EVALUATION OF ANTICONVULSANT ACTIVITIES OF ETHANOL STEM BARK EXTRACT OF HYMENOCARDIA ACIDA TUL (EUPHORBIACEAE) IN MICE AND CHICKS

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ABSTRACT

Hymenocardia acida commonly known as Jan yaro in hausa is used in traditional medicine for abdominal pain, menstrual pain, and epileptic fits. The main objective of this study is to investigate the anticonvulsant property of ethanol stem bark extract of H. acida in mice and chicks. The tests employed included maximal electroshock test in chicks, pentylenetetrazole (PTZ), 4-aminopyridine (4-AP) and strychnine-induced convulsion in mice. The intraperitoneal LD50 of the ethanol stem bark extract of H. acida was estimated to be 7 mg/kg body weight in mice. The extract produced no significant effect on the mean recovery time of convulsed animals in maximum electroshock test in chicks. The extract at 50 mg/kg and 200 mg/kg protected 33.3% and 50% of the mice respectively against pentylenetetrazole induced convulsion. The extract at 50 mg/kg protected 50% and at 100, 200 mg/kg protected 66.7% of seizures induced by 4-aminopyridine in mice. The extract (50 and 200 mg/kg) produced significant (p < 0.05) increase in mean onset of convulsion induced by 4-aminopyridine in mice. The extract at doses of 50 mg/kg and 100 mg/kg protected 33.3% of the mice against strychnine induced convulsion. Phytochemical screening revealed the presence of alkaloids, steroids, glycosides, saponins, tannins and flavonoids. The results revealed that ethanol stem bark extract of Hymenocardia acida possess bioactive compounds useful in the management of epilepsy.

Keywords: Hymenocardia acida, anticonvulsant, MEST, PTZ, 4-AP, strychnine

INTRODUCTION

Epilepsy is a major neurological disorder that accounts for 0.75% of the global burden of disease (WHO, 2016). World Health Organization estimates that proportions of the general population with active epilepsy (i.e. continuing seizures or the need for treatment) at a given time is 4 to 10 per 1,000 people. However, some studies in developing countries suggest that the proportion is 7 to 14 per 1,000. In developed countries, annual new cases are 30 to 50 per 100,000 people in the general population. In developing countries, this figure is often close to twice as high due to the higher risk of experiencing conditions that can lead to permanent brain damage (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, "kwarto" in Tiv, "emela" in Etulo, "Uchuo" in Igede, "enanche" in...
Idoma (Dalziel, 1937). *Hymenocardia acida* is a dioecious tree up to 6-10 m tall, often stunted. It is found in Savanna region of Nigeria (Schmelzer and Gurib-Fakim, 2008). 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). It is one of the most common plants used in the management of sickle cell anaemia in Nigeria (Ibrahim *et al.*, 2007). The Hausa tribe in Northern Nigeria has over the years used the decoction of leaves and stems bark or root bark for the treatment of pain of various categories such as migraine, sickle cell crisis and menstrual pain (Olotu *et al.*, 2010). Despite improvements in establishing newer antiepileptic drugs and other measures in the management of epilepsy such as surgery, vagus nerve stimulation, deep brain stimulation and so forth, about three fourth of people with epilepsy in low- and middle-income countries do not get the treatment they need (WHO, 2016). WHO encourages the integration of herbal medicines of proven safety in the healthcare programs of developing countries (WHO, 2014). The leaves, stem bark and root bark of *Hymenocardia acida* are used to treat epilepsy among the Idoma and Igede people of North Central Nigeria. Thus, scientific researchers are needed to provide evidences of the efficacy and safety of this beneficial medicinal plant. This study is designed to investigate the anticonvulsant potential of ethanol stem bark extract of *H. acida* in mice and chicks.

**MATERIAL AND METHODS**

**Plant Material**
The stem bark of *Hymenocardia acida* was collected from Dajin tohu around Shika dam, Zaria in the month of June, 2015. It was 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.

**Plant Extraction**
The stem bark (carefully scraped) of *H. acida* was cleaned and air dried under shade until constant weight was obtained. It was then powdered using pestle and mortar and then sieved. About 805g of the stem bark was then macerated with 70% ethanol at room temperature with occasional stirring for two weeks. This was filtered and the clear filtrate evaporated to dryness on a water bath maintained at 60°C until a brown solid residue was obtained. The residue was powdered and stored in a desiccator until required for use.

**Animals**
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. The animals were maintained in a well-ventilated room, fed on standard animal feed and water provided *ad libitum* under standard laboratory conditions in accordance with National Academy of Science, Guides for the Care and Use of Laboratory Animals (1996).

**Drugs and Chemicals**
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). Solvents used include ethanol, butanol,
chloroform and ethyl acetate (Sigma Chemical Co. St Louis USA), other reagents were ferric chloride, dragendorff’s reagent, wagner’s reagent, sulphuric acid, sodium hydroxide, hydrochloric acid and magnesium chips.

**Route of drug administration**
The ethanol stem bark extract of *H. acida*, phenobarbitone, phenytoin, and sodium valproate were administered intraperitoneally. Pentylenetetrazole, 4-aminopyridine and strychnine were administered subcutaneously.

**Phytochemical Screening**
Preliminary phytochemical screening was carried out as described by Trease and Evans (2009). Acute toxicity of ethanol stem bark extract of *Hymenocardia acida* was investigated in mice using the method of Lorke (1983).

**Maximal electroshock-induced convulsion test in chicks**
The method of Swinyard and Kupferberg (1985) and of Browning (1992) was employed. Fifty day old cockerels were randomly divided into five groups of ten each. The first group was pre-treated with normal saline (10 ml/kg) *i.p.*, the second, third and fourth groups were pre-treated with 50, 100 and 200 mg/kg of the ethanol stem bark extract of *H. acida* *i.p.* and the fifth group was pre-treated with 20 mg/kg phenytoin *i.p.* Thirty minutes later, maximum electroshock was administered to induce convulsion in the chicks using Ugobasile electroconvulsive machine (model 7801) connected to a stabilizer with corneal electrodes placed on the upper eyelids of the chicks after dipping them in normal saline. A current (80 mA) which induced tonic seizures in 90% of the control groups of chicks was used. The shock duration, frequency and pulse width was set and maintained at 0.8 secs, 100 pulse/sec and 0.6 ms respectively which were used throughout the study. Seizures were manifested as hind limb tonic extension (HLTE). The ability of the extract to prevent this feature or reduce the mean recovery time of convulsion was considered as an indication of anticonvulsant activity.

**Pentylenetetrazole (PTZ)-induced convulsion in mice**
The method of Swinyard *et al.*, (1989) was employed. Thirty mice were divided into five groups of six each. The first group was pre-treated with normal saline 10 ml/kg *i.p.* The second, third and fourth groups were pre-treated with 50, 100 and 200 mg/kg of the ethanol stem bark extract of *H. acida* and the fifth group pre-treated with 200 mg/kg body weight valproic acid *i.p.* Thirty minutes later, mice in all the groups were injected with PTZ 100 mg/kg body weight subcutaneously and observed for a period of thirty minutes. The absence of tonic extension of limbs for at least 5 seconds duration indicates a compound’s ability to abolish the effect of PTZ on seizure threshold.

**4-aminopyridine (4-AP)-induced convulsion in mice**
The method described by Yamaguchi and Rogawski (1992) was employed. Thirty mice were divided into five groups of six each. The first group was pre-treated with normal saline 10 ml/kg *i.p.* The second, third and fourth groups were pre-treated with 50, 100 and 200 mg/kg of the ethanol stem bark extract of *H. acida* and the fifth group pre-treated with 20 mg/kg body weight phenobarbitone *i.p.* Thirty minutes post treatment, 4-aminopyridine was administered at a dose of 14 mg/kg body weight intraperitoneally to each mouse and observed for a period of thirty minutes for characteristic behavioral signs, such as hyperactivity, trembling, intermittent forelimb extension, tonic seizures and death.
Ability of the extract to protect the mice from lethality within 30 minutes observation period was considered as an indication of anticonvulsant activity.

**Strychnine (STN)-induced convulsion in mice**
The method described by Porter et al., (1984) was employed. Thirty mice were divided into five groups of six each. The first group was pre-treated with normal saline 10 ml/kg i.p, the second, third and fourth groups were pre-treated with 50, 100 and 200 mg/kg of the ethanol stem bark extract of *H. acida* and the fifth group pre-treated with 20 mg/kg body weight phenobarbitone i.p. Thirty minutes post treatment, strychnine 0.25 mg/kg body weight was administered to each mouse subcutaneously and observed for a period of thirty minutes. The proportion of mice presenting convulsion as well as the onset of tonic convulsion was recorded. Abolition of tonic extension jerks of the hind limbs within 30 minutes after strychnine administration was considered as an indication of anticonvulsant activity.

**Statistical Analysis**
Results were expressed as Mean ± Standard Error of Mean (SEM), and percentage. Statistical analysis for difference between means were carried out using One Way Analysis of Variance (ANOVA) followed by Dunnett’s post hoc test. The statistical analysis was performed using SPSS software version 20, values of p ≤ 0.05 were considered significant.

**RESULTS**
The ethanol stem bark extract obtained from *Hymenocardia acida* were dark brown semi solids with pleasant odour. The extract percentage yields was 14.12% w/w. The preliminary phytochemical screening of ethanol stem bark extract of *Hymenocardia acida* revealed the presence of alkaloids, glycosides, saponins, tannins and flavonoids. The intraperitoneal median lethal dose (LD₅₀) of the ethanol stem bark extract of *Hymenocardia acida* in mice was estimated to be 775 mg/kg body weight.

The ethanol stem bark extract of *Hymenocardia acida* at all doses tested did not protect the chicks against convulsion induced by maximal electroshock. The extract at all doses did not produce significant decrease in mean recovery time of convulsed animals. 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 HLTE induced by maximal electroshock.

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

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>% Protection against Seizure</th>
<th>Mean Recovery Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS (10ml/kg)</td>
<td>0.0</td>
<td>9.5 ± 1.3</td>
</tr>
<tr>
<td>ESHA (50)</td>
<td>0.0</td>
<td>6.5 ± 0.5</td>
</tr>
<tr>
<td>ESHA (100)</td>
<td>0.0</td>
<td>7.5 ± 1.3</td>
</tr>
<tr>
<td>ESHA (200)</td>
<td>0.0</td>
<td>10.9 ± 1.4</td>
</tr>
<tr>
<td>PHN (20)</td>
<td>90.0</td>
<td>19.0 ± 0.0*</td>
</tr>
</tbody>
</table>

Values are presented as Mean ± SEM and percentages. 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, ESHA- Ethanol Stem bark Extract of *Hymenocardia acida*, PHN- Phenytoin.
The ethanol stem bark extract of *Hymenocardia acida* produced no significant effect on mean onset of seizures induced by PTZ in mice. However, the stem bark extract at dose of 200 mg/kg body weight produced significant (p<0.01) increase in mean latency to death and produced 50.0% protection against PTZ induced convulsion in mice. The standard anticonvulsant (sodium valproate, 200 mg/kg) produced 66.7% protection against PTZ-induced convulsion and protected 83.33% of mice from mortality.

The ethanol stem bark extract (50 and 200 mg/kg) of *Hymenocardia acida* produced significant (p < 0.05) increase in mean onset of convulsion induced by 4-aminopyridine in mice. Maximal protection (66.7%) against 4-aminopyridine-induced convulsion in mice was recorded with the stem bark extract at doses of 100 and 200 mg/kg body weight. Phenobarbitone (20 mg/kg), the standard anticonvulsant used produced 100% protection against convulsion and mortality.

### Table 2: Effect of Ethanol Stem Bark Extract of *Hymenocardia acida* on Pentylentetrazole (PTZ) - Induced Seizure in Mice

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>% Protection</th>
<th>Mean Onset of Seizure (min)</th>
<th>% Mortality</th>
<th>Mean Latency to Death (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS (10ml/kg)</td>
<td>0.0</td>
<td>4.5 ± 0.8</td>
<td>100</td>
<td>5.8 ± 0.8</td>
</tr>
<tr>
<td>ESHA (50)</td>
<td>33.33</td>
<td>4.3 ± 0.3</td>
<td>50</td>
<td>11.3 ± 1.2</td>
</tr>
<tr>
<td>ESHA (100)</td>
<td>0</td>
<td>4.8 ± 0.8</td>
<td>66.7</td>
<td>7.5 ± 0.9</td>
</tr>
<tr>
<td>ESHA (200)</td>
<td>50</td>
<td>7.7 ± 1.5</td>
<td>50</td>
<td>18.0 ± 1.2**</td>
</tr>
<tr>
<td>SV (200)</td>
<td>66.7</td>
<td>8.0 ± 4.0*</td>
<td>16.7</td>
<td>16.5 ± 12.5**</td>
</tr>
</tbody>
</table>

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, ESHA- Ethanol Stem extract of *Hymenocardia acida*, SV- Sodium Valproate.

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

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>% Protection</th>
<th>Mean Onset of Seizure (min)</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS (10ml/kg)</td>
<td>0.0</td>
<td>11.0 ± 1.1</td>
<td>100.0</td>
</tr>
<tr>
<td>ESHA (50)</td>
<td>50</td>
<td>17.3 ± 0.3*</td>
<td>50.0</td>
</tr>
<tr>
<td>ESHA (100)</td>
<td>66.7</td>
<td>17.5 ± 2.5</td>
<td>33.3</td>
</tr>
<tr>
<td>ESHA (200)</td>
<td>66.7</td>
<td>19.5 ± 0.5*</td>
<td>33.3</td>
</tr>
<tr>
<td>SV (200)</td>
<td>100</td>
<td>0.0 ± 0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

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, ESHA- Ethanol stem extract of *Hymenocardia acida*, PHEB- Phenobarbitalone.
Table 4: Effect of Ethanol Stem Bark Extract of *Hymenocardia acida* on Strychnine Induced Convulsion in Mice

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>% Protection</th>
<th>Mean Onset of Seizure (min)</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS (10ml/kg)</td>
<td>16.7</td>
<td>7.2 ± 1.2</td>
<td>83.3</td>
</tr>
<tr>
<td>ESHA (50)</td>
<td>33.3</td>
<td>12.5 ± 66.7</td>
<td></td>
</tr>
<tr>
<td>ESHA (100)</td>
<td>33.3</td>
<td>12.5 ± 66.7</td>
<td></td>
</tr>
<tr>
<td>ESHA (200)</td>
<td>0.0</td>
<td>9.0 ± 100.0</td>
<td></td>
</tr>
<tr>
<td>SV (200)</td>
<td>100</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

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, ESHA- Ethanol stem extract of *Hymenocardia acida*, PHEB- Phenobarbitone.

**DISCUSSION**

The LD$_{50}$ values for the ethanol stem bark extract of *Hymenocardia acida* following intraperitoneal administration suggested that the extract was relatively less toxic according to classification of LD$_{50}$ values by Matsumura, (1975) and Corbett et al., (1984)

MEST is a standard AED test that evaluates the testing material’s ability to protect against hind limb tonic extension (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). Such protection projects anticonvulsant activity of AEDs that prevent spread of epileptic seizure discharge from an epileptic focus during seizure activity (Raza et al., 2001). Currently available AEDs that are clinically effective in the treatment of generalized tonic-clonic and partial seizures such as carbamazepine, oxcarbazepine and lamotrigine also suppress HLTE in MEST (Browning, 1992). 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 stem bark extract of *Hymenocardia acida* did not produce significant effect on maximal electroshock induced convulsion.

Anticonvulsant activity in PTZ test identifies compounds that can raise the seizure threshold in the brain (Raza et al., 2001). Antiepileptic drugs effective in the therapy of generalized seizure of petit mal type exhibit dose dependent suppression of PTZ induced seizure (McNamara, 2006) e.g. phenobarbitone, valproate, ethosuximide and benzodiazepines. PTZ has been shown to interfere with GABA neurotransmitter and the GABA$_A$ receptor complex (DeDyn et al., 1992). Antagonism of PTZ-induced seizure suggests potentiating effect on GABAergic neurotransmission. The increase in the latency of seizures and some levels of protection by *Hymenocardia acida* extract against threshold seizure induced by PTZ suggests it could be effective in the therapy of absence or myoclonic seizures.

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). Drugs like phenytoin, which block seizure spread are effective antagonists of K$^+$ channel, while those with specific actions on other cellular targets may be weak or inactive, presumably because they are unable to attenuate the spread of intense (non-NMDA receptor mediated) excitation evoked by 4-aminopyridine (Yamaguchi and Rogawski, 1992). The ability of the ethanol stem bark extract 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 stem bark extract of *Hymenocardia acida* did not produce effect on strychnine induced convulsion. Strychnine directly antagonizes the inhibitory reflexes mediated by glycine (Sayin et al., 1993).
Phytochemical screening of the ethanol leaf and stem bark extracts of *Hymenocardia acida* revealed the presence of phytochemical constituents among which flavonoids and saponins have been reported to possess anticonvulsant activity (Kavvadias *et al.*, 2004) and may therefore be responsible for anticonvulsant potential of the stem bark extracts of *Hymenocardia acida*.

**CONCLUSION**

The study revealed that ethanol stem bark extract of *Hymenocardia acida* possesses anticonvulsant activity against pentylenetetrazole induced seizures, hence may support the ethnomedicinal use of the plant in the treatment of epilepsy of petitmal (absence) type.

**REFERENCES**


