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In vivo Evaluation of Peripheral Analgesic and Diarrhea Retardation Effects of Crude Methanolic Extracts from Diverse Potential Spice *Cinnamomum zeylanicum* L. Barks on Swiss Albino Mice

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Abstract

The crude methanolic extracts from the bark of *Cinnamomum zeylanicum* (*C. zeylanicum*) (Family: Lauraceae) were carried out for the evaluation of peripheral analgesic activity and anti-diarrheal activity *in vivo* bioassays using a group of Swiss Albino mice. Two different doses containing 400 mg/kg and 200 mg/kg with respect to the weight of the test animals were administered in both the cases for the assessment of action in comparison with the standards. In the estimation of analgesic effect peripherally, both the extracts of *C. zeylanicum* exhibited extremely significant pain relieving action with percent inhibition of licking responses of 63.89% at 400 mg/kg and 66.67% at 200 mg/kg close to the value of standard Acetyl Salicylic Acid with 75% inhibition of response induced by Acetic Acid at a dose of 0.1 ml/kg of body weight to each mouse. The bark extracts of *C. zeylanicum* at lower dose of 200 mg/kg showed extremely statistically significant anti-diarrheal activity with 68.18% reduction of diarrhea compared to the standard loperamide 59.09% in castor oil induced antidiarrheal assay protocol.

Keywords: *Cinnamomum zeylanicum*; Peripheral analgesic; Anti-diarrheal; Licking; Barks

Introduction

Traditional medicine is the application of theories, beliefs and experiences indigenous to different cultures through the knowledge, skill, and practices, may be definable or not, for the betterment of health in order to prevent, diagnose, improve or treat any type of ailment of human body [1]. Sheng-Ji narrated ancient information containing description of 67 species of plants in about 450 to 600 B.C. from the Vedas [2].

Additionally, the World Health Organization (WHO) has estimated that about 80% of the populations use the extracts of plant and their active components for treating their primary health problems [3,4]. These active herbal medicines are environmentally obtainable essence from plant-originated substances having pharmacological or other human health related effects with little or no processing that have been used to treat diseases locally or regionally as per the definition of World Health Organization [5,6]. Consequentially, increment of the number of individuals seeking herbal medicines lies on the generation of the expertise of practicing physicians who are known as Kavirajes for thousand years on the aboriginal medicine network [7].

About 75-80% people of the world with majority in the developing countries depends on plant originated drugs for main health care due to less side effects besides being cheap accessible and better cultural acceptability [8,9].

The scientific search for biologically active substance is quite common from time immemorial in Bangladesh and growing rapidly from the acknowledgement of 500 plant species with medicinal values according to the observatory book of the Asiatic Society, Bangladesh [10,11]. From the age immemorial the name Cinnamon has been established as a flavouring agent commonly expressed as spice. Barks of *Cinnamomum zeylanicum* Linn; commonly called as cinnamon in English and as dal chini, darchini, dal cheeni in hindi and Bangla from Lauraceae family is almost absolutely cultivated in lands. It is a small evergreen tree which is acquired from the brown bark

forming quills with longitudinal striations possessing handy trade related usefulness [4].

About 70% people of Western countries uses anti-nociceptive drugs regularly for headaches, other specific pains and febrile illness [12] which is much higher in the developing countries. The insulin-potentiating effect showed the prospect and likelihood of being a spice of day to day life having the quality and probability for the ailment of thousands of people of the developing countries [13,14]. Besides, Diarrhoea is a leading cause of malnutrition and death among children in the developing countries of the world which is characterized by increased frequency of bowel movement, wet stool and abdominal pain [15,16]. The plausibility of commonly used spices having Diarrhoea reducing activity would bring new dimension for the people with lower income countries. As a result, bark of the plant was taken into account for the exploration of the above mentioned medicinal properties.

Materials and Methods

Collection and extraction of plant barks

The barks of *C. zeylanicum* of were collected from Sher-e-Bangla Agriculture University area, Dhaka and sent to Bangladesh National Herbarium, Dhaka, Bangladesh for recognition. A voucher specimen with Accession number 44916 represents authenticity of the collected plant. The fresh barks were dehydrated for 15 days in order to have a proper grinding. The properly powdered material (500 g) which was obtained from desiccated barks after passing through the grinding machine was rinsed in 1.5 L of methanol. The Amber glass bottle with capacity of 5 L containing powder and 98% methanol was made secure by sealing with bottle cap and kept for a period of 21 days accompanying occasional shaking and stirring. A rotary evaporator was used to concentrate the 98% methanolic extract using low temperature and reduction of pressure after filtering through a cotton plug and with a filter paper respectively.

Experimental animals

Swiss-albino mice (18-24 g) aged 4-5 weeks of either sex was obtained from the Animal Resource Branch of the International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR,B). They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 2ºC; 60-70% relative humidity) in a 12 h light-dark cycle and fed ICDDR,B formulated rodent food and water (ad-libitum). As these animals are very sensitive to environmental changes, they are kept before the test for at least 3-4 days in the environment where the experiment will take place.

Chemicals

1% solution of acetic acid, Acetyl Salicylic Acid and Loperamide (Collected from Square Pharmaceuticals Limited, Bangladesh) were used in the experiment. Analytical grade reagents and solvents for conducting the studies were provided by Phytochemical Research Laboratory of State University of Bangladesh.

Evaluation of anti-diarrheal activity

The anti-diarrheal activity using the methanolic extracts of *C. zeylanicum* barks was assayed using the method of castor oil initiated frequent watery discharge from bowel in mice [17-19]. Castor oil induced Swiss albino mice were separated into control, positive control and test groups containing three mice in each group. Control group received vehicle {Tween 80 (0.5 ml/kg)} in normal saline] at dose of 10 mg/kg orally as demonstrated by Shoba and Thomas. The positive control group received Loperamide (Imotil- Loperamide hydrochloride 2 mg/capsule) from Square Pharmaceuticals Limited at the dose of 50 mg/kg b.w. orally. The two test group received extracts of *C. zeylanicum* barks at the doses of 200 mg/kg and 400 mg/kg b. w. respectively. Each animal was placed in a discrete cage and the floor lining was changed at every hour. The number of diarrheic feces excreted by the animals was recorded during an observation period of 5 hours.

The percentage hindrance of defecation in mice was estimated by the following equation:

% Inhibition of defecation = [(D1-D2)/D1] × 100

D1 = Number of Mean defecation induced by castor oil

D2 = Number of Mean defecation induced by drug or extract

Peripheral analgesic activity

Peripheral analgesic activity can be assayed by using acetic acid induced writhing test [20,21]. Physical sensation pain is exerted by injecting 0.1 ml/kg Acetic Acid subcutaneously into the right hind paw of the test animals. As a result writhing response at regular intervals due to distressed feeling by external stimuli occurs [22-25]. The incorporation should continue as long as the pain sensation exists. The enumeration of response due to licking and biting experience is performed by each writhing and contrasted with the control group [26]. Acetylsalicylic acid (Carva-Aspirin 75 mg/tablet) was taken as standard to compare effect at both higher and lower dose of 200 mg and 400 mg/kg body weight. Twelve randomly selected experimental animals were divided into four groups where they received a particular treatment. Prior to any treatment, each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly. As it was difficult to observe the biologic response of the mice at a time receiving same treatment, it was necessary to identify individual animal of a group during the treatment as M1, M2, and M3.

In order to administer the extract at doses of 400 mg/kg body weight and 200 mg/kg body weight of mice, the exactly weighed extracts were measured respectively and triturated in unidirectional way by adding of small amount of Tween-80 (a suspending agent). After proper mixing of extract and suspending agent, normal saline was slowly added. The final volume of the suspension was made up to 3.0 ml. To stabilize

the suspension, it was stirred well by vortex mixture. Acetylsalicylic acid at the dose of 50 mg/kg body weight was made for incorporation to the positive control group. At zero hour test samples, control {Tween-80 (0.5 ml/kg)} solution in saline) and Acetylsalicylic acid were administered orally by means of a long needle with a ball-shaped end. After 30 minutes Acetic Acid (0.1 ml/kg) at a dose of 10 ml/kg body weight was administered subcutaneously to each of the animals of all the groups. Each mouse of all groups were observed individually for counting the number of writhing responses they made in 5 minutes commencing just after the subcutaneous administration of acetic acid solution.

% Inhibition of Writhing response= [(P1-P2)/P1] ×100

P1= Control writhing response

P2= Test writhing response

Statistical analysis

Representation of values were performed as (mean ± SD) and ANOVA was utilized for contrasting the level of significance between the control group and experimental groups, where the p values and statistical significance were inscribed below the table.

Results and Discussion

From **Table 1,** the test was performed by taking samples at doses 400 and 200 mg/kg body weight. Statistical evaluation of the data confirmed that both doses of extract showed significant peripheral analgesic activity with percent inhibition of licking responses was 63.89% (400 mg/kg) to 66.67% (200 mg/kg). The reasons for this anti-nociceptive action may be due to the presence of tannins, flavonoids, saponins and steroids [27,28]. Hence, the extracts may either create hindrance in the biosynthesis of prostaglandins or exertion of activity by the combination with receptor.

The methanolic extracts of bark of *C. zeylanicum* were subjected to castor oil induced anti-diarrheal test and the following data are collected and recorded in **Table 2.** The methanolic extracts of bark of *C. zeylanicum* (200 mg/kg) exhibited statistically significant anti-diarrheal activity with a 68.18% reduction of diarrhea compared to the standard loperamide. Flavonoids could have the potential against diarrheal effect [29] which might be present in *C. zeylanicum*.

Table 1: Test materials used in the evaluation of peripheral analgesic activity of crude extract of *C. zeylanicum* (The values are represented as mean±SEM; n=3, *p<0.0003, **p<0.002, ***p<0.0007, indicates significant compared to control).

Test Samples (Code no.)	Dose	Number of writhing reaction (Mean ± SEM)	t-test value	% Inhibition writhing response
Tween-80 (0.5 ml/kg) in normal saline (Control)	0.1 ml/10 g of body weight	24 ± 1.15	_	_
Acetyl Salicylic Acid (Standard)	50 mg/kg body weight	6 ± 0.58*	13.9427	75.00
Fractionated Methanolic extract (FME 400)	400 mg/kg body weight	8.67 ± 1.67**	7.5624	63.89
Fractionated Methanolic extract (FME 200)	200 mg/kg body weight	8 ± 1.15***	9.798	66.67

Table 2: Test materials used in the evaluation of anti-diarrheal activity of crude extract of *C. zeylanicum* (The values are represented as mean±SEM; n=3, *p<0.003, **p<0.004, ***p<0.0005 indicates significant compared to control).

Test Samples (Code no.)	Dose	Number of Diarrheal feces (Mean ± SEM)	t-test value	% Reduction of diarrhea
Tween-80 (0.5 ml/kg) in normal saline (Control)	0.1 ml/10 g of body weight	7.33 ± 0.33	_	_
Lopeiramide (Standard)	50 mg/kg body weight	3 ± 0.58*	6.5	59.09
Fractionated Methanolic extract (FME 400)	400 mg/kg body weight	4.33 ± 0.33**	6.364	40.91
Fractionated Methanolic extract (FME 200)	200 mg/kg b.w.	2.33 ± 0.33***	10.6066	68.18

Conclusion

First time reporting of *in vivo* activities of *C. zeylanicum* for both the studies stipulate the possession of peripheral analgesic and anti-diarrheal activities while assessing the methanolic fractions. However, more secure cost effective active compounds responsible for those specific bioactivities will bring science to new horizon in the treatment of pain and

diarrhoea through day to day used multipotential herb. Further study is required by using HPLC for precise identification.

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