZ | 200 | 4.3 ± 0.21 | 4.7 ± 0.21 | 5.6 ± 0.21 | 6.2 ± 0.36* |
| V | HAECZ | 400 | 5 ± 0.33 | 6 ± 0.33** | 8.3 ± 0.19** | 10.2 ± 0.19** |
| **Note:** Each value is represented as Mean ± SEM, n=6, *p<0.05, **p<0.01 as compared to control group. | | | | | | |

## DISCUSSION

Analgesics are the drugs used to relieve pain without affecting the consciousness of the patient. There are many synthetic and herbal drugs which are used as analgesics. In this study we have taken the hydroalcholic extract of *Cinnamomum zeylanicum* (HAECZ) to evaluate the analgesic activity. The phytochemical study of HAECZ bark shows presence of carbohydrates, terpenoids, tannins, saponnins, flavonoids, alkaloids and steroids (Table 1). The bark of cinnamon mainly contains active chemical constituents like essential oil, cinnamaldehyde, euginol, camphene, cinnamyl acetate and cinnamyl alcohol [6].

From the literature review it was confirmed that *Cinnamomum zeylanicum* has shown different pharmacological activity like anti-inflammatory, antioxidant, antimicrobial, anti-diabetic, and memory enhancing activity. In the present study we have evaluated the analgesic activity of HAECZ bark using two most commonly used methods like Tail Immersion test and Hot plate test. The hot plate test and tail immersion test were used to evaluate central analgesic effect. The HAECZ bark shows a significant analgesic effect in both tail immersion and hot plate test (Tables 2 and 3).

As per Rahmtullah et al., *Curcuma longa* showed analgesic and anti-inflammatory activity due to presence of chemical constituents like saponins, Tannins, flavonoids and alkaloids [12]. Therefore in the present study HAECZ bark shown analgesic activity in two most popularly method may be due to presence of the above mention chemicals. Further studies are required to investigate the possible mechanism of analgesic effect of HAECZ and to isolate the individual phytoconstituent and evaluate its analgesic activity using above methods.

## CONCLUSION

From the above study it was concluded that hydroalcoholic extract of *Cinnamomum zeylanicum* bark have analgesic activity in dose depended manner. It is not showing any toxic effect up to the dose of 1000 mg/kg of body weight. Further study required to confirm the mechanism and exact phytoconstituent responsible for its analgesic activity.

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