

Evaluation of the anticonvulsant activity of ethanol leaf extract of *Panicum maximum* in pentylenetetrazole and strychnine induced seizure in mice.Uchechukwu L. Iyanyi^{1*}, Ikenna Oparah¹, Jude E. Okokon²¹Department of Pharmacology and Toxicology, Madonna University, Elele, Nigeria.²Department of Pharmacology and Toxicology, University of Uyo.Submitted: 9th July., 2023; Accepted: 5th August., 2023; Published online: 31st Aug., 2023DOI: <https://doi.org/10.54117/jcbr.v3i4.4>*Corresponding Author: Uchechukwu L. Iyanyi; uchechukwuiyanyi@yahoo.com; 08135008001**Abstract**

Epilepsy is a chronic neurological condition affecting around 50 million people worldwide. This study evaluated the anticonvulsant effect of *Panicum maximum* in pentylenetetrazole (PTZ) and strychnine (STN) induced convulsions in mice. For each model, 25 mice were divided into five groups; group 1 received 10 ml/kg distilled water, groups 2-4 received 100 mg/kg, 200 mg/kg and 400 mg/kg of extract p.o respectively. Group 5 received 3 mg/kg of diazepam p.o. Convulsion was induced intraperitoneally using PTZ and STN, thirty minutes after the extract was administered. The onset of myoclonic, tonic-clonic seizure, time of death within 60 minutes of PTZ and STN administration were monitored. Data were statistically analyzed using one-way ANOVA followed by Dunnett's comparison tests. In STN-induced seizure model, 100 mg/kg of *P. maximum* exhibited a significant ($p < 0.001$) anticonvulsant activity by delaying myoclonic seizure onset in mice when compared to the control. Significant ($p < 0.001$) activity was also observed in the onset of tonic clonic seizure at same dose when compared to control. In PTZ-induced seizure model, 200 mg/kg exhibited more significant ($p < 0.001$) activity followed by 100 mg/kg ($p < 0.05$) by delaying myoclonic seizure onset in mice when compared to the control. Significant ($p < 0.001$) activity were observed in tonic clonic phase at same doses respectively. The different doses administered couldn't protect

the mice from death. Diazepam, standard drug used, protected all the animals without any signs of convulsions. The results provide evidence that the extract possesses anticonvulsant activity.

Keywords: Anticonvulsant, mice, *Panicum maximum*, pentylenetetrazole, strychnine.

Running title: Evaluation of the anticonvulsant activity of ethanol leaf extract of *Panicum maximum* in mice.

Introduction

People of all ages are affected by the persistent neurological condition, epilepsy (Devinsky *et al.*, 2018). Epilepsy affects around 50 million people worldwide. Recurrent seizures caused by excessive electrical discharges in a cluster of brain cells are its defining feature (WHO, 2023). The majority of patients continue to experience seizure episodes in addition to the substantial side effects of current antiepileptic medications. According to estimates, 90% of epilepsy patients reside in developing nations, and the majority of them do not receive any medication to treat their condition (Sucher and Charles, 2015). Investigations exploring the effects of various medicinal plants that have traditionally been used to treat seizures have been inspired by this therapy gap.

Natural plant products are fast becoming the mainstay of managing different ailments. *Panicum maximum* Jacq. (Poaceae) is a perennial grass which is distributed widely in Africa and other tropical regions of the world. The Ibibios of Akwa Ibom State, Nigeria, have used the leaves in ethnomedicine to cure a variety of illnesses; including malaria, microbiological infections, rheumatic pain, inflammation, and diabetes (Antia et al., 2010). There have been reports of the leaf extract's effects in malarial and pain (Okokon et al., 2012), bacterial infections (Gothandam et al., 2010; Doss et al., 2011a; Doss et al., 2011b), inflammation (Okokon et al., 2011), and management of leishmania (Okokon et al., 2014). This research aimed to evaluate the anticonvulsant activity of *Panicum maximum* in mice using pentylenetetrazole and strychnine induced seizure models.

Materials and methods

Materials

The drugs used were chemoconvulsants, Pentylenetetrazole (PTZ) (Sigma, Germany), Strychnine (Sigma, Germany) and Diazepam (Valium) 5 mg/ml injection (F-Hoffman-La roche, Switzerland).

Experimental animals

Adult albino mice (18 – 30 g) of both sexes were used for the study. The animals were gotten from the animal house of the Department of Pharmacology and Toxicology, Madonna University, Elele, Rivers State. The animals were provided with standard laboratory pellets and water *ad libitum*.

Twenty-five animals (both sexes) were used for each seizure model. The animals were divided into 5 groups with 5 animals in each group. Group I received 10 ml/kg of distilled water p.o. Group II-IV received 100, 200 and 400 mg/kg of extract p.o respectively while group V received diazepam 3 mg/kg, i.p. Convulsion was induced via intraperitoneal

route using pentylenetetrazole and strychnine. The study was carried out under the approval of the Animal Research Ethics Committee, Madonna University, Elele, Nigeria number (MUN/FP/AE/23/022). The experimental procedures by the National Institutes of Health Guide for the Care and Use of Laboratory Animals (2011) was observed.

Methods

Collection of plant material

The leaves of *Panicum maximum* were randomly collected from the compound of Madonna University, Elele, Nigeria between February and April, 2022. The plant was identified by a Botanist, Mr. Uwakwe B. and the specimen deposited at the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Madonna University, Elele, Rivers state, Nigeria (MUFP/58).

Preparation of plant extract

The leaves were cut into smaller pieces, dried and coarsely powdered with a manual blender. Extraction of plant was done by cold maceration using ethanol (absolute) for 72 hr. The filtrate was pooled together to obtain the ethanol extract (EE) and concentrated to a dry mass by drying at 40 °C in a water bath.

Pharmacological tests

Median lethal dose

The median lethal dose (LD₅₀) of the ethanol extract of *P. maximum* was performed in rats using the method described by Lorke (1983).

Pentylenetetrazole-induced convulsion

Albino mice were randomly divided into five groups (n =5). Group I (control) received the vehicle (10 ml/kg, distilled water, p.o.). Groups II–IV received the extract (100, 200 and 400 mg/kg, p.o. respectively while group V received diazepam (Hoffman-la Roche, 3 mg/kg, p.o). Thirty minutes later, PTZ (Sigma,

60 mg/kg, i.p) was injected to all the animals. The extract and diazepam were administered through oral route and PTZ, i.p for quick absorption and activity. The animals were observed for the time of onset of myoclonic spasms, tonic-clonic phases of seizures and time of death. Animals devoid of seizure/convulsion without subsequent death during the 60 min observation period were considered protected (Nogueira and Vassillief, 2000; Okoye *et al.*, 2008).

Strychnine-induced convulsion

Albino mice were randomly divided into five groups (n = 5). Control (group I) animals were treated with the vehicle (10 ml/kg, distilled water, p.o.). Mice in groups II–IV were treated with the PM 100, 200, 400 mg/kg, p.o. respectively while group V received diazepam (Hoffman-la Roche, 3 mg/kg, p.o). These treatments were carried out thirty minutes before the administration of strychnine (Sigma-Aldrich, 2 mg/kg, i.p.) to all the groups. The extract and diazepam were administered through oral route and STN, i.p for quick absorption and activity. The animals were observed for 60 min after injection of strychnine for myoclonic spasms, tonic clonic seizures and time to death. The time for the animals to exhibit myoclonic, tonic clonic seizures and time of death were recorded. An animal that showed neither of these effects was considered protected (Ogbonnia *et al.*, 2003; Yemitan and Adeyemi, 2003).

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) followed by post-tests, to compare replicate means using Graph pad

version 5.1. p values < 0.05, 0.01 and 0.001 were considered significant, very significant and highly significant respectively.

Results

Median lethal dose (LD₅₀)

The median lethal dose of the leaf extract of *P. maximum* in rats was estimated to be 2,154 mg/kg suggesting a relatively safe extract.

Effects of *P. maximum* on strychnine-induced seizure

The result of the study showed that the ethanol extract of *Panicum maximum* showed significant anticonvulsant activity by delaying the myoclonic and tonic-clonic seizure onset and time of death in the animals induced with strychnine at the lowest and medium doses administered (100 mg/kg and 200 mg/kg). At doses of 100 mg/kg and 200 mg/kg, the extract showed significant (p<0.001; 0.01) anticonvulsant activity respectively by delaying the myoclonic seizure onset when compared to the negative control. (Figure 1). A significant (p < 0.001; 0.05) delay in the onset of tonic clonic seizure was also observed at doses of 100 mg/kg and 200 mg/kg respectively whereas no significant anticonvulsant activity was observed at 400 mg/kg when compared with the negative control (Figure 2). There was a significant delay in time of death at a dose of 100 mg/kg when compared to the negative control but none of the doses administered prevented the animals from STN-induced seizure death. The diazepam treated group did not show any sign of convulsion and protected all the animals from death within 60 mins in all the parameters investigated (Figure 3).

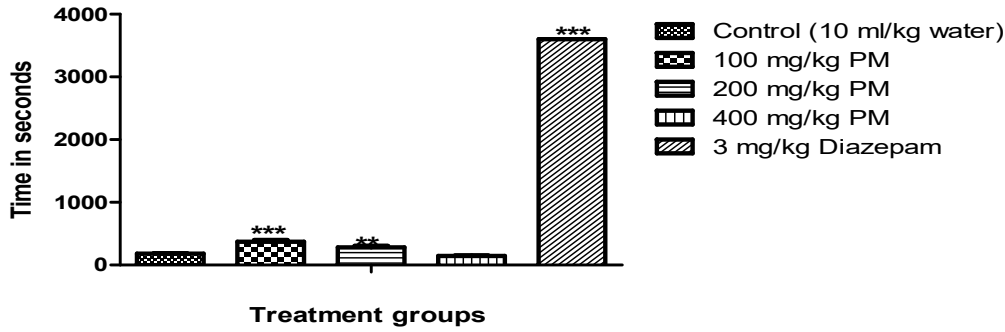


Figure 1: Effect of *Panicum maximum* on myoclonic seizure onset in strychnine- induced seizure in mice.

The values are expressed as mean ± SEM; **p < 0.01, ***p < 0.001 when compared with control group, n=5.

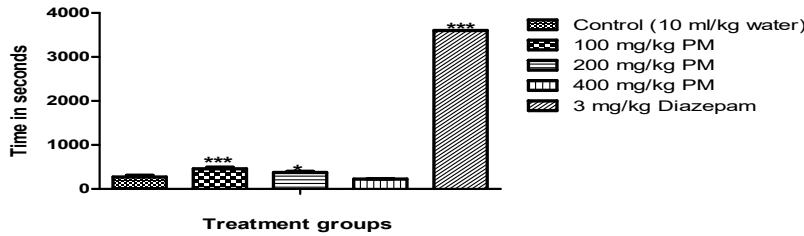


Figure 2: Effect of *Panicum maximum* on tonic- clonic seizure onset in strychnine- induced seizure in mice.

The values are expressed as mean ± SEM; *p < 0.05, ***p < 0.001 when compared with control group, n=5.

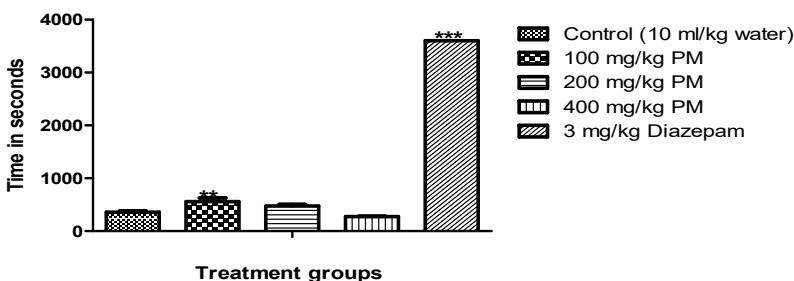


Figure 3: Effect of *Panicum maximum* on time of death in strychnine- induced seizure in mice.

The values are expressed as mean \pm SEM; ** $p < 0.01$, *** $p < 0.001$ when compared with control group, $n=5$.

Effect of *P. maximum* on pentylenetetrazole-induced seizure

In the pentylenetetrazole induced seizure model, the extract significantly ($p < 0.001$; 0.05) delayed the onset of myoclonic seizure and time to death at doses of 100 and 200 mg/kg respectively when compared to the negative control group while at a dose of 400 mg/kg, there was no significant activity when compared to the negative control (Figure 4). A high significant delay ($p < 0.001$) in tonic-clonic seizure onset was observed at doses of 100 and 200 mg/kg when compared to the negative control. (Figure 5). A high significant ($p < 0.001$) delay in time of death of animals was also observed at doses of 100 and 200 mg/kg. None of the treatment groups protected the animals from PTZ-induced seizure death. The diazepam treated group did not show any sign of convulsion and protected the animals from death within 60 mins in all the parameters investigated (Figure 6).

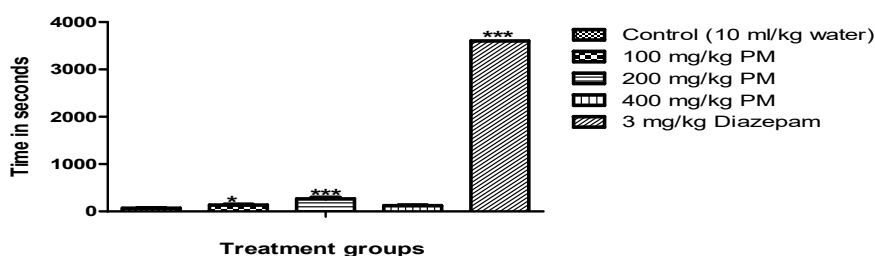


Figure 4: Effect of *Panicum maximum* on myoclonic seizure onset in pentylenetetrazole-induced seizure in mice.

The values are expressed as mean \pm SEM; * $p < 0.05$, *** $p < 0.001$ when compared with control group, $n=5$.

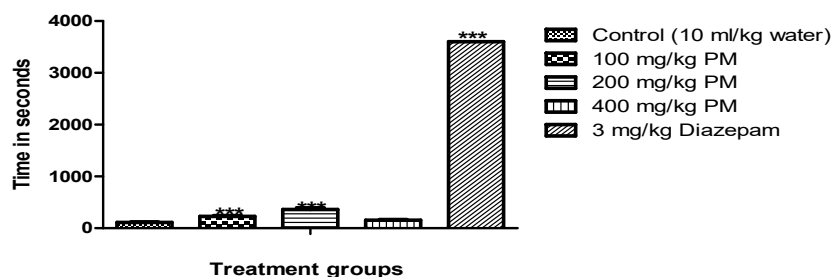


Figure 5: Effect of *Panicum maximum* on tonic clonic seizure onset in pentylenetetrazole-induced seizure in mice.

The values are expressed as mean \pm SEM; *** $p < 0.001$ when compared with control group. $n=5$

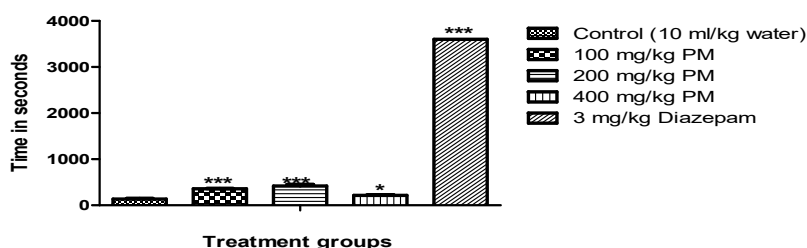


Figure 6: Effect of *Panicum maximum* on time of death in pentylenetetrazole-induced seizure in mice.

The values are expressed as mean \pm SEM; * $p < 0.05$, *** $p < 0.001$ when compared with control group, $n=5$.

Discussion

Panicum maximum extract exhibited significant anticonvulsant activity by delaying the onset of myoclonic and tonic-clonic seizure and increasing time to death in extract treated animals at the lowest and medium doses (100 and 200 mg/kg) in STN-induced seizure model. Comparable to the positive control, diazepam, the extract did not prevent strychnine (STN)-induced seizure death in mice, but it prolonged duration of seizure before death. The ability of *P. maximum* to delay the onset of seizure or shorten the duration of seizure was considered an indication of anticonvulsant activity.

Strychnine is a strong convulsant that is known to selectively block inhibitory glycine receptor inputs, primarily in the spinal cord, in order to cause excitatory responses in the central nervous system (CNS) (Nicol, 2001). According to reports, strychnine inhibits glycine's inhibitory actions at all glycine receptors (Pamar and Shiv, 2006). Agents that prevent STN-induced seizures work by boosting glycine's inhibitory impact. The *P. maximum* extract showed substantial action against this STN seizure model, suggesting that it may have anticonvulsant properties as glycine agonists or by improving the binding of glycine to its receptors. The observed anticonvulsant activities of the lower doses as

opposed to that of the highest dose (400 mg/kg) may have resulted from the partial agonist activity of the extract by acting as glycine agonist at lower doses and an antagonist at higher doses.

P. maximum leaf extract partially protected mice from PTZ-induced seizures. Medications that prevent PTZ-induced seizures are typically effective against absence seizures and, this could be used as index of their potential effectiveness against absence seizures (White, 1997; Rang *et al.*, 2007), suggesting that the extract may be beneficial in the treatment of absence seizure.

PTZ is a convulsant agent used in anticonvulsant drug screening (Nicoll, 2001; Kwan and Brodie, 2006). By blocking the gamma aminobutyric acid (GABA) pathway at GABA_A receptors, PTZ causes seizures (Amabeoku *et al.*, 2007; Salaudeen *et al.*, 2022), which is a major inhibitory neurotransmitter involved in epilepsy. Glutamatergic mechanism is also involved in its activity (Chindo *et al.*, 2014) by activation of N-methyl-D-aspartate (NMDA) receptor which appears to be involved in the initiation and generalization of the PTZ-induced seizures (Yudkoff *et al.*, 2006; Parmar *et al.*, 2022). The inhibition of NMDA receptor/glutamatergic neurotransmission and potentiation of GABAergic neurotransmission are common ways to combat PTZ-induced seizures (Son and Yen, 2014; Ofokansi *et al.*, 2021). It is believed that substances that prevent mice from having PTZ-induced seizures block T-type Ca²⁺ current. (Rahimi *et al.*, 2019) and are effective in treating human myoclonic and absence seizures (McNamara, 2006; Ofokansi *et al.*, 2021; Sarfo *et al.*, 2022). The ability of the extract to exert inhibitory effect against PTZ-induced seizures suggests that its effect may be caused by the activation of GABA and/or suppression of NMDA receptor/glutamatergic neurotransmissions. The extract treated

groups did not produce complete protection against PTZ-induced seizure compared to the positive control, diazepam. Diazepam, the standard drug used, protected the animals without any signs of convulsions in both PTZ and STN- induced convulsion groups within the 60 min observation period. Diazepam inhibits seizure frequency and severity by acting as a positive modulator of GABA_A receptors, increasing the GABA_A receptor mediated Cl⁻ conductance (Dhir *et al.*, 2006).

Reported phytochemical screening results revealed that alkaloids, flavonoids, tannins, terpenes, saponins, anthraquinones, reducing sugars, cardiac glycosides are present in *P. maximum* (Antia *et al.*, 2010). Steroids, flavonoids, and saponins have all been linked to have anticonvulsant properties (Chindo *et al.*, 2014; Prathima *et al.*, 2016). Therefore, these constituents may have contributed to the observed anticonvulsant effects.

Conclusion

The findings of this investigation indicate that *P. maximum* leaf extract has considerable anticonvulsant activity against pentylentetrazole and strychnine-induced seizures at lower doses. This could possibly be as a result of the partial agonist activity of the leaf extract. Further research is recommended to elucidate the precise anticonvulsant mechanism of action of the extract and identify the active ingredient responsible for this activity.

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Conflict of Interests

The authors declare no conflict of interest.

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