SUPPLEMENTARY MATERIAL

Title: *Cinnamomum cassia*: an implication of serotonin reuptake inhibition in animal

models of depression

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Abstract

The aim of the study was to explore the traditional use of *Cinnamomum cassia* against depression. The standardized methanolic extract of the bark of *C. cassia* was evaluated for antidepressant action using various behavioral tests i.e. tail suspension test (TST), forced swim test (FST) and locomotor activity test. The serotonergic and noradrenergic modulation was assessed using 5-hydroxytryptophan (5-HTP) induced head twitches and yohimbine potentiation tests, respectively. The fluoxetine and phenelzine was used as positive control in the study. The *C. cassia* extract significantly decreased the immobility time in TST (Maximum effective dose tested was 50 mg/kg) while no effect was observed in FST and locomotor activity test. The extract significantly increased the 5-HTP induced head twitches while yohimbine induced lethality remain unaltered. The aforementioned results resembles to that caused by fluoxetine. The standardized methanolic extract of *C. cassia* demonstrated antidepressant activity that can be attributed to rise in serotonin levels.

Key words: *C. cassia*; fluoxetine; antidepressant; serotonin

Experimental

Animals

Sprague-Dawley rats (150–200 g) and Balb/c mice (25-30g) were obtained from the Animal house of the COMSATS institute of information technology (CIIT), Abbottabad. The animals were housed under standard environmental settings at ($25 \pm 2^{\circ}\text{C}$) with a 12 hour light/dark cycle with free access to food and water. The experiments were carried out according to the recommendations of the ethical care committee of CIIT which is in accordance with the guidelines provided by the National Institute of Health (NIH publication no. 85-23, revised 1985) for the care and use of laboratory animals.

Chemicals

The following chemicals were used: 5-Hydroxytryptophan supplement (100 mg capsules, Natrol, USA), Ethanol (BDH laboratory, England), Cinnamaldehyde, fluoxetine phenelzine and yohimbine (Sigma Aldrich, USA). Distilled water was used during experiments

Preparation of extract

Bark (1 kg) of *C. Cassia* (identified by Mr. Sher Wali, Herbarium Department, University of Karachi; Specimen voucher No. 20051) was cut into minute pieces and extracted repeatedly four times through 70% methanol at room temperature. The extract was concentrated under vacuum to give thick syrup that was freeze dried leading to the crude methanolic extract. The extract was standardized against cinnamaldehyde using HPLC-DAD (Agilent 1200, USA). Briefly, the methanolic extract of *Cinnamomum cassia* (25 mg/10ml) and cinnamaldehyde (6.9 mg/10ml) were diluted (1ml/10ml) and 5 μl of each sample was injected in the HPLC containing C18 column (4.6 x 50 mm, 1.8 μm , Zorbax SB, Agilent Technologies, USA) set at 20°C . Acetonitrile and 0.04% aqueous acetic acid (25:75) was used as mobile phase and flow rate was 1.0 ml/min.

Tail Suspension Test (TST)

The test was performed as explained by Steru (Steru et al. 1985). Male mice were separated into four groups i.e. plant extract (25, 50 and 100 mg/kg), fluoxetine (30mg/kg), phenelzine (30mg/kg) and vehicle (normal saline, 10ml/kg). One hour after administration (intraperitoneal),

animals were suspended (15cm above the ground) from the tail for a period of 6 min. The immobility time was recorded for the last 5 minutes providing first minute for acclimatization.

Locomotors activity test

The test animals were divided into three groups i.e. *Cinnamomum cassia* (IC₅₀ dose), phenelzine (30mg/kg) and vehicle (10ml/kg). Preceding the administration test substances, the mice were placed (5 minutes) in the activity cage (1.5×1.5 feet box having marking of 2 × 2 inches on the floor) for familiarization. After 1 hour of administration, the animals were again placed in an activity cage and behavior was videotaped. The number of boxes crossed by the test animal were counted and compared with the control.

Forced swim test (FST)

FST was executed as described by Porsolt (Porsolt, Le Pichon and Jalfre 1977). The test was performed in two sessions, pre-test session and a test session. In pre-test session, rats were placed in FST tank (18 × 18 × 50 cm) filled with water (25 ± 2 °C) up to 20 cm depth. After 24 hours, the animals were treated (*i.p.*) with plant extract (Maximum effective dose tested), phenelzine (30mg/kg), fluoxetine (30mg/kg) and vehicle (5ml/kg). After 1 hour, the treated animals were again exposed to FST for six minutes and immobility time was recorded for last 5 minutes giving the 1st minute for familiarization.

5-hydroxytryptophan induced head twitches

Three groups were made from male mice (n= 5 / group) i.e. 5-HTP alone (200 mg/kg) or in combination with potent extract (Maximum effective dose tested) or fluoxetine (30 mg/kg). 5-HTP was administered to 1st group and head twitches were counted for 30 minutes. In combination groups, 30 minutes before the 5-HTP administration, the animals were treated with respective test substances to evaluate their effect on the head twitches count (Sallinen, Haapalinna, Viitamaa, Kobilka and Scheinin 1998).

Yohimbine potentiation test

The mice were divided into three groups i.e. yohimbine (40 mg/kg dissolved in ethanol) alone or in combination with potent plant extract (Maximum effective dose tested) or phenelzine (30 mg/kg). After 24 hours, animals were observed for mortality and compared with control (Quinton 1963).

Statistics

Data was presented as means $\pm$ S.E.M (n = 5 / group for FST and TST while n = 10 / group for 5-HTP induced head twitches and Yohimbine potentiation test). Comparison between groups was computed using one-way ANOVA followed by post hoc test (Least Significant Difference) using SPSS software.

References

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Figure S1. Standardization of *Cinnamomum cassia* methanolic extract

The bark extract was standardized against cinnamaldehyde using HPLC-DAD. Figure (a) shows the chromatogram of active cinnamaldehyde (6.9 mg/10ml, retention time = 15.474 min) while figure (b) shows the chromatogram of *C. cassia* extract (25 mg/10ml) showing the cinnamaldehyde peak at 15.429 min which is equivalent to 0.22 mg.