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ANTICONVULSANT EFFECTS OF THE ETHANOL ROOT EXTRACT OF *Setaria megaphylla* (STEUD) T. DUR AND SCHINZ IN MICE.

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ABSTRACT

Background: *Setaria megaphylla* (Steud) T. Dur and Schinz (Poaceae), is a medicinal plant used in South-South Nigeria to treat malaria, hemorrhoids, urethritis, inflammation, diabetes, fevers and various pains.[1] While some publications have been found on *Setaria megaphylla* leaves, not much work has been done on its roots. **Objectives:** This work was therefore designed to investigate anticonvulsant effects of *Setaria megaphylla* roots to authenticate ethnomedicinal claims of its medicinal usefulness. **Methodology:** The root extract (150, 300, 450 mg/kg) was investigated for anticonvulsant effect against aminophylline and pentylenetetrazole-induced convulsion in mice using standard procedures. **Results**: Administration of root extract (150 – 450 mg/kg) of *S. megapylla* caused a dose dependent, significant ($p < 0.05 – 0.001$) delay in the onset of clonic and tonic convulsions induced by aminophylline and pentylenetetrazole (PTZ). The effect of pentylenetetrazole at the highest dose (450 mg/kg) was higher than that of the standard drug diazepam 2 mg/kg. The extract (150 – 450 mg/kg) also caused significant ($p < 0.001$) prolongation of time of death when compared to the standard drug diazepam 2 mg/kg. **Conclusions:** The results of this study revealed that the ethanol root extract of *S. megaphylla* possesses anticonvulsant effect through its phytochemical components. This therefore justifies its ethnomedicinal usage in the treatment of convulsions and epilepsy.

KEYWORDS: Analgesic, Anticonvulsant, *Setaria megaphylla*, medicinal plant.

1.0 INTRODUCTION

Setaria megaphylla is a perennial broad-leafed bristle grass, with robust roots usually about 30 cm diameter at the base. The leaves are large, soft to touch and bluish grey green in colour, usually about 1 m long and 10 cm broad. It is glabrous but scarbrid on the margins and has compressed and more or less keeled leaf sheaths (Bromilow, 1995).[2] It is found along rivers and in areas where there is plenty of moisture, like tropical and subtropical areas of Africa, America and India (Van Oudtshoorn, 1999).[3]

Leaf-decoction is put into a bath or given by mouth in Ivory Coast to babies suffering convulsions or fits of epilepsy, used to treat blennorhoea, given to a pregnant woman to ease delivery and is abortifacient (Burkill, 1985).[4] Pressed juice of the leaves of *Setaria megaphylla* is used for anuria and the ashes of the leaves with kaolin face makeup is used for psychosomatic disorders (Mbuta, *et al*, 2012).[5] It is also used for psychosis, debility, psychological problems, neurasthenia and insanity (Mbuta, *et al*, 2012).[5]

The plant possesses antiplasmodial activity (Clarkson *et al*, 2004)[6], anti-inflammatory[7] and anti-nociceptive effects (Okokon, Antia and Ita, 2006)[8], significant anticancer and moderate antileishmanial activity (Okokon, Dar and Choudhary, 2012).[9]

2.0 MATERIALS

2.1 Collection and Identification of Plant Sample

Setaria megaphylla roots collected from Anwa forest in Uruan, Uruan Local Government Area of Akwa Ibom State, Nigeria were Identified and authenticated in the Department of Botany and Ecological Studies, University of Uyo. A voucher specimen was deposited in the Faculty of Pharmacy Herbarium, University of Uyo, Uyo.

2.2 Animal Stock

Adult Swiss albino mice obtained from the Animal House of the University of Uyo, Uyo, Akwa Ibom State were maintained in the University of Uyo Animal House and fed with growers pellet feed with water given *ad libitum.*

3.0 METHODS

3.1 Extraction

Setaria megaphylla roots were washed and air-dried to get a constant weight, cut into small pieces and pulverized to powder. The powder (1.5 kg) was macerated in 70% ethanol for 72

hours and the liquid filtrate concentrated and evaporated to dryness in a vacuo at 40 °C using rotary evaporator. The dried extract was weighed and stored in a refrigerator at -4 °C.

3.2 Acute Toxicological Study

Acute toxicity study was carried out to determine the median lethal dose (LD_{50}) of the root extract using the method of Miller and Tainter (1944.[10] as reported by Udobang, Okokon and Etuk (2016).[7] The mice were treated with various doses (1000 - 5000 mg/kg) of the ethanol extract and observed for 24 hours. Physical signs of toxicity such as writhing, decreased motor activity, decreased body/limb tone, decreased respiration and deaths were recorded.

3.3 Qualitative Phytochemical Screening

The ethanol root extract of *Setaria megaphylla* was subjected to phytochemical screening to reveal the presence of chemical constituents in the plant such as saponins, alkaloids, tannins, flavonoids, terpenes and cardiac glycosides using the methods described by Odebiyi and Sofowora (1978).[11] and Trease and Evans (2002).[12]

3.4 EVALUATION OF ANTICONVULSANT ACTIVITY

3.41 PTZ-induced Convulsion: Anticonvulsant effect of the extract was assessed using a modified method of Vellucci and Webster (1984)[14] on mice fasted overnight. The mice were divided into five groups of six animals each. Control group (Group 1) received normal saline. Groups 2, 3 and 4 received *S. megaphylla* 150, 300 and 450 mg/kg p.o. respectively and group 5 received diazepam 2 mg/kg one hour before induction of convulsion. Seizure was induced in each set of mice with PTZ (70 mg/kg i.p.). The onset of clonic/tonic convulsion and the mortality rates were 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 (Vellucci and Webster, 1984[14] Amabeoku and Chikuni, 1993).[15]

3.42 Aminophylline-induced Convulsion: *Setaria megaphylla* root extract was evaluated for activity against aminophylline – induced convulsion using the method of Juliet, Subramanyan and Suresh (2003).[13] The adult Swiss albino mice were divided into five groups of six animals each. Group 1 received normal saline. Groups 2, 3 and 4 received *S. megaphylla* 150, 300 and 450 mg/kg p.o. respectively and group 5 received diazepam 2 mg/kg one hour before induction of convulsion. The seizure was induced using aminophylline (280 mg/kg,

i.p). The animals were observed for 120 minutes after the administration of aminophyline and the following parameters were noted.

1. Time to onset of myoclonic jerks in minutes.
2. Time to onset of tonic convulsions in minutes.
3. Time to death during experimental time of 120 minutes.
4. Number of mice dead/alive at 24 hours.

4.0 RESULTS

Pentylenetetrazole (PTZ)-induced convulsion in mice: Administration of root extract (150 – 450 mg/kg) of *S. megapylla* caused a dose dependent delay in the onset of clonic and tonic convulsions when compared to control (Table: 1). This effect was significant ($p < 0.05$ – 0.001) and at the highest dose (450 mg/kg) was higher than that of the standard drug diazepam 2 mg/kg. The extract (150 – 450 mg/kg) also caused a significant ($p < 0.001$) prolongation of time of death when compared to control. The highest dose (450 mg/kg) of the extract produced effects that were higher than that of the standard drug diazepam (2 mg/kg) (Table:1).

Aminophyline-induced Convulsion in Mice: The result in Table:2 shows the effect of *Setaria megaphylla* root extract on aminophyline-induced convulsion in mice. The extract (150 – 450 mg/kg) raised the threshold of seizure and exerted a statistically significant ($p < 0.001$) dose-dependent delay on the onset of clonic and tonic convulsions when compared to control. There was also a significant ($p < 0.001$) prolongation of time of death when compared to control and this was short compared to that of the standard drug diazepam (2 mg/kg).

Table 1: Effect of extract on PTZ-induced convulsion in mice.

Treatment mg/kg	Latency of clonic convulsion	Latency of tonic convulsion	Time of death (mins)	Mortality (%)
Control	33.83 ± 3.0	94.83 ±4.44	186.83 ± 9.81	100
Extract 150	35.16 ± 1.93	101.33 ± 8.77	324.16 ± 12.87^{c}	100
Extract 300	42.33 ± 1.87	131 ± 6.53^{a}	343.66 ± 6.09^{c}	100
Extract 450	58.83 ± 3.0^{c}	193.83 ± 4.44^{c}	476 ± 6.62^{c}	100
Diazepam 2	44.16 ± 1.74^{a}	159.66 ± 6.13^{c}	323 ± 13.57^{c}	100

Data were expressed as mean ± SEM. significant at $^{a}p < 0.05$; $^{c}p < 0.001$ when compared to control. n = 6.

Table 2: Effect of extract on aminophylline-induced convulsion in mice.

Treatment mg/kg	Latency of clonic convulsion	Latency of tonic convulsion	Time of death (mins)	Mortality %
Control	1.00 ± 0.00	3.66 ± 0.21	5.66 ± 0.51	100
Extract 150	1.5 ± 0.22	6.66 ± 0.55	9.16 ± 0.40	100
Extract 300	1.00 ± 0.00	10.00 ± 1.12^{c}	26.00 ± 1.09^{c}	100
Extract 450	1.00 ± 0.00	11.16 ± 0.74^{c}	33.00 ± 0.96^{c}	100
Diazepam 2	3.50 ± 0.56^{c}	49.00 ± 1.34^{c}	109.66± 6.49^{c}	100

Data are expressed as mean ± SEM. significant at $^{c}p < 0.001$ when compared to control n = 6.

5.0 DISCUSSION

In the two anticonvulsant models (aminophylline and pentylenetetrazole) evaluated, the extract raised the threshold of seizures and exerted a statistically significant dose-dependent delay on the onset of clonic and tonic convulsions and caused a significant prolongation of time of death. The effect of the extract (450 mg/kg) on the PTZ- induced convulsion was higher than that of the standard drug, diazepam (2 mg/kg) and the prolongation of time of death by the extract (150 – 450 mg/kg) was quite comparable to that of the standard drug, diazepam (2 mg/kg). The aminophylline test represents an adenosine-receptor antagonist model and the PTZ test a valid model for human generalized myoclonic seizures (Ahmadiani, Mandgary and Sayyah, 2003).[16]

Mechanisms of aminophylline-induced convulsion have not yet been sufficiently clarified. It is thought that blockade of adenosine receptors, inhibition of phosphodiesterase, mobilization of intracellular calcium ions, or finally, enhanced excitatory amino acids release may be of importance (Wlaź, Roliński, Kleinrok and Czuczwar, 1994).[17] Studies have also implicated oxidative stress due to the generation of free radicals and reactive oxygen species to be responsible for the aminophylline-induced seizures (Ray, Gulati, Anand and Vijayan, 2005).[18]

Some studies have indicated that PTZ diminishes the GABAergic tone (Macdonald and Barker, 1997)[19] probably by a competitive antagonist action on the benzodiazepine receptors (Rehavi, Skolnick and Paul 1982).[20] Correspondingly, drugs that reduce T-type Ca^{2+} currents, such as ethosuximide (Coulter, Hugenard and Prince, 1989)[21] and drugs that enhance $GABA_A$-receptor neurotransmission, such as benzodiazepines (Macdonald and

Kelly, 1995[22]; White, Smith and Wilcox, 2007)[23] can block seizures induced by PTZ. Phenobarbitone and diazepam which are standard antiepileptic drugs have been reported to exert their antiepileptic effects by enhancing GABA-mediated inhibition in the brain (Porter and Meldrum, 2001[24]; Rang, Dale, Ritter and Moore, 2003).[25] Therefore the extract may be acting from one or more of the above mechanisms to exert anticonvulsant effect.

Terpinen-4-ol and other monoterpenes such as α-terpineol, citronellol and linalool present in this extract[7] have shown anticonvulsant activity in different convulsion animal models (De Sousa, Nóbrega, de Morais, de Almeida, 2009).[26] Other monoterpenes present in this extract such as borneol, astaxanthin, cervacrol and methone with hexadecanoic acid[7] have also been shown to possess antioxidant properties.[7] It is therefore possible that these identified phytoconstituents may in part be responsible for the anticonvulsant activities of this extract.

6.0 CONCLUSION

The results of this research work reveals that *Setaria megaphylla* ethanol root extract through its phytochemical constituents possess significant anti-convulsant activity and also validates its ethnomedicinal use in the treatment of convulsion and epilepsy. Further investigation to identify, elucidate and isolate the active components with their possible mechanisms of actions in order to standardize them is recommended.

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