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ANTICONVULSANT SCREENING OF THE METHANOLIC LEAF EXTRACT OF *TAPINANTHUS DODONEIFOLIUS* DC DANSER (LORANTHACEAE) IN PENTYLENETETRAZOLE INDUCED SEIZURE MODEL IN ALBINO RATS

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ABSTRACT

Tapinanthus dodoneifolius DC Danser (Loranthaceae) also known as African mistletoe is a medicinal plant found in Nigeria and has diverse ethno-medical uses including the management of epilepsy. The aim of this study was to evaluate the anticonvulsant activity of the methanolic leaf extract of *Tapinanthus dodoneifolius*. The leaves of the plant were collected from *Azadirachta indica* trees in Borno state, Nigeria. They were air dried, and then size reduced into a coarse powder which was defatted with petroleum ether and then extracted by maceration using methanol. The anticonvulsant activity of the extract obtained was investigated by studying its effect on seizures induced by pentylenetetrazole in Wistar rats with diazepam and sodium valproate as standard controls. *Tapinanthus dodoneifolius* (125 and 250 mg/kg) dose dependently delayed the onset of clonic convulsion induced by pentylenetetrazole ($p < 0.01$). However, 500 mg/kg of the extract offered 100% protection against pentylenetetrazole induced clonic convulsion. The extract dose dependently protected the rats against mortality caused by pentylenetetrazole induced convulsion. The data obtained suggest that *Tapinanthus dodoneifolius* possesses significant anticonvulsant activity.

KEYWORDS: Epilepsy, Anticonvulsant, Methanol, PTZ, *Tapinanthus dodoneifolius*



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INTRODUCTION

The use of medicinal plants in curing diseases is as old as man^{1,2}. It has been shown by records from ancient Egypt, Assyria, China and India that the use of plants for medicinal purposes extends back to earliest recorded history³. The World Health organization has long recognized and drawn the attention of many countries to the ever increasing interest of the public in the use of medicinal plants and their products in the treatment of various ailments⁴. The family of the mistletoes (Loranthaceae) to which *Tapinanthus dodoneifolius* belongs is a large family of about 75 genera and over 900 species⁵. Phytochemical screening of *Tapinanthus dodoneifolius* (DC) Danser a species of African mistletoe showed the presence of anthraquinones, saponins and tannins⁶. Uses of African mistletoe in folkloric medicine include: the treatment of epilepsy, hypertension, headache, infertility, cancer, menopausal syndrome and rheumatism⁷. Anti-epileptic drugs (AEDs) currently available do not provide cures nor prevent relapse and they are often associated with serious side effects, including teratogenicity, chronic toxicity and adverse effects on cognition and behaviour⁸. This study, therefore focuses on the Anticonvulsant screening of the methanolic leaf extract of *Tapinanthus dodoneifolius* leaf extract.

MATERIALS AND METHODS

Experimental site, Source of Plant Material, Collection and Authentication

All Experiments were conducted in the laboratories of the Faculty of Pharmacy, University of Maiduguri, Borno state, Nigeria. The leaves of *Tapinanthus dodoneifolius* DC DANSER (Loranthaceae) (Plate 1) were collected from *Azadiracta indica* trees at 202 housing estates, Jere local government area of Borno state, Nigeria. The collection was made in the month of February 2013. The plant was identified and authenticated by a Taxonomist

with the Department of Biological sciences, University of Maiduguri, Borno State Nigeria.

Preparation of plant leaf Extract

The leaves of *Tapinanthus dodoneifolius* DC DANSER (Loranthaceae) after collection were washed with clean water, and then air dried for two weeks. After drying the leaves were size reduced into a coarse powder using a mortar and pestle. The powdered material (300 g) was defatted using petroleum ether (200 ml) for 24 hours in a maceration bottle. The marc was then air-dried and then macerated with (400 ml) methanol for two days with occasional shaking. It was then filtered with a filter paper. The filtrate was then evaporated in a rotary evaporator and the resulting thick, viscous liquid obtained was transferred to a stainless steel plates and air dried until a constant weight of 5.6 g of amorphous dark coloured extract was obtained giving a percentage yield of 1.87%. The extract was then stored in a dry air-tight container in a cool dry place.

Experimental animals

Male and female Swiss albino rats (weighing 100-190 g) which were bred in the animal house of the Faculty of Pharmacy, University of Maiduguri were used for the study. They were housed in well ventilated and regularly sanitized rooms and were fed with standard animal feed and given water ad libitum.

LD₅₀ determination

Lorke's method⁹ was modified and used for the determination of LD₅₀ of the crude extract of *Tapinanthus dodoneifolius* leaves. The method was biphasic in nature and a total of 15 mice of both sexes was used. The LD₅₀ was calculated by calculating the geometric mean of the lowest lethal dose and the highest non-lethal dose.

Pilot Studies

Eight Swiss albino rats were divided into four groups of two mice each. The first three groups received subcutaneous PTZ (80 mg/kg, 100 mg/kg and 120 mg/kg respectively) and the

fourth group received 0.2 ml of sterile water for injection as control. All the animals were observed for onset of seizures and death. The dose of PTZ that caused convulsion but no death in 97% of experimental animals was noted, and was used to induce convulsions in the study.

Anticonvulsant screening using Pentylenetetrazole induced seizures in albino rats

Thirty rats (100-180 g) were divided into six groups of five rats each. The first group received 0.2 ml distilled water for injection, the second, third and fourth groups received the plant extract [TDE] (500, 250, 125 mg/kg respectively). The fifth group received sodium valproate 150 mg/kg, and the sixth group received Diazepam 10 mg/kg. All doses were administered intraperitoneally. After 30 minutes all the rats in all the groups received 80 mg/kg of pentylenetetrazole subcutaneously and were observed over a period of 30 minutes. Parameters measured/observed were: time for onset of myoclonic jerk, loss of righting reflex, and hind limb extension and presence or absence of convulsion and death. Absence of a clonic spasm of at least 5 seconds duration indicated a compounds ability to abolish the effect of Pentylenetetrazole seizure threshold¹⁰.

Statistical analysis

The results were analyzed by Computer software GraphPad InStat® @ USA, 2003 for statistical significance using one way ANOVA followed by Dunnet's test. A $p < 0.05$ was considered significant and $p < 0.01$ extremely significant.

RESULTS

The dose of pentylenetetrazole that produced clonic seizures lasting for a period of at least five seconds in 97% (CD_{97}) of animals tested without being lethal was 80 mg/kg body weight. The extract at a dose of 500 mg/kg protected all the rats from clonic convulsions. The extract at dose levels of 250 mg/kg and 125 mg/kg body weight showed a statistically significant

increase in the onset of clonic convulsion ($p < 0.01$). The extracts also exhibited a dose dependent significant reduction in various phases of epileptic seizures when compared with the negative control. Diazepam and Sodium valproate used as positive controls protected all the albino rats from clonic convulsion. The percentage protection of the extracts against the mortality of PTZ induced epileptic seizures was also dose dependent (Table 1 and Figure 1).

DISCUSSION

The extract (250 and 125 mg/kg) of *Tapinanthus dodoneifolius* (DC) Danser exhibited significant anticonvulsant effect by delaying the onset of clonic convulsions ($p < 0.01$). The extract (500 mg/kg) of *Tapinanthus dodoneifolius* protected all the rats from clonic convulsions. The extract also exhibited a dose dependent significant reduction in various phases of epileptic seizures when compared with the negative control ($p < 0.05$). Diazepam and sodium valproate used as positive controls protected all the rats from clonic convulsions (Table 1). The result of this finding agrees with the reports of Nwude and Ibrahim⁷ in which antiepileptic activity of *Tapinanthus dodoneifolius* was reported. Since the extract was able to prevent PTZ induced convulsion, it is possible that the extract may be acting via GABA receptors modulation. However, more studies are encouraged that can determine the exact mechanism of action of the plant extract.

The percentage protection of the extract against mortality due to pentylenetetrazole induced epileptic seizures was also dose dependent. There was no statistically significant difference ($p > 0.05$) between the effect of Diazepam (10 mg/kg) and the extract at a dose of 500 mg/kg in protecting the rats from the onset of clonic convulsions. The effect of the extract at dose level of 500 mg/kg in protecting the rats from pentylenetetrazole induced epileptic seizures was comparable with that of Sodium valproate (150 mg/kg). This study therefore confirms folkloric claims of

antiepileptic activity of *Tapinanthus dodoneifolius* as reported by Nwude and Ibrahim⁷. This study also validates the antiepileptic property of African mistletoe that warrants its prescription in African ethno-medicine for management of epilepsy as reported by Bassey¹¹. The results of this study

suggest that the methanolic leaf extract of *Tapinanthus dodoneifolius* (DC) Danser contains pharmacologically active constituents which possess anticonvulsant activity which may be effective in management of epileptic seizures and supports the folkloric use of this plant in management of epilepsy.

Table 1
Effect of methanolic leaf Extract of *Tapinanthus dodoneifolius* in Pentylentetrazole-Induced Seizure in Wistar rats

Group (Treatment)	Dose (mg/kg)	Mean Onset of myoclonic jerk (seconds)	Mean Onset of clonic convulsions (Mean ± SEM)	Mean time of hind limb extension (seconds)	Percentage Protection (%)	Mortality (%)
Negative Control	Vehicle	153.4	174.2±3.292	203.4	00	60
Diazepam	10	A	A	A	100	00
Valproic acid	150	310	A	A	100	00
TDE	500	394.7	A	A	100	00
TDE	250	322	338.4±9.277**	367	80	20
TDE	125	181.6	205.8±3.980**	237.6	40	60

Key: *P < 0.05, **P < 0.01 compared with control, n = 5. TDE = *Tapinanthus dodoneifolius* methanolic leaf extract, vehicle: water for injection, A= absence of myoclonic jerk, clonic convulsions, or hind limb extension.

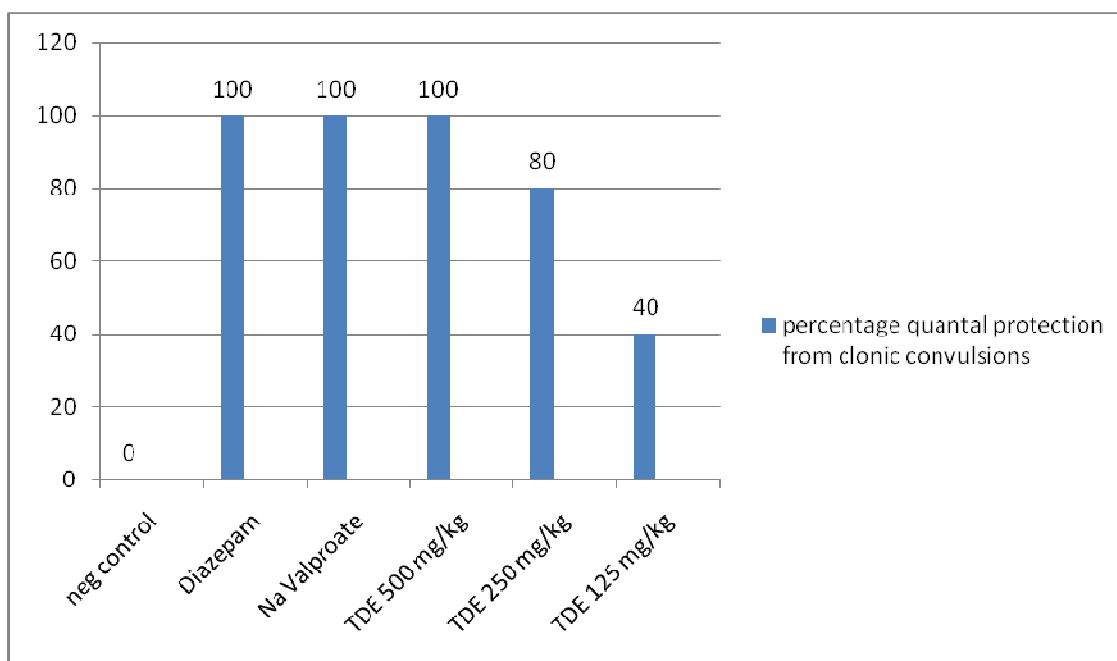


Figure 1
Effect of methanolic leaf Extract of *Tapinanthus dodoneifolius* in Pentylentetrazole-Induced Seizure in Wistar rats (percentage quantal protection from clonic convulsions).



Plate 1
Picture of Tapinanthus dodoneifolius DC Danser.

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