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Research Article

In Vivo Antidepressant and Anxiolytic Effects of Methanol Extract of Leaves of *Zingiber rubens* Roxb. in Mice Model

Nazmul Huda, A F M Shahid Ud Daula, Md Abdul Barek and Mohammad Anwarul Basher*

Department of Pharmacy, Noakhali Science and Technology University, Noakhali, Bangladesh

***Corresponding Author**: Mohammad Anwarul Basher, Associate Professor of Pharmacy, Noakhali Science and Technology University, Noakhali, Bangladesh.

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Abstract

Background: *Zingiber rubens* Roxb. (Family: Zingiberaceae) is a plant found in tropical regions of Asia, East Himalaya, India, Bangladesh, Myanmar, Thailand, Vietnam. There have no pharmacological studies on leaves have been performed. The present study was aimed to investigate antidepressant and anxiolytic effect of methanol extract of leaves of *Z. rubens.*

Methods: The anxiolytic activity was evaluated with the adult mice by open field test, hole cross test, hole board test, elevated plus maze test and the antidepressant activity was investigated in a forced swim test. The efficacy of the plant extract (100, 200 and 400 mg/kg) was compared with the standard anxiolytic drug diazepam (1 mg/kg).

Results: The leaves extract of *Z. rubens* demonstrated significant stronger effect ((13.83, $p < 0.001$ and 12.17, $p < 0.001$ for 200 and 400 mg/kg respectively) than diazepam and seemed the most potent causing fewer spontaneous movements in open field test. On the other hand, for leaf extracts in all doses, the animals become sluggish demonstrating single or no transition at 120 minutes (1.17, $p < 0.05$, 1.0, $p < 0.001$ and 0.17, $p < 0.001$ for 100, 200 and 400 mg/kg respectively) in the hole cross test. There significantly increased entries ($P < 0.001$) into and time spent ($P < 0.001$) on the open arms of the elevated plus maze test. In the hole board test, the extract significantly increased head-dip counts (28.33, $p < 0.05$ and 48.67 $p < 0.001$) for 200 and 400 mg/kg respectively. Both diazepam and 400 mg/kg doses produced equivalent effects and demonstrated immobility time of 55.67 s ($p < 0.001$) and 54.27 s ($p < 0.001$) respectively in forced swim test.

Conclusions: Based on the results from in vivo activities, the leaves of Z. rubens was showed greater anxiolytic and antidepressant effects than diazepam in mice and that this effect might be mediated by benzodiazepine receptors.

Keywords: *Anxiolytic; Antidepressant; Open Field Test; Hole Cross Test; Elevated Plus Maze Test; Benzodiazepine Receptors*

Introduction

Medicinal plants were used by people of ancient cultures without knowledge of their active ingredient. In last few years, there has been great focus on the possible health benefits of natural substances with antioxidant, antimicrobial, analgesic, antipyretic, sedative, antidepressant, anxiolytic, antipsychotic, anticancer, anti-diabetic and others activities [1]. Zingiberaceae**,** the ginger family of flowering plants, made up of about 52 genera with a total of about 1600 known species [2,3]. The family is mainly distributed throughout tropical African countries, China, Nepal, India, Bangladesh, Thailand, Indonesia, Malaysia, Singapore, Brunei, the Philippines and Papua New Guinea [2-4]. Various reports have been found revealing potential therapeutic potentials of the species of Zingiberaceae. For example, plant extracts of *Zingiber officinale* showed antidepressant [5], anxiolytic [6], analgesic, anti-inflammatory and antiemetic effects [7,8]; antioxidant [9], antipyretic [10], anti-amoebic [11], anti-tumor [12] and anti-hyperglycaemic effects of *Z. zerumbet* [13]; antioxidant effect of *Z. chrysanthemum* [14]; cytotoxic effect of *Z. ligulatum* [3]; antibacterial effect of *Z. cernuum* [15]; thrombolytic effect of *Z. cassumunar* [16]. Zingiber rubens Roxb., locally called "Bengal Ginger", is a wild medicinal herb. The plant is widely distributed in Bangladesh, India, Myanmar, Thailand, Vietnam [17,18]. The plant has extensive uses in traditional herbal preparations [19,20]. Despite its wide anecdotal uses, only few reports were published on phytochemical studies of root and rhizome [21,22] and no pharmacological studies on leaves have been performed. The aim of our research was therefore to examine the effect of leaf extract of *Zingiber rubens* on anxiety and depression and it was tested by methanol extract of its leaf in mice model.

Materials and Methods

Drugs and chemicals

All chemicals and reagents used in this study were of analytical grade. Methanol (Merck, Germany) was used as a solvent during extraction. Diazepam used as positive control was purchased from Square Pharmaceuticals Limited, Bangladesh.

Plant materials

Zingiber rubens was collected on May 2017 from Mowlavibazar- a district situated in the eastern part of Bangladesh and the plant is identified by National Herbarium Institute, Dhaka, Bangladesh (Accession number: DACB-44932). The selected plant part was then thoroughly washed with water, dried and powdered. About 400 g of the powdered materials was taken in a clean, flat bottomed glass container and soaked in 1500 ml of 80% methanol at room temperature for two weeks with occasional shaking and stirring. Then the solution was filtered using filter cloth and Whatman filter paper (Bibby RE200, Sterilin Ltd., UK) and concentrated with a rotary evaporator (RE-EV311-V, LabTeck S.R.L, Italy). It produced a gummy concentrate of greenish black color; this concentrate was our crude methanol extract.

Experimental animals

For experiments, swiss albino mice of both sexes (6 - 7 weeks old) with mean body weight 25 ± 5.0 g were collected from International Centre from Diarrheal Diseases Research (ICDDR, B), Bangladesh. All mice were kept in polycarbonate cages (6 in each box) in a temperature and humidity controlled room (23 ± 1°C, 55 - 60% humidity). Animals were fed with commercial mice pellet diet ad libitum throughout experimental period.

Neuropharmacological effects

Open field test

For open field test, a box (72 cm x 72 cm x 36 cm) made of plywood and clear Plexiglas was used. The floor of the box was divided and marked into sixteen 18 x 18 cm squares. The animals were divided into control, positive control and three test groups containing five mice each. Before performing the experiment, controls and extracts were administered orally 30 minutes prior to the test. Each mouse was then kept into the apparatus and the number of square blocks visited by each mouse was calculated for 5 minutes on 0, 30, 60, 90 and 120 minutes intervals [22,24].

Hole cross test

Hole cross test was performed in a steel cage (30 cm x 20 cm x 14 cm) with partition fixed in the middle of the cage having a hole of 3 cm diameter at a height of 7.5 cm. After administration of sample, each mouse was introduced into the cage and the number of transitions from one chamber to other through the hole inside the cage was counted for 3 minutes on 0, 30, 60, 90 and 120 minutes intervals [25].

Elevated plus maze test

For elevated plus maze test, the apparatus was comprised of two open arms (35 cm x 5 cm) across each other and perpendicular to two closed arms (35 cm x 5 cm x 15 cm) with a centre platform (5 cm x 5 cm) [26-29]. The open arms were exposed having no wall whereas the closed arms were enclosed by walls 15 cm high. Groups of mice were treated with controls and extracts 1 hour prior to experiment. In the experiment, the animals were placed at the center area of the maze with its head directed toward a closed arm and allowed to move freely in the maze for five minutes. Here, number of entry and total duration in the open arm were recorded.

Hole board test

A wooden box (40 x 40 x 25 cm) with 16 evenly distributed holes (diameter 3 cm) in the floor resting to a height of 35 cm was used for hole board test [30]. Control and extracts were administered 1 hour prior to test. In the test, each mouse was placed at the center of the box and allowed to move freely in the box for 5 minutes. Due to innate nature, mouse explores the holes by dipping its head and number of head dips was recorded. Dipping the head at least to the eye level was considered and no repeated dips into the same hole were counted unless separated by exploration of another hole.

Forced swimming test

A cylindrical tank (height 50 cm, width 70 cm) filled with water was used for forced swimming test. Each mouse was dropped into the tank and monitored for six minutes in such inescapable situation. It is usually assumed that it takes two minutes for mice to habituate with the situation; thus, immobility of the mouse in later four minutes was recorded. A mouse was considered immobile when it stopped swimming but made small movements to keep itself floated in an upright position for two seconds.

Statistical analysis

Data were calculated as mean ± SEM values. One-way ANOVA with Dunnett's test was performed using GraphPad Prism (version 8.0) A probability level of 0.05 (adjusted P value according to GraphPad Prism) or less was accepted as significant; ap < 0.05, bp < 0.01, cp < 0.001 vs. vehicle; αp < 0.05, βp < 0.01, γp < 0.001 vs. diazepam.

Result

Open field test

In open field test, number of square travelled was recorded after 0, 30, 60, 90 and 120 minutes and the results were shown in figure 1A. For all samples, square crossed was highest at 0 minutes that gradually decreased showing lowest number at 120 minutes. Number of squares crossed at 0 minutes were 62.33 and 58.33 for vehicle and diazepam respectively; their movements were gradually decreased and finally showed 47.17 ($p < 0.001$) and 27.5 ($p < 0.001$). Effect at 100 mg/kg extract were found nearly equal of diazepam throughout the observation period and showed 25.17 ($p < 0.001$) squares travelled at 120 minutes. At the onset of experiment i.e. at 0 minutes, doses at 200 mg/kg and 400 mg/kg had equivalent effects as diazepam but as the time elapsed, the movements were sharply dropped and finally demonstrated greater effect than diazepam (13.83, $p < 0.001$ and 12.17, $p < 0.001$ for 200 and 400 mg/kg respectively).

Hole cross test

In the hole cross, number of holes instead fo squares (in open field test) were counted at 0, 30, 60, 90 and 120 minutes were recorded (Figure 1B). At 0 minute, vehicle showed 21.17 ($p < 0.001$) entries through hole; at this time, dose at 100 mg/kg extract did not produce any effect demonstrating nearly equal movements (20.33, $p < 0.001$) as vehicle. On the other hand, diazepam and extracts at 200 mg/kg and 400 mg/kg produced immediate effects showing 14.0 ($p < 0.001$), 7 ($p < 0.001$) and 6 ($p < 0.001$) transitions respectively. Number of movements were gradually declined for all samples. Vehicle had 5.17 ($p < 0.05$) movements whereas diazepam showed 2.83 ($p < 0.05$) at 120 minutes. On the other hand, for leaf extracts in all doses, the animals become sluggish demonstrating single or no transition at 120 minutes (1.17, $p < 0.05$, 1.0, $p < 0.001$ and 0.17, $p < 0.001$ for 100, 200 and 400 mg/kg respectively.

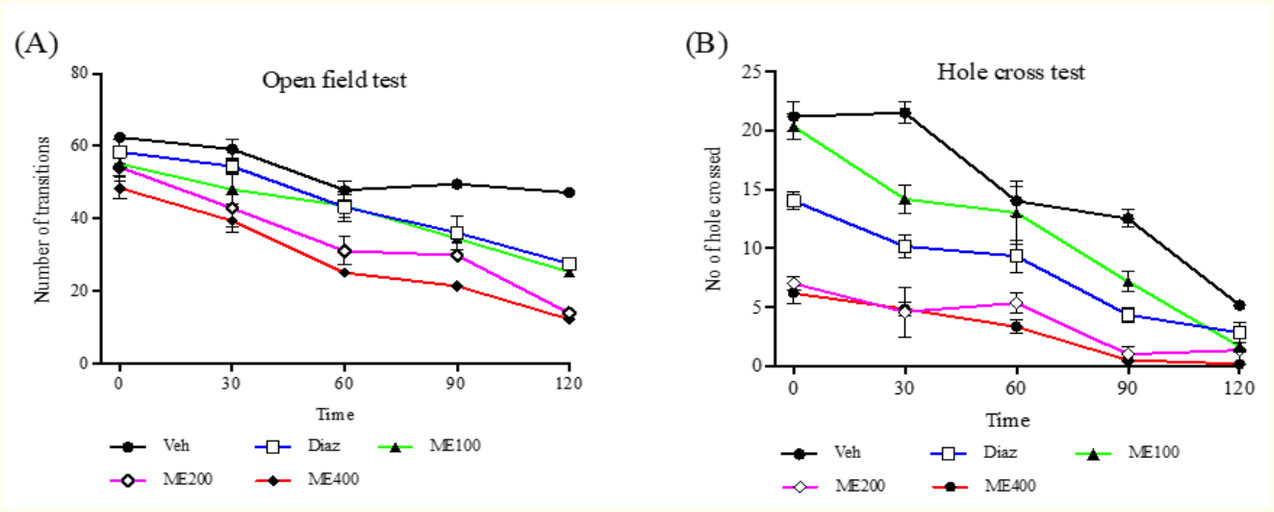

Figure 1: *Effect of methanol extract of leaves of Zingiber rubens on neuropharmacological tests. 'Veh' stands for vehicle or control; 'Diaz' for diazepam and 'ME' for methanolic extract. Data are expressed as mean ± SEM of six animals.* $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ *vs. vehicle;* $^{\alpha}p < 0.05$, $^{\beta}p < 0.01$, $^{\gamma}p < 0.001$ *vs. diazepam.*

Elevated plus maze test

The leaves extract of *Z. rubens* produced anxiolytic-like effect by the increase of time spent in the open arms compared to control showed in figure 2A. Time spent in open arms of vehicle and diazepam were 32.17s ($p < 0.001$) and 64.67 s ($p < 0.001$) respectively. Extract at 100 mg/kg showed higher spent time (50.50s, $p < 0.05$) than vehicle but lower than diazepam. Both 200 and 400 mg/kg doses produced better effects than diazepam and demonstrated open arms spent time of 80.50s ($p < 0.001$) and 118.33s ($p < 0.001$) respectively.

Hole board test

The result of hole board test was shown in figure 2B. Diazepam (37.33, $p < 0.001$) showed more than double number of head dipping than vehicle (17.83, $p < 0.001$). Extract at 100 mg/kg dose produced slightly higher dipping (21.0, $p < 0.001$) than vehicle; as the dose increased to 200 mg/kg, the effect was greater causing 28.33 head dipping ($p < 0.05$). On the other hand, the highest dose 400 mg/kg showed pronounced effects having 48.67 ($p < 0.001$) which was greater than diazepam.

Forced swimming test

Effects of the extract on immobility time in forced swim test was shown in figure 2C. Immobility times of vehicle and diazepam were 89.17s ($p < 0.001$) and 55.67s ($p < 0.001$) respectively. Extract at 100 mg/kg showed reduced immobility (74.17s, $p < 0.05$) than vehicle but greater than diazepam. Both 200 and 400 mg/kg doses produced better effects than diazepam and demonstrated immobility time of 61.0s ($p < 0.001$) and 54.27s ($p < 0.001$) respectively.

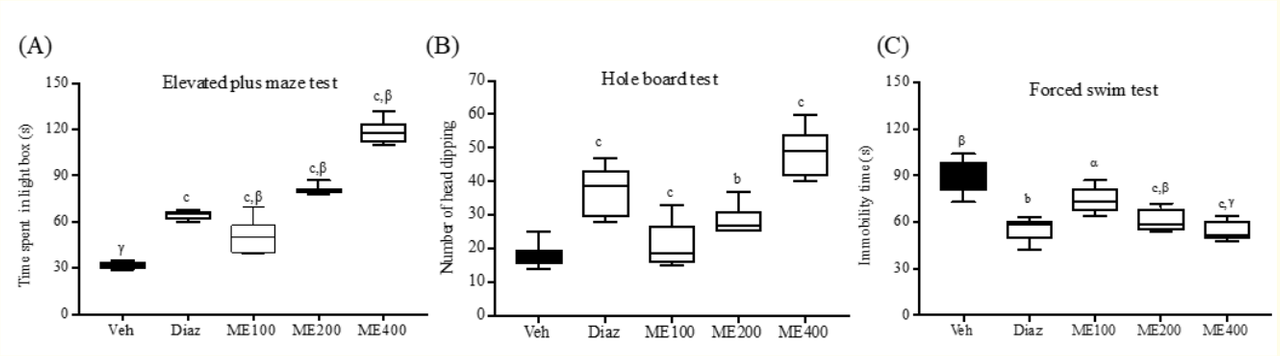

Figure 3: *Effect of methanol extract of leaves of Zingiber rubens on neuropharmacological tests. 'Veh' stands for vehicle or control; 'Diaz' for diazepam and 'ME' for methanolic extract. Data are expressed as mean ± SEM of six animals.*
$^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ vs. vehicle; $^{\alpha}p < 0.05$, $^{\beta}p < 0.01$, $^{\gamma}p < 0.001$ vs. diazepam.

Discussion

The present study was performed to evaluate neuropharmacological effects such as anxiolytic and antidepressant-like effects of methanol extracts of leaves of *Zingiber rubens* Roxb. on mice model. In the open field test, leaves extract of *Z. rubens* at 200 and 400 mg/kg doses demonstrated significant stronger effect than diazepam and seemed the most potent causing fewer spontaneous movements. A decrease in spontaneous movements is the result of a decrease in excitability of central nervous system indicating anxiolytic effect of the extracts [31,32]. In the hole cross test, the extract (200 and 400 mg/kg both) showed significant decrease ($p < 0.005$) in spontaneous motor activity from the first observation (0 minute) ($p < 0.001$) and continued up to 5th observation period (120 minutes) ($p < 0.001$) which indicates profound sedative activity of the plant extracts. The light-dark transition test is one of the most widely used tests to measure anxiety-like behavior in mice based on the natural aversion of mice to brightly illuminated areas and on their spontaneous exploratory behavior in novel environments [33]. Mice are allowed to move freely between the two chambers. In this study, 200 and 400 mg/kg doses significantly showed the time spent in light box and number of transitions; which was found more potent than diazepam. In elevated plus maze, innate trait of mice to avoid the exposed open areas and preference for sections enclosed by protective walls are utilized [34,35]. Effectiveness of plant extracts as anxiolytics is evaluated by a statistically significant increase in mice activity in the open arms. Anxiogenic compounds cause a reduction of these indices while an increase is observed in case of anxiolytics. Both 200 and 400 mg/kg extracts of *Z. rubens* significantly increased time spent and transitions in the open arms in a dose-dependent manner and found greater effects than diazepam. The number of entries into the open arms and the time spent in the open arms are used as indices of open space-induced anxiety in mice. Thus, the actions of *Z. rubens* in the elevated plus maze model in this study suggest anxiolytic property. The hole board test is based on assumption that head-dipping of mice is inversely proportional to their anxiety state and therefore increased number of head dips into the holes on the board means reduced anxiety state [35,36]. In the present study, leaves of *Z. rubens* significantly increased head-dip counts than diazepam at 400 mg/kg which also compared with control group. Thus, the results suggest that, methanolic extracts of *Zingiber rubens* has anxiolytic effect. The forced swim test is one of the most commonly used assays for the study of depressive-like behavior in mice's. Treatment with antidepressants is expected to reduce immobility during the test [37-39]. In our study, both diazepam and leaves

extract (400 mg/kg) produced equivalent effects indicating potential anti-depression effect of the extracts. Neuropharmacological effects of plant extracts are definitely due to presence of different phytocompounds. Various plant secondary metabolites have been isolated and found to influence the action of noradrenaline, serotonin, GABA, benzodiazepine neurotransmitters, etc.; thus, are used in psychotic disorders. Alkaloids, terpenoids, glycosides from different plant species were found to demonstrate anxiolytic and antidepressant effect [40-43]; some natural flavonoids [44,45] show affinity for benzodiazepine receptors rendering CNS effect. Thus, neuropharmacological activities of leaves of *Z. rubens* observed in this study may be due to the presence of one or a combination of phytoconstituents present in the extracts [46].

Conclusion

The results obtained in this study suggest that methanol extracts of leaves of *Zingiber rubens* possess anxiolytic and antidepressant activities due to one or a combination of phytoconstituents identified in the extracts. Further investigations are planned for isolation and identification of the chemical principle(s) responsible for the observed biological effects of the extracts.

Conflict of Interest

The authors declare they have no competing interests.

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Volume 7 Issue 3 March 2019