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Research Article

COMPARATIVE STUDY OF AQUEOUS AND ETHANOLIC EXTRACT OF AMORPHOPHALLUS PAEONIIFOLIUS TUBER ON CENTRAL NERVOUS SYSTEM ACTIVITY IN MICE

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ABSTRACT

Objective: The objective of the present study was to perform phytochemical screening and to carry out a comparative study on aqueous and ethanolic extract of *Amorphophallus paeoniifolius* tuber on central nervous system (CNS) activity in Swiss albino mice.

Methods: *A. paeoniifolius* tuber was collected, shed dried, and powdered. Extracts of the powder were prepared separately using ethanol and water. Phytochemical screening was also done from the extract. The animals were divided in six groups. Group 1 received DMSO solution (Control). Group 2 animals received Diazepam (1.5 mg/kg, standard). Groups 3 and 4 received *A. paeoniifolius* aqueous extract (200 mg/kg and 400 mg/kg, respectively). Groups 5 and 6 received *A. paeoniifolius* ethanolic extract (200 mg/kg and 400 mg/kg, respectively). *In vivo* CNS activities of the extracts were checked by actophotometer, rotarod, and forced swim test.

Results: The extracts show presence of alkaloids, flavonoids, steroids, terpenoids, reducing sugar, carbohydrate, and tannin. The aqueous and ethanolic extract of *A. paeoniifolius* tuber showed significant decrease in locomotor activity when compared to control. Among the extracts, the 400 mg/kg ethanolic extract shows maximum CNS depression. All the extracts showed significant muscle relaxant similar to the standard drug. The extracts also exhibited significant increase in immobility time in a dose-dependent manner in forced swim test.

Conclusion: The aqueous and ethanolic extract of *A. paeoniifolius* tuber (200 mg/kg, 400 mg/kg) shows significant CNS depressant and muscles relaxant and anxiolytic properties.

Keywords: *Amorphophallus paeoniifolius*, Locomotor activity, Muscle relaxant activity, Central nervous system depressant activity, Anxiolytic activity.

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INTRODUCTION

Central nervous system (CNS) activity refers to depression of central nervous system, anti-anxiety, local or general anesthesia, sedation, anticonvulsant activities, muscle relaxant properties, and memory enhancement effects. The drugs affecting CNS do so by modulation of neuronal communication. CNS depressants and anesthetics affect neurotransmitters like gamma aminobutyric acid (GABA). They showed their activity on N-methyl D-aspartate (NMDA) receptor, μ opioid receptor, and cannabinoid receptor or by blocking sodium channels, decreasing calcium influx.

Since ages plants have been used as the source of medicinal agents. In particular, more than 50% of medicinal agents have natural source [1]. Although there is a great advancement in modern medicine, traditional medicine is still the primary form of treatment for several chronic diseases in developing countries including India. Nowadays, Ayurveda, traditional Chinese medicine, Kampo, traditional Korean medicine, and Unani medicines are getting popularity worldwide for health promotion as well as adjuvant therapy [2]. Recent popularity of traditional medicine has provided excellent opportunity to India to look for newer therapeutic compounds from our traditional medicinal system Ayurveda.

Amorphophallus paeoniifolius also known as Elephant foot yam is mainly cultivated in Indonesia, Sumatra, Philippines, Java, China, Malaysia, Bangladesh, and India. In India, it is known as suran or jamikand and is cultivated in Andhra Pradesh, Kerala, Tamil Nadu, Maharashtra, Uttar Pradesh, Gujarat, Jharkhand, and West Bengal [3]. The corms are consumed as food and widely used in several ayurvedic preparations. The corms are traditionally used as carminative, digestive, stomachic, anodyne, anti-inflammatory, anthelmintic, and aphrodisiac. They are useful in arthralgia, tumors, elephantiasis, hemorrhoids, hemorrhages, cough, bronchitis, asthma, anorexia, vomiting, amenorrhea, dysmenorrhea, fatigue, anemia, and general debility [4].

Various extracts of the *A. paeoniifolius* tuber shown to have pharmacological activities such as analgesic activity [5,6], anthelmintic activity [7], antimicrobial activity [8-10], hepatoprotective activity [11], and anti-inflammatory activity [12]. The pet ether extract shows significant CNS depressant activity [13]. However, limited reports about the pharmacological activity are available for the plant. Therefore, the aim of the present study was to determine the central nervous system activity of the aqueous and ethanolic extract of *A. paeoniifolius* tuber in healthy mice.

MATERIALS AND METHODS

A. paeoniifolius tuber was purchased on September 2021 from local market of Asansol, West Bengal, India. Male Swiss albino mice were obtained from animal house of Gupta College of Technological Sciences, Asansol. The animals were fed with standard diet and water ad libitum and housed under standard environmental condition (25°, 12 h light and 12 h dark cycle). The Animal Ethics Committee of Gupta College of Technological Sciences approved the experimental protocol (Protocol No.: GCTS/IAEC/2021/AUG/01).

Preparation of the extracts

The tuber of the plant is shade dried and grounded to powder using a mixer grinder. The powder is then extracted with distilled water using maceration technique to get the aqueous extract (APAE). The tuber powder is also extracted with ethanol (Merck) by soxhlation to get the ethanolic extract (APEE). Phytochemical screening of both the extracts was done.

Evaluation of CNS activity

Evaluation of CNS activity was done using actophotometer (Technoworld, Delhi, India), rotarod (Biological Museum, Agra, India), and forced swim test. All the experiments were carried out in isolated and noiseless condition. Animals were subjected to minimum pain. All the ethical guidelines are strictly followed during the experiments. The LD50 of ethanolic extract was reported to be 2000 mg/kg [14] and the approximate LD50 of aqueous extract was reported to be more than 2500 mg/kg [15]. $1/10^{th}$ and $1/5^{th}$ of the LD50 was taken as the dose for the experiments.

Experimental design

The animals were divided into six groups, with six animals in each group.

- Group 1: DMSO solution by oral route
- Group 2: Diazepam (1.5 mg/kg, IP)
- Group 3: APAE (200 mg/kg, in distilled water by oral route)
- Group 4: APAE (450 mg/kg, in distilled water by oral route)
- Group 5: APEE (200 mg/kg, in DMSO solution by oral route)
- Group 6: APEE (400 mg/kg, in DMSO solution by oral route).

Locomotor activity using actophotometer

Animals were first placed in actophotometer and the locomoteor activity is recorded for 10 min before treatment. Then, the mice were treated with APAE (200 mg/kg, 400 mg/kg, oral) and APEE (200 mg/kg, 400 mg/kg, oral) and standard drug diazepam (Ranbaxy, India) (1.5 mg/kg IP) and DMSO (Sigma-aldrich) solution based on respective group 30 min before being placed in the actophotometer again. The locomotor activity was recorded for 10 min for individual animal after treatment. The percentage change in locomotor activity was calculated.

Muscle relaxant property using rotarod

Animals were first placed on rotarod apparatus individually and fall of time before treatment was recorded. Then, mice were treated with APAE (200 mg/kg, 400 mg/kg, oral) and APEE (200 mg/kg, 400 mg/kg, oral) and standard drug Diazepam (1.5 mg/kg IP) and vehicle based on respective group. After 30 min, the animals were placed one by one on rotarod apparatus and their fall of time was recorded.

Forced swim test

The standard method of forced swim test was followed [16]. A glass cylinder filled 10cm high with water (25±2°C). The mice were treated with APAE (200 mg/kg, 400 mg/kg, oral) and APEE (200 mg/kg, 400 mg/kg, oral) and standard drug Diazepam (1.5 mg/kg IP) and vehicle based on respective group 30 min before being placed individually placed in the glass cylinder and were forced to swim for 6 min and the duration of immobility was measured during final 4 min interval of the test. The time spent by each mice floating in the water without any struggle making only those movement to keep its head above the water is regarded as immobility period. Each animal were used only once for the test.

Statistical analysis

The data obtained in pharmacological experiments were expressed as mean±SD. In the experiments, difference between control and treatments was tested for significance using one-way ANOVA. Values of $p<0.05$ for n=6 mice in each group were considered statistically significant.

RESULTS

Phytochemical screening

APAE shows presence of alkaloid, tannin, carbohydrate, and reducing sugar. APEE shows presence of alkaloid, flavonoid, steroid, tannin, carbohydrate, and reducing sugar when appropriate qualitative phytochemical tests were carried out in our laboratory.

Locomotor activity using actophotometer

A. paeoniifolius shows significant decrease in locomotor activity in dose-dependent manner. APAE showed a significant decrease in locomotor activity in mice ($p<0.01$, n=6). APEE showed more significant decrease in locomotor activity in mice ($p<0.01$, n=6). The APEE 400 mg/kg dose shows maximum decrease in % inhibition in locomotor activity among the test samples. However, diazepam found to be more potent CNS depressant than APAE and APEE, as shown in Fig. 1.

Muscle relaxant property using rotarod

A. paeoniifolius shows significant muscle relaxation in dose-dependent manner. Both the extracts show significant decrease in fall of time in rotarod. APAE showed a significant muscle relaxant property in mice ($p<0.01$, n=6). APEE also showed significant muscle relaxant property in mice ($p<0.01$, n=6). However, diazepam found to be more potent muscle relaxant than APAE and APEE, as shown in Fig. 2.

Forced swim test

A. paeoniifolius extracts both show anxiolytic property. They show significant increase in immobility time in forced swim test. APAE showed a significant increase in immobility time in mice ($p<0.01$, n=6) when compared to control. APEE also showed significant increase in immobility time in mice ($p<0.01$, n=6). However, diazepam found to be more potent anxiolytic than APAE and APEE, as shown in Fig. 3.

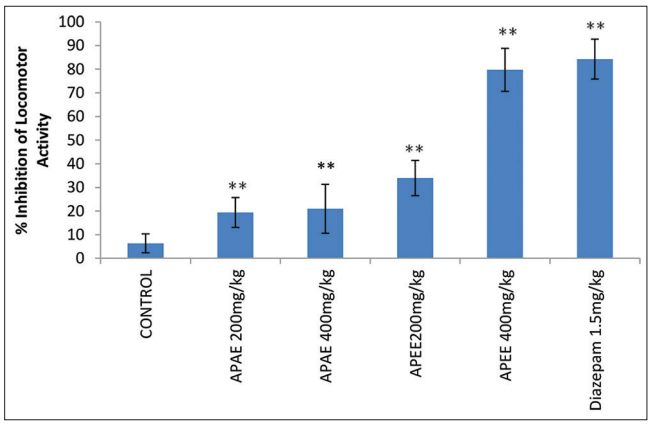

Fig. 1: Effect of *Amorphophallus paeoniifolius* on locomotor activity of mice. *A. paeoniifolius* aqueous extract (APAE) (200 mg/kg and 400 mg/kg) and ethanolic extract (APEE) (200 mg/kg and 400 mg/kg) show significant decrease ($p<0.01$) in locomotor activity in mice as compared to control. Diazepam (1.5 mg/kg) shows similar decrease in locomotor activity in mice. **$p<0.01$ when compared to control

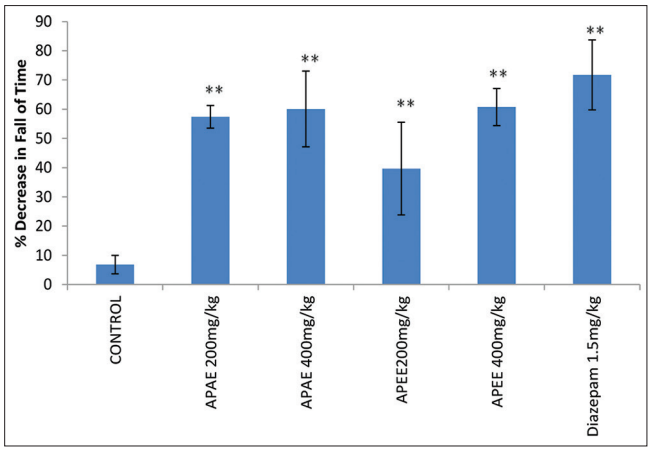

Fig. 2: Effect of *Amorphophallus paeoniifolius* on muscle relaxant property of mice. *A. paeoniifolius* aqueous extract (APAE) (200 mg/kg and 400 mg/kg) and ethanolic extract (APEE) (200 mg/kg and 400 mg/kg) show significant decrease ($p<0.01$) in fall of time in mice as compared to control. Diazepam (1.5 mg/kg) also shows similar decrease in fall of time in mice. **$p<0.01$ when compared to control

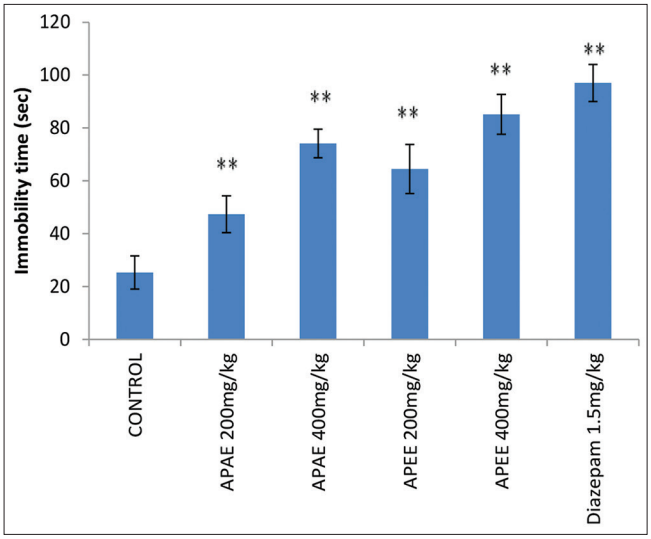

Fig. 3: Effect of *Amorphophallus paeoniifolius* on immobility time in mice. *A. paeoniifolius* aqueous extract (APAE) (200 mg/kg and 400 mg/kg) show significant increase (p<0.01) in immobility time in mice as compared to control. Diazepam (1.5 mg/kg) also shows similar increase in immobility time in mice. **p<0.01 when compared to control

DISCUSSION

CNS depression and anxiolysis are principally mediated in the CNS by the $GABA_A$ receptor complex. Many centrally acting drug like barbiturates increase chloride conductance by allosteric modification of $GABA_A$ receptor [17]. CNS depression can be also produced by direct inhibition of chloride conductance or simultaneous inhibition of voltage activated Ca^{++} currents [18].

The present work demonstrates A. paeoniifolius aqueous and ethanolic extracts possess CNS depressant activity when compared to control. Measurement of locomotor activity using actophotometer is a popular model for evaluation of CNS depressant property. In this test, if the activity score of the animal is reduced after drug treatment; then, it denotes CNS depression caused by the drug. APAE and APEE both significantly reduced the activity score of the mice in this test. Thus, both the extracts were showing CNS depression in mice. The ethanolic extract shows higher level of depression than aqueous extract of *A. paeoniifolius*. We can measure skeletal muscle relaxant activity of any drug using rotarod. If the fall of time decreases in mice after drug treatment that means that drug is showing skeletal muscle relaxant property. APAE and APEE both were showing significant decrease in fall of time in rotarod when compared to control. Forced swim test is a popular model for testing anxiolytic properties of a drug. In this test, the mice after they are placed in the water filled cylinder shows immobility. During this immobility time, they are only doing the movements which are essential to keep its head above the water. This immobility time is increased after drug treatment if the drug is anxiolytic. Both the extracts APAE, APEE shows an increase in immobility time when compared to control in forced swim test. Thus, they are proved to have anxiolytic activity. Thus, A. peoniifolius extracts involved in this study that shows significant CNS depression, muscle relaxation, and anxiolytic property.

Earlier researches on plant materials and phytoconstituents show that flavonoids and neuroactive steroids are reported as ligands for $GABA_A$ receptor which led to the assumption that they can act as CNS depressant drugs like benzodiazepines [19]. Many plants are found to possess CNS depressant and anxiolytic activity due to presence of certain phytoconstituents such as saponins, triterpenoids, and flavonoids [20,21]. *A. paeoniifolius* aqueous extract shows presence of alkaloid, tannin, carbohydrate, and reducing sugar. *A. paeoniifolius* ethanolic extract shows presence of alkaloid, flavonoid, steroid, tannin, carbohydrate, and reducing sugar. May be due to presence of flavonoid and steroids the extracts are showing significant CNS depressant, muscle relaxant, and anxiolytic activity.

CONCLUSION

From the results, we can conclude that *A. paeoniifolius* aqueous and ethanolic extracts show significant CNS depressant, muscle relaxant, and anxiolytic properties. Flavonoids and steroids may be the phytoconstituents responsible for these activities. The present study was carried out using crude extract. Therefore, further studies are needed to find out the main phytoconstituents responsible for these pharmacological actions.

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AUTHORS' CONTRIBUTIONS

Titin Debnath: Contributed to formulate the question, carry out the study, curate the data, and analyze it. Malini Sen: Contributed to conceptualize the study, formulate the question, design the study, and write the article and review.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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