Original Research Article

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Psychopharmacological study on ethanol root extract of *Panicum maximum*

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Abstract

Panicum maximum is used in Ibibio ethnomedicine for the treatment of various diseases such as CNS disorders. The ethanol root extract of *Panicum maximum* (137 - 547 mg/kg) was investigated for antidepressant and anticonvulsant activities in mice using open field and forced swimming tests for depression models and aminophylline and pentylene tetrazol-induced convulsion for anticonvulsant activity evaluation. The extract was found to significantly ($p < 0.05$ - 0.01) increase the frequency of line crossing, rearing and walling activities of mice in open field test. The extract also significantly ($p < 0.05$ - 0.001) increased the duration of immobility of mice in forced swimming test. The root extract also protected the mice significantly ($p < 0.05$) against aminophylline and PTZ-induced convulsions. The root extract of *P. maximum* has depressant and anticonvulsant activities and this supports its use in ethnomedicine for the treatment of central nervous system disorders.

Keywords: *Panicum maximum*; Root extract; Anticonvulsant; CNS stimulant; Depressant

1. Introduction

Panicum maximum Jacq (Poacace) is a perennial, tuft grass with a short, creeping rhizome regarded as the most valuable fodder plant and extensively used to make hay. The stem of this robust grass can reach a height of up to 2 m, the leaf sheath are found at the bases of the stems and are covered in fine hairs. It is a tropical grass and widely distributed in Africa and other tropical regions of the world [1]. The Ibibios of Akwa Ibom State, Nigeria use the leaves ethno-medically in the treatment of various ailments such as malaria, microbial infections, rheumatism pain, inflammation and diabetes. Antidiabetic [2], antimalarial and analgesic [3], antibacterial [4,5,6], anti-inflammatory and antipyretic [7], antifungal [8], anticancer, antioxidative burst, and antileishmanial [9] activities of the leaf extract have been reported. Also, *Panicum maximum* root extract has been reported to possess analgesic and antimalarial properties [10] with LD_{50} value of 2738.1 mg/kg. Phytochemical components such as alkaloid, flavonoid, tannins, terpenes, saponin, and cardiac glycosides [10]. In this study, we investigated the antidepressant and anticonvulsant activities of ethanol root extract of *Panicum maximum.*

2. Materials and Methods

2.1. Plants collection

The plant material *Panicum maximum* (root) was collected in a farmland in Uyo area, Akwa Ibom State, Nigeria in August, 2018. The plant was identified and authenticated by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. Hebarium specimen was deposited at Department of Pharmacognosy and Natural Medicine Herbarium.

2.2. Extraction

The plant parts (root) were washed and shade-dried for two weeks. The dried plants' materials were reduced to powder using mortar and pestle. The powdered material was soaked in 50% ethanol. The liquid filtrate was concentrated and evaporated to dryness in vacuo at 40˚C using rotary evaporator and stored in a refrigerator at - 4˚C.

2.3. Animals

Albino Swiss mice (19-28 g) of either sex were obtained from the University of Uyo animal house. They were maintained on standard animal pellets and water *ad libitum*. The animal were cared humanely and experiments were

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conducted in accordance with the guidelines described in U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines (http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm).

2.4. Evaluation of Antidepressant Activity

2.4.1. Open Field Test

Swiss albino mice of either sex were randomly divided into groups of 5 mice each and treated as follows for 5 days before open field test; control (normal saline, 2 ml/kg *p.o.*), imipramine (5.0 mg/kg, *p.o.*) and ethanol root extract of *Panicum maximum* (137, 273 and 547 mg/kg, *p.o.*). The open field arena was made of acrylic (transparent walls and black floor, L 30 × W 30 × H 15 cm), divided into nine squares of equal areas (100 cm^2). The open field was used to evaluate the exploratory activity of the animal [11]. The observed parameters were the number of squares crossed (with the four paws) and number of grooming and rearing, recorded for 5 min testing period.

2.4.2. Forced Swimming Test

Swiss albino mice of either sex were randomly divided into groups of 5 mice each and treated as follows for 5 days before the behavioural test; control (normal saline, 2 ml/kg *p.o.*), imipramine (5.0 mg/kg, *p.o.*) and *Panicum maximum* ethanol root extract (137, 273 and 547 mg/kg *p.o.*). For assessing antidepressant activities, we employed the method described by Porsolt *et al.,* [12, 13]. The development of immobility when mice were placed inside an inescapable cylinder filled with water reflects the cessation of persistent escape-directed behavior. Briefly, mice were individually placed in a circular tank (46 cm tall × 20 cm in diameter) filled with tap water (25 ± 1°C) to a depth of 20 cm and left there for 5 min. During this period, the behavior of the animals was recorded by an observer. Mice were considered immobile when remained floating without struggling and making only slight movements necessary to maintain the head above the water.

2.5. Anticonvulsant activity

2.5.1. Pentylene tetrazol-induced convulsion

Anticonvulsant effect of the extract was assessed using a modified method of Vellucci and Webster [14] on overnight fasted mice. The mice were divided into five groups of six animals each and treated with 137, 273 and 547 mg/kg of the root extract respectively, phenobarbitone (standard drug), 40 mg/kg one hour before induction of convulsion. Seizure was induced in each set of mice with pentylene tetrazol (PTZ) (70 mg/kg i.p). Control group received normal saline. The onset of clonic/tonic convulsion and the mortality rate was recorded and compared with the respective control group. The ability of the plant extract to prevent or delay the onset of the hind limb extension exhibited by the animals was taken as an indication of anticonvulsant activity [15].

2.5.2. Aminophylline-induced Convulsion

The extract was evaluated for activity against aminophylline-induced convulsion using the method of Juliet et al., [16]. The mice were divided into 5 groups of six animals each and treated with 137, 273 and 547 mg/kg of the extract respectively and phenobarbitone, 40 mg/kg one hour before induction of convulsion. Seizure was induced using aminophylline (280 mg/kg, i.p). The animals were observed for 120 min after the administration of aminophylline and the following parameters were noted: 1. Time to onset of myoclonic jerks in min; 2. Time to onset of tonic convulsions in min; 3. Time to death during experimental time of 120 min; 4. Number of mice dead/alive at 24 h.

2.6. Data and Statistical Analysis

The results were presented as mean and SEM and comparisons among groups for statistical significant differences were done by analysis of variance (ONE-WAY ANOVA) followed by Tukey Kramer's multiple comparison tests using GraphPad Prism 5.3 application software. The *p*-values of less than 0.05 were considered as indication of significance.

3. Results

3.1. Open Field Test

Administration of root extract of *Panicum maximum* (137-547 mg/kg) for 5 days caused considerable dose-dependent increases in the frequencies of line crossing, rearing and walling activities. These increases were only significant ($p < 0.05$-0.01) at the highest dose (547 mg/kg) of the extract when compared to control. The standard drug, imipramine (5 mg/kg), caused a significant ($p < 0.001$) increase in the locomotor activity of the mice as evident in the frequency of the line crossing, walling and rearing activities (Table 1).

Table 1. Effect of ethanol root extract of *Panicum maximum* on locomotive behavior of mice during open field test

Treatment	Dose (mg/kg)	Line crossing	Walling	Rearing
Control normal saline	-	35.25 ± 3.53	10.75 ± 1.50	1.25±0.25
Imipramine	5	93.75 ± 5.72[c]	20.25 ± 1.25[a]	7.50±0.53[c]
Crude extract	137	40.26 ± 1.92	13.33 ± 1.26	2.20±1.72
	273	49.66 ± 8.98[c]	15.33 ± 3.40	3.00±1.00[a]
	547	97.33 ± 3.66[c]	20.0 ± 2.51[a]	3.00±1.15[a]

Data are expressed as mean ± SEM; Significant at [a]$p < 0.05$, [c]$p < 0.001$, when compared to control (n=5)

3.2. Effect on Forced Swimming Test

Administration of the ethanol root extract of *Panicum maximum* (137- 547 mg/kg) to mice for five days

Table 2. Effect of ethanol root extract of *Panicum maximum* on behavior of mice during forced swimming test

Treatment	Dose (mg/kg)	Latency of immobility (s)	Duration of immobility (s)
Control normal saline	-	70.0 ± 3.72	130.0 ± 5.53
Imipramine	5	77.25 ± 5.19	106.25 ± 3.33 [a]
Crude extract	137	165.0 ± 9.15 [c]	154.33 ± 5.49 [a]
	273	179.66 ± 10.32 [b]	140.0 ± 12.00
	547	130.9 ± 3.18 [a]	172.66 ± 7.51 [b]

Data are expressed as mean ± SEM; significant at [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$, when compared to control. (n=5)

significantly ($p < 0.001$) increased latency and duration of immobility in mice during forced swimming test when compared to control. These increases were non dose-dependent. The standard drug, imipramine (5 mg/kg), produced a significant ($p < 0.001$) reduction in the immobility time of the mice when compared to control (Table 2).

3.3. Aminophylline-induced convulsion

Administration of root extract of *P. maximum* (137-547 mg/kg) provided a considerable degree of protection for the mice against seizure induced by aminophylline. The extract prolonged the time for onset of myoclonic convulsion in a dose-dependent fashion and this was only significant ($p <$

Table 3. Effect of ethanol leaf extract of *Panicum maximum* on aminophylline-induced convulsion in mice

Treatment	Dose mg/kg	Onset of myoclonic convulsions (min)	Onset of tonic convulsions (min)	No. of deaths
Control normal saline	-	6.47 ± 0.83	8.26 ± 1.11	6/6
Phenobarbitone	40	23.00 ± 1.20[c]	0.00 ± 0.00[c]	6/6
Crude extract	137	8.34 ± 0.50	13.26 ± 0.58	6/6
	273	8.56 ± 1.72	17.45 ± 2.93[a]	6/6
	547	10.62 ± 2.70[c]	17.14 ± 2.27[a]	6/6

Data are expressed as mean ± SEM; significant at [a]$p < 0.001$, when compared to control, (n=6)

0.001) at the highest dose (547 mg/kg) but not comparable to that of the standard drug, phenobarbitone (Table 3). The lower doses (137 and 3273 mg/kg) could not offer any significant protection against onset of myoclonic convulsion. Similarly, the extract exerted a significant ($p <$ 0.05) prolongation of time for onset of tonic convulsion in a dose-dependent manner (Table 3). The standard drug, phenobarbitone also offered 100% protection to the animals treated with it.

3.4. PTZ–induced convulsion

Administration of root extract of *P. maximum* (137-547 mg/kg) provided a considerable degree of protection for the mice against seizure induced by pentylene tetrazol. The extract prolonged the time for onset of myoclonic convulsion in a dose-dependent fashion and this was only significant ($p < 0.001$) at the highest dose (547 mg/kg) but not comparable to that of the standard drug, phenobarbitone

Table 4. Effect of ethanol root extract of *Panicum maximum* on pentylene tetrazol-induced convulsion in mice

Treatment	Dose mg/kg	Onset of myoclonic (s)	Onset of tonic (s)	No. of deaths
Control normal saline	-	0.49 ± 0.07	1.14 ± 0.02	6/6
Phenobarbitone	40	1.26 ± 0.28[c]	0.00 ± 0.00[c]	6/6
Crude extract	137	0.66 ± 0.18	3.03 ± 0.56 [a]	6/6
	273	0.59 ± 0.21	4.36 ± 0.56 [b]	6/6
	547	0.85 ± 0.21[c]	5.74 ± 0.31[c]	6/6

Data are expressed as mean ± SEM; significant at [a]$p < 0.001$, when compared to control, (n=6).

(Table 4). The lower doses (137 and 273 mg/kg) could not offer any significant protection against onset of myoclonic convulsion. Similarly, the extract exerted a significant ($p <$ 0.05-0.001) prolongation of time for onset of tonic convulsion in a dose-dependent manner (Table 4). The standard drug, phenobarbitone also offered 100% protection to the animals treated with it.

4. Discussion

In this study, evaluation of the effect of ethanol root extract of *Panicum maximum* on experimentally-induced depression and convulsion was carried out in mice using different models; Open field and forced swimming tests for depression as well as PTZ- and aminophylline-induced convulsion. The root extract (137-547 mg/kg) was found to cause significant dose-dependent increases in the frequency of line crossing, walling and rearing activities of the pretreated mice. It also increased latency and duration of immobility time in mice significantly in forced swimming test.

Monitoring of locomotor activity of animals has been used to assess the effect of drug on the CNS. An increased movement is a measure of the level of excitability of the CNS [17], while its decrease may be resulting from depression of the CNS [18]. Central nervous system stimulants are known to increase locomotor activity, while agents with depressant activity cause reduction in movements [19]. The root extract was found to increase significantly line crossing, walling and rearing activities during open field test suggesting stimulatory effect on the CNS.

The root extract further exhibited CNS depressant effect by its potential to increase immobility time of mice during forced swimming test. Forced swimming test is one of the most commonly used animal models of depression for antidepressant screening. In the forced swimming test, the

development of immobility when mice are placed into an inescapable cylinder of water reflects the cessation of persistent escape-directed behavior [20]. Various antidepressants are able to reverse the immobility and promote the occurrence of escape-related behavior. This model of depression is widely used to screen new antidepressants [12, 13, 21]. This test is quite sensitive to major antidepressant drugs including tricyclics, serotonin-specific reuptake inhibitors, MAO inhibitors, and atypical antidepressants [12, 21, 22].

Forced swimming test represents the behavioural despair model, claimed to reproduce a condition similar to human depression [12, 21, 23]. The test is based on the observation that animals, following initial escape oriented movements, develop an immobile posture when placed in an inescapable chamber. The immobility is thought to reflect either a failure of persistence in escape-directed behaviour (i.e. behavioural despair) or the development of passive behaviour that disengages the animal from active forms of coping with stressful stimuli [20]. It is well known that clinically effective antidepressants (such as imipramine) typically increases the swimming efforts of the animal seeking a solution to the problem and, therefore, they decrease the duration of immobility in the forced swimming test [12]. The reverse was observed in this study as the immobility time was significantly increased.

The results of this study suggest that the root extract exhibited significant depressant activity with a strong psychomotor stimulation. The root extract was reported to contain chemical constituents such as alkaloids, flavonoids, tannins, terpenes, saponins, anthraquinones, reducing sugars, phenol, and cardiac glycosides [10]. These phytochemical constituents may be responsible for the observed activity of the root extract in this study.

The root extract was also observed in this study to offer significant protection to mice against PTZ and aminophylline-induced convulsions. The exact mechanisms of seizures induced by aminophylline appear to be diverse, multiple and complex, and also unclear. It has been suggested that seizures induced by aminophylline, could be the result of adenosine receptor antagonism or due to inhibition of cerebral nucleotidase activity [24, 25], which lowers the adenosine content in the brain and eventually lead to a process of disinhibition. However, a report indicates that di-phenylhydantoin, a potent inhibitor of adenosine uptake was ineffective in preventing these seizures [26]. Apart from nonspecific adenosine receptor antagonism [27], aminophylline is thought to have inhibitory influence on adenosine synthesis. At higher doses, inhibition of phosphodiesterase activity including mobilization of intracellular calcium ions from labile stores are said to be implicated in aminophylline-induced seizures [28, 29]. However, a report by Ray *et al.,* [30], has implicated oxidative stress due to the generation of free radicals and reactive oxygen species to be responsible for the seizures induced by aminophylline. It is likely that the root extract maybe acting in one of these pathways to protect the mice from aminophylline-induced convulsion.

According to De Sarro *et al.,* [31], pentylene tetrazol (PTZ) is suggested to exert its anticonvulsant effect by inhibiting the activity of gamma aminobutyric acid (GABA) at $GABA_A$ receptors. Gamma aminobutyric acid is the major inhibitory neurotransmitter which is implicated in epilepsy. The enhancement and inhibition of the neurotransmission of GABA will attenuate and enhance convulsion respectively [32, 33]. Phenobarbitone and diazepam, standard antiepileptic drugs, have been shown to exert their antiepileptic effects by enhancing GABA-mediated inhibition in the brain [34, 35]. These drugs are reported to antagonise PTZ-induced convulsion [36] by enhancing GABA neurotransmission. Phenytoin was unable to prevent PTZ-induced seizure because it exerts its antiepileptic effect by blocking sodium ions into brain cells thus inhibiting generation of repetitive action potential [34]. Since the root extract of *P. maximum* was able to delay PTZ-induced convulsion, this also confirms its CNS depressant effect.

The results of this study show that ethanol root extract of *Panicum maximum* possess depressant and anticonvulsant activities which justifies its use in ethnomedicine for the treatment of central nervous system disorders.

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