Original Paper

Evaluation of *Mitragyna inermis* (wild) leaf extract as an anticonvulsant agent in pentylenetetrazole induced seizures in mice.

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Abstract

Epilepsy is a neurological setback in developing countries especially in Nigeria. Current drug therapies are either expensive or have associated side effects. *Mitragyna inermis* (Rubiaceae) leaves are used in northern Nigeria traditionally for managing epilepsy. The methanolic extract of *Mitragyna inermis* was found to contain alkaloids, glycosides, tannins, carbohydrate, cardiac glycosides, saponins and terpenoids. The extract at 1200 and 600 mg/kg significantly (p<0.05) increased the mean time for onset of convulsion, the mean time for duration of convulsion (latency) and offers a relative protection from death in PTZ induced seizures suggesting it as a therapeutic potential for managing epilepsy.

Keywords: *Mitragyna inermis*, Pentylenetetrazole, Convulsions, Epilepsy.

Introduction

Epilepsy is one of the severe neurological disorder affecting about 50 million people worldwide, majority of which live in developing countries [1]. The seizures occur due to excessive electrical discharge in the brain and the pattern depends not only on the cause but also the origin, extent, intensity and thereof epileptic discharge in the brain [2]. Epilepsy in Nigeria has been found as psychosocial burden [3]. In Nigeria and other parts of Africa, there is a traditional belief that epilepsy is contagious and/or caused by supernatural factors [4, 5]. These negative beliefs contribute to the stigma and segregation experienced by affected persons, with significant social, economic and medical implications [6].

Current antiepileptic drugs (phenytoin, sodium valproate, diazepam, paraldehyde) have been used to manage epileptic patients in developing countries with little success and a lot of gap (Number of individuals going untreated) as high as 90% [7] and factors which contribute to these huge gaps in drug management include; lack of prioritization, well-structured health care system, price control and a limited and often erratic supply of antiepileptic drugs [8] which has led to profound social consequences for sufferers in the developing countries. [8]. Treatment gap together with cultural beliefs and attitudes about the source and treatment of epilepsy, for example, the attribution of its cause to evil spirit, may influence individuals in the developing countries to seek help from traditional healers rather than from western medicine [9, 10]. Traditional healers make use of herbs for the treatment of epilepsy.

*Mitragyna inermis* (Family Rubiaceae) is one of the plants used to manage epilepsy in Northern Nigeria and is proven to have alkaloids as its major bioactive substances which is structurally similar to clinically useful anticonvulsant [11].
This work seeks to evaluate the antiepileptic effects of methanolic leaf extract of *Mitragyna inermis* in pentylenetetrazole induced seizures in mice in order to ascertain the activity of the plant as used by most Hausa/Fulani Community of Northern Nigeria.

**Materials and Methods**

**Collection and preparation of plant material**

Fresh leaves of *Mitragyna inermis* were collected from a bush in Damboa, Damboa Local Government Area Borno State with the help of a traditional medical practitioner and was identified by a botanist in the Department of Biological Sciences, University of Maiduguri where voucher specimen number(20032010) was assigned and deposited in the herbarium within the Faculty of Pharmacy, University of Maiduguri.

**Extraction of the plant materials**

The collected fresh leaves were shade dried and later grinded into powdered form using a grinding machine. Grinded powder was weighed until a constant weight was obtained. About 400 g of the powder was weighed and subjected to maceration and further 1000 ml of methanol was added and left for 24 hours. The mixture was then filtered and the residue was soaked in another 500 ml of methanol for another 24 hours. The procedure was repeated for 48 hours and then the filtrate was evaporated under reduced pressure using rotary vacuum evaporator at 45°C, the content was air-dried and the methanolic extract was obtained. The methanolic extract was then used for the preliminary phytochemical screening, acute toxicity and anticonvulsant evaluation.

**Animal model**

Mice weighing 25-35 g were obtained from the Disease Free Animal House of Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Maiduguri, Nigeria. The animals were allowed to acclimatize to the laboratory environment for two weeks and were provided with water and food continuously. After the animals were acclimatized, they were divided into five groups of 5 animals each for experiments.

**Preliminary Phytochemical screening**

A preliminary study of methanolic leaf extract of *Mitragyna inermis* was done to investigate it for presence of alkaloids, saponins, tannin, glycosides, flavonoids, steroids, terpenes, carbohydrates, anthraquinones, and reducing sugar using the standard laboratory procedures described by Trease and Evans [12] and Sofowora [13].

**Pharmacological Studies**

**Acute toxicity**

The acute toxicity (LD<sub>50</sub>) of the methanolic leaf extract of *Mitragyna inermis* was determined using modified Lorke’s method [14] through intraperitoneal routes in mice. The dose levels of 10, 100, 1000 and 2000 mg/kg were used in phase 1. Mortality was observed for 24 hours.

**Pilot Studies**

Two mice each (25-35 g) were administered Pentylenetetrazole (PTZ) (SIGMA-ALDRICH, Co., 3050 Spruce street, St. Louis, MO 63103 USA 314-771-5765) 30 mg/kg, 60 mg/kg, 120 mg/kg and 10 ml/kg of DMSO (IP) and observed for onset of jerk, onset of convulsions and death. The appropriate convulsive dose of PTZ was determined and used for the study.

**Pentylenetetrazole Induced Seizure in mice.**

Twenty five mice (25-35 g) were grouped into 5 groups of 5 mice each. The first group received DMSO 10 ml/kg, the second group received sodium valproate ( Sigma life Science; SIGMA-ALDRICH, MO 63103 USA 314-771-5765) 200 mg/kg, the third, fourth and fifth groups received the methanolic leaf extract of *Mitragyna inermis* 1200, 600 and 300 mg/kg respectively. Thirty minutes after pretreatment with the extract all the mice were administered PTZ 60 mg/kg and observed for onset of jerk, onset of convulsion and death. Absence of convulsions for at least 5 seconds duration indicates ability of the extract to attenuate the effects of pentylenetetrazole on seizure threshold [15].

**Statistical Analysis**

The results were analyzed using Graph Pad Instat software. Pooled students t-test was used to determine the level of significance of the variable data. p<0.05 was considered significant, p<0.01 was considered highly significant and p<0.001 was considered extremely significant.

**Results**

**Preliminary phytochemical screening**

The preliminary phytochemical screening of the methanolic leaf extract of *Mitragyna inermis* showed the presence of carbohydrates, tannins, cardiac glycosides, saponin glycosides, flavonoids and alkaloids. However, anthraquinone was found to be absent in the extract. Additionally no results were found in hydrochloric acid and sodium hydroxide tests for tannins and alkaloids respectively (Table 1).
Acute toxicity studies of Mitragyna inermis

Acute toxicity studies of methanic leaf extract of Mitragyna inermis was found to be relatively very less or nontoxic in mice when compared with the lethal dose (that can kill 50% of the mice) (LD$_{50}$), which was found to be greater than 2000 mg/kg (intraperitoneally) after the phase 1. (Table 2).

Table 1: Quantitative phytochemistry of methanolic leaf extract of Mitragyna inermis

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Test</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>General test (Molisch’s test)</td>
<td>Red colour</td>
<td>++</td>
</tr>
<tr>
<td>Test for monosaccharide (Barfoed’s)</td>
<td>Precipitate formed</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Test for reducing sugar (Fehling’s)</td>
<td>Red Precipitate</td>
<td></td>
<td>+ +</td>
</tr>
<tr>
<td>Test for ketosis (Salivanoff’s test)</td>
<td>Red Colour</td>
<td></td>
<td>+ +</td>
</tr>
<tr>
<td>Test for pentose</td>
<td>No colour</td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric test</td>
<td>Blue-green precipitate</td>
<td>+ + +</td>
</tr>
<tr>
<td>Lead acetate</td>
<td>Precipitate formed</td>
<td></td>
<td>+ +</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td>No colour</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>Test for free (Borntrager’s)</td>
<td>No colour</td>
<td>-</td>
</tr>
<tr>
<td>Test for combined</td>
<td>No colour</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Cardiac Glycoside</td>
<td>Salkowski test</td>
<td>Reddish-brown colour</td>
<td>+</td>
</tr>
<tr>
<td>Lieberman-Burchard test</td>
<td>Violet to blue colour</td>
<td></td>
<td>+ + +</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Reddish-brown colour</td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>Saponin Glycosides</td>
<td>Frothing test</td>
<td>Frothing formation</td>
<td>+ + +</td>
</tr>
<tr>
<td>Fehling test</td>
<td>Brick-red precipitate</td>
<td></td>
<td>+ +</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>Shinoda’s test</td>
<td>Red colour</td>
<td>+ +</td>
</tr>
<tr>
<td>Ferric chloride test</td>
<td>Violet colour</td>
<td></td>
<td>+ + +</td>
</tr>
<tr>
<td>Lead acetate</td>
<td>Precipitate formed</td>
<td></td>
<td>+ +</td>
</tr>
<tr>
<td>Sodium hydroxide test</td>
<td>No colour</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Dragendorff’s reagent</td>
<td>Orange precipitate</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Mayers reagent</td>
<td>Buff-coloured precipitate</td>
<td>+</td>
</tr>
</tbody>
</table>

- = absent, + = present in small quantity, ++ = present in moderate quantity, +++ = present in large quantity,

Table 2: Acute toxicity studies of methanolic leaf extract of Mitragyna inermis in mice

<table>
<thead>
<tr>
<th>Phases</th>
<th>Dose levels (IP)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phase 1</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 mg/kg</td>
</tr>
<tr>
<td></td>
<td>100 mg/kg</td>
</tr>
<tr>
<td></td>
<td>1000 mg/kg</td>
</tr>
<tr>
<td></td>
<td>2000 mg/kg</td>
</tr>
<tr>
<td><strong>LD$_{50}$</strong></td>
<td>&gt;2000 mg/kg</td>
</tr>
</tbody>
</table>

IP = Intraperitoneal route, LD$_{50}$ = Lethal Dose that can kills 50% of mice
Anti-convulsant activity of *Mitragyna inermis* leaf extracts in pentylenetetrazole induced seizures

The methanolic extract of *Mitragyna inermis* exhibited a significant anticonvulsant activity in a dose dependent manner compared to the negative control. Sodium valproate was used as a positive anti-convulsant agent as was found to be statistically significant at 300 mg/kg (p<0.001) and 600 mg/kg (p<0.01). However, there was no significant difference between the anticonvulsant activity of the extract at the highest tested dose (1200 mg/kg) and sodium valproate (p>0.05). The percentage protection of the methanolic leaf extract against PTZ induced epileptic seizures was increased with increase in the extract dose. (Table 3).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Onset of Clonic Convulsion in Seconds (Mean±SEM)</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (DMSO)</td>
<td>Vehicle (10 ml/kg)</td>
<td>31.60±3.20</td>
<td>00</td>
</tr>
<tr>
<td>Sodium Valproate</td>
<td>200</td>
<td>417.60±20.80</td>
<td>100</td>
</tr>
<tr>
<td>MI extract</td>
<td>1200</td>
<td>408.20±31.50</td>
<td>40</td>
</tr>
<tr>
<td>MI extract</td>
<td>600</td>
<td>250.40±5.90**</td>
<td>20</td>
</tr>
<tr>
<td>MI extract</td>
<td>300</td>
<td>100.50±4.30***</td>
<td>00</td>
</tr>
</tbody>
</table>

Vehicle = Dimethyl sulphoxide (DMSO), MI = *Mitragyna inermis*, Student T-test, n = 5,
***= p˂0.001 was considered extremely significant when compared with sodium valproate,
**= p˂0.01 was considered highly significant when compared with sodium valproate,
*= p˂0.05 was considered significant when compared with sodium valproate

### Discussion

The phytochemical constituents detected in this study are in agreement with several literature reports [16-19] in which similar active principles have been detected. However, the findings of Timothy and colleagues [16] in which anthraquinone was found to be present did not agree with the present study which may be due to the difference in plant part or solvent system used Uthman and coworkers [17] did not observe the presence of terpenoids in their study which is contradiction towards the current study. Wakirwa *et al* [18] and Konkon *et al* [19] did not detect saponins and tannins despite the use of the same part of plant and solvent systems with the present study. The presence of these active phytochemical constituents in the methanolic leaf extract of *Mitragyna inermis* as reported in present study may be responsible for its antimicrobial, antidiabetic, antimalarial, antihypertensive, analgesic, antiarthritis and anticonvulsant activities[16, 20].

The acute toxicity studies of methanolic leaf extract of *Mitragyna inermis* in mice indicates that the extract is relatively less toxic which is in agreement with the reports of Timothy and colleagues [16, 21], Uthman *et al* [17], Adoum *et al* [20] and Konkon *et al* [19] were different plant extracts have also been found to be less toxic in respective test animals.

The presence of phytochemical constituents in the plant such as alkaloids [16, 22], tannins [17, 21], saponins and flavonoids [17] may be responsible for the observed anticonvulsant effects. Alkaloids have been shown to potentiate GABA effect, while tannins, saponins and flavonoids have been shown to modulate CNS activities which can easily be linked to anticonvulsant activity [17, 21]. PTZ used as convulsant agent in this study has been reported to produce seizures by inhibiting GABA neurotransmission & by blocking chloride channel linked.
to GABA<sub>A</sub> receptors [23, 24]. Therefore, the protection afforded by the Mitragyna inermis in PTZ induced convulsion may be attributable to its ability to potentiate GABA<sub>A</sub> receptors [25].

**Conclusion**

The results of this study suggest that methanolic leaf extract of Mitragyna inermis contains bioactive principles which may be responsible for the observed anticonvulsant activity and may be beneficial in the management of epilepsy. Additionally the observations are in support of the traditional use of this plant in the management of epilepsy. However further studies are wanted to determine the exact mechanism of the action.

**Acknowledgement**

The authors are sincerely thankful to Mr Maspalma I. Dauda and Mr Sibiya Usman of Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Maiduguri for their technical assistance and support.

**Conflict of interest**

The authors declare that there is no conflict of interest to reveal.

**References**


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