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# EVALUATION OF ANTICONVULSANT ACTIVITIES OF ETHANOL LEAF EXTRACT OF *Hymenocardia acida* TUL (EUPHORBIACEAE) IN MICE AND CHICKS

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**ABSTRACT**
***The aim of the research is to investigate the anticonvulsant property of ethanol leaf extract of H. acida (ELHA) in mice and chicks. The work employed maximal electroshock test (MEST) in chicks, pentylenetetrazole (PTZ), 4-aminopyridine (4-AP) and strychnine-induced convulsion in mice. The results showed the intraperitoneal $LD_{50}$ of ELHA to be 3808 mg/kg in mice. The extract produced no significant effect on mean recovery time in MEST. ELHA at 250 and 500 mg/kg protected 50% of mice against seizures by 4-AP. ELHA at 1000 mg/kg produced significant ($p < 0.05$) increase in mean onset of seizures induced by 4-AP. ELHA at 500 mg/kg protected 33.3% of mice against PTZ induced convulsion, however no significant effect was observed on mean onset of seizures induced by PTZ and strychnine. Phytochemical screening revealed the presence of alkaloids, steroids, glycosides, saponins, tannins and flavonoids. In conclusion, results revealed that the ethanol leaf extract of H. acida possess bioactive compounds that could be useful in the management of epilepsy.***
***Keywords: Hymenocardia acida, MEST, PTZ, 4-AP, Strychnine***

## INTRODUCTION

About 50 million people worldwide have epilepsy of which nearly 80% are found in developing countries. Epilepsy is a major neurological disorder that accounts for 0.75% of the global burden of disease (WHO, 2016).

*Hymenocardia acida* (Euphorbiaceae) is very popular in African Traditional medicine. It is called "*Heart-fruit*" in English, "*jan yaro*" in Hausa, "*yawa satoje*" in Fulani, "*ikalaga*" in Igbo, and "*Orunpa*" in Yoruba, "*Uchuo*" in Igede, "*enanche*" in Idoma. *Hymenocardia acida* is a dioecious tree up to 6-10 m tall, often stunted. It is found in Savanna region of Nigeria. *H. acida*has been used in folk medicine for many years in Nigeria and some other parts of Tropical Africa. The leaves, bark and roots of *H. acida* are used either in infusion or powdered form to treat hypotension, diabetes, sickle cell, epilepsy, schizophrenia (Dalziel, 1937). The work investigated the anticonvulsant potential of ethanol leaf extract of *H. acida* in mice and chicks.

Plate I: *Hymenocardia acida* in its natural habitat (Zaria, Nigeria)

**MATERIALS AND METHODS**

Leaves of *Hymenocardia acida* were collected from Dajin tohu around Shika dam, Zaria in the month of June, 2015, identified and authenticated by a taxonomist with the Department of Biological sciences, Ahmadu Bello University, Zaria by comparing with specimen voucher (number 1275) already deposited in the herbarium.

Albino mice (21 to 25g) and one day old ranger cockerels obtained from the Animal House of the Department of Pharmacology and Therapeutics, ABU, Zaria and National Animal Production and Research Institute (NAPRI), Shika Zaria respectively were used for the study. Chemicals such as Pentylenetetrazole (Sigma chemical Co. St Louis, USA), 4-aminopyridine (Merck-Schuchardt, Germany) and Strychnine (Sigma Chemical Co. St Louis, USA) were the chemical agents used to induce seizure in the experimental animals. The standard drugs used for the experiment were Phenytoin sodium (Hospira, UK Limited), Phenobarbitone (Lab Renaudin, France) and Sodium valproate (Sanofi Aventis, U.S).

The ethanol leaf extract of *H. acida*, phenobarbitone, phenytoin, and sodium valproate were administered intraperitoneally. Pentylenetetrazole, 4-aminopyridine and strychnine were administered subcutaneously.

**Anticonvulsant Studies**

The anticonvulsant studies were carried out using electrically induced method (Swinyard and Kupferberg,1985; Browning,1992) as well as using chemical convulsants such as pentylenetetrazole (Swinyard *et al.*,1989), 4-aminopyridine (method described by Yamaguchi and Rogawski, 1992) and Strychnine (Porter *et al.*, 1984). Doses of 250, 500 and 1000 mg/kg of the ethanol leaf extract of *H. acida i.p* were used. Normal saline (10 ml/kg) and standard drugs were used as the negative and positive control respectively.

**RESULTS AND DISCUSSION**

The preliminary phytochemical screening of ethanol leaf extract of *Hymenocardia acida* revealed the presence of alkaloids, glycosides, saponins, tannins and flavonoids among which flavonoids and saponins have been reported to possess anticonvulsant activity (Kavvadias *et al.*, 2004) and may therefore be associated with anticonvulsant potential of the leaf extract of *Hymenocardia acida*.

The intraperitoneal median lethal dose ($LD_{50}$) of the ethanol leaf extract of *Hymenocardia acida* in mice was estimated to be 3808 mg/kg body weight suggesting that the extract is relatively less toxic according to classification of $LD_{50}$ values by Matsumura, (1975) and Corbett *et al.*, (1984).

The ethanol leaf extract of *Hymenocardia acida* at all doses did not protect the chicks against convulsion induced by maximal electroshock, and no significant decrease in mean recovery time of convulsed animals is observed. Whereas the standard anticonvulsant drug Phenytoin (20 mg/kg) produced significant effect ($p < 0.05$) on the mean recovery time and protected 90% of the chicks against Hind Limb Tonic Extension (HLTE) induced by Maximal Electroshock Test (MEST) (Table 1). MEST is a standard AED test that evaluates the testing material's ability to protect against HLTE (DeLorenzo *et al.*, 2001).

It is a model for generalized tonic clonic seizure, which is highly reproducible with a consistent end point (Stables and Kupferberg, 1997). Protection against HLTE also indicates the ability of a testing material to inhibit or prevent seizure discharge within the brainstem seizure substrate (Browning, 1992).

The ethanol leaf extract of *Hymenocardia acida* produced no significant effect on mean onset of seizures induced by PTZ in mice. The standard anticonvulsant (sodium valproate, 200 mg/kg) gave 66.7% protection against PTZ-induced convulsion and protected 83.33% of mice from mortality (Table 2). Anticonvulsant activity in PTZ test identifies compounds that can raise the seizure threshold in the brain (Raza *et al.*, 2001). Protection by *Hymenocardia acida* extract (ELHA 500) against threshold seizure induced by PTZ suggests it could be useful in the therapy of absence or myoclonic seizures.

The ethanol leaf extract at 1000 mg/kg produced significant ($p < 0.05$) increase in mean onset of convulsion induced by 4-aminopyridine in mice. Phenobarbitone (20 mg/kg), the standard anticonvulsant used produced 100% protection against convulsion and mortality (Table 3). 4-aminopyridine is a $K^+$ channel antagonist and it interferes with all aspect of neuronal excitability, including resting membrane potential, responsiveness to synaptic inputs, frequency adaptation and neurotransmitters release (Wickenden, 2002). The ability of the ethanol leaf and stem bark extracts of *Hymenocardia acida* to protect the mice from the convulsant effect of 4-aminopyridine suggests that it interacts with $K^+$ channel to produce the anticonvulsant activity.

The ethanol leaf extract of *Hymenocardia acida* produced no significant effect on mean onset of seizures induced by Strychnine in Mice. Phenobarbitone (20 mg/kg) the standard anticonvulsant produced 100% protection against seizure and mortality (Table 4).Strychnine directly antagonizes the inhibitory reflexes mediated by glycine (Sayin *et al.,* 1993).

**Table 1: Effect of Ethanol Leaf Extract of *Hymenocardia acida* on Maximal Electroshock Test (MEST) in Chicks**

| Treatment (mg/kg) | %Protection against seizure | Mean recovery time of seizure (min) |
|---|---|---|
| NS 10 ml/kg | 0.0 | 9.5 ± 1.3 |
| ELHA (250) | 0.0 | 9.1 ± 1.3 |
| ELHA (500) | 0.0 | 8.5 ± 0.4 |
| ELHA (1000) | 0.0 | 12.5 ± 1.8 |
| PHN (20) | 90.0 | 19.0 ± 0.0* |

Values are presented as Mean ± SEM and percentages. *P< 0.05, Mean recovery time of seizure compared to normal saline group using One way ANOVA followed by Dunnett's post hoc test, n=10, NS- Normal saline, ELHA- Ethanol Leaf Extract of *Hymenocardia acida*, PHN- Phenytoin.

**Table 2: Effect of Ethanol Leaf Extract of *Hymenocardia acida* on Pentylenetetrazole (PTZ) - Induced Seizure in Mice**

| Treatment (mg/kg) | % Protection seizure (min) | Mean onset of | % Mortality death (min) | Mean latency to |
|---|---|---|---|---|
| NS 10 ml/kg | 0.0 | 4.5 ± 0.8 | 100.0 | 5.8 ± 0.8 |
| ELHA (250) | 0.0 | 6.3 ± 0.9 | 66.7 | 9.5 ± 0.9 |
| ELHA (500) | 33.3 | 4.5 ± 0.9 | 66.7 | 7.0 ± 1.6 |
| ELHA (1000) | 0.0 | 7.8 ± 1.7 | 66.7 | 7.5 ± 1.8 |
| SV (200) | 66.7 | 8.0 ± 4.0* | 16.7 | 16.5 ± 12.5** |

Values presented as Mean ± SEM and percentages, *P< 0.05,**P< 0.01 compared to normal saline group using One way ANOVA followed by Dunnett's post hoc, n=6, NS- Normal Saline, ELHA- Ethanol leaf extract of *Hymenocardia acida,* SV- Sodium Valproate.

**Table 3: Effect of Ethanol Leaf Extract of *Hymenocardia acida* on 4- aminopyridine Induced Seizure in Mice**

| Treatment (mg/kg) | % Protection | Mean onset of seizure (min) | % Mortality |
|---|---|---|---|
| NS 10 ml/kg | 0.0 | 11.0 ± 1.1 | 100.0 |
| ELHA (250) | 50.0 | 16.3 ± 1.5 | 50.0 |
| ELHA (500) | 50.0 | 12.3 ± 0.9 | 50.0 |
| ELHA (1000) | 33.3 | 19.0 ± 2.7** | 66.7 |
| PHEB (20) | 100.0 | 0.0 ± 0.0 | 0.0 |

Values presented as Mean ± SEM and percentages, *P< 0.05, **P<0.001 compared to normal saline group using One way ANOVA followed by Dunnett's post hoc, n=6, NS- Normal Saline, ELHA- Ethanol leaf extract of *Hymenocardia acida,* PHEB- Phenobarbitone.

**Table 4: Effect of Ethanol Leaf Extract of *Hymenocardia acida* on Strychnine Induced Convulsion in Mice**

| Treatment (mg/kg) | % Protection | Mean onset of seizure (min) | % Mrtality |
|---|---|---|---|
| NS 10 ml/kg | 16.7 | 7.2 ± 1.2 | 83.3 |
| ELHA (250) | 16.7 | 9.8 ± 1.7 | 83.3 |
| ELHA (500) | 0.0 | 9.8 ± 1.5 | 100.0 |
| ELHA (1000) | 16.7 | 8.8 ± 1.4 | 83.3 |
| PHEB (20) | 100.0 | 0.0 ± 0.0 | 0.0 |

Values presented as Mean ± SEM and percentages, onset of seizure( treated groups) was compared to normal saline group using One way ANOVA followed by Dunnett's post hoc, n=6, NS- Normal Saline, ELHA- Ethanol leaf extract of *Hymenocardia acida,* PHEB- Phenobarbitone.

**CONCLUSSION**

The results of this study suggest that the ethanol leaf extract of *Hymenocardia acida* contain phytochemical constituents that possess anticonvulsant action. This could explain Ethnomedicinal use of the plant in the treatment of epilepsy.

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