Evaluation of the Anticonvulsant Effect of the Methanol Extract of Evolvulus alsinoides in Mice

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Abstract: The purpose of this research work is to study the anticonvulsant activity of Evolvulus alsinoides Linn (convolvulaceae) used in traditional medicine for the treatment of neurodegenerative diseases, amnesia, mental illness and epilepsy. In this study, the anticonvulsant activity of the crude methanolic extract was investigated at doses of 50, 100, 200 and 400 mg/kg using the Pentyleneetetrazole (PTZ) induced seizure (chemically induced seizure) and maximal electroshock seizure (electrically induced seizure) models in mice. The sedative hypnotic effect was also studied using the Diazepam induce sleep model. The onset of diazepam induced sleep was decreased dose dependently; there was a marked increase in duration of sleep to 4-7 times the duration of sleep in the control group. The extract at doses of 100-400 significantly increased the latency of PTZ induced seizure; there was a 100% protection against seizure at the highest dose of 400 mg/kg. In the MEST test a dose dependent decrease in the duration of seizure was also observed with all the doses administered.400 mg/kg of the extract and 30mg/kg Diazepam shows the highest activity in this test. These findings suggest that the methanol extract of the plant contains bioactive principles that may be beneficial in the treatment of epilepsy.

Keywords: Diazepam, epilepsy, seizure, Evolvulus alsinoides, methanol, pentyleneetetrazole.

INTRODUCTION

Traditional, alternative or herbal medicine refers to medical/medication practices other than orthodox medicine/medical practice. The name as called in different places symbolizes safety in contrast to synthetic or orthodox medicines [1]. The use of herbal medicine as an adjunct or substitute to orthodox medicine has gained much popularity worldwide. The WHO reported that about 80% of people in the third world countries depend wholly or partly on herbal medicines for their medications [2]. Most researchers have tried to provide a scientific link/basis to explain the traditional claims of efficacy of most herbs by using in vitro and in vivo experimental models.

Neurological disorders such as epilepsy, Parkinson disease, Alzheimer disease, psychiatric disorders and depressive illnesses have been studied extensively [3,4,5]. The link between herbs and the management of these diseases have been investigated through researches using various in vitro and in vivo models. Anticonvulsant activity of plants have been studied by a lot of researchers, example includes Casimiroa edulis [6], Rhus chirindensis stem bark [7], Cotyledon orbiculata leaf extract [8], Marsilea quadrifolia [9], aqueous root extract of Ficus religiosa [10] and a lot of other studies which we cannot mention. The present study aims to investigate the preliminary anticonvulsant activity of Evolvulus alsinoides by using the Maximal electroshock seizure and the Pentyleneetetrazole induced seizure models. These models have been described as the gold standard for the evaluation of new antiepileptic drug [11].

Evolvulus alsinoides is often prostrate, slender and wiry with long hairs. It occurs throughout the region from India to west Cameroon, and widely dispersed elsewhere in tropical Africa and worldwide. Local names of the plant includes, Indian: Vishnukiranthi, sankhapuspi, Hausa: kaafi fi-mallam or matakin kuciya, Fulani: Ndottithon, Senegal: Blue winged dove and Yoruba: Efunje.

The plant is found in waste places and around villages [12]. In Sri Lanka, roots and stem extract of the plant are used to treat dysentery and depression. Leaves are recommended for asthma and mental disturbances [13]. It is used in insanity, epilepsy and nervous debility [14]. The plant is used in Ayurveda as a brain tonic in the treatment of neurodegenerative diseases, asthma and amnesia [15]. Other traditional uses include treatment of fever, loss of memory, syphilis and to promote hair growth [15].
Previous studies on the plant includes Antibacterial and antihelminthic activity [16, 17], antiulcer and anticonvulsant activity [18], antioxidant, antistress and antiamnesic activity [5] and immunomodulatory activity [19].

Epilepsy, a heterogeneous syndrome characterized by recurrent and spontaneous seizures affects approximately 1% of the world population [20]. About 10% of the patients are refractory and continue to have seizures at interval of one month or less, which severely disrupts their life and work [21]. Epilepsy is the third most common neurological disorder in the U.S. after Alzheimer's disease and stroke. Its prevalence is greater than cerebral palsy, multiple sclerosis and Parkinson's disease combined [22]. Antiepileptic drugs (AEDs) currently in use notably Phenytin, Carbamazepine, Phenobarbital and Valproate, referred to as older AEDs were (drugs introduced before 1990). These drugs induce hepatic microsomal enzymes, cytochrome P450 (CYPs) thereby complicating the use of multiple anti-seizure drugs as well as impacting metabolism of oral contraceptives, warfarin and many other drugs [23]. The drugs also enhance metabolism of endogenous compounds including gonadal steroids and vitamin D [23]; These shortcomings has led to a renewed reawakening of interest in the search for medicinal plants that will be effective for the management of diseases including epilepsy.

MATERIALS AND METHODS

Plant collection

The plant material was collected in the month of October, 2012 in Illela local Government Area of Sokoto state, Nigeria. The plant was identified by Mallam Abubakar Umar of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences Usman Danfodiyo university Sokoto Nigeria. A voucher specimen (No EA058) was deposited for future reference.

Extraction

The plant material was cleaned; air dried and crushed into coarse powder using mortar and pestle. 500g of the powdered crude drug was extracted with 2 Litrre of methanol (70%) using maceration procedures for 5 days. Thereafter, it was decanted, filtered and the filtrate concentrated at room temperature. The concentrate was then dried in the oven at a temperature not exceeding 40°C. The same procedure was repeated two more times; this gave a yield of 10% w/w. The extract was stored in a refrigerator until required for experiment. Fresh solutions of the extract were prepared for each study.

Animals

Swiss albino mice (16- 30g) of either sex were obtained from the animal facility centre of the Department of Pharmacology and Toxicology, Usman Danfodiyo University Sokoto, Nigeria. The animals were maintained in a well ventilated room, under ambient laboratory conditions of temperature and humidity. They were fed with standard mice chow and water ad libitum. All experimental protocols were in accordance with the Usman Danfodiyo University Research policy; and ethic and regulations governing the care and use of experimental animals.

Drugs /Chemicals and treatment

Pentylenetetrazole (Sigma USA Batch No: SLBD3876V) , Diazepam (Swiss pharma Nigeria Limited), normal saline and different doses of E. alsinoides extract were administered via the intraperitoneal route.

Phytochemical screening

Crude methanol extract of Evolvulus alsinoides was screened for the presence of secondary metabolites such as alkaloids, tannins saponins, glycosides, flavonoids, steroids and triterpenoids. The screening was done using standard protocol previously described by[24,25].

Acute toxicity studies

A median lethal dose (LD50) for the crude methanol extract in mice was estimated using the intraperitoneal route. Briefly, the method was divided into two phases. In the initial phase, 3 groups of three mice each were treated with the fraction at doses of 10, 100 and 1000 mg/kg body weight i.p. and observed for signs of toxicity and death for 24 hours. In the second phase, 4 groups each containing one mouse was injected with four more specific doses of the extract. The LD50 value was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived [26].

Diazepam-induced sleep in mice

Swiss albino mice of either sex were divided into five groups of six mice each. Group 1-3 received 100, 200, and 400mg/kg of E. alsinoides extract respectively via i.p route; group 4 received normal saline which served as control, in equivalent volume of the extract. Thirty minutes post administration all the groups were administered diazepam 10mg/kg i.p. The onset and duration of sleep was observed and recorded with the mice placed in individual cages. Loss of rightening reflex was considered as the criterion for sleep [27] while the interval between the loss and the recovery of straightening was regarded as the duration of sleep [28].

Pentylenetetrazole-induced seizure in mice

The method described by [29, 30] was employed for this study. Clonic seizures were induced in mice (n=6) by treatment with 70 mg/kg pentylenetetrazole. Thirty minutes before treatment with the convulsant drug, mice were treated with normal saline E. alsinoides (50,100,200 and 400mg/kg) and diazepam 30mg/kg. Absence of an episode of clonic spasm of at least 5 seconds is indicative of protection against pentylenetetrazole induced seizure.
Maximal electro shock seizure test
The method of [31] was used with some modifications; thirty six mice were fasted and divided into six groups of six mice per group. Animals in each group were stimulated through corneal electrodes by a 60 cycle (60 Hz) alternating current until MES indicated by hind limb tonic- extensor spasm was elicited, before and 30 min post treatment of animals in groups 1 - 4 with E. alsinoides (50,100,200 and 400 mg/kg), group 5 mice were treated with distilled water (10 ml/kg) while group 6 received diazepam (30 mg/kg, i.p.). The duration of electrically induced convulsion was noted for each mouse [31].

Statistical analysis
Results were expressed as mean ± standard error of mean. Statistical analysis was performed by analysis of variance (ANOVA); when a statistically significant result was obtained with ANOVA, a post hoc Dunnett t-test was performed for multiple comparisons. Values of p<0.05 were considered significant.

RESULTS
Preliminary Phytochemical screening
The phytochemical screening of the methanol extract of E. alsinoides revealed the presence of carbohydrate, saponins, steroids, triterpinoids, glycosides and tannins in the extract.

Acute Toxicity studies
The intraperitoneal median lethal doses of the crude methanol root bark extract was estimated to be 3312 mg/kg. The animal presented with abdominal constriction and respiratory depression before death.

Diazepam induced sleep in mice
The extract significantly (p<0.05) decreased the onset of diazepam induced sleep at all the dose levels; the duration of sleep was markedly increased dose dependently (figure 1).

Fig. 1: Effect of the methanol fraction of Evolulus alsinoides on diazepam induced sleep seizure in mice; N/sal: normal saline, E. als: Evolulus alsinoides; n=6

Pentylenetetrazole induced seizure in mice
Evolvolus alsinoides extract produced a dose dependent protection against pentylenetetrazole induced seizure with the highest dose (400mg/kg) producing 100% protection. Seizure latency was also increased dose dependently. The protection offered by E. alsinoides 400mg/kg was comparable to diazepam (30mg/kg), a known anticonvulsant.

Table 2: Effect of E.alsinoides on PTZ induced seizure

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Latency of seizure (sec)</th>
<th>Quantal protection</th>
<th>Percentage Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/saline</td>
<td>10</td>
<td>44.40±3.36</td>
<td>0/6</td>
<td>0</td>
</tr>
<tr>
<td>Diazepam</td>
<td>30</td>
<td>-</td>
<td>6/6</td>
<td>100</td>
</tr>
<tr>
<td>E. alsinoides</td>
<td>50</td>
<td>144±0.00</td>
<td>2/6</td>
<td>33.3</td>
</tr>
<tr>
<td>E. alsinoides</td>
<td>100</td>
<td>289±0.17</td>
<td>2/6</td>
<td>33.3</td>
</tr>
<tr>
<td>E. alsinoides</td>
<td>200</td>
<td>983±1.21</td>
<td>4/6</td>
<td>66.7</td>
</tr>
<tr>
<td>E. alsinoides</td>
<td>400</td>
<td>-</td>
<td>6/6</td>
<td>100</td>
</tr>
</tbody>
</table>
Maximal electroshock seizure in mice

Evolvulus alsinoides extract decreased the duration of the maximal electroshock seizure. There was 100% protection against mortality at 400mg/kg which is comparable to diazepam, a known anticonvulsant. Normal saline and 50mg/kg of the extract did not protect the mice against convulsion and death.

Table 3: Effect of E. alsinoides on MEST induced seizure

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Duration of TCS (sec.)</th>
<th>Protection against TCS (%)</th>
<th>Protection against mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/saline</td>
<td>10</td>
<td>28±1.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diazepam</td>
<td>30</td>
<td>-</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>E. alsinoides</td>
<td>50</td>
<td>19.2±0.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E. alsinoides</td>
<td>100</td>
<td>17.1±0.5</td>
<td>0</td>
<td>33.33</td>
</tr>
<tr>
<td>E. alsinoides</td>
<td>200</td>
<td>12.4±1.2</td>
<td>66.7</td>
<td>66.7</td>
</tr>
<tr>
<td>E. alsinoides</td>
<td>400</td>
<td>7.0±0.5</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

TCS= tonic clonic seizure

DISCUSSION

Phytochemical screening of the extract revealed the presence of secondary metabolites such as saponins, tannins and flavonoids. Anticonvulsant effect of saponins and flavonoids has been reported by [32, 33]. The intraperitoneal median lethal dose (LD50) was found to be 3312mg/kg, and this was suggested to be slightly toxic [34]. The highest dose used in the study 400mg/kg was less than 30% of the LD50 as was reported to be safe in ethno pharmacological studies [35].

The extract dose dependently reduced the onset of diazepam induced sleep, diazepam acts by increasing GABA mediated synaptic inhibition either by directly activating GABA receptors or, more usually, by enhancing the action of GABA on GABAA receptors [21]. The total sleep time was potentiated more than 4 times at the dose 200mg/kg suggesting that the extract of E. alsinoides has sleep potentiating properties [36].

Evolvulus alsinoides extract produced a 50-100% protection of the mice against PTZ induced seizure at doses of 100-400mg/kg. The protection of the extract against PTZ induced convulsion suggested that the extract interacts with GABAergic neurotransmission. The PTZ test is assumed to identify anticonvulsant drugs effective against myoclonic and absence seizures. E. alsinoides significantly attenuated electrically induced seizure in mice. Electroshock seizures are characterized by tonic extension of the hind limb and abolition of this activity is taken as anticonvulsant action. The ability of E. alsinoides to decrease the duration of tonic clonic seizure in the MEST test shows its activity against generalized tonic clonic seizures [37-40]. E. alsinoides has demonstrated potent activity against PTZ and MEST seizures and it would generally be right to say that it will be effective against absence seizures and generalized tonic-clonic seizures. Since the MEST test identifies agents with activity against generalized tonic-clonic seizures, whereas the PTZ test identifies compounds that are efficacious against generalized absence and myoclonic seizures [39].

CONCLUSION

In conclusion the result of the present study indicates that the methanol extract of Evolvulus alsinoides contain bioactive principles that may be of benefit in the treatment of epilepsy. This further provides validity for the use of the plant for the management of epilepsy in traditional medicine. Further research is encouraged in the area of isolation and characterization of the bioactive compounds responsible for the anticonvulsant activity.

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