Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
https://www.phytojournal.com
JPP 2023; 12(1): 135-140
Received: 12-11-2022
Accepted: 23-12-2022

Sarmin Akter Shochona
Department of Pharmacy, Stamford University Bangladesh, 51, Siddeswari Road, Dhaka, Bangladesh

Md. Abdul Mannan
Department of Pharmacy, Stamford University Bangladesh, 51, Siddeswari Road, Dhaka, Bangladesh

Corresponding Author:
Md. Abdul Mannan
Department of Pharmacy, Stamford University Bangladesh, 51, Siddeswari Road, Dhaka, Bangladesh

Assessment of central nervous system (CNS) depressant activity of methanolic extract of *Alpinia calcarata* in mice

Sarmin Akter Shochona and Md. Abdul Mannan

Abstract
In a recent study, experimental mouse models were used to assess the central nervous system (CNS) depressive action of methanolic extract of *Alpinia calcarata*. The methanolic extract of *Alpinia calcarata* was tested for central nervous system (CNS) depressant action in adult mice using the open field, hole cross, tail suspension, forced swimming, and thiopental sodium-induced sleeping duration tests at dosages of 50, 100, and 200 mg/kg. Deionized water served as the control group, and three test groups each got a different dose of MEAC in addition to a conventional medicine called diazepam (1 mg/kg). Gavage was used to deliver all drugs orally. In both mouse models of depression, such as the open field and hole cross test, the plant extract at dosages of 50, 100, and 200 mg/kg significantly reduced locomotor activity (p<0.05). When tested in the tail suspension and forced swimming tests, the extract enhanced the immobility period at dosages of 100 and 200 mg/kg considerably (p<0.05). However, as compared to the control group, the extract also significantly (p< 0.05) delayed the start of thiopental sodium-induced sleep and prolonged the amount of time spent sleeping in the test animals. In all mouse studies, MEAC demonstrated potent and dose-dependent depressing action. The current study's findings suggest that *A. calcarata's* methanolic extract exhibits central nervous system (CNS) depressive action. However, further investigation is underway to determine the exact phytoconstituents that are responsible for CNS depressant activity of MEAC.

Keywords: *Alpinia calcarata*, depressant, extract, diazepam

Introduction
One such significant fragrant medicinal plant that belongs to the Zingiberaceae family is *Alpinia calcarata* Rosc. In Bangladesh, they refer to it as "Sugondha Boss." It is grown in tropical nations including Bangladesh, China, India, Malaysia, Sri Lanka, and Timor. The herb is utilized in this region as a digestive tonic, carminative, stomachic, expectorant, and antifungal agent to treat a variety of illnesses [1]. It is also used as a tonic, aphrodisiac, diuretic, and for disorders of the liver and kidney as well as headache, lumbago, diabetes, chest discomfort, rheumatic pain, bronchitis, and dyspepsia. It is known for being very effective for chest complaints [2]. Additionally, dyspepsia, blood impurities, throat pain, voice enhancement, and marinating for youthful vitality are also advised uses [3].
According to reports, 1, 8-cineole and -pinene are the main components of the plant's leaf, blossom, and rhizome oils as well as -fenchyl acetate from the root oil [4]. Protocatechinic acid, 1,8-cineole, quercetin, -pinene, 4-O-methyl-syringic acid, methyl cinnamate, vanillic acid, and a variety of diterpenes have all been found in the roots, rhizomes, and leaves of *A. calcarata* [5-7]. Numerous benzenoids, including flavonoids, alkaloids, protocatechuic acid, vanillic acid, and syringic acid, were discovered in Indian *A. calcarata* leaves [8]. From the rhizomes of *A. calcarata* cultivated in China, certain diterpenes, including calcaratarins A through E, sesquiterpenes, including shyobunone, and coumarins, including herniarin, have been identified. In vitro tests with human KB cells revealed cytotoxic action for the two bis-labdanic diterpenoids [9]. The essential oil of *A. calcarata's* rhizomes, roots, and leaves contains at least 18 volatile chemicals, among which 1,8-cineol (42%), camphene (7.6%), and -pinene (11.30%), -fenchyl acetate (14.7%), camphor (5%), and borneol (2.5%) are the primary constituents [5]. Additionally, *A. calcarata* rhizomes have been found to contain tannins, polyphenols, steroids, glycosides, flavonoids, and alkaloids [10]. The potential application of this plant in pharmaceutics or as an agricultural resource can be revisited given the wide range of phytochemical variety and pharmacological capabilities of *A. calcarata*. Both the ethanolic and aqueous extracts of *A. calcarata* rhizomes have been found in studies to have antibacterial [11], anthelmintic [12], antifungal [13], antinociceptive [14], antioxidant

[10], gastroprotective [15], aphrodisiac [16], antidiabetic [17], and anticancer [18] properties. *A. calcarata* extract has recently been proven to have anti-inflammatory and antioxidant effects [19]. The American Cockroach, Periplanata Americana, is another pest that *A. calcarata* essential oil has been shown to be effective against [20].

However, there is no scientific evidence to support this plant's potential as a CNS depressant. Therefore, the primary goal of this research was to assess the central nervous system (CNS) depressant effect of methanolic extract of *Alpinia calcarata* in experimental mouse models, with an emphasis on its usage in traditional medicine.

Materials and methods

Plant Material

Alpinia calcarata plant material (Rhizomes) was gathered from the plantation area of Dhaka, Bangladesh. Bushra Khan, Principal Scientific Officer, Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh, then identified the samples that had been gathered. For future reference, a voucher specimen was placed in the herbarium.

Preparation of extraction

By removing the soil, fresh rhizomes of the *Alpinia calcarata* plant were harvested. Then, to thoroughly remove the dust, the rhizomes were rinsed in water that was at normal temperature. Rhizomes of *A. calcarata* were cleaned, then air dried for 12 to 15 days in the shade. Rhizomes were chopped into little pieces and blended by a machine once they had sufficiently dried. 900 mL of methanol were used to soak 200 g of powdered rhizomes for three days. Throughout this time, the maceration process was occasionally aided by stirring. The extract was filtered using clean cloth and then cotton after three days. 8.90 g of extract (Yield 4.45%) were produced using a rotary evaporator (BC-R 201 Shanghai Biochemical Equipment Co. Ltd.) to remove the solvent. The unprocessed extract was kept in a beaker and kept cold and out of the sun. Following that, tests on the extract's central nervous system (CNS) depressant action, acute toxicity, and phytochemical screening were conducted.

Drugs and chemicals

The following medicines and substances were employed in this research: methanol, thiopental sodium, and diazepam (all from Sigma Chemicals Co., USA) (Merck, Germany).

Experimental animals

In this investigation, Swiss albino mice measuring 20–25 g at 3–4 weeks of age were employed. These mice were gathered at the Jahangirnagar University's Pharmacology Laboratory in Savar, Dhaka. Animals were housed in polyvinyl cages with bedding made of soft wood. Under typical climatic circumstances (25°C, 55–65% relative humidity, and a 12-hour light/dark cycle), animals were kept in good condition. Prior to doing the studies, the animals spent 14 days becoming used to the lab setting. All experimental mice were handled in accordance with the Swiss Academy of Medical Sciences' and Swiss Academy of Sciences' Ethical Principles and Guidelines for Scientific Experiments with Animals (1995). All experimental guidelines were accepted by the Stamford University Bangladesh Institutional Animal Ethical Committee.

Drugs and treatments

The standard medication diazepam (1 mg/kg) was employed in central nervous system (CNS) depressant activity studies. The sample was prepared by dissolving in deionized water at the doses of 50, 100, and 200 mg/ kg body weight. The control group was given 0.1mL/mouse deionized water, while the test groups were given extract orally 30 minutes prior to the experiments. All the groups received medicines and samples by gavage. All other chemicals and reagents were analytical grade and thoroughly purified.

Acute toxicity test

Mice were divided into control and six test groups (n=5). The MEAC doses administered orally to the test groups were 500, 1000, 2000, 3000, 4000, and 5000 mg/kg body weight. After gavage the animals were kept in separate cages and were allowed to food and water ad *libitum*. The animals were then monitored for the following 72 hours for potential behavioral changes, allergic reactions (skin rash, itching), and mortality [21].

Phytochemical screening

Alkaloids, flavonoids, saponins, tannins, cardiac glycosides, carbohydrates, reducing sugars, proteins, glucosides, terpenoids, and steroids were all detected in methanolic extract of *A. calcarata* through qualitative testing [22].

Pharmacological test

Open field test

The open field test is a common measure of exploratory behavior and general activity in mice. The experiment reveals the animals' emotional reactions as well. For this test, a box was used which is made of wood. It is generally square, rectangular, or circular in shape with surrounding walls that prevent escape. It was divided into squares that measured 4 by 4 half-square meters each in black and white. According to how mice are treated, the test was carried out in three stages: deionized water in the first, diazepam in the second, and methanol extract of *A. calcarata* in the third. At 0, 30, 60, 90, and 120 minutes after the treatments, mice movements were recorded for three minutes [23].

Hole cross test

The hole cross test is typically used to evaluate the hypnotic and sedative effects on lab animals. The wooden box was used for hole cross test had a dimension of 30×20×14 cm3 with partition in the middle of the box. The partition had a hole that is 3cm in diameter and 7cm in height. Three groups of mice were created, and each group received a different treatment—control, diazepam, or extract. To take the reading, mouse was placed in one side of the box and its movement from one side to another side through the hole was watched for 3 min at 0, 30, 60, 90, and 120 min after the treatments [23].

Tail suspension test

The tail suspension test was carried out following by the instruction of Steru et. al. with slight modification. To conduct this test, an adhesive tape was attached around 1 inch from tip of the tail of mouse to hang it 50 cm above the floor by the assistance of a stand. Passively hung and motionless mice were observed by treated with control, diazepam, and extract. Each mouse's immobility time was recorded for 6 minutes. The exam was done between 1-3 p.m. [24].

Forced swimming test

With a few minor modifications, the forced swimming test is conducted in accordance with Porsolt *et al*. For swimming at 25°C, a glass cylinder with a 20 cm diameter and a height of 45 cm was filled with fresh water to a height of 17 cm. When

there was less movement and wriggling in the cylinder, mice were classed as immobile. After five minutes, the surplus water was carefully wiped away with a clean, dry cloth, and the mouse was then placed in a case under light to dry off fast. All groups were tested during the hours of 1:00 and 3:30 p.m. [25].

Thiopental sodium-induced sleeping time test

The creatures were divided into five groups of five mice each at random. Each group of mice was given either a control, diazepam, or extract before being put in a box. Each mouse received an intraperitoneal injection of thiopental sodium (40 mg/kg) 30 minutes later to put them to sleep. After the treatments, the latent period (the interval between thiopental administrations and loss of righting response) and the sleeping period (the interval between loss and recovery of righting reflex) were observed [26].

Statistical analysis

The findings were shown as mean SEM. Using the SPSS 18.00 program, one-way analysis of variance (ANOVA) was used for the statistical analysis, followed as necessary by Dunnett's post hoc test. At a threshold of 0.05, differences between groups were deemed significant.

Results

Phytochemical screening

Alkaloids, flavonoids, saponins, tannins, cardiac glycosides, terpenoids, and steroids were found in phytochemical screens of the crude extract of *A. calcarata* (Table 1).

Table 1: Preliminary qualitative phytochemical screening of methanolic extract of *A. calcarata* (MEAC).

Plant constituents	Inference
Alkaloids, flavonoids, saponins, tannins, cardiac glycosides, terpenoids, steroids	Present
Carbohydrates, reducing sugar, proteins, glucosides	Absent

Acute toxicity

No mortality was seen following oral administration of MEAC at dosages ranging from 500 to 5000 mg/kg, however behavioral abnormalities were noted over the course of a 72-hour observation period. It is safe to conclude that MEAC has a low toxicity profile because its LD_{50} is greater than 5000 mg/kg.

Open field test

Mice that were given treatments with plant extract at dosages of 50, 100, and 200 mg/kg had considerably less activity when moving about (p 0.05). In the perimeter, fewer squares were crossed between extract-treated groups than in the control groups, a difference that was statistically significant (p 0.05). From the initial 30 minutes of observation to the last 120 minutes of observation, the strong depressant impact was shown (Table 2).

Table 2: Effects of *A. calcarata* extract and diazepam on the open field test

Treatment	Dose (mg/kg)	Number of squares crossed				
		0 min	30 min	60 min	90 min	120 min
Control	0.1mL/mouse	78.20±1.4	71.40±0.87	59.20±1.15	49.40±0.67	34.00±1.37
Diazepam	1	76.80±2.51	36.80±1.28*	20.40±0.81*	10.80±0.58*	4.20±0.58*
MEAC	50	71.00±1.30	51.60±1.07*	32.20±1.20*	19.80±0.97*	12.80±0.86*
MEAC	100	76.00±1.64	44.00±1.51*	28.40±0.92*	15.20±1.06*	10.40±0.51*
MEAC	200	72.60±2.56	38.20±0.97*	23.40±1.40*	12.40±0.67*	5.40±0.81*

Values are presented as mean ± SEM (n= 5). MEAC= Methanolic extract of *A. calcarata*.
* p< 0.05, vs. control (Dunnett's test).

Hole cross test

In the hole cross test, MEAC demonstrated a substantial (p 0.05) decrease in movement from its value at 30 minutes to 120 minutes at the test dosages of 100 and 200 mg/kg, which was equivalent to the control group (Table 3). However, during the course of the monitoring period, the common medication diazepam similarly reduced the number of holes traversed.

Table 3: Effects of *A. calcarata* extract and diazepam on hole cross test.

Treatment	Dose (mg/kg)	Number of holes crossed				
		0 min	30 min	60 min	90 min	120 min
Control	0.1mL/mouse	14.60±1.03	11.8±0.37	9.80±0.37	7.20±0.58	5.00±0.54
Diazepam	1	13.80±0.58	6.4±0.51*	4.2±0.37*	2.0±0.31*	0.8±0.37*
MEAC	50	14.6±1.03	10.0±0.31	8.20±0.37	4.4±0.51*	2.2±0.37*
MEAC	100	13.60±0.60	8.80±0.37*	6.00±0.44*	3.60±0.51*	1.60±0.24*
MEAC	200	14.20±0.97	7.00±0.44*	4.60±0.51*	2.80±0.37*	1.20±0.37*

Values are presented as mean ± SEM (n= 5). MEAC= Methanolic extract of *A. calcarata*.
* p< 0.05, vs. control (Dunnett's test).

Tail suspension test

The mobility of the experimental mice was considerably (p 0.05) reduced after oral administration of MEAC at dosages of 100 and 200 mg/kg. At 50 mg/kg, plant extract had no appreciable effects on this test. During the observation period, diazepam considerably lengthened the immobility time (Table 4).

Table 4: Effects of *A. calcarata* extract and diazepam on tail suspension test.

Treatment	Dose (mg/kg)	Immobility Time(s)
Control	0.1mL/mouse	95.40±1.91
Diazepam	1	219.40±2.50*
MEAC	50	84.00±2.09
MEAC	100	133.60±2.89*
MEAC	200	188.60±1.88*

Values are presented as mean ± SEM (n= 5). MEAC= Methanolic extract of *A. calcarata*.
* $p< 0.05$, vs. control (Dunnett's test).

Forced swimming test

When compared to the control group, the immobility duration was substantially (p 0.05) lengthened by the crude MEAC extract at dosages of 100 and 200 mg/kg. Similar to this, diazepam prominently increased the immobility time (Table 5).

Table 5: Effects of *A. calcarata* extract and diazepam on forced swimming test.

Treatment	Dose (mg/kg)	Immobility Time (s)
Control	0.1mL/mouse	60.80±2.55
Diazepam	1	179.80±3.02*
MEAC	50	74.00±1.76
MEAC	100	122.00±2.56*
MEAC	200	166.80±3.55*

Values are presented as mean ± SEM (n= 5). MEAC= Methanolic extract of *A. calcarata*.
* $p< 0.05$, vs. control (Dunnett's test).

Thiopental sodium-induced sleeping time test

In a test where thiopental sodium was used to induce sleep, MEAC at dosages of 50, 100, and 200 mg/kg lowered sleep at an earlier stage. When compared to the control group, it significantly (p 0.05) delayed the onset of thiopental sodium-induced sleep and increased sleep duration in test animals (Table 6).

Table 6: Effects of *A. calcarata* extract and diazepam on thiopental sodium-induced sleeping time test

Treatment	Dose (mg/kg)	Onset of action (min)	Duration of sleeping time (min)
Control	0.1mL/mouse	6.72±0.25	43.20±1.56
Diazepam	1	2.90±0.15*	149.60±1.77*
MEAC	50	5.69±0.19	49.80±1.46*
MEAC	100	4.71±0.21*	91.20±1.35*
MEAC	200	3.37±0.13*	131.20±1.31*

Values are presented as mean ± SEM (n= 5). MEAC= Methanolic extract of *A. calcarata*.
* $p< 0.05$, vs. control (Dunnett's test).

Discussion

The central nervous system (CNS) is crucial to how the entire human body is physiologically organized. People in the modern world frequently experience sadness, anxiety, epilepsy, and restlessness [27]. The majority of over-the-counter medications, including benzodiazepines such as diazepam, zolpidem, zopiclone, and zaleplon, induce negative side effects such as fatigue, weight gain, nausea, dry mouth, sexual dysfunction, forgetfulness, drowsiness, headache, and wooziness [28]. According to earlier research, herbal remedies were crucial in treating CNS diseases [29]. The goal of the current study was to assess the methanolic extract of *A. calcarata's* central nervous system (CNS) depressant properties in experimental mouse models.

Terpenoids, flavonoids, alkaloids, steroids, saponins, cardiac glycosides, and tannins were found in the early phytochemical screening of MEAC (Table 1). Terpenoids appeared to have CNS depressive properties, according to a number of research studies [30]. As a result, the CNS activity in MEAC could be caused by the presence of terpenoids. According to reports, flavonoids function as ligands for the brain's GABAA receptor in a manner similar to that of benzodiazepines [31]. Alkaloids' effects on the brain's regulatory system for sleep may be the cause of the depressive impact [32]. The tannin may also act as a general CNS depressive [33].

The locomotor activity is a test to determine the CNS's level of excitability [34], and any decline in this activity may be closely tied to drowsiness brought on by CNS depression [35]. The methanolic extract of *A. calcarata* was investigated for various neuro-pharmacological effects in the current investigation. The reduction in mice's exploratory behavior suggested that the plant extract had CNS-depressant properties. Additionally, the analysis of locomotor activity using hole cross and open field tests revealed that *A. calcarata* extracts reduced the frequency and amplitude of movements. The impact of reducing locomotor activity was noticeable in the second observation (30 min) and persisted until the fifth observation period (120 min). Between the third (60 min) and fifth (120 min) observation periods, there was a maximum drop of locomotor activity (Table 2 and 3). The primary inhibitory neurotransmitter in the central nervous system is gamma-amino-butyric acid (GABA). GABA helps explain how certain sedative-hypnotic, muscle relaxant, and anxiolytic medications work. Therefore, extracts of *A. calcarata* may operate by directly activating the GABA receptor or by potentiating GAB Aergic inhibition in the CNS via membrane hyperpolarization, which results in a reduction in the firing rate of cortical neurons in the brain [36].

Behavior tests for behavioral despondency, including as the forced swimming and tail suspension tests, are helpful for examining the pathological basis of depression and assessing

the efficacy of antidepressant medications [37]. Tricyclics, serotonin reuptake inhibitors, monoamine oxidase inhibitors, and atypical antidepressants are among the key groups of antidepressant medications that these tests are sensitive to [38]. The immobility commonly scored in both tasks reflects the behavioral despondency associated with clinical sadness [39]. Drugs that lowered noradrenergic release (adenosine or clonidine) and depleted brain monoamines (reserpine) have been found to lengthen immobility duration (despair behavior) [40–42]. Similar to this, we found in our study that *A. calcarata* methanolic extract considerably lengthened immobility duration as measured by TST and FST (Table 4 and 5).

In the thiopental sodium-induced sleeping time test, MEAC enhanced sleep length and dose-dependently induced sleep at an early stage compared to control (Table 6). When administered at the proper dose, the benzodiazepine-type hypnotic drug thiopental causes hypnosis or drowsiness in animals by amplifying GABA-mediated postsynaptic inhibition via an allosteric alteration of GABA receptors [43]. Since MEAC's pharmacological profiles were comparable to those of bezodiazepines in the current experiment, it is also likely that they may interact with the benzodiazepine receptors that are next to the GABA receptor. The time it takes for sleep to start can be shortened, the length of sleep can be extended, or both might happen when a substance has CNS depressive effects. The findings of this test suggest that MEAC may have a depressive effect on the central nervous system. Overall, the findings appear to be indicative of the central nervous system depressive effects of this methanolic extract of *A. calcarata*.

Conclusions

We draw the conclusion that *A. calcarata's* methanolic extract has potent, dose-dependent central nervous system (CNS) depressive action based on the findings of the current investigation. To explore the underlying processes of central nervous system (CNS) depressive effects and to identify the active substances in charge of these pharmacological actions, more research is required.

Ethics

All experimental mice were handled in accordance with the Swiss Academy of Medical Sciences' and Swiss Academy of Sciences' Ethical Principles and Guidelines for Scientific Experiments with Animals (1995). All experimental guidelines were accepted by the Stamford University Bangladesh Institutional Animal Ethical Committee.

Conflict of interest

Regarding this inquiry, there are no conflicts of interest for the writers.

Acknowledgements

The authors are appreciative to the Chairman of the Department of Pharmacy at Stamford University Bangladesh for making the resources of the Pharmacology and Phytochemistry Laboratory available to them.

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