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Investigation of *in vivo* neuropharmacological effect of *Alpinia nigra* leaf extract

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PEER REVIEW

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Comments

It is an interesting paper in which the authors tried to focus on the neuro-pharmacological effect of *A. nigra* leaf extract on mice. The anxiolytic activity was determined by well-established protocols that signify an anti-depressant effect found in this leaf extract. It is stimulating enough for further research works to develop a new therapeutic agent for anxiety and depression.

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ABSTRACT

Objective: To analyze *in vivo* neuro-pharmacological effects of *Alpinia nigra* as anxiety is a particular form of behavioral inhibition that occurs in response to novel environmental events.
Methods: In present study, the extract of *Alpinia nigra* was evaluated for its central nervous system depressant effect using mice behavioral models, such as hole cross, open field and thiopental sodium induced sleeping time tests for its sedative properties and an elevated plus-maze test for its anxiolytic potential, respectively.
Results: In anxiolytic study, the extract displayed increased percentage of entry into open arm at the dose of 400 and 200 mg/kg. The extract produced a significant ($P<0.01$) increase in sleeping duration and reduction of onset of sleep compared to sodium thiopental at both doses (200 and 400 mg/kg). The extract (200 and 400 mg/kg) also showed a dose-dependent suppression of motor activity and exploratory activity of the mice in both open field and hole cross test.
Conclusion: This study demonstrates that the treated extract has significant central nervous system depressant effect. Further studies on active constituent of the extract can provide approaches for therapeutic intervention.

KEYWORDS
Sedative, Elevated plus maze, Anxiolytic, Antidepressant activity, *Alpinia nigra*

1. Introduction

Anxiety and depression are the most common psychiatric disorders. Over 20% of the adult population suffer from these illnesses at some time during their lives[1-3]. It has become an important area of research interest in psychopharmacology during this decade[4].

Benzodiazepines are among the most prescribed and effective antianxiety drugs used worldwide[5]. But these are being slowly replaced by antidepressants, which are not only efficacious in depression, but also in the acute and long-term treatment of several anxiety disorders[6]. Consumption of these drugs is believed to double every five years[7]. Most of these drugs, however, have an unfavorable risk and benefit ratio, and their prominent side effects still represent a barrier to long-term treatment with these drugs[8]. In addition, the risk of interaction with other substances is high, particularly with alcohol[9]. Hence, there

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is an urgent need to search for newer, better-tolerated, and more efficacious therapeutic agents, for better management of anxiety and depression.

Alpinia nigra (*A. nigra*) (Gaertn.) Burtt (Zingiberaceae) commonly known as galangal, black-fruited, kala is an aromatic, perennial and rhizomatous herb. It is closely related to the galangal curcuma and ginger[10]. It is widely distributed in Yunnan and Hainan Province of China, Thailand and other Southeast Asian countries[11].

A. nigra has two flavone glycosides, astragalin (1) and kaempferol-3-O-glucuronide (2)[12]. A number of studies revealed that 1 and 2 possesses several biological activities, *e.g.*, antibacterial[13], antioxidant[14-16], antiprotozoal[17], hepato-protective[18,19] and glycation inhibitory effects[20]. The crude shoot extract is reported to cause destruction of surface tegument leading to paralysis and death of intestinal parasite[21]. The aqueous extract of shoot and rhizome of *A. nigra* (common name "Tora") has been used in Assam for curing health problems like bone weakness, irregular menstruation, jaundice and gastric ulcers[22].

However, so far, its effect on central nervous system (CNS) activity has not been studied. Therefore, we undertook the study to evaluate the anxiolytic potential of *A. nigra*, by using different animal models and studying the effect of the plant on their exploratory behavior.

2. Materials and methods

2.1. Preparation of leaf extract

Leaves of *A. nigra* were collected at their full mature form from Bangladesh Centre for Scientific and Industrial Research (BCSIR). The plant was identified and authenticated by standard taxonomical method at BCSIR. The collected leaves were thoroughly washed with distilled water and dried in the sun and mechanical dryer at 60-70 °C. The dried sample was coarsely powdered and extracted with 800 mL methanol for 3 d to allow total extraction process. After that the plant extract was filtered with sterilized cotton filter and the filtrate was collected in a beaker. The plant extract then kept in a water bath at 60 °C to evaporate the solvent from the solution. The container allowed to airtight for 72 h and filtrate thus obtained was concentrated by using a rotary evaporator. The extract was stored in refrigerator at 4 °C until used for treatment[23].

The study was performed to find out if the extract had any effect on central nervous system. Elevated plus maze test was conducted for determination of anxiolytic activity whereas thiopental sodium induced sleeping time test was for sedative activity. Effect on exploratory behavior of mice was evaluated by hole cross test and open field test.

2.2. Drugs and chemicals

The drugs and chemicals used for the experiments were diazepam (Square Pharmaceutical Ltd., Bangladesh), thiopental sodium (Gonoshastho Pharmaceuticals Ltd., Bangladesh), methanol (Sigma Chemicals Co., USA).

2.3. Experimental animals

Swiss albino mice of either sex, weighing between 20-25 g, were collected from Animal Research Branch of BCSIR. Animals were maintained under standard environmental conditions [(24.0±1.0) °C, relative humidity: (55-70)% and 12 h light/12 h dark cycle] and free access to feed and water *ad libitum*. Prior to experimentation, the animals were acclimatized to laboratory condition for one week. The research was approved by the Institutional Ethics Committee.

2.4. Anxiolytic activity

2.4.1. Elevated plus maze test

In elevated plus maze test, the apparatus was made of wood with two open and two closed arms across each other respectively forming a plus-sign figure. The elevated plus maze (EPM; 30 cm×6 cm×6 cm, each arm) was situated 50 cm above the floor. After administration of the drug, each animal was placed at the center of the maze facing one of the closed arms. The number of open and closed arm entries, plus time spent in open and closed arms was recorded for 5 min at 0, 30, 60, 90, 120 min after administration of the extract (200 and 400 mg/kg), diazepam (1 mg/kg) and vehicle (1% Tween 80 in water). The whole test was carried out in a sound attenuated room[24]. Entry into an arm was defined as the point when the animal placed all four paws onto the arm.

$$\text{Percent of time spent in open arm} = \frac{\text{Time in open arm}}{\text{Time in the open arm+time in closed arm}}$$

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This test has been widely validated for measuring anxiolytic- and anxiogenic-like activities in rodents[25,26].

2.5. Sedative activity

2.5.1. Thiopental sodium induced sleeping time test

For the experiment, the animals were randomly assigned to four groups, each with 5 mice. The test groups were given