



Anticonvulsant Potential of Ethanolic Extract of *Aspilia africana* Leaf in Mice

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Authors' contributions

This work was collaboration between the two authors. Authors OJK and HDK designed the study and performed the statistical analysis. Author OJK managed the literature searches and wrote the first draft of the manuscript. While author HDK proof-read the manuscript to make corrections. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JALSI/2018/38808

Editor(s):

(1) Purnachandra Nagaraju Ganji, Department of Hematology and Medical Oncology, Emory University School of Medicine, USA.

Reviewers:

(1) Suprakash Chauhdury, Dr. D. Y. Patil Vidyapeeth, India.

(2) Syed Mohd Abbas Zaidi, HSZH Govt. Unani Medical College, India.

Complete Peer review History: <http://www.sciedomain.org/review-history/23137>

Original Research Article

Received 14th October 2017
Accepted 4th January 2018
Published 12th February 2018

ABSTRACT

Aim: This study was carried out to determine the anticonvulsant potential of *A. africana* leaf extract in its polar (ethanol) constituents.

Study Design: The study was adapted from three methods of seizure stimulation of the *in vivo* animal model using the maximal electroshock (MES), pentylenetetrazole (PTZ), strychnine (STC) methods and in addition, motor coordination.

Place and Duration of Study: The study was carried out in the laboratory of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria between August to October, 2017.

Methodology: The powdered leaf of *A. africana* (2000 g) was extracted cold in 10 litres of ethanol (50%). The extraction was made using rotary evaporator at a maximum temperature 45°C. The total dried extract obtained from the 2000 g powdered leaves of *A. africana* was 216.4 g given 10.8% w/w yield. The dose was established based on preliminary data that proof ratio 1/33 of the

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LD50 (6.6 g/kg) safe in mice using intraperitoneal route; hence dose 200, 100, 50 and 25 mg/kg applied according to the study design.

Results: Computer software Graph pad PRISM[®] version 5.00 was used for data analysis. The marked delayed onset of seizure indicated an anti-seizure potential of ethanolic extract of *A. africana* leaf with the maximal delayed onset of seizure and abolished hind limb tonic extension largely recorded in the least and medium doses, 25, 100 mg/kg respectively significant, $P = .05$ compared with the normal control in the applied methods. The study also indicated reduced motor planning potential of *A. africana* leaf extract significantly, $P = .05$ compared with normal control.

Conclusion: This study suggests antiseizure potential of *A. africana* leaf of ethanolic extract.

Keywords: *A. africana*; Antiseizure; Pentylenetetrazole (PTZ); Strychnine (STC); Maximal electroshock (MES).

1. INTRODUCTION

Epileptic seizure has been described as chronic neurological condition with history across all ages, race and gender with prevalence variance across countries. It is characterized by uncontrolled muscle contraction and relaxation of the affected body part resulting in bladder and bowel incontinent, which is also commonly known as convulsion [1]. Convulsion often a symptom of an epileptic seizure. However, not all epileptic seizures lead to convulsions, and not all convulsions are caused by epileptic seizures [2,3].

It has been described by authorities to be the third most common chronic neurological disorders after stroke and Alzheimer's disease [4,5]. According to the WHO, 2017 fact sheet report about 50 million people suffer epilepsy worldwide especially 75-80% of the population live in developing countries owning little or no access to medical services or treatment [6,7]. The prevalence of epilepsy in Nigeria is rated about 6.2 to 20.8 per 1000 [8,9]. However, it has been reported not less than 30% of epileptic seizure associated individual do not have seizures control even with the best available medications [10,5,11,6]. Furthermore, undesirable side effects such as psychosis, agitations, aggression, allergies, sedation, blood dyscrasias, teratogenesis, changes in mood, memory problem and intolerance of anticonvulsant drugs used clinically often render treatment difficult. Thus, search for new, safer and affordable antiepileptic drugs is geared towards naturally-occurring compounds, which is thought to belong to new structural, chemical type or other mechanism of action, especially from herbal sources.

It is acknowledged by several authorities that natural products remained the keystone in drug discovery and maintain important role in

antiepileptic seizure even in the future [12]. Various medicinal plants are known for their anticonvulsant value and their extracts are important source of chemicals for the development of better and safer drugs for the treatment of epilepsy as earlier stated. Similarly, the use of herbal medicine in the management of epilepsy is widely accepted among rural dwellers in most Nigerian communities and their efficacies are well acclaimed. Some of these medicinal plants remain unexplored for their value as sources of antiepileptic drugs and therefore, research is encouraged to validate the folkloric claims of these medicinal plants so as to provide scientific evidence of their safety and efficacy [6].

The plant *Aspilia africana* (wild sunflower), also called iodine or haemorrhage plant because of its characteristics. It is an *Asteraceae* species, evaluated for its medicinal potential. But no ethnomedicinal information is available on its antiseizure/anticonvulsant activities as at the time of this study.

2. STUDY LIMITATION

This study was limited by the fact that there is no record of behavioral study including antiseizure evaluation of any *A. africana* extract.

3. MATERIALS AND METHODS

3.1 Plant Material and Preparation of *Aspilia africana* Extraction

Fresh leaves of *Aspilia africana* were sourced from Wilberforce Island rainforest around the Niger Delta University, Bayelsa State, Nigeria. On the 17th of February, 2017, and were authenticated by DR. Gideon Alade of the Department of Pharmacognosy and Herbal medicine, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State,

Nigeria. The voucher specimen of the plant leaves was prepared and deposited at the Herbarium Unit of the Department of Pharmacognosy and Herbal medicine, Faculty of Pharmacy, Niger Delta University, Wilberforce Island Bayelsa State. Fresh leaves of *A. africana* were collected, air-dried for 14 days and milled into powder with the aid of industrial grinder. The powdered leaf of *A. africana* (2000 g) was extracted cold in 10 litres of alcoholic water (50%) with daily shaking for 96 hours. The mixture was filtered and the filtrate concentrated using a rotary evaporator at a maximum temperature of 45°C to obtain ethanolic extract of *A. africana* leaf. Further drying of the extract was carried out using the freeze-dryer to obtain powder extract. The total dried extract obtained from the 2000 g leaves of *A. africana* was 216.4 g given 10.8% w/w yield. The semi-solid paste of the extract of the *A. africana* leaf was then stored in the refrigerator at 4°C till needed for use. Based on preliminary data that proof ratio 1/33 of the LD50 (6.6 g/kg) [13,14] safe in mice using intraperitoneal route (i.p), hence 200, 100, 50 and 25 mg/kg applied in the study.

3.2 Experimental Animals

Male mice weighed between 23 g and 30 g were secured and acclimatized for two weeks at the Animal Breeding and Research Unit, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Wilberforce Island Bayelsa State, Nigeria. The animals were kept under conducive laboratory conditions and fed with standard animal feed (Grower's pelletized), and water ad libitum. The principle of laboratory animal care as prescribed by the National Institute of Health-NIH (publication No. 85-23) guidelines and procedures were followed in this study [15,16]. The research study was subjected to the Postgraduate Research Ethical Committee, University of Port Harcourt, Nigeria, for approval.

3.3 Administration of Extracts

The ethanolic extract was administered to the male mice according to their respective group through the i.p route. The volume of extract administered intraperitoneally was 10 mL/kg in all cases. The i.p route is considered to be faster, more consistent with its outcome and readily reproducible. This route is preferred in the central nervous system related studies because of the possibility of interference of metabolic processes

with the test agent given through the oral route [17].

3.4 Anti-seizure Study Design

The study design was adapted from three methods of seizure induction of the *in vivo* animal model as described by Kasthuri et al. [18].

3.5 Maximum Electroshock Induced Convulsion in Mice

The method of Swinyard and Kufferberg [19,20] was employed. Twenty four male mice were used and randomly allotted into six group of four mice each. The first group received i.p 10 mL per kg body weight of normal saline the second group received i.p 60 mg/kg phenobarbitone (analytical grade) while the third, fourth, fifth and sixth group received 25, 50, 100 and 200 mg of the ethanolic extract per kg body weight i.p. Thirty minutes later, maximum electroshock was administered to induce seizure in the mice using Ugo Basile electroconvulsive machine (Model 18182 v 220) with an electrode clipped to each ear of the mice. The current, shock duration, frequency and pulse width set and maintained at 60 mA, 2.0 s, 100 pulses per second and 2.0 ms respectively. Abolition of hind limb tonic extension (HLTE) was considered as protection from the electroshock [21]. The standard drug, phenobarbitone was preferred based on existing record of its suppressive effect against generalized tonic-clonic seizure as characterized in MES, [18]. Chloride ion influx and direct blockade of excitatory glutamate signaling is the core mechanism of action for hypnotic/anticonvulsant effect as widely recognized.

3.6 Strychnine-induced Convulsion in Mice

The method of Porter et al. [22] was employed. As stated in the MES method earlier, the first group received i.p 10 mL per kg body weight normal saline and the second, third, fourth, and fifth group received i.p 25, 50, 100 and 200 mg per kg body weight of ethanolic extract, and the sixth group received i.p 1.0 mg/kg of diazepam (Swipha Nig. Ltd.). Thirty minutes later, mice in all group received i.p 2.0 mg/kg of strychnine (Sigma chemicals Co. St Luis U.S.A). Abolition of tonic extensor jerks of the hind limbs was considered an indicator that the testing materials could prevent strychnine-induced convulsion.

Diazepam was used in this method based on its record of acceptable application especially in some chemical methods of animal seizure coupled to the fact that seizures in this method are not fully relieved by acceptable doses of any classical anticonvulsants including benzodiazepines [18].

3.7 Pentylenetetrazole (PTZ) Induced Convulsion in Mice

The method of Swinyard et al. [23] was employed. As treated in the STC method, thirty minutes later, mice in all group received i.p 80 mg/kg pentylenetetrazole (Sigma chemicals Co. St Luis U.S.A). An absence of an episode of clonic spasm of at least 5 seconds duration indicated a compound's ability to abolish the effect of pentylenetetrazole on seizure threshold. Diazepam was used following its well known mechanism, as an increasing factor of the neurotransmitter gamma-aminobutyric acid, GABA which is the target site of PTZ seizure stimulation.

3.8 Motor Coordination Evaluation

Test for motor incoordination potential of *A.africana* leaf on twenty four (24) male mice was adopted from Swinyard and Kufferberg [19] using Ugo Basile, Italy Rota Rod (for mice 47600 V04). The rota rod is set at 6 revolutions per minute (rpm) for 120 s with baseline record established by placing the mice on the rota rod for at least 120 s constant grip were selected, weighed and divided into the normal control group, phenobarbitone group and test group of 25, 50, 100 and 200 mg/kg respectively with four mice per group. Thirty minutes after the control and test substances have been administered. The falling time from the rotating rod was recorded for each mouse per group. Phenobarbitone is a choice of a standard drug of motor coordination in this study because of its hypnotic/anti-anxiety profile coupled to its suggested mechanism of action as chloride ion influx and direct blockade of excitatory glutamate signaling.

3.9 Method of Data Analysis

The laboratory data were expressed as mean, standard error of the mean ($M \pm SEM$). Statistical difference for parametric data was determined by one-way analysis of variance (ANOVA) followed by a post hoc test (Student Newman-Keuls Test,

SNK). Difference considered statistically significant with $P = .05$ for all comparisons. Computer software Graph pad PRISM® version 5.00 was used for the analysis.

4. RESULTS AND DISCUSSION

4.1 Results

The outcome of the study proved abolition of HLTE in the MES method and delayed onset of seizure in the PTZ and STC methods of seizure stimulation as well as reduced motor coordination with ethanolic extract of *A. africana* leaf pretreated group as shown in the tables and figures below.

Table 1. Anti-seizure evaluation of ethanolic extract of *A. africana* leaf in MES

Treatment	Dose	Incidence of HLTE seizure (%)
NSc	10 mL/kg	100
PHB	60 mg/kg	0*
EEAA1	25 mg/kg	75
EEAA2	50 mg/kg	50
EEAA3	100 mg/kg	25*
EEAA4	200 mg/kg	75

*N=4, * denotes significant $P = .05$ compared to control value, NSc= normal saline control, PHB=Phenobarbitone, EEAA= ethanol extract of *A. africana* leaf*

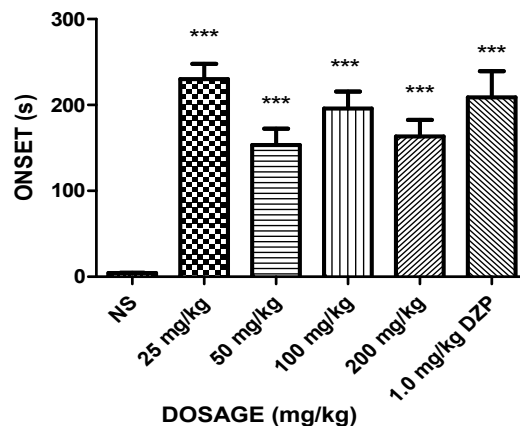


Fig. 1. Ethanolic extract of *A.africana* leaf of STC graph showing delayed onset in animal seizure

*Data analyzed using Newman-Keuls Multiple Comparison Test showed, *** denotes statistically significant ($P = .01$). NS = Normal control, DZP = Diazepam*

Table 2. Effect of ethanolic extract of *A. africana* leaf on motor coordination

Treatment	Dose	Rota rod performance (s)	% inhibition of motor coordination
NSc	10 mL/kg	120.0 ± 0.0	0
PHB	60 mg/kg	85.0 ± 24.0	29.2
EEAA1	25 mg/kg	68.0 ± 30.1	43.3
EEAA2	50 mg/kg	22.3 ± 3.47	81.5*
EEAA3	100 mg/kg	87.5 ± 25.9	27.1
EEAA4	200 mg/kg	52.8 ± 17.0	56.0

Values are presented as the Mean ± SEM ($n = 4$). NSc= normal saline control, PHB = phenobarbitone, EEAA = ethanol extract of *Aspilia africana*; * denotes significant $P = .05$ compared with the normal saline control group (one-way ANOVA followed by Newman-Keuls Multiple Comparison Test)

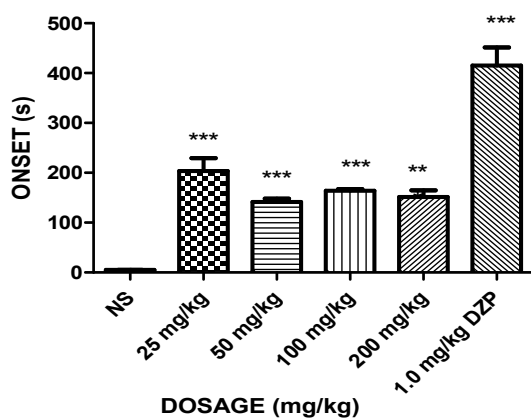


Fig. 2. Ethanolic extract of *A. africana* leaf in PTZ method with graph showing delayed onset animal seizure

Data analyzed using Newman-Keuls Multiple Comparison Test showed,*** denotes statistically significant ($P = .01$). NS = Normal control, DZP = Diazepam

4.2 Discussion

The outcome of the maximal electroshock method conform to the chemical methods (Pentylentetrazole and Strychnine) used in this study. The data in Table 1 above prove ethanolic extract of *A. africana* leaf to possess antiseizure potential, reducing seizure frequency in both cerebral hemispheres of the brain usually characterized as generalized seizure. From Table 1, all the test group's doses have indications of reduced incidence of seizure with the highest percentage reduction found in 50 mg/kg and 100 mg/kg with a protection rate of 50% and 75% respectively. This study outcome contrast an evaluation of anticonvulsant activity studied by Kar et al. [24] and similar to Gollapalle et al. [25] because of its dose independent response mimicking GABAergic transmission following it riches in

phytoconstituents such as flavonoids among others [13] well known and reported for its antiseizure /anticonvulsant role.

The PTZ method indicated highest delayed onset period of over 200s in 25 mg/kg was statistically significant ($P = .05$) compared to the normal control as well as the delayed onset period of the standard group as seen in figure 1 above. It is worthy of note that the least dose of *A. africana* leaf extract used in this study scored anti-seizure potential response in a dose-independent manner contrary to Gollapalle et al. [25].

The strychnine method also indicated delayed onset period of at least 200 s with statistically significant ($P = .05$) when compared with normal control, see Fig. 2. The dose independence response appears contrary to antiseizure evaluation data using strychnine [24].

The crude drug, *A. africana* leaf extracts tested positive to reduced motor coordination using rota rod means of evaluation complimenting the various models of seizure evaluation. The mean value of the pretreated group with ethanolic extract of *A. africana* leaf showed calmness among all the doses with 50 mg/kg extract statistically significant ($P = .05$) compared to the normal control. Further expression was made by estimating the percentage of motor coordination inhibition which reflects similar to the mean score. This is a clear pointer or indication of anxiolytic, sedative and antiseizure potential with the earlier not scientifically measured in this study but apparent. It is also interesting to note that the crude drug reduced muscular tone was more pronounced in the test substances (ethanolic extract of *A. africana* leaf) than the standard drug, phenobarbitone see table 2 above. The mean value of the motor evaluation with the rota rod also showed dose independence. This can be attributed to the fact that the test substance is not pure nor isolate

corresponding with antiseizure models used in this study and several other plant extract studies especially similar to that of the Nazifi et al. [6] including other studies with similar pattern of outcome compared to an evaluation of central nervous system effect study by Lidianne et al. [26] as well as *in vivo* sedative and muscle relaxants activity study by Rauf et al. [27].

5. CONCLUSIONS

The study has provided and establishes scientific evidence of the presence of antiseizure potential in ethanolic extract of *A. africana* leaf as well reduced motor coordination pointing to muscular relaxant potential. The study has also proven polar constituents of *A. africana* leaf to be sensitive to seizure control at the least dose 25 mg/kg in the electrical and chemical methods as well as reduced motor coordination.

6. IMPLICATION OF STUDY

The outcome of the study has shown antiseizure potential in ethanolic extract of *A. africana* leaf in lower concentrations as well as reduced motor coordination. Further study is needed to investigate the molecular mechanism(s) by which ethanolic extract of *A. africana* leaf act to control seizure in mice.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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