Effect of ethanolic extract of *Zingiber officinale* Roscoe on Central Nervous System activity in mice

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*Zingiber officinale* Roscoe, commonly known as ginger, is a traditional herb used to treat various disorders. In this study, we evaluated potential pharmacological effects of ethanolic extracts of *Z. Officinale* with respect to central nervous system (CNS) activity in mice. Role of ethanolic extract of ginger on CNS activity in mice was studied using models of elevated plus maze test, barbiturate-induced sleeping time, tail suspension test, hot-plate and tail-flick test. Ginger extract was administered to mice at single doses of 50 and 200 mg/kg, perorally while diazepam (1 mg/kg), morphine (5 mg/kg) and imipramine (30 mg/kg) intraperitoneally were used as standard drugs. The results showed that the ginger extract at all dose levels significantly exhibited anxiolytic activity increased the sleeping latency but reduced the sleeping time. Tail suspension test showed that the extract at both the doses was able to induce a significant decrease in the immobility time, similar to imipramine, a recognized antidepressant drug. Tail-flick and hot-plate tests demonstrated antinociceptive property of ginger extract, similar to morphine, a recognized antinociceptive agent. Higher dose level (200 mg/kg) showed better protective effects. Phytochemical screening of ethanolic extract revealed the presence of various phytoconstituents such as phenolic compounds, flavonoids, tannins, anthocyanins, carbohydrates, glycosides, proteins, resins and volatile oils. The possible mechanism by which ginger exhibited the significant beneficial effects on various CNS models in mice could be attributed to its antioxidant potential.

Keywords: Antidepressant activity, Antinociceptive activity, Antioxidant, Anxiolytic activity, Depression, Diazepam, Ginger, Imipramine, Locomotor activity, Morphine

Central nervous system (CNS) disorders include abnormalities in both physical and psychological domains. Many drugs used for treatment of CNS disorders have side effects and/or poor efficacy and thereby non-compliance. Moreover, for many CNS drugs such as antidepressants and antipsychotics, it takes time to determine whether a particular drug is efficacious in an individual patient.

It is by now commonly accepted that under situations of oxidative stress, reactive oxygen species (ROS) such as superoxide (O$_2^-$), hydroxyl (OH$^-$), perhydroxyl (OOH$^-$) and peroxyl (ROO$^-$) radicals are generated. These reactive oxygen species play an important role in degenerative or pathological processes, such as aging, cancer, coronary heart disease, Alzheimer’s disease, neurodegenerative disorders, atherosclerosis, cataracts and inflammation. Oxidative damage was considered a likely cause of age associated brain dysfunction as the brain is vulnerable to oxidative stress due to a relatively high rate of free radical generation without commensurate levels of antioxidative defenses.

Plants are potential source of natural antioxidants that continue to serve as leads for development of novel drugs. *Zingiber officinale* Roscoe (Zingiberaceae), commonly called Ginger, is an important plant with several ethnomedicinal and nutritional values. is used extensively worldwide as a spice, flavouring agent and herbal remedy. Traditionally, ginger is used in Ayurveda, Siddha, Chinese, Arabian, Africans, Caribbean and many other medicinal systems to cure a variety of diseases viz., asthma, constipation, cough, dyspepsia, indigestion, inflammation, loss of appetite, nausea, palpitation, pain and vomiting. *Z. officinale* has been reported already for its analgesic, anticancer, antidiabetic, anti-inflammatory, antimicrobial, antioxidant, hepatoprotective, immunomodulatory, larvicidal, nephroprotective and neuroprotective.
Here, we investigated the psychopharmacological effect of ethanolic extract of dried *Zingiber officinale* rhizome by studying its influence on central nervous system (CNS) activity in Swiss albino mice.

**Materials and Methods**

**Plant material and preparation of extract**

Rhizomes of *Z. officinale* were purchased from local market and plant material was identified and authenticated by Dr. K.C. Bhatt, at National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi. The pieces of dried rhizome were extracted by ethanol in Soxhlet extractor. The ethanol extract was evaporated under reduced pressure to obtain dry masses. The extract was then stored in a desiccator for phytochemical and pharmacological evaluations.

**Drugs and Chemicals**

Diazepam (Ranbaxy, India), morphine (Sigma Chemicals, USA), imipramine (Torrent, India), pentobarbital (Neon, India), phenytoin (Sigma Chemicals, USA) and gum acacia (CDH, India) were other chemicals or drugs used in the study.

**Phytochemical screening**

Phytochemical investigation of ethanolic extract for the presence of various phytoconstituents was carried out using the methods previously described by Trease & Evans.\(^{20}\)

**Animals**

Adult Swiss albino mice (*Mus musculus albinus*), of either sex, weighing 20-25 g obtained from the animal house of R.V. Northland Institute, Dadri, G.B. Nagar and Jamia Hamdard, New Delhi, India. They were housed in an environmentally regulated room on a 12 h light: 12 h dark cycle with 25±2°C and had free access to food and water. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC, Reg. No.1149/ac/07/CPCSEA) of R.V. Northland Institute, Greater Noida, G.B. Nagar and experiments were conducted according to the CPCSEA, India guidelines on the use and care of experimental animals.

**Experimental protocol**

The animals were tested during the light period and observed in a closed room with constant temperature and poorly illuminated room with a red light. All tests were performed in different days with distinct groups of animals. The ethanolic extract of *Z. officinale* rhizome was suspended in 1% carboxy methyl cellulose (CMC) and was administered to mice at single doses of 50 and 200 mg/kg perorally 60 min before the start of observation, while diazepam (1 mg/kg), morphine (5 mg/kg) and imipramine (30 mg/kg) as standard drugs were used intraperitoneally 30 min before the observations recorded.

**Antinociceptive activity**

The antinociceptive activity of the under study substances was performed in mice by tail-flick and hot-plate responses\(^{21,22}\), i.e., the reaction time (in seconds) of each animal, was measured at 60 min after extract administration, and 30 min of morphine administration (5 mg/kg, i.p.) for 4 h.

**Tail-flick test**

The painful stimulus of tail was produced by a heating wire and cut-off time was 15 s using tail-flick analgesiometer.

**Hot-plate test**

The parameter evaluated was the latency time for licking of legs and jumping responses after exposure on the hot-plate surface. The hot-plate temperature was kept at 55±0.5°C and the cut-off time was 30 s.

**Anxiolytic activity**

The elevated plus maze test was used to evaluate antianxiety activity, following the procedure described by Itoh *et al.*\(^{23}\). Briefly, the apparatus consisted of two open arms (16×5 cm\(^2\)) and two enclosed arms (16×12×5 cm\(^2\)). The arms extended from a central platform (5×5 cm\(^2\)), and maze was elevated to a height of 25 cm from the floor. The zinger extract was administered perorally in varying doses 60 min before the evaluation of antianxiety activity. At the time of experiment, each mouse was placed at the centre of maze, facing one of the enclosed arms. During a 5 min test period the time spent on open arms and in closed arms was recorded.

**Effect on sleeping time**

After 60 min of oral administration of ginger extract (50 and 200 mg/kg, po) or vehicle (1% CMC), all groups received sodium pentobarbital (40 mg/kg, i.p.). The time elapsed between the administration of pentobarbital until the loss of the righting reflex was recorded as the sleep latency and the time elapsed between the loss and voluntary recovery of the righting reflex was recorded as sleeping time\(^{24}\).

**Antidepressant activity**

Antidepressant activity was assessed by tail suspension test as per Steru *et al.*\(^{25}\). For the test, mice were suspended on the edge of a shelf, 58 cm
above the ground with adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was recorded for a period of 5 min after the 60 min of ginger extracts and 30 min of imipramine administration.

**Effect on locomotor activity**

Locomotor activity of mice was evaluated by means of the actophotometer apparatus\(^\text{26}\). The animals were placed individually in the actophotometer immediately subsequent to administering, and ambulation was recorded at 30, 60 and 120 min. The locomotor activity was expressed in terms of total photobeam count/5 min/animal.

**Statistical analysis**

All the statistical calculations were performed by Jandel Sigma Stat 2.0, statistical software. Data are presented as the mean±SE. ANOVA was applied for the analysis of the results. Differences between groups were regarded as significant at a level of \(P < 0.05\).

**Results**

**Phytochemical screening**

Phytochemical screening of ethanolic extract revealed the presence of various phytoconstituents such as phenolic compounds, flavonoids, tannins, anthocyanins, carbohydrates, glycosides, proteins, resins and volatile oils (Table 1).

**Antinociceptive activity**

The reaction time to nociceptive effect produced by the hot-plate or the tail-flick test was significantly \((P < 0.05)\) in the extract administered (50 and 200 mg/kg, po) group. The antinociception caused by the extract was lower compared to that of morphine in mice (Fig. 1).

**Anxiolytic activity**

The extract (50 and 200 mg/kg, po) and diazepam (1 mg/kg, i.p.) induced significant \((P < 0.01)\) increase

| Table 1—Phytochemical analysis of ethanolic extract of *Zingiber officinale* |
|------------------|------------------|
| Bioactive Principles | Ethanolic extract of ginger |
| Alkaloids | +++ |
| Proteins | ++ |
| Tannins | ++ |
| Carbohydrates | ++ |
| Glycosides | ++ |
| Saponins | +++ |
| Steroids | + |
| Flavonoids | ++ |
| Terpenoids | + |
| Phenolic compounds | + |

+++ abundantly present; ++ moderately present; and + fairly present

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Fig. 1—Effect of *Zingiber officinale* (ZO) extract (50 and 200 mg/kg, p.o.) and morphine (5 mg/kg, i.p.) on tail-flick test and hot plate test in mice. [Statistical analysis was done using ANOVA followed by Scheffe test. Values are expressed as mean±SE (n=8). *\(P < 0.05\), **\(P < 0.01\)]
Fig. 2—Effect of Zingiber officinale (ZO) extract (50 and 200 mg/kg, p.o.) and diazepam (1 mg/kg, i.p.) on elevated plus maze test in mice after 60 min of extract administration. [Statistical analysis was done using ANOVA followed by Scheffe test. Values are expressed as mean ±SE (n=8). *P <0.05, **P <0.01]

in the occupancy in the open arms. The extract treated and diazepam group showed a decreased preference for the closed arms. However, mice treated with extract @ 200 mg/kg were more effective (Fig. 2).

Effect on sleeping time

The absolute values of sleep latency and sleeping time demonstrate that the mice treated with extract (50 and 200 mg/kg), 60 min before the injection of pentobarbital, presented an significant (P <0.05) increase in the sleep latency and reduction of pentobarbital-induced sleeping time (Fig. 3)

Antidepressant activity

In this test, ginger extract at both the doses (50 and 200 mg/kg) significantly (P <0.05) decreased the immobility time in mice, as compared to the respective controls. The mice treated with imipramine (30 mg/kg), as expected of an antidepressant drug, also showed decreased immobility time (Fig. 4).

Effect on motor activity

In order to check the general locomotor performance of mice, a 5 min activity test was performed for each mouse. No significant alteration in the total locomotor activity was observed in mice after 60 min of administration of ginger extract (data not shown).

Discussion

In the present study, the effects of ethanolic extract of Zingiber officinale rhizome was studied in several behaviour models such as tail-flick test, hot-plate test, elevated plus maze test, barbiturate-induced sleeping time, motor activity test and tail suspension test to investigate its possible central activity in mice. These tests are classical models for screening central nervous system (CNS) actions providing information about anxiolytic, psychomotor performance, myorelaxant activity, antidepressant and analgesic activity. The present study results are supported by Smith et al., in that the antioxidant attenuate oxidative stress induced brain disorders in animals. Despite the widespread traditional use of ginger for treating various disorders, information on CNS activities is limited. The present work demonstrated that the ethanolic extract of ginger has anxiolytic effects in mice. Earlier reports on the chemical constituents of plants and their pharmacology suggest that plant containing flavonoids, saponins, resins and...
tannins possess activity against many CNS disorders\textsuperscript{2}. Phytochemical tests of ethanolic extract of ginger revealed the presence of phytoconstituents such as phenolic compounds, flavonoids, tannins, anthocyanins, carbohydrates, glycosides, proteins, resins and volatile oils. The different doses of ginger extract and imipramine in mice was able to induce antidepressant effect in tail suspension test (Fig. 4). The tail suspension test is the most widely validated tests for assaying new antidepressant agents. This was supported by other workers who found that flavonoids and tannins were found to have antinociceptive and antioxidant activity\textsuperscript{14-29}. Pentobarbital induced sleeping time test was also used to evaluate the possible antidepressive-like effects observed with ginger extract here in this study (Fig. 3). Increased sleep latency and decreased sleeping time are classically related to CNS stimulant drugs. The antinociceptive effect of ginger extract and morphine was assessed by the use of two common tests, tail-flick, specific for spinal reflex and hot-plate, which reflects a more complex, centrally integrated process. Both, the extract as well as morphine exhibited antinociceptive activity (Fig. 1). The ginger extract might involve an action on opioid receptors; however, further studies are needed to ascertain this mechanism. The antinociceptive activity of ginger extract (200 mg/kg) was almost equivalent to that of morphine in mice at spinal and supra spinal level.

In conclusion, results of the present study revealed that administration of ethanolic extract of \textit{Zingiber officinale} @ 50 and 200 mg/kg significantly influenced the CNS activities in mice. It demonstrated significant increase in sleeping latency and decrease in sleeping time; decrease in the immobility time (anxiolytic activity), comparable to imipramine, the standard antidepressant drug; and also antinociceptive property similar to morphine. These activities demonstrated by the ethanolic extract of \textit{Z. officinale} could be attributed to the antioxidants present in the extract.

References


