Phytochemical and Anticonvulsant Activity of the Ethanol Root Bark Extract of *Mimosa pigra* L. (Fabaceae) in Laboratory Animals


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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

**Background and objectives**: Various parts of *Mimosa pigra* (MPG) are used in traditional medicines to treat convulsive disorders. The objective of this study was to investigate the anticonvulsant properties of *Mimosa pigra* ethanol root extract (EREM).

**Methods**: The acute toxicity of the extract was investigated using OECD 423 protocol of 2002. The anticonvulsant properties of EREM at 200, 400, and 800 mg/kg were evaluated using Maximal Electroshock Test (MEST) in chicks; strychnine (SCN−) and pentylenetetrazole (PTZ)-induced seizures in mice.

**Results**: The extract at 400 and 800 mg/kg significantly (p<0.05) prolonged the mean onset of clonic and tonic convulsions in mouse model of SCN-induced seizure. In PTZ-induced seizure, the extract at 400 mg/kg significantly (p<0.05) increased the mean onset of clonic seizure, while at 800 mg/kg, there was significant (p<0.05) prolongation in the mean onset of clonic and tonic seizure compared to control. The extract did not protect the chick against MEST but significantly (p < 0.05) reduced the mean recovery time at the of 200, 400 and 800 mg/kg. The extract offered 60 and 100% protection at 400 and 800 mg/kg respectively in SCN-induced seizure. Similarly, EREM offered 20 and 40% protection at 400 and 800 mg/kg respectively in PTZ-induced seizure. Diazepam (10 mg/kg), a reference drug significantly (p<0.05) prolonged the onset of clonic-tonic seizure and protected against SCN−, and PTZ-induced convulsion in mice.

**Conclusion**: These findings indicated that EREM may possess anticonvulsant activity in SCN−, and PTZ-induced seizure in mice. Thus, lend scientific credence to the anticonvulsant claim of EREM in ethnomedicine.

**Keywords**: *Mimosa pigra*; Ethnomedicinal claim; Maximal Electroshock Test; Pentylenetetrazole; Strychnine
INTRODUCTION

Epilepsy is a heterogeneous group of disorders characterized by neuronal hyper-excitability and hyper-synchronous neuronal firing. About 5% of the global population have experienced at least one epileptic attack once in their lifetime (Kumar et al., 2014). The mainstay approach to the management of epileptic attacks involves the use of antiepileptic drugs which are only able to control or reduce the frequency of seizures in about 50% of patients on them (Bora & Singh, 2020). In addition to this, most of these drugs are expensive and cause undesirable adverse effects. This emphasizes the need to design new agents to treat convulsive and related neurological disorders. Medicinal plants represent a very rich source of therapeutic phytoconstituents and some of which have been scientifically validated for their anticonvulsant potentials (Adebayo et al., 2020; Akinpelu et al., 2023).

*Mimosa pigra* L is a plant indigenous to tropical African, Northern American, and warm oceanic island regions. It is leguminous in nature, and it belongs to Fabaceae (Leguminosae) plant family. It is commonly referred to as ‘Oniwa-agogo’ in Yorubaland (Okonkwo et al., 2016). In ethnomedicine, the different parts of this plant have been reported to be used in the treatment of asthma, respiratory diseases, diarrhoea, gonorrhea, typhoid fever, and genitourinary tract infection (Sonibare & Gbile, 2008; Mbatchou et al., 2011). Pharmacologically, the anti-diabetic, anti-inflammatory and analgesic activities have been reported (Shorinwa et al., 2015) Phytochemical studies of the genus Mimosa revealed the presence of flavonoids, steroids, saponins, alkaloids, coumarins, tannins (Rizwan et al., 2022). Report has also shown that the root bark of this plant is used to treat epilepsy and other convulsive disorders. However, the anticonvulsive action of the root of this plant has not been scientifically proven despite the marked anticonvulsant properties of the Mimosa family such as *Mimosa pudica* (Ngo Bum et al., 2004)

The current study aims to investigate the anticonvulsant potential of the ethanolic root bark of *Mimosa pigra* in mice and chicks.

METHODOLOGY

Equipment and Drugs

Ugo Basile electroshock machine, pentylenetetrazole (PTZ), Strychnine (SCN) (Sigma Aldrich, UK), Phenytoin sodium (Pfizer Global Pharmaceuticals, USA), Normal saline (Juhel Nigeria Ltd, Enugu), Diazepam (Valium®- Roche products Ltd, Canada).

Collection and Plant Material

The plant material used in this study was based on the result of literature search on the ethnomedicinal uses of plants that belong to the Fabaceae family used in neurodegenerative disorders. *Mimosa pigra* (MEP) root bark was obtained from the medicinal plant garden at the University of Ibadan and authenticated by Mr. Bolu Ajayi of the Department of Plant Biology, Faculty of Life Sciences, University of Ilorin (Voucher number: UILH/002/1561/2022).

Processing and Extraction of Plant Material

The fresh roots of MPG were washed with water and the root bark excised, and dried under the shade until a constant weight was achieved. The barks were size reduced and subsequently ground into coarse powder. This was followed by extracting approximately 500 g of the powdered root barks in 1000 ml of 90% ethanol for 72 h until extraction is completed. The extract was filtered and the filtrate was evaporated to dryness and theType equation here. dried extract was stored in the refrigerator until the time of use.

Experimental Animals

Swiss Albino mice and day-old rangers cockerels were employed in this study. The mice were obtained from the Animal House Facility of the Department of Pharmacology and Toxicology, University of Ilorin, while the day-old rangers cockerels were obtained from the National Animal Production Research Institute (NAPRI), Kaduna State Nigeria. The mice were conserved at ambient temperature (22°C) and light, fed with standard rodent feed, and water was provided ad libitum. All experimental protocol complied with the National Institute of Health Guide for the Care and Use of Laboratory animals (Publication No 5-23, revised 1996). The study protocol was approved by the ethics committee of the Faculty of Pharmaceutical Sciences, University of Ilorin, with ethics approval number of FPS-ERS/ASN/2023/3 assigned to the study.

Phytochemical Screening

The phytoconstituents of the root bark extract of the plant was evaluated using previously reported standard methods (Harborne, 1973; Sofowora, 1993; Evans, 2009).
Acute Toxicity Study of the MPG Extract

The acute toxicity of the ethanolic root bark extract of MPG was determined using standard methods described in the OECD 423 guideline (OECD, 2008). Overnight fasted mice were divided into two groups (n=3 per group) and administered with 2000 mg/kg oral dose of the extract and continuously monitored for different evidence of autonomous central nervous system functions toxicity or death, and behavioral changes within a period of 2 hours, and after a period of 24 hours, 72 hours, and up to 14 days after administration of the extract. Half maximal (LD₅₀) lethal dose was thereafter determined. The same procedure was adopted to determine the 50% lethal dose LD₅₀ in chicks.

Maximal Electroshock-induced seizure in chick

The method described by (Swangard & Kupferber, 1985) as modified by (Sayyah et al., 2002) was employed in this study. A total of fifty day-old white cockerels divided into five groups (n=10 per group) were employed in this study. Each of the five groups were coded either as negative control consisting of 10 ml/kg of normal saline, 200 mg/kg, 400 and 800 mg/kg of MPG extract, and a positive control (20 mg/kg phenytoin). The mice were pre-treated with the different solutions in the above groups via the intraperitoneal route of administration. Following pre-treatment, maximal electroshock seizures was induced in the chicks using the Ugo Basile electroconvulsive machine (Model 7801) connected to Claude Lyons stabilizer with corneal electrodes placed on the upper eyelids of the chicks. The shock duration, frequency and pulse width were set and maintained at 0.80s, 200 pulse per second and 0.8ms respectively. A current of about 90mA, which produced tonic seizures in 90% of the control chicks, was used throughout the study. Seizures were manifested as tonic hind-limb extension (THLE). The ability to prevent this feature or prolong the latency and or onset of the THLE was considered as an indication of anticonvulsant activity (Sayyah et al., 2002).

Strychnine-induced seizure in mice

Strychnine-induced seizure was induced in a total of 25 mice using a previously reported method (Porter et al., 1984). The animals were divided into five groups (n=5 per group) and each group was pre-treated with one of the negative control (10 ml/kg of normal saline) orally, 200 mg/kg, 400 mg/kg , 800 mg/kg or the positive control (10 mg/kg diazepam intraperitoneally). All animals were injected with 2 mg/kg of strychnine 1 hour after pre-treatment via the sub-cutaneous route of administration. The animals in each group were monitored for evidence of tonic-clonic convulsion for thirty minutes. Animals that survive beyond thirty minutes were considered protected. The percentage protection was calculated as follows:

% protection = (number of animals that survived in each group/total number of the animals in each group) x 100

Statistical analysis

The results were expressed as Mean ± Standard Error of Mean (S.E.M). The significance of difference between the treatment groups and control group were analyzed using One-way Analysis of Variance (ANOVA) followed by Dunnett’s post-hoc test against a single control using Graphpad InStat® Biostatistics software (GraphPad Software, Inc.,L. Jolla, USA). P-values lesser than 0.05 were considered statistically significant.
RESULTS

Phytochemical analysis of *Mimosa pigra* ethanol root bark extract
The presence of biologically active phytoconstituents such as alkaloids and phenols were identified in the root bark of MPG root extract. Table 1 summarizes the results of the phytochemical screening of the plant extract.

Table 1: Phytochemical constituents of the ethanol root bark extract of *Mimosa pigra* (EREM).

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>Present</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Absent</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Present</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Present</td>
</tr>
<tr>
<td>Steroids</td>
<td>Absent</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Present</td>
</tr>
<tr>
<td>Phenol and Tannins</td>
<td>Present</td>
</tr>
</tbody>
</table>

Maximal Electroshock Test
Relative to the negative control (normal saline), *Mimosa pigra* ethanolic root bark extract significantly (P<0.05) reduced the average time required to recover from seizure. The mean recovery time was shortest at the 800 mg/kg dose level. Detailed result of the electroshock test is presented in Table 2.

Table 2: Effect of EREM and Phenytoin on maximal electroshock–induced seizure in chicks.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Onset of action(sec)</th>
<th>Recovery(sec)</th>
<th>Duration of action(sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-S 10ml/kg</td>
<td>768.10±0.53</td>
<td>761.10±11.34</td>
<td>660.00±12.19</td>
</tr>
<tr>
<td>EREM 200mg/kg</td>
<td>129.20±0.25a</td>
<td>137.60±106a</td>
<td>504.00±62.09</td>
</tr>
<tr>
<td>EREM 400mg/kg</td>
<td>118.50±0.34a</td>
<td>126.10±0.72a</td>
<td>456.00±49.95</td>
</tr>
<tr>
<td>EREM 800mg/kg</td>
<td>113.80±0.33a</td>
<td>110.70±8.18a</td>
<td>534.00±62.89</td>
</tr>
<tr>
<td>Phenytoin 20mg/kg</td>
<td>0.00±0.00a</td>
<td>0.00±0.00a</td>
<td>0.00±0.00a</td>
</tr>
</tbody>
</table>

Data presented as Mean±S.E.M. *a* Significantly (p < 0.05) different compared to control group, where N-S = Normal saline and n=5. EREM- Ethanol root bark extract of Mimosa pigra.

Effect of diazepam on strychnine and pentylenetetrazole –induced seizures in mice
The results of the effect of diazepam on strychnine and pentylenetetrazole -induced seizures in mice is presented in Tables 3 and Table 4, respectively. The ethanol root bark extract of the *Mimosa pigra* significantly prevented strychnine-induced seizures in a dose dependent manner. A dose-dependent substantial reduction in the onset time of jerking and convulsion was observed at all dose levels relative to the negative control. The percentage protection rose from 60% to 100% when the administered dose of the extract was increased from 400 mg/kg to 800 mg/kg. A similar trend was observed in the pentylenetetrazole -induced mice. However, the protective effect of extract on the pentylenetetrazole -induced mice was lower compared to the strychnine-induced mice.

Table 3: Effect of EREM and Diazepam on Strychnine-induced seizures in mice.

<table>
<thead>
<tr>
<th>Treatment Group (n=5 per group)</th>
<th>Seizure onset ± S.E.M. (min.)</th>
<th>Mortality Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clonic</td>
<td>Tonic</td>
</tr>
<tr>
<td>Control-NS (1 ml/kg)</td>
<td>3.72±0.41</td>
<td>4.77±0.51</td>
</tr>
<tr>
<td>EREM (200 mg/kg)</td>
<td>2.52±0.26</td>
<td>3.30±0.65</td>
</tr>
<tr>
<td>EREM (400 mg/kg)</td>
<td>10.58±1.77a</td>
<td>8.65±4.17a</td>
</tr>
<tr>
<td>EREM (800 mg/kg)</td>
<td>7.61±4.95a</td>
<td>0.00±0.00a</td>
</tr>
<tr>
<td>Diazepam (10 mg/kg)</td>
<td>30.00±0.00a</td>
<td>30.00±0.00a</td>
</tr>
</tbody>
</table>

Data presented as Mean±S.E.M. *a* Significantly (p < 0.05) different compared to control group, where N-S = Normal saline, EREM- Ethanol root bark extract of Mimosa pigra.
The inhibition of pentylenetetrazole-induced seizures by EREM also suggests that the extract may compete with pentylenetetrazole for GABA receptor, particularly GABA\textsubscript{A} receptor, therefore altering GABAergic neurotransmission. On the other hand, EREM may also exert its anticonvulsant effect via antagonistic effect on glutamate via the NMDA receptor since PTZ has been shown to exert its chemoconvulsant effect via agonistic action on glutamate via the NMDA receptor neurotransmission. The presence of flavonoid and alkaloid in EREM may at least in part be attributed to the anticonvulsant effect of EREM as GABA agonist and NMDA receptor antagonist in a similar fashion with antiepileptic drugs (Sharifi-Rad et al., 2021).

Maximal electroshock seizure threshold (MEST) is a classical measure of seizure sensitivity with a wide range of utilization (Ferraro et al., 2002). It provides an indication of a compound’s ability to prevent seizure spread when all neuronal circuits in the brain are maximally active (Zuliani et al., 2010). Maximal electroshock test is a model for generalized tonic-clonic (grand mal) seizures, and one of the well validated methods of determining antiepileptic activity in preclinical studies. It involves the induction of an electrical stimulus of sufficient intensity to produce maximal seizures of hind limbs, with tonic extension as the endpoint of the test. By reducing the rate at which voltage-activated sodium channels recover from inactivation, antiepileptic drugs can reduce the recurrent firing of action potentials and decrease hind limb tonic extension in maximum electroshock seizures (Ferraro et al., 2002; Zuliani et al., 2010).

Thus, the above findings possibly indicates that EREM does not act via this mechanism. Hence, its use may be limited in the treatment of generalized tonic-clonic seizures.

Although there was no observed anticonvulsant potential of the extract in MEST but worth mentioning is the reduction in the mean recovery time from seizure among the groups of chicks administered with varying doses of the plant extract relative to the baseline, with highest recovery time observed at the 400 mg/kg dose.

### DISCUSSION

The anticonvulsant potential of the root bark of *Mimosa pigra* extract was investigated using mouse models of pentylenetetrazole (PTZ-) and strychnine (SCN)-induced convulsion as well as maximal electroshock (MEST) test in this study. Our results showed that the ethanol root bark extract of *Mimosa pigra* (EREM) may possess anticonvulsant effects in PTZ- and SCN-induced convulsion models. The acute oral toxicity study showed that the extract was not toxic at 2000 mg/kg suggesting the safety of this extract since no mortality was recorded at this concentration in both mice and chicks. Therefore, doses of 200, 400 and 800 mg/kg were used to evaluate the anticonvulsant potential of EREM in this study.

The elongation of the mean onset of tonic-clonic convulsion and protection offered by EREM in strychnine-induced convulsion model suggests that the extract may possess anticonvulsant effect. The anticonvulsant potential of EREM may be mediated via enhancement of the inhibitory actions of glycine in the spinal cord since Strychnine exerts its effect by blocking glycine from binding to the glycine-gated chloride channel thereby reducing the ability of the spinal cord to propagate nerve signals (Zehra et al., 2021). This finding is in line with previous studies of medicinal plants showing anticonvulsant effect in strychnine-induced convulsion model in mice (Ngo Bum et al., 2004; Adebayo et al., 2020; Akinpelu et al., 2023).

Pentylenetetrazole induce seizure by acting as GABA\textsubscript{A} receptor antagonist and PTZ may induce seizure as NMDA receptor agonist, since the excitotoxicity of glutamate neurotransmission on the brain is mediated through the activation of NMDA receptors (Löschler et al., 1993; Ghasemi et al., 2014; Wang & Reddy, 2017). Therefore, GABA\textsubscript{A} receptor agonist and NMDA receptor antagonist are well known to exert anticonvulsant effects (Ghasemi et al., 2014; Löschler et al., 1993; Nasiri-Boroujeni et al., 2021; Wang & Reddy, 2017). The inhibition of pentylenetetrazole-induced seizures by EREM also suggests that the extract may compete with pentylenetetrazole for GABA receptor, particularly GABA\textsubscript{A} receptor, therefore altering GABAergic neurotransmission.
CONCLUSION
The present study concluded that the root bark extract of *Mimosa pigra* possess anticonvulsant properties, thus leading credence to its traditional use in the management of convulsive and related disorders. This study further established the safety of the root extract of plant in mice and chicks. The safety of the plant in human may require further investigation.

REFERENCES


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Conflict of Interest: None declared

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