Central nervous system stimulant actions of *Alpinia galanga* (L.) rhizome:  
A preliminary study

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Methanolic and ethyl acetate extract of *A. galanga* showed significant central nervous system (CNS) stimulant activity in mice using actophotometer and rotarod test. CNS stimulation at a dose of 500 mg/kg was comparable with standard drugs caffeine and amphetamine derivative modalart. The extracts did not shown any depressant effect in forced swim or tail suspension tests. It can be concluded that *A. galanga* rhizome may have stimulant activity in mice and the active constituents responsible for this effect is present both in crude methanolic extract as well as in ethyl acetate fraction of methanolic extract of this plant species.

**Keywords:** *Alpinia galanga*, Mice, Rhizome, Stimulant

Stimulants are drugs that exert their action through excitation of the central nervous system. CNS stimulants include caffeine, cocaine, and various amphetamines. These drugs are used to enhance mental alertness and reduce drowsiness and fatigue. They usually increase alertness and energy, which are accompanied by increases in blood pressure, heart rate, and respiration\(^1\). Stimulants may also be used in treating health conditions like sleep disorders\(^2\), attention-deficit hyperactivity disorder\(^3\), and depression\(^4\). Some recent studies show the use of stimulant drugs in short-term treatment of obesity and asthma\(^5,6\).

The use of herbal medicine worldwide has provided an excellent opportunity for India to look for therapeutic lead compounds from the ancient systems of therapy, i.e. Ayurveda, Siddha and Unani which can be utilized for development of new drug\(^7\). The plant *Alpinia galanga* L. (*Zingiberaceae*) is a herb primarily used in cooking. Commonly called greater galangal, this rhizomatous herb is distributed in various parts of India and throughout Southeast Asia. It is also a primary herb in both Ayurvedic and Tibetan system of medicine. The herb grows to a height of about 5 feet; the leaves are long, like narrow blades, and the flowers of curious formation, growing in a simple, terminal spike, with white petals and deep red veining. The branched pieces of rhizome are 1.5-3 inches long and seldom more than 0.75 inch thick. The rhizomes exhibit many pharmacological properties, including antitumour\(^8\), antiallergic\(^9\), antiulcer\(^10\), antifungal\(^11\), antibacterial\(^12\) and antiviral activities\(^13\). Ethnobotanically, the rhizomes are used to treat a variety of sicknesses including coughs, headache, asthma, bronchitis, inflammation, rheumatoid arthritis and colic\(^14,15\). Essential oil from *A. zerumbet* has also been shown to have CNS depressant activity\(^16\). Traditionally the rhizome has been reported to have stimulant activity\(^17,18\). The preliminary communication reports CNS stimulant activity of crude methanolic extract as well as ethyl acetate fraction of *A. galanga* using a battery of pharmacological tests.

**Materials and Methods**

Collection and drying—The rhizomes of *Alpinia galanga* were collected from the campus of North Bengal University, Shivmandir, Siliguri, West Bengal, India. The plant material was authenticated by Shibpur Botanic Garden, Botanical survey of India, Howrah, West Bengal, India for authentication (authentication No. CNH/111/2011/Tech. II/628). The rhizomes were shade dried and made into a coarse powder.
Extraction—The ground powder was extracted in Soxhlet apparatus using methanol. The methanol was evaporated by heating in water bath. Fractionation of the extract was done further using petroleum ether, chloroform and ethyl acetate respectively and the solvent was evaporated each time after fractionisation by heating on a water bath.

Animals—Male Swiss Albino mice (20-25 g) were obtained from animal house of Gupta College of Technological Sciences. The animals were housed under standard environmental condition (25 °C, 12:12 h L: D cycle) and fed with standard diet (Tetragon Chemie Private limited, Bangalore, India) and water ad libitum. All animal experiments were carried out in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Forests and Environment, Government of India and the study was approved by the Institutional Animal Ethics Committee (955/A/06/CPCSEA).

Preparation and administration of the tests and standards—The crude methanolic (250 and 500 mg/kg) extract as well as ethyl acetate fraction (250 and 500 mg/kg) of A. galanga was administered orally in the form of suspension, in water for injection. The standard drug modalert (50 mg/kg Sunpharma, Mumbai) was administered ip and caffeine (30 mg/kg) was administered orally in the form of suspension in water for injection. The negative control diazepam (4 mg/kg Ranbaxy Laboratories Ltd, Mumbai) was administered ip.

Experimental design to measure CNS stimulant and depressant activity—Animals were weighed and randomly divided into the following 8 groups with 6 animals in each group:

Gr I: Control animals received water for injection equivalent volume.
Gr II: Animals received standard caffeine at the dose of 30 mg/kg,
Gr III: Animal received standard modalert at the dose of 50 mg/kg,
Gr IV: Animal received negative control diazepam at the dose of 4 mg/kg,
Gr V: Animal received crude methanol extract at the dose of 500 mg/kg,
Gr VI: Animal received ethyl acetate fraction at the dose of 500 mg/kg,
Gr VII: Animal received crude methanol extract at the dose of 250 mg/kg,
Gr VIII: Animal received ethyl acetate fraction at the dose of 250 mg/kg.

All eight groups were used for CNS stimulant activity while groups I to VI were used to determine CNS depressant action.

Acute toxicity study—The crude methanolic extract of A. galanga and its partitioned ethyl acetate fraction were administered orally in the form of suspension in distilled water at 500, 1000, 2000 mg/kg to different groups of male swiss albino mice, as per OECD test guidelines (425) for oral acute toxicity study and mortality was observed for up to 7 days.

Measurement of locomotor activity using Actophotometer—Each animal was placed in the actophotometer for 10 min and the initial locomotor reading was taken. Animals showing comparable locomotor activity were selected for the study. The mice were divided into groups as described under experimental design. 1 hr after administration of the drug/placebo, the animals were placed in the actophotometer for 10 min and locomotor activity was measured.

Measurement of motor coordination using Rotarod—After placing each animal on the rota-rod (20 RPM) initial gripping time were measured. Animals showing comparable gripping time were selected for further study. After 1 hr of administration of drug/placebo, the animals were placed in rotarod and gripping time was recorded.

Forced swim test—Mice were individually forced to swim inside a vertical Plexiglas cylinder (height: 40 cm; diameter: 18 cm, containing water up to a height of 15 cm maintained at 25 °C). Each mouse was placed into the cylinder and forced to swim. The initial time taken by each animal to become immobile was noted. Animals showing comparable immobility time were used for further experiments. 24 h after the initial trial the mice were treated with the drug/placebo and 1 hr post-drug administration they were placed in the cylinder and total duration of immobility was measured for a period of 5 min. An animal was judged to be immobile whenever it remained floating passively in water in a slightly hunched but upright position with its nose just above the water surface.

Tail suspension test—The mice were suspended using the tail suspension test apparatus. The duration of immobility was recorded for a period of 5 min. Mice were considered immobile when they hang passively and completely motionless for at least
1 min. Animals showing comparable duration of immobility was used for further studies. Animals were divided into various treatment groups as explained in the experimental design. 1 hr after administration of the drug, the animals were suspended and observed for the time taken to become immobile.

**Statistical analysis**—The data are presented as mean±SEM. The difference between groups was calculated by one way ANOVA followed by Tukey’s multiple comparison test. Probability values less than 0.05 (P<0.05) were considered as statistically significant. All statistical tests were conducted using Graph Pad Prism 4.0 software (GraphPad Software, Inc, San Diego CA).

**Results**

**Phytochemical screening**—Phytochemical screening of crude methanolic extract as well as the ethyl acetate fraction of rhizomes of *A. galanga* indicated the presence of flavonoids, tannins and terpenoids. While the petroleum ether and chloroform fractions of the rhizome only showed the presence of tannins. Hence further studies were conducted using the methanolic extract as well as its ethyl acetate fraction.

**Acute toxicity study**—Both methanolic extract and ethyl acetate fraction did not show any mortality at the maximum dose of 2 g/kg. Hence pharmacological studies were conducted at a dose of 250 and 500 mg/kg.

**CNS depressant activity**

Since 500 mg/kg was found to be the optimal dose at which both methanolic extract and ethyl acetate fraction of *A. galanga* rhizomes showed CNS stimulant activity subsequent studies related to CNS depressant activity were conducted using this particular dose.

**Forced swim test**—The methanolic extract and ethyl acetate fraction of *A. galanga* at 500 mg/kg as well as standard drugs caffeine and modalert did not show any change in immobility time compared to control groups. Diazepam showed significant increase in immobility time due to its potent CNS depressant activity (Fig. 2A).

**Tail suspension test**—The ethyl acetate fraction and methanolic extract of *A. galanga* at the dose level of 500 mg/kg exhibited significant decrease in immobility time compared to control animals, while...
diazepam showed significant increase in immobility time. The decrease in immobility time by A galanga was comparable with standard drugs caffeine and modalert (Fig 2B).

Discussion

A number of plants and herbs with CNS stimulant action are known through folklore but their introduction into modern therapy awaits pharmacological testing by modern research methods. One of such agents is A. galanga which has been mentioned in ayurveda for nervous stimulation while galanga is used by homeopaths as a stimulant.

The present study investigates the effects of methanolic extract as well as its ethyl acetate subfraction for possible CNS stimulant activity using various animal behavioural models of CNS stimulation and depression. The results obtained from the present study have shown that administration of methanolic extract and ethyl acetate fraction of rhizomes of A. galanga to the swiss albino mice at 500 mg/kg produced significant stimulation of the CNS (locomotor activity and motor coordination). This effect was comparable with standard drugs caffeine (30 mg/kg) and modalert (50 mg/kg). The results suggest that the methanolic extract and ethyl acetate fraction of A. galanga may have CNS stimulant activity. Since both methanolic extract and ethyl acetate fraction derived from the rhizomes of A. galanga showed dose dependant increase in CNS stimulant activity it confirms the presence of pharmacologically active compounds in both the fractions.

Earlier studies have shown that plants containing flavonoids, saponins, and tannins may protect against various CNS disorders. Phytochemical tests of methanolic extract and its ethyl acetate fraction revealed the presence of flavonoids, tannins and terpinoids which may be responsible for the CNS stimulant actions of A. galanga. Previous studies related to phytochemical analysis of A. galanga have shown that the rhizome may also contain essential oils like kampferide, alpinin, galangin, methyl cinnamate, cincole and tannins like phlobaphines. Future studies need to address the main active constituents and molecular mechanisms associated with stimulant activity of A. galanga rhizome.

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