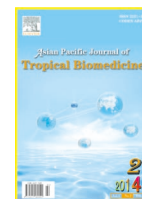




Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb



Document heading doi:10.1016/S2221-1691(14)60222-7 © 2014 by the Asian Pacific Journal of Tropical Biomedicine. All rights reserved.

Investigation of *in vivo* neuropharmacological effect of *Alpinia nigra* leaf extractFarjana Sharmen¹, Adnan Mannan¹, Md. Mominur Rahman², Md. Ashraf Uddin Chowdhury², Muhammad Erfan Uddin², A. M. Abu Ahmed^{1*}¹Department of Genetic Engineering and Biotechnology, University of Chittagong, Chittagong-4331, Bangladesh²Department of Pharmacy, International Islamic University Chittagong, Chittagong, Bangladesh

PEER REVIEW

Peer reviewer

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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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Foundation Project: Supported by Department of Genetic Engineering and Biotechnology, University of Chittagong. (Grant No. Ref No. GEB/AC-54/18-09-2010).

Article history:

Received 18 Oct 2013

Received in revised form 27 Oct, 2nd revised form 11 Nov, 3rd revised form 7 Dec 2013

Accepted 26 Jan 2014

Available online 28 Feb 2014

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.) Burt (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 (Gonosastho 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.

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

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

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

the leaf extract of *A. nigra* at doses of 200 and 400 mg/kg body weight, while the positive control was treated with diazepam (1 mg/kg) and control group with vehicle (1% Tween 80 in water). Thirty minutes later, thiopental sodium (40mg/kg) was administered to each mouse to induce sleep. The animals were observed by placing them on separate chambers for the latent period (time between thiopental administrations to loss of righting reflex) and duration of sleep *i.e.* time between the loss and recovery of righting reflex. The onset of sleep and total sleeping time were recorded for control, positive control and test groups[27].

2.6. Exploratory activity

2.6.1. Open field test

The method was adopted as described by Gupta *et al*[28]. In open field test, the animals were divided into control, positive control and test groups containing 5 mice each. The test groups received extract of *A. nigra* at the doses of 200 and 400 mg/kg body weight orally whereas control group received vehicle (1% Tween 80 in water). The floor of half square meter open field was divided into a series of squares each alternatively colored black and white. The apparatus had a 40 cm height wall. The number of squares traveled by the animals was counted for 5 min at 0, 30, 60, 90, 120 min after oral administration of both doses of the extract.

2.6.2. Hole cross test

The apparatus was a cage of 30 cm×20 cm×14 cm with a steel partition fixed in the middle, dividing the cage into two chambers. A hole of 3.5 cm diameter was made at a height of 7.5 cm in the center of the cage. Animals were randomly divided into control, positive control and test groups containing 5 mice each. The test groups were treated with extract of *A. nigra* at the doses of 200 and 400 mg/kg body weight orally whereas positive control group with diazepam (1 mg/kg) and control group with vehicle (1% Tween 80 in water). Number of passages of the animals through the hole from one chamber to the other was counted for 5 min at 0, 30, 60, 90 and 120 min after oral administration of the extract as well as diazepam and vehicle[29]. The apparatus was thoroughly cleaned after each trial.

2.7. Statistical analysis

The data were expressed as mean±standard error of mean (S.E.M.). Statistical comparisons were performed using One way ANNOVA followed by Dunnett's multiple comparison test. The values obtained were compared with the vehicle

control group and were considered statistically significant when $P<0.05$.

3. Results

In the EPM, the behavior of mice model, as observed, confirmed the anxiolytic activity of diazepam as reported previously. The methanol extract of *A. nigra* at the dose of 400 mg/kg ($P<0.01$), significantly increased the percentage of entries of mice into the open arms, and the percentage of time spent in the open arms of the EPM as shown in Table 1. The effects of treatment of mice at the dose of 200 mg/kg on open arm entries and time spent in open arms were dose-dependent. The number of closed arm entries and time spent in the closed arms were decreased significantly in the extract treated groups which was comparable with the standard diazepam. The effect of the methanolic extract of *A. nigra* on sodium thiopental induced hypnosis in mice is shown in Table 2.

Table 1

Effect of methanolic extract of *A. nigra* on EPM test during 5 min test session.

Animal group	% of number of entry into open arm	% of Time (in seconds) spent in open arm
Control	55.880±1.908	51.930±7.372
Diazepam	76.280±1.652**	79.390±5.182*
Extract 400 mg/kg	80.270±2.257**	86.130±4.790**
Extract 200 mg/kg	65.710±2.332*	68.330±3.757

Values are expressed as mean±S.E.M., (n=5); * $P<0.05$, ** $P<0.01$, Dunnet test as compared to control (Vehicle=0.4 mL/mouse).

Table 2

Effect of methanolic extract of *A. nigra* on thiopental sodium induced sleeping time.

Groups	Onset of sleep (min)	Duration of sleep (min)
Control	40.20±1.655	47.00±0.949
Diazepam	14.80±0.860**	149.80±3.441**
Extract 400 mg/kg	10.40±2.112**	180.00±4.324**
Extract 200 mg/kg	18.20±1.655**	120.00±8.591**

Values are expressed as mean±S.E.M., (n=5); * $P<0.05$, ** $P<0.01$, Dunnet test as compared to control (Vehicle=0.4 mL/mouse).

In the thiopental induced hypnosis test, the extract at doses, 200 and 400 mg/kg showed a significant reduction in the time of onset of sleep in a dose-dependent manner (Table 2). The effect of the extract (200 and 400 mg/kg) on the onset of sleep were comparable to that of standard. Both doses of the extract potentiated the duration of thiopental sodium induced sleeping time in test animals compared to controls (Table 2).

Open field test of *A. nigra* treated groups (200 and 400 mg/kg body weight) showed significant and dose-dependent

reduction of movement from its initial value at 0 to 120 min (Figure 1). The number of squares travelled by the mice was decreased significantly from its initial value at 0 to 90 min at the dose level of 400 mg/kg body weight ($P < 0.01$) of the methanol extract from the leaves of *A. nigra* (Figure 1).

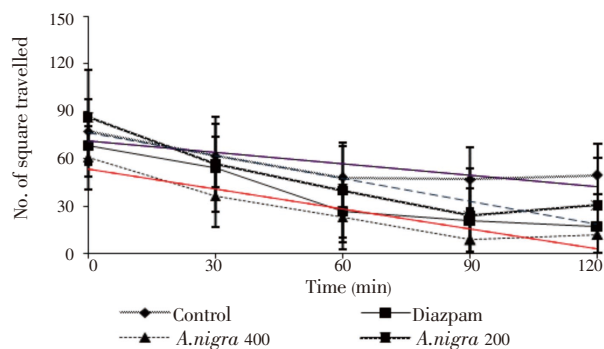


Figure 1. Effect of methanolic extract of *A. nigra* on exploratory behaviour open field test in mice.

Values are mean \pm S.E.M., ($n=5$); * $P < 0.05$, ** $P < 0.01$, Dunnet test as compared to control (Vehicle=0.4 mL/mouse).

The number of hole crossed from one chamber to another by mice of the control group was similar from 30 to 120 min (Figure 2). Hole cross test of *A. nigra* treated groups showed decrease of movement from its initial value at 0 to 90 min. But, at doses of 400 mg/kg ($P < 0.01$), maximum suppression of locomotor activity was displayed which was comparable to the reference drug diazepam (Figure 2).

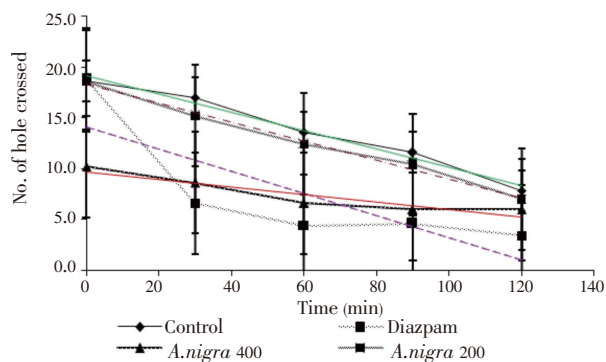


Figure 2. Effect of methanolic extract of *A. nigra* on exploratory behaviour (hole cross test).

Values are mean \pm S.E.M., ($n=5$); * $P < 0.05$, ** $P < 0.01$, Dunnet test as compared to control (Vehicle=0.4 mL/mouse).

4. Discussion

This study examined some neuropharmacological effects of *A. nigra* and established that it has anxiolytic- and antidepressant-like activities. The EPM is one of the most widely validated tests and is highly sensitive to the influence of both anxiolytic and anxiogenic drugs acting at the gamma aminobutyric acid type A (GABAA)-benzodiazepine complex^[30]. In EPM, normal mice will

normally prefer to spend much of their allotted time in the closed arms. This preference appears to reflect an aversion towards open arms that is generated by the fears of the open spaces. Drug like diazepam that increases open arm exploration are considered as anxiolytic and the reverse holds true for anxiogenics^[31]. In this study, we observed that the administration of different doses (200 and 400 mg/kg body weight) of methanolic extract of *A. nigra* induced an anxiolytic-like effect in mice, as it increased open arm entries and the time spent in the open arms of the EPM when compared to the control animals.

Earlier reports showed a significant decrease in the locomotor score for diazepam when compared to the control animals. Locomotor activity is considered as an index of alertness and a decrease in that indicates a sedative effect^[32]. Both the doses (200 and 400 mg/kg body weight) of the *A. nigra* showed a decrease in the locomotor score and produced a significant increase in the hypnotic effect induced by the thiopental sodium, in a dose-dependent manner, thus suggesting a profound sedative activity. Thiopental is basically a hypnotic agent, given at appropriate dose, induced hypnosis by potentiating GABA mediated post synaptic inhibition through allosteric modification of GABAA receptors. Substances which possess CNS depressant activity either decrease the time for onset of sleep or prolong the duration of sleep or both^[33,34]. In addition, the study on locomotor activity, as measured by hole cross and open field tests, showed that both doses of methanol extract from the leaves of *A. nigra* decreased the frequency and the amplitude of movements.

The method employed for this assay is considered as a very sensitive way to detect agents with CNS depressant activity^[35]. The sedative effect recorded here may be related to an interaction with benzodiazepines and related compounds that bind to receptors in the CNS and have already been identified in certain plant extracts. Phytochemical analysis of the plant extract reveals that *A. nigra* contains alkaloids, glycosides, cardiac-glycosides, flavonoids, steroids, tannins, anthraquinone glycosides and saponins. Many flavonoids and neuro-active steroids were found to be ligands for the GABAA receptors in the CNS; which led to the hypothesis that they act as benzodiazepine-like molecules^[36–38]. This is supported by the present study on the behavioral effects in animal models of anxiety and sedation.

It may possible that the mechanism of anxiolytic action of *A. nigra* methanol extract could be due to the binding of any of the phyto-constituents to the GABAA-BZD complex. In support of this, it has been found that flavones bind with

high affinity BZD site of the GABAA receptor^[39].

The locomotor activity is a measure of the level of excitability of the CNS and sedation resulting from depression of the central nervous system^[40]. The result indicated that the extract significantly decreased the locomotor activity as shown by the results of the open field and hole cross tests. The results were also dose-dependent and statistically significant. Therefore, the use of *A. nigra* in folkloric medicine may be due to its CNS action validated by our findings. However, further investigation is necessary to determine the exact phyto-constituents and mechanism of action that are responsible for the biological activities of the methanol extract of *A. nigra*.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

Authors would like to thank Department of Genetic Engineering and Biotechnology, University of Chittagong for thesis financial support for conducting this research work (Ref No. GEB/ AC-54/ 18-09-2010). Special thanks to Dr. Md. Atiar Rahman, Assistant Professor, Dept. of Biochemistry and Molecular Biology, University of Chittagong for his guidance during the study.

Comments

Background

Anxiety and pain are neurologic disorders. CNS depressants can reduce muscle tension, pain, insomnia and acute stress reactions. Natural products can be good sources of potent and effective CNS depressants that are needed for various medicinal purposes.

Research frontiers

This study was aimed to investigate the *in vivo* neuropharmacologic effect of *A. nigra* leaf extract at different doses using mice behavioral models. Four different experiments were conducted to observe the CNS depressant effect.

Related reports

Previous studies also claimed similar medicinal impacts of *A. nigra*. It contains several compounds having roles

in metabolic pathways. It has been reported to possess antioxidant potential besides having folklore use against pain.

Innovations and breakthroughs

Neuropharmacological studies are very significant nowadays due to the lack of effective drugs and medications. Identification of neuropharmacologically active compounds in plants can help to develop new drugs. This research-based study shows the possible role of a medicinal plant in this regard.

Applications

This will help to observe the relation of the identified compounds from this plant with other neurotransmitters and metabolic pathways. Researchers can become interested to find out the active component(s) responsible for anxiolytic effect of this plant.

Peer review

It is an interesting paper in which the authors tried to focus on the neuropharmacological effect of *A. nigra* leaf extract on mice. The anxiolytic activity was determined by well-established protocols that signify an antidepressant 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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