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Neuropharmacological evaluation of methanolic extract of *Costus speciosus* Linn. rhizome in Swiss albino mice

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ABSTRACT

Objective: To evaluate the neuropharmacological properties of *Costus speciosus* (*C. speciosus*) rhizome using different experimental mouse models.

Methods: The anxiolytic effect was investigated by hole-board test, elevated plus maze and light/dark test, while central nervous system (CNS) depressant effect was evaluated by thiopental sodium-induced sleep test. Finally, antidepressant effect was evaluated by forced swimming test and tail suspension test.

Results: In both elevated plus maze and hole board test, 400 mg/kg *C. speciosus* showed more significant CNS depressant effect than 1 mg/kg diazepam. Both 200 mg/kg and 400 mg/kg *C. speciosus* extract produced a significant dose-dependent decrease in onset of sleep. In forced swimming test, *C. speciosus* rhizome showed a decrease in duration of immobility in a dose-dependent manner. Imipramine (10 mg/kg) and *C. speciosus* extract at 400 mg/kg dose exhibited a significant reduction in duration of immobility in tail suspension test which provided additional evidence of antidepressant effect of *C. speciosus* rhizome.

Conclusions: Our study indicates that *C. speciosus* rhizome possesses CNS depressant, anxiolytic and antidepressant-like activities. Further studies are warranted determine the exact phytoconstituents and mechanism of action responsible for the neuropharmacological effect.

1. Introduction

Among the stress-related mental issues responsible for different physiological disorders and early death, anxiety and depressive disorders are most common. It has been assumed and confirmed that more than 20% adult populations suffered these mental conditions during their life[1]. According to the WHO report on mental health, over 1.1 billion people worldwide suffering mental disorder, and people with anxiety disorder account for almost 4 percent of the world population[2]. It is assumed that the total incidence of the disease will increase from 12.3% to 15.0% within the year 2020[3]. Because of the upward push of this alarming anticipation, WHO speculates that depression will become the second place among causes for premature death and disability within 2020[2]. Depression is considered as a burden for every country and is more severe in third world countries where diagnosis and treatment are inadequate and relatively more expensive[4]. This burden has a significant effect on the total economic and health of every country[5]. Because of

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various pharmacological side effects and resistance to the chronic treatment, approximately two-thirds of depressed or anxious patients are reacting poorly to the currently available treatment[6]. This situation has led researcheres to investigate medicinal plants which can combat anxiety and depression[7]. Medicinal plant researches have been standardized constantly to search the potent new therapeutic products from plant sources for the remedy of neurological diseases using different experimental animal models[8].

Our study focused on *Costus speciosus* (*C. speciosus*), a flowering plant also known as crepe ginger which belongs to the family Costaceae under the order of Zingiberales[9]. *C. speciosus* is native to Southeast Asia, commonly grown as medicinal and ornamental plants[10]. The rhizomes of *C. speciosus* are significant sources of saponins, diosgenin and tigogenin. Previous studies reported that methanolic extract of the rhizome revealed the presence of alkaloids, cardiac glycosides, saponins, and sterols[11,12]. Five new compounds were reported to isolate from the rhizomes, tetradecyl 13-methylpentadecanoate, tetradecyl 11-methyltridecanoate, 14-oxotricosanoic acid, 14-oxoheptacosanoic acid and 15-oxooctacosanoic acid[9]. The rhizomes are bitter and have antidiabetic, anti-dyslipidemic, hepatoprotective, antibacterial, antifertility, anti-inflammatory, antipyretic, anti-asthmatic, antifungal and estrogenic activities[13–17]. According to the literature survey, there is no evidence of neuropharmacological effects of methanolic extract of *C. speciosus* rhizomes. Therefore, this study was performed in order to explore the neuropharmacological potentiality of *C. speciosus* rhizome.

2. Materials and methods

2.1. Collection and identification of plant parts

The rhizome of *C. speciosus* was collected from Maheshkhali, Bangladesh. Identification and authentication of this plant part were confirmed by National Herbarium of Bangladesh (Authentication number: 43651).

2.2. Drying, grinding and extraction of rhizome of C. speciosus

The collected rhizomes were separated from unwanted materials, flowers and other plant parts. After grind, coarse powders of this plant were dried by a consecutive air drying process without any direct exposure to sunlight. Then, 500 g of ground fine powders was emerged in 1.5 L methanol in a suitable jar and sealed properly for 15 d, while occasional shaking and stirring was employed to agitate the mixture for proper extraction. The filtrate (methanol extract) was obtained after filtration by a piece of smooth, white cotton material, then by Whatman filter paper and finally evaporation by spontaneous natural vaporization method. At the end, 30.2 g of gummy concentrate slight golden color extract was obtained.

2.3. Experimental animals

Swiss-albino mice were used for this study. They were collected from the animal house of Jahangirnagar University, Savar, Dhaka and kept at 20-25 ℃ and (55±10)% relative humidity. All experimental animals were fed by ICDDRB formulated rodent food and water *ad libitum*. They were allowed to acclimatize to the laboratory conditions at the least three to four days earlier the date conducting the experiment[18]. All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the ethical committee of Noakhali Science and Technology University (Approval no. R-2018070045).

2.4. Study design

Swiss albino mice were randomized into four groups (with five mice in every group). Group-1: Distilled water 10 mL/kg orally, Group-2: Diazepam 1 mg/kg orally in case of anxiolytic test; Diazepam 2 mg/kg orally in case of thiopental induced sleep test; Imipramine 10 mg/kg in case of antidepressant test, Group-3: *C. speciosus* rhizomes dissolved in distilled water with few drops of Tween 80, 200 mg/kg orally, Group-4: *C. speciosus* rhizomes dissolved in distilled water with few drops of Tween 80, 400 mg/kg orally.

2.5. Anxiolytic evaluation

2.5.1. Elevated plus maze test

This anxiolytic test was done with the reference of method described by Lister[19], here an entry was defined as having all 4 paws inside the arm[20].

2.5.2. Hole board test

Hole board test is an experimental model used for screening anxiolytic activity based on number of head dips in holes. This test was performed with the reference of method described by by File and Wardill[21].

2.5.3. Light dark test

The light/dark test was performed according to the Bourin and Hascoët[22] method, where rodents were subjected to brightly illuminated regions and their exploratory behaviour in response to slight stressors with subsequent innate aversion was observed.

2.6. CNS depressant evaluation

Thiopental induced sleep test was performed according to the previously defined method[23]. The time to lose righting reflex, without delay after thiopental sodium injection (latent period) and the length of sleep (time between the loss and recuperation of reflex) were observed.

2.7. Antidepressant evaluation

2.7.1. Tail suspension test

Tail suspension test was employed to measure stress in mice. It is primarily based on the theory that if a mouse is subjected to inescapable stress, it would become motionless, which represents the state of depressive nature[24]. Steru *et al*.[25] described this method for the very first time, on which this experiment was performed.

2.7.2. Forced swimming test

Forced swimming test is another behavioral test to evaluate antidepressant activity[26] and this study used the method described by Porsolt *et al*[27].

2.8. Statistical analysis

All the experimental data were analyzed statistically by SPSS version 19 and expressed as mean ± SEM. One-way analysis of variance (ANOVA) followed by Dunnett's *t*-test were used for analysis, and $P<0.05$ was considered to be statistically significant.

3. Results

3.1. Anxiolytic evaluation

3.1.1. Elevated plus maze test

Methanolic extract of *C. speciosus* produced anxiolytic-like effect at both doses level of 200 and 400 mg/kg respectively, which was confirmed by increased time in open arm circumstances and reduced time compared to the control subjects (Table 1).

3.1.2. Hole board test

The methanolic extract of *C. speciosus* increased the number of head dips significantly ($P<0.01$) at two doses level of 200 and 400 mg/kg (33.6 ± 3.69 and 57.4 ± 2.23, respectively) compared with control group (12.80 ± 1.68), and 400 mg/kg showed more significant result even compared to standard drug group (34.20 ±2.01).

3.1.3. Light dark test

The methanolic extract of *C. speciosus* increased the time spent in light compartment significantly ($P<0.01$) at 200 and 400 mg/kg compared with control group (Table 2).

Table 1. Effect of *C. speciosus* rhizome on elevated plus maze test in mice.

Groups	Time spent in open arm	Time spent in closed arm
Control (10 mL/kg distilled water)	20.42 ± 2.04	279.58 ± 2.04
Diazepam (1 mg/kg)	85.76 ± 3.26**	214.24 ± 3.26**
C. speciosus (200 mg/kg)	38.08 ± 9.12	261.91 ± 9.12*
C. speciosus (400 mg/kg)	50.74 ± 2.35*	249.26 ± 2.35**

All values are represented as mean ± SEM (n = 5), **$P<0.01$, *$P<0.05$ when compared with control.

Table 2. Effect of methanolic extract of *C. speciosus* rhizome on light dark test.

Group	Time spent in light box(s)	No. of transitions
Control (distilled water 10 mL/kg)	85.60 ± 3.89	14.20 ± 0.86
Diazepam (1 mg/kg)	205.96 ± 19.73***	15.00 ± 1.58
C. speciosus (200 mg/kg)	138.05 ± 9.86*	12.60 ± 0.81
C. speciosus (400 mg/kg)	151.91 ± 8.03**	12.20 ± 1.39

All values are represented as mean ± SEM (n = 5) where, level of significance stated as ***$P<0.001$, **$P<0.01$, *$P<0.05$ when compared with the control group.

3.2. CNS depressant evaluation

Both 200 mg/kg and 400 mg/kg doses of the extract induced a significant decrease in onset of sleep and significantly increased the duration of sleep compared with control group (Table 3). Extract at 400 mg/kg showed more significant effect than 200 mg/kg.

3.3. Antidepressant evaluation

Tail suspension test and forced swimming test all showed that methanolic extract of *C. speciosus* at two doses and imipramine significantly decreased immobility time compared with control groups ($P<0.05$) (Table 4).

4. Discussion

Anxiety and depressive disorders are pathological conditions with high prevalence and play a remarkable role in global morbidity and mortality[28,29]. The present study successfully validates the neuropharmacological effects of methanol extract of *C. speciosus* rhizome.

Elevated plus maze model is a broadly used *in vivo* method for screening anxiolytic potentiality, in which experimental animals avoiding uncovered open areas of the maze and having a desire for sections enclosed by protecting wall are presumed to be aversive[29]. If tested animals treated with plant extracts showed any statistically significant changes in open arms, it is considered as the sign of anxiolytic effectiveness of this particular plant extract. In this experiment, methanolic extract of *C. speciosus* produced anxiolytic-like effect at both doses level of 200 and 400 mg/kg, which was confirmed with the increased time spent in open arm circumstances and the reduced time spent in the closed arms.

Table 3. Effect of methanolic extract of *C. speciosus* rhizome on thiopental-induced sleeping time test.

Group	Onset of sleep (min)	Duration of sleep (min)
Control (distilled water 10 mL/kg)	9.80 ± 0.66	55.00 ± 5.90
Diazepam (2 mg/kg)	7.20 ± 0.73*	173.60 ± 4.73**
C. speciosus (200 mg/kg)	5.20 ± 0.58**	65.60 ± 3.31
C. speciosus (400 mg/kg)	3.00 ± 0.31**	79.80 ± 4.10**

All values are represented as mean ± SEM (n = 5), **$P<0.01$ and *$P<0.05$ when compared with the control group.

Table 4. Effect of methanolic extract of *C. speciosus* rhizome on tail suspension test and forced swimming test in mice.

Groups	Immobility time	
	Tail suspension test	Forced swimming test
Control (10 mL/kg distilled water)	232.53 ± 8.39	206.53 ± 2.82
Imipramine (10 mg/kg)	199.68 ± 2.91*	144.64 ± 2.16**
C. speciosus (200 mg/kg)	177.46 ± 5.18**	201.97 ± 15.33
C. speciosus (400 mg/kg)	124.67 ± 8.00**	142.33 ± 2.99**

All values are represented as mean ± SEM (n = 5), $^{**}P<0.01$, $^{*}P<0.05$ when compared with the control group.

The anxiolytic effect of *C. speciosus* is just like diazepam which is a popular drug of benzodiazepine group. The effect of anxiolytic agent is associated with facilitating the opening of the Gamma amino butyric acid $(GABA)_A$-activated chloride channels. Therefore, it can be hypothesized that *C. speciosus* could act by inducing a benzodiazepine-like substance[29]. Other ways to test anxiolytic effect are hole board test and light dark test. The hole board model is based on the head-dipping behavior of test animals which are sensitive to changes in the emotional state and have increase in head–dipping behavior in anxiolytic-like state[30,31]. Crude methanolic extract of *C. speciosus* at doses of 200 mg/kg and 400 mg/kg showed a significant increase in the number of head dips (33.6 and 57.4, respectively) when compared to the control group (12.8). According to Barua *et al*[32], in light/dark test, rodents are subjected to brightly illuminated regions and their exploratory behaviour in response to slight stressors with subsequent innate aversion is observed. This test is useful to determine anxiolytic-like or anxiogenic-like activity of mice. Transition is regarded as an index of activity-exploration because of habituation over time, and the time spent in every compartment is regarded a reflection of aversion[33]. Different classical models are used[34] for different neuropharmacological tests, namely forced swimming, tail suspension, and thiopental-induced sleep tests[34]. GABA is the foremost inhibitory neurotransmitter in the CNS[35]. Involvement of GABA in various neurological disorders such as epilepsy, depression, Parkinson syndrome, and Alzheimer's disease has been explored[36]. Diverse drugs utilized in various neurological and psychological problems may modify the GABAergic actions, which is acted by increasing synthesis of GABA through potentiating the GABA-mediated postsynaptic inhibition *via* allosteric changes in GABA receptors. It at once increases the chloride conductance or indirectly potentiates GABA-triggered chloride conductance with simultaneous depression of voltage activated Ca^{2+} channel like barbiturates[37,38]. Therefore, it is presumed that the plant extract may act by potentiating GABAergic inhibition *via* membrane hyper polarization which leads to a decrease in the firing rate of critical neurons in the brain[39]. Thus an increase in the duration of the GABA-gated ion channel opening and enhanced affinity for GABA may also be responsible for CNS depressant effect[40].

Thiopental sodium belonging to barbiturate group induces sleep in both human and rodents. Thiopental sodium-induced sleep test is used to investigate sedative-hypnotic drugs. By binding with GABA receptor complex, it exerts GABA mediated hyperpolarization of postsynaptic neurons[41]. All doses of the crude methanolic extracts (200 mg/kg and 400 mg/kg) produced a significant decrease in onset of sleep and increase in duration of sleep when compared to the control group.

For assessment of antidepressant-like effect, the forced swimming test is widely used. In this model, shorter immobility time indicates antidepressant-like activity while extended immobility time indicates CNS depressant-like effect[42]. In forced swimming test, crude methanolic extract of *C. speciosus* rhizome produced a significant reduction in duration of immobility. Standard imipramine (10 mg/kg) and *C. speciosus* methanolic extract of 400 mg/kg showed a significant reduction in duration of immobility which confirmed antidepressant-like effect[43]. In tail suspension test, the time of immobility is indicative of a behavioral despair which reflects a depressive state[44]. Crude methanolic extracts at two doses of 400 and 200 mg/kg were found to have significantly reduced immobility time compared to the control group. These results support that the crude methanolic extract of *C. speciosus* rhizome could possess antidepressant-like effect.

The results of our study demonstrate that methanolic extract of *C. speciosus* rhizome produces remarkable neuropharmacological effects on experimental mice models. Further investigation is recommended to determine the responsible phytoconstituents and the exact biomedical pathway.

Conflict of interest statement

The authors declare they have no competing interests.

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