Trop J Nat Prod Res, February 2018; 2(2):99-102 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Anti-seizure activity of Extract of *Jatropha gossypiifolia* Linn (Euphorbiaceae)

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ARTICLE INFO

Article history:
Received 13 January 2018
Revised 27 January 2018
Accepted 02 February 2018
Published online 07 February 2018

ABSTRACT

The leaves of *Jatropha gossypiifolia* has been widely used for the treatment of epilepsy and childhood convulsions in ethnomedicinal practice of Northern Nigeria. In this study, we investigated the anti-seizure claim of this plant part using established scientific protocols. Phytochemical screening and acute toxicity evaluation of the methanol leaf extract were performed while maximal electroshock-induced seizures in cockerels (MEST), pentylenetetrazole (PTZ), strychnine (STN) and 4-aminopyridine (4-AP) induced seizure models were employed in mice. The methanol leaf extract contains cardiac glycosides, steroids, triterpenes, tannins and flavonoids, while the median lethal dose of the extract was estimated to be 1131 mg/kg. In the anti-seizure studies, the extract at all the tested doses (75, 150 and 300 mg/kg) did not delay the recovery time of convulsed cockerels in MEST model nor delay the mean onset of seizures in PTZ and 4-AP models in mice in comparison with controls. Conversely, the extract at 150 mg/kg dose significantly ($p \leq 0.05$) delayed the mean onset of seizures in mice in STN model compared to control treatments. In conclusion, this study provided the possible pharmacological basis for the use of *Jatropha gossypiifolia* leaf in treating seizure conditions.

Keywords: Seizure, *Jatropha gossypiifolia*, Strychnine, 4-aminopyridine.

Introduction

Epilepsy is a syndrome comprising of varied symptoms, it is a neurological disorder that involves disturbances of electrical transmission in the brain with excessive depolarization of the neuronal cells of the brain, manifesting as motor, sensory or psychological malfunction with or without loss of consciousness.[1] The prevalence of epilepsy in the world is increasing and thus posing a huge challenge in mental health management. It has been estimated that about 2.4 million people are diagnosed each year with epilepsy.[2] About 50 million people are reported to be epileptic with about 12 million living in Sub-Saharan Africa.[2]

Plant parts have been used extensively for the treatment of neurological disorders in West Africa, however pharmacological validation of such use is poorly documented.[3] Moreover, epileptic seizures are accompanied by oxidative activities which contribute to the neurodegeneration sequelae seen in long-term epileptics within the brain cells and most plants possess inherent antioxidant actions. We hypothesize that plants that have been used in folkloric medicine to treat seizure-like disorders could be good candidates in treating epilepsy with added benefits of antioxidant actions than conventional anti-seizure drugs.

Jatropha gossypiifolia belongs to the family Euphorbiaceae commonly known as bellyache bush, "Binidazugu" in Hausa, "Lapalapa" in Yoruba and "Wluluidi" in Igbo. It is a small shrub with dark green purplish leaves; useful in seizure disorders among others.[4] Different parts of the plant have been reported to have anti-inflammatory, antidiarrheal, analgesic, antipyretic, antimicrobial, antidiabetic and antihaemorrhagic activities among others.[5]

Animal models have been in existence for almost a century for the discovery and profiling of potential anti-seizure lead compounds.[6] These models provide a correlation between experimental seizure and the processes of seizure initiation in humans.[7] The popularity and clinical utility of these models in Antiepileptic Drug Development have been unparalleled.

Materials and Methods

Source of Plant Materials

The leaves of *Jatropha gossypiifolia* were collected from the wild in Giwa town, Kaduna state in October 2015. It was identified and authenticated by Malam Muhammad Namadi of the Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria. A specimen with voucher number 2767 was deposited in the herbarium.

Experimental Animals

Swiss Albino mice of either sex were obtained from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, while day old cockerels were procured from National Animal Production Research Institute (NAPRI), Shika. The animals were housed in appropriate cages and were acclimatized to the laboratory environment before the commencement of the study. They were given free access to standard feeds and water *ad libitum*. Principles of laboratory animal care, NIH publication No.85-23 revised in 1985, were followed and experimental procedures were approved by the institutional ethical committee.

Chemicals and Drugs

Methanol (Sigma-Aldrich, St. Louis U.S.A), 4-Aminopyridine (Merck-Schuchadt), Pentylenetetrazole (Sigma Chemical Co.Ltd, USA), Strychnine (Sigma Chemical Co. St Louis USA), Phenobarbitone (Bayer, UK), Phenytoin sodium (Parke-Davis and Co. Ltd), Sodium valproate (Sanofi Synthelabo, Onslow St.Surrey,Canada).

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Citation: Yaro AH, Aliyu M, Garba K, Hassan S. Anti-seizure activity of Extract of *Jatropha gossypiifolia* Linn (Euphorbiaceae). Trop J Nat Prod Res. 2018; 2(2):99-102. doi.org/10.26538/tjnpr/v2i2.8

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Preparation of Plant Extract
The leaves of *Jatropha gossypiifolia* were washed and air-dried at room temperature until completely dried (when constant weight was obtained). The air-dried plant material was crushed into a coarse powder with the aid of a mortar and pestle. One hundred and eighty grams (180 g) of the resultant powdered plant material was macerated with 1.5 L (70% methanol: 30% water) for 72 h. The extract was filtered using Whatman filter paper No.25. The solvent was evaporated from the resulting filtrate over a water bath set at a low temperature (40ºC).

Qualitative Phytochemical Screening
Preliminary phytochemical screening was performed on the dried extract using standard procedure.[8]

Pharmacological Studies
Median Lethal Dose (LD_{50}) Determination
The method described by Lorke[9] was adopted. The study was divided into two phases. In the first phase, three groups of three mice were treated intraperitoneally (i.p) with the extract at doses of 10, 100 and 1000 mg/kg body weight. The animals were subsequently observed for signs of toxicity and death for a period of 24 h after treatment. In the second phase, four groups each comprising one mouse were treated intraperitoneally with the extract of the plant using four specific doses (200, 400, 800, and 1600 mg/kg) of the extract which depended on the outcome of the first phase. The LD_{50} value was determined as the geometric mean of the lowest dose that caused death and the highest dose at which all the animals survived.
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Anticonvulsant Studies
Maximal Electroshock-induced Seizure Test (MEST) in Chicks
The method described by Swinyard and Kupferberg[10] was adopted. Fifty (50) one-day old ranger cockerels were randomly divided into five groups each containing ten cockerels. The first group received distilled water (10 mL/kg i.p) while the second, third and fourth groups were administered the extract (75, 150 and 300 mg/kg body weight i.p) and the fifth group was treated with 20 mg/kg phenytoin sodium (i.p). Thirty minutes later, maximal electroshock was administered to induce seizure in the chicks using Ugo Basile Electroconvulsive Machine (Model 7801) connected to Claude Lyons stabilizer with corneal electrodes placed on the upper eyelids of the chicks. The shock duration, frequency, current and pulse width were set and maintained at 1.6 s, 150 pulse/sec, 80 mA and 0.8 ms, respectively. Seizures were manifested as hind limb tonic extension (HLTE). The ability to prevent this feature or prolong the latency and/or onset of the HLTE or reduce the recovery time was considered as an indication of anticonvulsant activity.[11]

Pentylenetetrazole-induced Seizure in Mice
Mice were divided into five groups of six mice each and received different treatments. Group I was treated with distilled water (10 mL/kg i.p). Groups II-1V were treated with 3 graded doses of the extract (75, 150 and 300 mg/kg i.p) while group V received standard drug sodium valproate (200 mg/kg i.p). Thirty minutes post administration, all groups received 100 mg/kg pentylenetetrazole (PTZ) subcutaneously. Animals were observed for the presence or absence of threshold seizures (an episode of clonic spasm of at least 5-second duration) and latency of convulsion.[12]

Strychnine-induced Seizures in Mice
Mice were divided into five groups of six mice each and received different treatments. Group I was treated with distilled water (10 mL/kg i.p). Groups II-1V were treated with 3 graded doses of extract (75, 150 and 300 mg/kg i.p) while group V received phenobarbitone sodium (30 mg/kg body weight i.p). Thirty minutes later, mice were administered 1 mg/kg body weight of strychnine (STN) subcutaneously and observed for incidence of convulsions. Prevention of tonic hind limb extensor jerk was considered as protection against seizures induced by strychnine. [13]

4-Aminopyridine-induced Seizures in Mice
Mice were divided into five groups of six mice each and received different treatments. Group I was treated with distilled water (10 mL/kg i.p). Groups II-1V were treated with the extract (75, 150 and 300 mg/kg i.p) while group V was treated with phenobarbitone sodium (30 mg/kg). Thirty minutes post-treatment, 4-Aminopyridine (4-AP) was administered at a dose of 15 mg/kg body weight to each mouse, subcutaneously. The absence of tonic hind limb extension or prolongation of the latency of the hind limb tonic extension was considered as indication for anticonvulsant activity. [14]

Statistical Analysis
Data were expressed as Mean ± SEM. Differences between means were analyzed by one-way analysis of variance (ANOVA), while statistical significance was obtained with ANOVA, Dunnett's post hoc test was performed. Values of $P \leq 0.05$ were considered significant.

Results and Discussion

The phytochemical screening of the leaf extract of *Jatropha gossypiifolia* revealed the presence of cardiac glycosides, steroids, triterpenes, tannins and flavonoids (Table 1). Flavonoids, saponins and alkaloids have been reported to demonstrate CNS modulating activities including anticonvulsant effects.[15]
The median lethal dose (LD_{50}) of the methanol leaf extract of *J. gossypiifolia* was estimated to be 1131 mg/kg. There was a significant reduction in locomotive activity in the mice before death. This suggests that it is relatively safe following intraperitoneal administration as profiled by Corbett *et al*.[16.] The doses employed in this study were lower than one-third of the median lethal dose, thus safe for ethnopharmacological studies.[17] The methanol leaf extract of *Jatropha gossypiifolia* did not delay the mean recovery time of convulsed cockerels when compared with distilled water treated group as against the standard drug (Phenytoin 20 mg/kg) which offered complete protection in MEST model (Table 2). The MEST seizure model is used to identify molecules that are effective against generalized tonic-clonic seizures, it is thought that agents that abolish seizure in this model act by limiting the spread of seizures within the brain.[18, 19] However, the leaf extract of *Jatropha gossypiifolia* did not exhibit potential in this type of epilepsy. Similarly, the leaf extract of *Jatropha gossypiifolia* did not delay the mean onset of seizures in both PTZ and 4-AP models when compared with distilled water group as against the significant protection offered by standard groups of sodium valproate (200 mg/kg) and phenobarbitone (30 mg/kg) (Tables 3 and 5). PTZ is a proconvulsant that acts by lowering seizure thresholds in experimental animals.[20] It is an antagonist of gamma-hydroxy butyric acid (GABA) receptor chloride complex which affects the release of GABA, adenosine and glutamine in the brain.[21, 22] In practice, agents effective in this model act by decreasing seizures, hence effective in absence or myoclonic seizures. The *Jatropha gossypiifolia* leaf extract did not significantly prolong the latency of seizures in mice, thus may be ineffective in the treatment of petit mal seizures. 4-Aminopyridine is a K^+ channel antagonist and produces seizures by evoking spontaneous excitatory neurotransmitter release in the brain.[23] It is a useful mechanistic model that profiles potential K^+ openers by increasing hyperpolarization of the neuronal cells. *Jatropha gossypiifolia* leaf extract did not open the K^+ channel based on this study. However, the extract at the dose of 150 mg/kg significantly ($p \leq 0.05$) delayed the mean onset of seizures when compared with distilled water group in the STN model (Table 4). Strychnine is a proconvulsant that inhibits glycinergic transmission to motor neurons and interneurons within the spinal cord.[24] The extract at dose of 150 mg/kg increased the latency of strychnine-induced seizures in mice. This suggests that the extract contains bioactive compounds that enhance the action of glycine as agonist or inhibiting its biosynthesis. MEST and PTZ models have been the primary screening methods for potential anti-seizure drugs. However, levetiracetam is available for treatment and/or as adjunct in epilepsy management, but did not pass the MEST and PTZ tests.[25] We therefore, suggest that *J. gossypiifolia* leaf may act in a similar manner as levitiracetam.

Table 1: Phytochemical Constituents of Methanol Leaf Extract of *Jatropha gossypiifolia*.

Constituents	Inference
Cardiac glycosides	+
Saponins	-
Steroids	+
Triterpenes	+
Tannins	+
Flavonoids	+
Alkaloids	-
Anthraquinones	-

Key: + = present, - = absent

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 2: Effect of Leaf Extract of *Jatropha gossypiifolia* on Maximal Electroshock Induced Seizures in Chick.

Treatment	Mean Recovery Time (min)
DW (10 mL/kg)	6.77 ± 1.23
JG (75 mg/kg)	8.33 ± 1.70
JG (150 mg/kg)	9.00 ± 0.82
JG (300 mg/kg)	6.77 ± 1.47
Phenytoin (20 mg/kg)	-

Data expressed as Mean ± SEM, JG = *Jatropha gossypiifolia*.

Table 3: Effect of Leaf Extract of *Jatropha gossypiifolia* on PTZ-Induced Seizures in Mice.

Treatment	Mean Onset of Seizures (sec)
DW (10 mL/kg)	6.83 ± 1.01
JG (75 mg/kg)	10.50 ± 2.83
JG (150 mg/kg)	9.00 ± 6.22
JG (300 mg/kg)	9.00 ± 2.25
SV (200 mg/kg)	-

Data expressed as Mean ± SEM, JG = *Jatropha gossypiifolia*, DS = Distilled water, SV = Sodium valproate.

Table 4: Effect of Leaf Extract of *Jatropha gossypiifolia* on STN-Induced Seizures in Mice.

Treatment	Mean Onset of Seizures (sec)
DW (10 mL/kg)	4.17 ± 0.31
JG (75 mg/kg)	6.33 ± 0.42
JG (150 mg/kg)	9.00 ± 1.75*
JG (300 mg/kg)	7.00 ± 0.63
PHB (30 mg/kg)	-

Data expressed as Mean ± SEM, n = 6. JG = *Jatropha gossypiifolia*, DW = Distilled water, PHB = Phenobarbitone, STN = Strychnine, * $P \leq 0.05$.

Table 5: Effect of Leaf Extract of *Jatropha gossypiifolia* on 4-AP-Induced Seizures in Mice.

Treatment	Mean Onset of Seizures (sec)
DW (10 mL/kg)	12.83 ± 3.42
JG (75 mg/kg)	7.17 ± 4.93
JG (150 mg/kg)	12.50 ± 4.23
JG (300 mg/kg)	8.83 ± 4.21
PHB (30 mg/kg)	-

Data expressed as Mean ± SEM, JG = *Jatropha gossypiifolia*, DW = Distilled water, PHB = Phenobarbitone, 4-AP = 4-Aminopyridine.

Conclusion

The data from this study suggests that methanol leaf extract of *Jatropha gossypiifolia* has anti-seizure actions and may be useful in the treatment of seizure-like disorders.

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.

Acknowledgements

The authors appreciate the contribution of Mr. Aminu Abdulsalam of the Department of Pharmacology Bayero Unuversity, Kano for his assistance during the laboratory work.

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