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Original Research Article

Evaluation of the Anti-Convulsant Activity of Aqueous Leaf Extract of *Jatropha curcas* (Euphorbiaceae) in Mice

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ABSTRACT

The prevalence of epilepsy is particularly high in Latin America and in several African countries notably Liberia and Nigeria. *Jatropha curcas* (*Euphorbiaceae*) is claimed to be used ethnomedicinally in the management of convulsion, however there are no scientific report on this hence the aim of the study. Dried and powdered leaves of *J. curcus* (500 g) was extracted by decoction. Preliminary phytochemical screening was done using standard methods. Male mice were randomly divided into five groups (n = 4). Group 1 (control) was given 0.2 mL each of normal saline orally while groups II, III, and IV received 100, 200, and 400 mg/kg of the aqueous leaf extract of *J. curcus* and Group V received 30 mg/kg phenobarbitone orally. After one hour, mice were electroshocked (current at 50 mA) for 0.2 s through a pair of ear clip electrodes. In similar grouping and administration, same doses of extract and diazepam (3 mg/kg) as the positive control were used and pentylenetetrazol (70 mg/kg) was administered intraperitoneally to induce convulsion. The onset of tonic leg extension and protection from mortality was noted. Doses of 100, 200, and 400 mg/kg significantly ($P < 0.05$) protected the mice against the maximal electroshock-induced convulsion while 400 mg/kg significantly ($P < 0.05$) protected the mice against pentylenetetrazol-induced seizure. The aqueous extract of the leaves of *Jatropha curcas* possesses some secondary metabolites that protected the mice against MES (all doses) and PTZ (400 mg/kg) induced convulsion hence may be useful in the management of epilepsy.

Keywords: Anticonvulsant**,** *Jatropha curcas*, Pentylenetetrazol, Phenobarbitone, Diazepam.

Introduction

The fascinating history of epilepsy is connected with the history of humanity. One of the first descriptions of epileptic seizures can be traced back to 2000 B.C. in ancient Akkadian texts, a language widely used in the region of Mesopotamia. The author described a patient with symptoms resembling epilepsy "*his necks turns left, his hands and feet are tense and his eyes wide open, and from his mouth, froth is flowing without having any consciousness".* The exorciser diagnosed the condition as *'antasubbu'* (the hand of sin) brought about by the god of the moon.[1]

Major advances in the understanding of epilepsy came in the 18th and 19th century; theories on epilepsy during this period were formulated on solid scientific basis and epileptics for the first time were treated as patients and not as lunatics or possessed.[2] The work of John Hughlings Jackson was preceded by a plethora of studies by the Dutch, German, English, and French physicians who evolved scientific thought and perform thorough studies of epilepsy. The advent of 20th century led to the in-depth understanding of the mechanisms of the disease, the development of effective drugs and neuro-imaging methods.[2]

As an alternative to orthodox medicine the World Health Organization (WHO) estimated that about 80% of the people living in developing countries use solely traditional medicines to treat their health problems.[3] A proper scientific evaluation of these herbal medicines with pertinent emphasis on established pharmacological and toxicological paradigms is necessary to determine their efficacy and safety.[4]

In ethnomedicine, febrile convulsions are treated with different methods and techniques. The treatment modality varies from one locality to the other but one common feature is the use of heat, strong lubrication with palm kernel oil along with plant decoctions and or cow urine.[5,6] *Jatropha curcas* L. (physic nut) is a drought resistant large shrub or small tree, belonging to the genus *Euphorbiaceae*, producing oil containing seeds.[7] *Jatropha curcas* L. is the commonest specie found in Nigeria, but many species exist in different parts of the world. Heller reported about 165-175 species that were known from the genus *Jatropha*.[8] Bhagat and Kulkarni report 14 wild and cultivated species in India; this is against the 12 species reported by Krishnan and Paramathma.[9] It is a multipurpose, drought resistant tree and can be cultivated in areas of low rainfall.[10] Jatropha is suitable for quick and efficient domestication compared with other woody species.[11]

Several pharmacological activities of *J. carcus* have been documented and they include anti-inflammmatory,[12] antioxidant,[13,14,15] antimicrobial[16] amongst others. Report on the anticonvulsant activity of this plant seems minuscule hence the rationale for this study. The aim of the study was to evaluate the acute toxicity profile and anticonvulsant effect of the aqueous leaf extract of *Jatropha curcas* L in mice.

Materials and Methods

Materials and Reagents

Acetic anhydride, alkaloidal reagents, alpha-naphthol, aluminium chloride, ammonium hydroxide, pentylenetetrazol, phenobarbitone,

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sulphuric acid, fehling's reagent (Sigma-Aldrich, Germany), petroleum ether, ethanol, chloroform (JHD, China), diazepam (Roche, Switzerland), ninhydrin, ferric chloride (Qualikems, India), hydrochloric acid (BDH, England), silver nitrate, sodium chloride, sodium hydroxide (Merck), treadmill device (UgoBasile Rota-rod, Model 7650, Italy).

Methods

Plant collection

Fresh leaves of *Jatropha curcas* were collected from Isiohor community in Benin City, Edo State, Nigeria on the 9th of February 2018. It was identified by Dr. Akinnibosun, a taxonomist in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Nigeria.

Preparation of Jatropha curcas extract

The dried and powdered leaves of *Jatropha curcas* were extracted using decoction method. The dried leaves (500 g) were weighed and mixed with 2 L of distilled water. It was allowed to boil for 30 min, filtered and the extract was concentrated over a regulated hot water bath (60ºC) using an evaporating dish. The percentage yield of the extract was determined.

Phytochemical screening

Phytochemical screening to detect for the presence or absence of alkaloids, tannins, saponins, carbohydrates, anthraquinones, and other phenolic compounds were done in accordance with standard methods.[17,18]

Experimental animals

Male albino mice weighing between 25 - 30 g were acclimatized in the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. The animals were kept in plastic cages and housed at room temperature and humidity. They were allowed free access to dry rodent pellet feeds (Top Feeds Limited, Ibadan, Nigeria) and water. The bedding materials (wood shavings) of the cages were changed daily. All experiments were carried out in accordance with the National Institute of Health Guidelines for the Care and Use of laboratory Animals (NIH Publications No. 80-23) revised in 2002.

Ethical approval

Ethical approval with reference number EC/FP/018/36 was obtained from the Ethics Committee of the Faculty of Pharmacy, University of Benin, Benin-City, Nigeria.

Oral acute toxicity test

Oral median lethal dose (LD_{50}) of *Jatropha curcas* extract was determined using a Modified Lorke (1983) method.[19] In the first phase, three groups of three mice each were administered 10, 100 and 1000 mg/kg of the extract orally. The second phase involved three groups of three mice each administered 1600, 2900 and 5000 mg/kg of the extract orally. In both phases, the animals were observed for signs of writhing, diarrhea, tremor and mortality within a 24 h period. Control animals received distilled water (0.2 mL).

Preparation of other drug solutions used in the study

Phenobarbitone stock solution

Phenobarbitone (15 mg) was dissolved in 5 mL distilled water to obtain a stock solution of 3 mg/mL phenobarbitone stock solution. This drug was used as the reference anticonvulsant agent against Maximal electroshock-induced seizures.

Pentylenetetrazol stock solution

A 700 mg powder of Pentylenetetrazol was dissolved in 10 mL of distilled water to obtain a stock solution of 70 mg/mL pentylenetetrazol stock solution. This agent was used to induce seizures.

Diazepam stock solution

Diazepam injection was serially diluted to give as stock solution of 0.1 mg/mL. A 1 in 10 dilution was done to give 0.1 mg/mL which was diluted further to give the required stock solution. A dose of 3 mg/kg was used. This agent was used as the reference anticonvulsant agent against pentylenetetrazol-induced seizures.

Motor coordination test

The rota-rod test described previously by Dunham and Miya (1957)[20] was used to evaluate motor coordination. The mice were screened on a treadmill device (UgoBasile Rota-rod, Model 7650, Italy) with slowly revolving rods of 0.5 cm diameter at 16 rpm for 120 s. Mice that remained on the rod for 120 s or longer were selected and allotted to five groups with four mice per group. The time each animal spent on the rod was noted and recorded.

Anticonvulsant studies

Anticonvulsant activity of the extract was evaluated by maximal electroshock (MES) and pentylenetetrazol (PTZ) induced seizure models.

Anticonvulsant effects of aqueous leaf extract of Jatropha curcas against maximal electroshock-induced seizure

The method described by Swinyard (1969)[21] was used. Twenty mice were divided into five groups of four mice each. Group 1 served as the control and mice were given 0.2 mL each of normal saline. Groups II, III, and IV were given 100, 200, and 400 mg/kg orally while Group V received 30 mg/kg phenobarbitone. After one hour, all animals were subjected to electroshock at a current of 50 mA for 0.2 s through a pair of ear clip electrodes. The onset of tonic leg extension as well as protection from mortality was noted.

Pentylenetetrazol-induced seizure

Twenty male mice were randomly allotted to five groups of four mice each. Group I served as the negative control and was given 0.2 mL while groups II, III, and IV were given 100, 200, and 400 mg/kg of the extract and Group V was given diazepam 0.1 mg/kg orally. After one hour, 70 mg/kg pentylenetetrazol was administered intraperitoneally to the mice in all the groups. The times of onset of tonic-clonic convulsions as well as protection against mortality were recorded.[22]

Statistical analysis

Data were expressed as mean ± standard error of mean (S.E.M). Statistical analysis was done using one-way analysis of variance followed by Dunnett's post-hoc test (Graphpad Prism® 6, San Diego, USA). $P < 0.05$ was considered statistically significant.

Results and Discussion

Phytochemical analyses

The plant extract of *Jatropha curcas* contained carbohydrates, alkaloids, saponins, proteins, flavonoids, terpenoids. However, anthraquinones, proteins and phytosterols were absent (Table 1).

Percentage yield of the aqueous leaf extract of Jatropha curcas

The percentage yield of the aqueous extract of the powdered plant was found to be 2.58% (12.89 g).

Oral acute toxicity test

Oral doses of 10, 100 and 1000 mg/kg the aqueous extract of *Jatropha curcas* produced neither noticeable toxic effects nor mortality in the mice (Table 2). In the phase 2, oral doses of 1600, 2900 and 5000 mg/kg the aqueous extract of *Jatropha curcas* produced neither marked toxic effects nor mortality in the mice (Table 3).

Effect of aqueous leaf extract of Jatropa curcas on maximal electroshock-induced convulsion in mice

At the dose of 100 mg/kg, the extract offered no protection against Maximal Electroshock-induced convulsion model, while 200 mg/kg offered 50% protection and 400 mg/kg offered 75% protection of the mice. Phenobarbitone (30 mg/kg) which served as the positive control completely protected the mice against convulsion while normal saline (negative control) offered no protection against MES-induced convulsion in mice (Table 4).

Effect of aqueous extract of jatropha curcas on pentylenetetrazol induced convulsion in mice

At dose of 100 and 200 mg/kg the aqueous extract significantly delayed onset of convulsion but offered no full protection against Pentylenetetrazol-induced convulsion. At 400 mg/kg, 75% protection

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of the mice against Pentylenetetrazol-induced convulsion was observed. Diazepam (3 mg/kg) which is the positive control completely protected the mice against convulsion while normal saline (negative control) offered no protection against PTZ-induced convulsion in mice.

Plants have always been used as medicine from ancient times. The use of *Jatropha curcas* in the management of febrile convulsion traditionally has been a trend in some parts of Nigeria hence, the need for this study which has given scientific authentication to the traditional claims. The treatment of febrile convulsion is still far from satisfactory and a number of new anticonvulsant remedies and measures have been introduced during the last century, some of which are related to compounds already known and the search for drugs with reduced toxic effect is continuing. The present investigation revealed that the aqueous extract of *Jatropha curcas* was without any lethal effect in a dose up to 5 g/kg and was fairly nontoxic and did not induce any change in animal behavior as food and water intake were normal during the acute toxicity study.

Assessment of the anticonvulsant activity revealed increased seizure latency, shortened duration of seizure and protection of treated mice from seizure-induced deaths. It is well known that drugs which provide protection against seizures induced by maximal electroshock are generally predicted to be effective against generalized tonic clonic seizures and partial seizures.[23] Maximal electroshock-induced convulsions can be effectively prevented by drugs that inhibit voltage dependent sodium channels such as phenytoin. The extract was effective against MES-induced convulsion at a dose of 200 mg/kg which showed 50% protection and 400 mg/kg which showed 75% protection probably by the mechanism stated above. Therefore, it could be possible that the extract elicits its anticonvulsant action via this mechanism.

The dose of 400 mg/kg of the aqueous extract of *J. curcas* showed significant (75%) protection against Pentylenetetrazol-induced convulsions while doses of 100 and 200 mg/kg of the aqueous extract of *J. curcas* delayed onset of convulsion significantly but there was no full protection against convulsion. PTZ-induced convulsion occurs through the antagonism of Gama-aminobutyric acid (GABA) receptor-chloride ion channel complex,[24] thereby attenuating GABA-dependent inhibition. Thus, there is also a possibility that the anticonvulsant activity of the aqueous extract could be mediated via GABAergic mechanisms. Agents that protect against tonic-clonic seizures induced by pentylenetetrazole are considered useful in humans.[25]

Table 1: Phytochemical composition of the aqueous leaf extract of *Jatropha curcas*.

Phytochemical	Inference
Alkaloids	+
Carbohydrate	+
Reducing Sugars	+
Deoxysugars	+
Saponins	+
Steroidal Saponins	-
Phenolics	+
Flavonoids	+
Proteins	-
Terpenoids	+

Table 2: Oral acute toxicity of whole extract of *Jatropha curcas* in Phase 1.

Dose (mg/kg)	Number of deaths	% Mortality
10	0/3	0
100	0/3	0
1000	0/3	0

Table 3: Oral acute toxicity of whole extract of *Jatropha curcas* in Phase 2.

Dose (mg/kg)	Number of deaths	% Mortality
1600	0/3	0
2900	0/3	0
5000	0/3	0

Table 4: The protection of mice from MES-induced convulsion by the aqueous leaf extract of *Jatropha curcas.*

Group	Rotarod Time (seconds)	Number of mice protected	% of mice protected
Normal Saline	124.00 ± 5.30	0/4	0
100 mg/kg A *J. curcas*	122.50 ± 2.50	0/4	0
200 mg/kg A *J. curcas*	125.00 ± 5.00	2/4	50**
400 mg/kg A *J. curcas*	127.50 ± 4.79	¾	75***
30 mg/kg Phenobarbitone	123.00 ± 4.06	4/4	100****

A *J. curcas* - Aqueous extract of *Jatropha curcas*; Values are mean ± SEM, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 compared to negative control, n = 4.

Table 5: The anti-convulsant effect aqueous leaf extract of *Jatropha curcas* against Pentylenetetrazol induced convulsion

Group	Onset of convulsion (seconds)	Rota rod Time (seconds)	Number of mice protected	% protection
Normal saline (neg. control)	210.00 ± 51.96	120.00 ± 0.00	0/4	0
100 mg/kg A *J. curcas*	555.00 ± 28.72***	125.00 ± 5.00	0/4	0
200 mg/kg A *J. curcas*	525.00 ± 96.05**	128.00 ± 4.26	0/4	0
400 mg/kg A *J. curcas*	75% Protected	127.50 ± 4.79	¾	75***
3 mg/kg Diazepam	100% Protected	125.00 ± 5.00	4/4	100****

A *J. curcas* - Aqueous extract of *Jatropha curcas*; Values are mean ± SEM, **P<0.01, ***P<0.001, ****P<0.0001 compared to negative control, n = 4.

Although, in practice, these types of seizures are distinct in their clinical manifestations and responses to therapy; only very few drugs can be effective against both seizures. The clinical aspect of generalized seizures is highly correlated with experimental seizures produced by subcutaneous administration of pentylenetetrazole while partial seizures in humans correlate positively with experimental seizures elicited by the maximal electroshock test.[26] These seizures respond differently to drugs through different mechanisms of action. Ethosuximide, for instance, which is effective against generalized seizures act through a reduction of Ca^{2+} entry into excitable membranes, while phenytoin which is effective against partial seizure produces anticonvulsant effects through alteration of ionic transport across excitable membrane.[27]

The present study provides evidence for the anticonvulsant activity of the aqueous extract of *Jatropha curcas* in mice being more effective against electroshock-induced (partial) convulsions than PTZ-induced (generalized) convulsion. Although in both versions of experimental convulsion, the time of onset of seizure significantly increased as the dose of the extract increased, but, the delay in onset was longer in maximal electroshock model than the PTZ-induced convulsion. It is, thus noteworthy that the aqueous extract of *Jatropha curcas* was effective against both seizure types and may be acting through synergistic effects in ionic movements across the seizure foci. The multiple mechanisms of *Jatropha curcas* might be due to the presence of different active components because the phytochemical screening of the aqueous extract revealed the presence of flavonoids, alkaloids, saponins, phenolics and terpenoids. The exact mechanisms will be better understood and elucidated in further studies.

Conclusion

The results from our study showed that the aqueous extract of the leaves of *Jatropha curcas* possesses anti-convulsant activity against Maximal electroshock and Pentylenetetrazol models at the dose of 400 mg/kg. The plant extract is remarkably safe at doses higher than 5 g/kg and the aqueous extract of the leaves of *Jatropha curcas* contains some active principles that can be useful in the treatment of epilepsy.

Conflict of interest

The authors declare no conflict of interest.

Author's 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.

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