

**Sedative and Anti-convulsant Activities of Methanol Extract of an African Edible Mushroom**

Kizito E. Bello^{1*}, Dina Yamin², Abdirasak S. Ali-Mude³, David A. Zakari¹, Obeimen C. Stella-Maris⁴, Itodo K. Janefrances⁴, Osazuwa O. Christopher⁵, Egbeja T. Idris⁶, Hadiza O. Abdulsalam⁷, Okpanachi M. Abdubala⁸, Audu A. Godwin⁹, Dorathy A. Ogohi⁹, Maji O. Okpanachi¹⁰, Momoh B. Theophilus¹⁰.

¹Department of Microbiology, Faculty of Natural Science, Kogi State (Prince Abubakar Audu) University, Anyigba. PMB 1008, Anyigba, Kogi State, Nigeria

²Department of Medical Laboratory Sciences, School of Science, The University of Jordan, Amman 11942, Jordan

³Faculty of Medicine and Health Sciences, SIMAD University, Mogadishu 252., Somalia

⁴Department of Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

⁵Department of Microbiology, Adekunle Ajasin University, Akungba, Ondo State, Nigeria

⁶Department of Animal and Environmental Science, Faculty of Natural Science, Kogi State (Prince Abubakar Audu) University, Anyigba. PMB 1008, Anyigba, Kogi State, Nigeria

⁷Department of Pure Chemistry, Faculty of Natural Science, Kogi State (Prince Abubakar Audu) University, Anyigba. PMB 1008, Anyigba, Kogi State, Nigeria

⁸Department of Animal and Environmental Biology, Faculty of Natural Science, Kogi State (Prince Abubakar Audu) University, Anyigba. PMB 1008, Anyigba, Kogi State, Nigeria

⁹Department of Biochemistry, Faculty of Natural Science, Kogi State (Prince Abubakar Audu) University, Anyigba. PMB 1008, Anyigba, Kogi State, Nigeria

¹⁰Department of Plant Science and Biotechnology, Faculty of Natural Science, Kogi State (Prince Abubakar Audu) University, Anyigba. PMB 1008, Anyigba, Kogi State, Nigeria

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Epilepsy and anxiety disorders continue to pose substantial neurological problem, which necessitate the search for safer and more efficacious treatment options. African edible mushrooms, recognized for their therapeutic attributes, have demonstrated potential in influencing the central nervous system, which has prompted inquiries into their sedative and anticonvulsant properties. This study aimed to assess the sedative and anticonvulsant activities of the methanol extract of *Pleurotus ostreatus* (POEXT) in mice. The acute toxicity of POEXT was assessed in accordance with the OECD Guideline 423 for acute oral toxicity evaluation of chemical substances. The sedative activity of POEXT was assessed using thiopental sodium (TS)-induced sleep model in mice. The anti-convulsant activity was assessed using Strychnine and Pentylene-tetrazole (PTZ)-induced seizure models in mice. The acute toxicity test revealed that POEXT is relatively safe at a dose up to 3200 mg/kg, with no evidence of morbidity or mortality. POEXT was found to have sedative and anticonvulsant activities. In the TS-induced sleep model, POEXT significantly ($p < 0.001$) reduced sleep latency in mice and prolonged the total sleep duration. POEXT exhibited dose-dependent protection against Strychnine and PTZ-induced seizures in mice. Therefore, the findings from this study provides the scientific basis for the traditional use of *Pleurotus ostreatus* in the management of epilepsy, and also revealed POEXT as a potential source of bioactive agent(s) for the management of epilepsy.

Keywords: *Pleurotus ostreatus*, Sedative, Anti-convulsant, Strychnine, Pentylene-tetrazole

Introduction

Epilepsy is among the most common forms of neurological disorder.¹ It is characterized by an abnormal surge of electrical activity in the brain.² According to the World Health Organization (WHO), epilepsy affects approximately 0.5 to 1% of the world population.³ Epilepsy is common among young children and the elderly.

*Corresponding author. Email: Bello.K@ksu.edu.ng
Tel.: +2348067122958

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Seizure is a major manifestation of epilepsy, and it usually results from a random misfiring of electrical impulses by a cluster of neurons in the brain.⁴ Two common types of seizures are known; these are partial (also known as focal or local), and generalized seizures. As the name implies, partial seizures occur in one part of the brain, usually in one of the hemispheres, while generalized seizures are touted to affect multiple parts of both hemispheres at the same time.^{5,6} A growing number of conventional drugs, such as antiepileptics, sedatives, and anxiolytics, are available for the management of epilepsy and other neurological disorders. However, the high cost of these drugs, inherent side effects, and difficulties associated with accessing them, especially in rural areas, and a number of other obstacles have made it difficult to rely completely on them.⁷⁻⁹ Consequently, there is a research focus on alternative therapeutic agents with better pharmacological profile in the management of epilepsy. Medicinal mushrooms have always played a key role in healthcare, especially for people dwelling in the developing world.^{10, 11} There are a number of these mushrooms used traditionally as antiepileptic agents. One of such mushrooms is *Pleurotus ostreatus*.

Pleurotus ostreatus is used in various countries across Africa as a vegetable.¹² In northern Nigeria, a decoction of *Pleurotus ostreatus* is used for the management of diabetes mellitus. The antibacterial potential of the ethanol extract of *Pleurotus ostreatus* has been reported.¹³ To the best of our knowledge, there is no scientific study on the sedative and anti-convulsant potentials of this mushroom. Hence, this study was carried out to evaluate the sedative and anti-convulsant potentials of the methanol extract of this mushroom.

Materials and Methods

Chemicals and Drugs

Ethanol (analytical grade), Strychnine, pentylenetetrazole, and picrotoxin were procured from Sigma Chemical Co. Ltd (USA) via a local supplier in Jos, Nigeria, while phenobarbital (Luminal) and sodium valproate (Convex) were sourced from Healthsealed Pharmacy Ltd., Abuja-FCT, Nigeria.

Collection and authentication of mushroom sample

Fresh *Pleurotus ostreatus* were harvested from Okene, Kogi State, Nigeria, on the 15th day of May, 2024. The mushroom samples were authenticated by a Plant Biologist Dr. T.B. Momoh at the Plant Science and Biotechnology Laboratory, Prince Abubakar Audu University, Anyigba, Kogi State, Nigeria. Herbarium specimen with voucher number PSB MUS O13 was deposited at the herbarium unit of the laboratory.

Preparation of extract

Pleurotus ostreatus mushrooms were shade-dried in the laboratory for seven days. The dried mushrooms were pulverized into a coarse powder with a hand mixer. Two kilograms (2 kg) of the powdered mushrooms were measured and placed in a 12 L glass container, and then macerated with 5 L of methanol at room temperature for 72 h with periodic stirring. The mixture was subsequently filtered through Whatman filter paper (Size No. 1), and the resulting filtrate was concentrated using a rotary evaporator at 40°C at a pressure of 204 mbar. The concentrated extract was labeled 'POEXT' and kept in the refrigerator at 4 – 8°C until used for the experiment.

Experimental Animals

Healthy adult male mice, weighing between 18 and 32 g, were used for the study. They were obtained from the Animal House Facility of the Department of Biochemistry, Prince Abubakar Audu University, Anyigba, Kogi State, Nigeria. Ethical approval for the study was granted by the Kogi State Ministry of Health with the approval reference number MOH/KGS/1376/1/89. The mice were housed in well-ventilated stainless-steel cages under proper sanitary conditions, the mice were fed with normal rodent pellets, and allowed free access to drinking water.

Acute toxicity study

The evaluation of acute toxicity for POEXT was conducted using a mouse model. The experiment complied with the OECD 423 guidelines of the Organization for Economic Co-operation and Development (OECD), which provides a systematic approach for assessing the toxicity of chemicals.¹⁴ The median lethal dose (LD₅₀) of the extract was established during the toxicity assessment.

Evaluation of sedative activity

Thiopental sodium-induced sleeping time test

This experiment was performed according to the procedure previously described by Ali *et al.* (2015).¹⁵ A total of 25 mice were randomly allocated into five groups (A – E) of 5 mice per group. The mice were treated as follows:

- Group A: Received normal saline at a dose of 10 mL/kg (negative control)
- Group B: Administered 100 mg/kg of POEXT
- Group C: Administered 200 mg/kg of POEXT
- Group D: Administered 400 mg/kg of POEXT
- Group E: Administered 1 mg/kg of Diazepam (positive control)

Thiopental sodium (40 mg/kg) was administered to all groups to induce sleep 30 minutes following the treatments. All treatments were

administered intraperitoneally. Post-administration, the mice were meticulously observed for sleep latency (time to onset of sleep) and total sleep duration.

Evaluation of anti-convulsant activity

Strychnine-induced seizure test

This study employed a modified version of the method outlined by Porter *et al.* (1984)¹⁶ to assess anticonvulsant activity. Twenty-five mice were randomly allocated to five experimental groups (A – E), each consisting of five animals, and were subjected to the following treatments:

- Group A: Administered 10 mL/kg of normal saline (negative control).
- Group B: Received 100 mg/kg of POEXT.
- Group C: Administered 200 mg/kg of POEXT.
- Group D: Administered 400 mg/kg of POEXT.
- Group E: Administered 30 mg/kg of phenobarbital (positive control).

All treatments were administered through intraperitoneal injection. After 30 minutes of the treatments, mice in all groups received a subcutaneous injection of 1.5 mg/kg strychnine. Observations were made to determine the number of mice exhibiting seizures and the duration of seizure. The lack of hind limb extension 30 minutes following strychnine injection was indicative of anticonvulsant efficacy.

The percentage of protection in the strychnine-induced seizure model was calculated based on the number of animals protected (those that did not exhibit convulsions or death) compared to the total number of animals tested as indicated in the formula below.

$$\% \text{ Protection} = \frac{\text{number of animals protected}}{\text{Total number of animals}} \times 100$$

Pentylenetetrazole (PTZ)-induced seizure test

The experiment was conducted following the procedure described by Venkateshwarlu *et al.* (2013).¹⁷ In this study, twenty-five mice were randomly assigned to five groups (A – E), each comprising five animals, and received the following treatments:

- Group A: Administered normal saline at a dose of 10 mL/kg (negative control)
- Group B: Received 100 mg/kg of POEXT
- Group C: Administered 200 mg/kg of POEXT
- Group D: Administered 400 mg/kg of POEXT
- Group E: Administered 200 mg/kg of Valproate (positive control)

All treatments were administered via the intraperitoneal route. After 30 minutes following treatment, each mouse was injected subcutaneously with pentylenetetrazol (PTZ) at a dose of 85 mg/kg. The animals were then observed for a period of 30 minutes to assess seizure activity. The extract was considered to have demonstrated anticonvulsant effects if no clonic spasms were observed in the mice for at least five seconds. This also indicate the extract's potential to counteract PTZ-induced seizures.

Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM). Data were subjected to one-way analysis of variance (ANOVA) to determine statistical significant difference among the group means, this was followed by Dunnett's post hoc test for multiple comparisons. A p-value of less than 0.05 (p < 0.05) was considered statistically significant. All data analyses were carried out using GraphPad Prism software.

Results and Discussion

Acute toxicity profile of POEXT

The findings from the acute toxicity evaluation of the methanol extract of *Pleurotus ostreatus* (POEXT) are summarized in Table 1. Administration of POEXT across all tested doses did not result in any observable toxic effects or mortality in mice. According to the Organization for Economic Cooperation and Development (OECD) classification system (2001), POEXT falls under category 5, indicating an LD₅₀ exceeding 3200 mg/kg, thereby suggesting a high safety margin.

Sedative effect of POEXT

The effects of POEXT on thiopental sodium-induced sleep in mice is presented in Table 2. The extract significantly reduced sleep latency in a dose-dependent fashion. Additionally, POEXT led to a significant ($p < 0.001$) prolongation of total sleep duration in comparison to the control group. Notably, at a dose of 400 mg/kg, POEXT exhibited effect comparable to that of diazepam, both in terms of reducing sleep onset and increasing sleep duration.

Table 1: Acute Toxicity profile of the Methanol Extract of *Pleurotus ostreatus* (POEXT) in Mice

Treatment dose (mg/kg)	Observed Signs of Toxicity	Mortality
10	-	0
200	-	0
400	-	0
800	-	0
1600	-	0
3200	-	0

Table 2: Effect of the Methanol Extract of *Pleurotus ostreatus* (POEXT) on Thiopental Sodium-induced Sleeping Time in Mice

Treatment	Onset of sleep (min)	Duration of sleep (min)
Control (10 mL/kg NS)	18.21 ± 0.63	58.21 ± 1.08
POEXT (100 mg/kg)	16.11 ± 0.28*	99.35 ± 2.19***
POEXT (200 mg/kg)	11.26 ± 0.14**	110.18 ± 1.31***
POEXT (400 mg/kg)	10.15 ± 0.73***	117.92 ± 1.45***
Diazepam (1 mg/kg)	9.21 ± 0.82***	120.11 ± 3.26***

Values are the mean ± SEM, n = 5. *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$ compared to the control. NS = Normal saline

In this study, thiopental sodium significantly shortened sleep onset time while extending total sleep duration in mice. This barbiturate induces sedation by enhancing the inhibitory function of gamma-aminobutyric acid (GABA) via binding to GABA-A receptors in the central nervous system.¹⁸ Our results demonstrated that POEXT similarly reduced sleep onset latency while prolonging sleep duration in a dose-dependent manner. The extract may exert its sedative effects through a mechanism similar to benzodiazepines, potentially interacting with GABA-A receptors and promoting hyperpolarization of postsynaptic neurons. Various bioactive compounds found in mushrooms, including triterpenoids and phenolics, have been implicated in potentiating barbiturate-induced sleep, which may explain the observed sedative effect of POEXT.¹⁹

Another plausible mechanism by which POEXT may exert its sedative effect may be through antagonism of excitatory neurotransmission, specifically by inhibiting glutamate-mediated signaling. This could involve the blockade of N-methyl-D-aspartate (NMDA) receptors, glycine receptors or metabotropic glutamate receptors.^{20, 21} These observations suggest that POEXT possesses sedative properties similar to those of tranquilizers and hypnotic agents, which could contribute to its potential anticonvulsant activity.

Anti-convulsant activity of POEXT

The protective effect of POEXT in a strychnine-induced seizure model in mice is presented in Table 3. At doses of 100 and 200 mg/kg, POEXT conferred a 20% protection rate against both seizures and mortality. A higher dose of 400 mg/kg markedly improved protection, achieving 80% survival rate against seizure-induced mortality.

Table 4 presents data from PTZ-induced seizure experiments. POEXT exhibited dose-dependent protective effects, significantly delaying both seizure onset and mortality compared to the control group ($p < 0.001$). At all tested doses, POEXT extended the latency to seizure onset and increased survival time in PTZ-exposed mice.

Table 3: Effect of the Methanol Extract of *Pleurotus ostreatus* (POEXT) on Strychnine-induced Seizures in Mice

Treatment	Onset of seizure/mortality (min)	Percentage Seizure/mortality protection (%)
Control (10 mL/kg NS)	6.21 ± 0.81	0.00
POEXT (100 mg/kg)	6.26 ± 0.21	20.00
POEXT (200 mg/kg)	8.38 ± 0.48	20.00
POEXT (400 mg/kg)	9.15 ± 0.29	80.00
Phenobarbital (30 mg/kg)	0.00	100.00

Values are the mean ± SEM, n = 5. NS = Normal saline

Table 4: Effect of the Methanol Extract of *Pleurotus ostreatus* (POEXT) on Pentylenetetrazole (PTZ)-induced Seizures in Mice

Treatment	Mean onset of seizure (min)	Percentage seizure protection	Mean onset of mortality (min)	Percentage mortality protection (%)
Control (10 mL/kg NS)	7.27 ± 0.26	0.00	5.11 ± 0.84	0.00
POEXT (100 mg/kg)	10.45 ± 1.21***	20.00	13.11 ± 1.45***	20.00
POEXT (200 mg/kg)	17.34 ± 1.36***	40.00	18.26 ± 1.48***	80.00
POEXT (400 mg/kg)	20.21 ± 2.11***	80.00	20.42 ± 1.23***	80.00
Valproate (200 mg/kg)	0.00	100.00	0.00	100.00

Values are the mean ± SEM, n = 5. *** $p < 0.001$ compared to the control. NS = Normal saline

Epileptic seizures result from abnormal, excessive neuronal discharges, often driven by alterations in synaptic transmission. One major cause of seizures is an imbalance between excitatory neurotransmitters, such as

glutamate, and inhibitory neurotransmitters like GABA.^{22,23} Antiepileptic drugs (AEDs) function by restoring this balance. In this study, strychnine was employed to induce seizures by inhibiting glycine-mediated neurotransmission. As a selective and competitive glycine receptor antagonist, strychnine disrupts glycine's inhibitory influence on motor and interneurons within the CNS.^{24,25} The significant, dose-dependent seizure protection provided by POEXT suggests that it may enhance glycine receptor activity, contributing to its anticonvulsant properties. PTZ is another well-established seizure-inducing agent, known to act by antagonizing GABA at GABA-A receptors, thereby reducing inhibitory neurotransmission.²⁶⁻²⁸ The protective effects of POEXT at all tested doses against PTZ-induced seizures indicate a potential interaction with the GABAergic system, either through direct receptor modulation or enhancement of endogenous GABA activity. This finding further supports the hypothesis that POEXT possesses anticonvulsant properties mediated through GABAergic pathways.

Conclusion

Pleurotus ostreatus possesses sedative and anti-convulsant activities, which may be a result of its interaction with the GABA-A and Glycine receptors in the central nervous system. This study, therefore, provides a scientific basis for its use in the management of epilepsy.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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