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Evaluation of anticonvulsant effects of methanolic extract of *Olax subscorpioidea* Oliv. leaves in chicks and mice

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Abstract
Preparations of *Olax subscorpioidea* have been used in the Nigerian traditional medicine for the management of convulsions, mental illness, pains, cancer and microbial infections. The efficacy of the leaves of this plant in management of convulsions has been widely acclaimed among the Igala communities of North-Central part of Nigeria and therefore, this study was aimed at examining the anticonvulsant effects of methanolic extract of *O. subscorpioidea* (MEOS) leaves in order to provide scientific basis for its use in management of convulsions. Phytochemical screening and evaluation of intraperitoneal median lethal dose of the extract was carried out. Anticonvulsant activity of MEOS was evaluated in chicks using maximal electroshock test, and in mice using pentylenetetrazole and strychnine-induced seizure models at doses of 100, 200 and 400 mg/kg. The intraperitoneal median lethal dose of MEOS was estimated to be 3800 mg/kg body weight in mice. MEOS at doses of 100 and 200 mg/kg provided 30 and 70% protection against maximum electroshock induced seizures respectively. The extract also significantly ($p < 0.05$) increased the mean latency to seizures in a dose dependent manner. MEOS at 100 mg/kg provided 50% protection against strychnine-induced seizures. A significant increase ($p < 0.01$) and ($p < 0.05$) in the mean onset of strychnine-induced seizures was also observed with MEOS at doses of 200 and 400 mg/kg respectively. These findings suggest that the methanolic extract of *Olax subscorpioidea* leaves possess anticonvulsant activity.

Keywords: Anticonvulsant, Maximal Electroshock, *Olax subscorpioidea*, Pentylenetetrazole, Strychnine

INTRODUCTION

Olax subscorpioidea – Oliv. (family: Olacaceae) is either a shrub or tree that grows up to 10 metres in height and is widely distributed in Africa especially in countries like Nigeria, Zaire, Senegal, Cameroon and Côte d'Ivoire (Burkill, 1997; Ayandele and Adebiyi, 2007). Traditionally, the roots have been used for the management of cancer (Soladoye *et al*., 2010) rheumatism (Ogunmefun and Gbile, 2012) and typhoid fever (Fadimu *et al*., 2014) while the stem bark has been used for microbial diseases (Ayandele and Adebiyi, 2007). The leaves have also been used the management of swelling and pains (Odoma *et al*., 2014), yellow fever, jaundice, venereal diseases and guinea worm infestation (Okoli *et al*., 2007). According to Oyedapo *et al*., (1997) the plant parts have been used in the management of convulsions in children, yellow fever and febrile symptoms.

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Scientific studies have been reported on the antimicrobial (Ayandele and Adebiyi, 2007), anti-ulcer (Ukwe *et al*., 2010), anthelminthic (Koné *et al*., 2012) and toxicological actions (Adebayo *et al*., 2014) of *Olax subscorpioidea*. The leaves have also been reported to posses' analgesic, anti-inflammatory and antidepressant-like properties (Odoma *et al*., 2014; Adeoluwa *et al*., 2015). To our knowledge, there is no scientific report on the anticonvulsant properties of the plant and therefore, the present study was aimed at providing scientific basis on the use of *Olax subscorpioidea* leaves in management of convulsions.

EXPERIMENTAL

Animals. Albino mice (18-24 g) of either sex obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, and one-day old ranger cockerels (30-40 g) obtained from the National Animal Production and Research Institute (NAPRI), Shika, Zaria, were used for the study. The animals were maintained in a well-ventilated room under ambient temperature and fed on animal feeds (Feeds Masters, Ilorin, Nigeria) and water *ad libitum*. All experimental protocols were approved by the Ahmadu Bello University Animal Ethics Committee which was in compliance with the Ahmadu Bello University Research Policy (Revised 2010).

Drugs and chemicals. Pentylenetetrazole and Strychnine obtained from Sigma chemical Co., USA, were used for the induction of seizure in the experimental animals while Methanol (Sigma Chemical Co., USA) was used for extraction. The standard drugs used for the experiments were Phenytoin sodium (Parker-Davis and Co Ltd. Detroit), Sodium Valproate (Sanofi-aventis, UK) and Phenobarbitone (Lab Renaudin, France). The drugs were freshly prepared to the desired concentrations with distilled water prior to use.

Plant material. The leaves of *Olax subscorpioidea* was collected from Anyigba, Kogi State, Nigeria, in the month of March 2013. It was identified by a taxonomist, Dr. Emmanuel I. Aigbokhan, of the Department of Biological Sciences, Faculty of Natural Sciences, Kogi State University, where a voucher specimen number (KSUH-277-2013-01) was deposited for future references.

Preparation of plant extract. The leaves of *Olax subscorpioidea* was shade dried until constant weight was obtained and then pulverized into fine powder with the aid of a mortar and pestle. About one hundred grams (100 g) of the powdered material was extracted exhaustively with 500 ml aqueous-methanol (1:4) using continuous soxhlet apparatus. The extract was concentrated under reduced pressure to yield a dark brown mass weighing 31.13 g referred to as methanol leaf extract of *Olax subscorpioidea* (MEOS). The extract was sealed in a bottled container and stored in a desiccator until required in the main study.

Phytochemical screening. Preliminary phytochemical analysis of methanolic extract of *Olax subscorpioidea* leaves was performed according to standard protocols as described by Evans, (2002). The extract was screened for the presence or absence of alkaloids, flavonoids, saponins, cardiac glycosides, tannins, anthraquinones and carbohydrates.

Acute toxicity studies. The intraperitoneal (*i.p*) median lethal dose (LD_{50}) of the methanolic extract of *Olax subscorpioidea* leaves was determined in mice using the method described by Lorke, (1983). The study was carried out in two phases; in the initial phase, three groups of three mice each received the extract at doses of 10, 100 and 1000 mg/kg and then observed for signs of toxicity and death within 24 hrs. In the second phase, three mice were treated with more

specific doses (which depended on the result of the first phase) of the extract and also observed for signs of toxicity and death within 24 hrs. The LD_{50} value was calculated as the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived.

Anticonvulsant studies.

Maximal electroshock (MES) induced seizures. The methods of Swinyard and Kupferberg (1985) and of Browning (1992) were employed using one day-old cockerels. A day old chicks have an underdeveloped blood brain barrier thereby facilitating easy passage of drugs and current into the brain (Browning, 1992). The apparatus used was the Ugo Basile Electroconvulsive Machine (Model 7801, Italy) with corneal electrodes placed on the upper eyelid of the chicks after dipping them in normal saline. A current which induced tonic convulsion in 90% of a control group (normal saline) of chicks was selected. The current, shock duration, frequency and pulse width was set and maintained at 80 mA, 0.8 sec, 100 pulse/sec and 0.6 ms respectively. A second group of ten chicks was pretreated with phenytoin (20 mg/kg) intraperitoneally and 30 minutes later, they were subjected to electrical stimulation as in normal saline treated group. Tests chicks were then intraperitoneally pretreated in groups of ten with 100, 200 and 400 mg/kg of *Olax subscorpioidea* extract before being subjected to electrical shock, 30 minutes later. Results were recorded as either positive or negative depending on whether hind limb tonic extension (HLTE) was produced or not. The onset and recovery period of convulsed chicks was also recorded and the percentage of convulsed animals calculated.

Pentylenetetrazole induced seizures. The method of Swinyard *et al*., (1989) was employed to induce convulsion in mice using PTZ. Thirty mice were divided into five groups of six mice each. The first group was pretreated with normal saline (10 ml/kg *i.p*.) and served as the negative control. The second, third and fourth groups were pretreated with 100, 200 and 400 mg/kg of the extract respectively, while the fifth group was pretreated with 200 mg/kg body weight of sodium valproate *i.p* (positive control). Thirty minutes later, mice in all the groups were injected with a convulsive dose of pentylenetetrazole (85 mg/kg) subcutaneously and were observed for a period of thirty minutes. The absence of a clonic spasm of at least five seconds duration indicates the extract's ability to abolish the effect of PTZ on seizure threshold.

Strychnine induced seizures. The method described by Porter *et al*., (1984) was employed to induce convulsion in mice. Thirty mice were divided into five groups of six mice each, with the first group being pretreated with normal saline (10 ml/kg *i.p.*). The second, third and fourth groups were pretreated with 100, 200 and 400 mg/kg of the extract respectively while the last group received 20 mg/kg of phenobarbitone all through the intraperitoneal route. Thirty minutes later, mice in all the groups were injected with a convulsive dose of strychnine (1 mg/kg) subcutaneously. Abolition of tonic extension jerks of the hind limbs within 30 minutes after strychnine administration was considered an indication that the extract prevented strychnine induced seizures.

Statistical analysis. Data were expressed as percentages and as mean ± standard error of mean (S.E.M.). Difference between means was analyzed by one way analysis of variance (ANOVA) followed by Dunnett's post hoc test. Values of $p< 0.05$ were considered significant.

RESULTS AND DISCUSSION

Percentage yield of *Olax subscorpioidea* leaf extract was 31.13% $^{w}/_{w}$, while preliminary phytochemical analysis on the extract revealed the presence of alkaloids,

flavonoids, saponins and tannins amongst other secondary metabolites (Table 1). These phytochemical compounds have been reported by researchers (Ayandele and Adebiyi, 2007; Odoma *et al*., 2014), some of which were responsible for its diverse pharmacological activities. Medicinal plant extracts are known to contain several phytochemicals with potentials for use as anticonvulsants (Kumar *et al*., 2012). For example, extracts from plants such as *Carissa edulis* - Vahl, *Randia nilotica* – Stapf and *Cissus cornifolia* – Planch have been reported for their strong anticonvulsant activities (Danjuma *et al*., 2009; Yaro *et al*., 2015; Ya'u *et al*., 2015). The results obtained from this study had also demonstrated potential anticonvulsant activity of methanolic extract of *Olax subscorpioidea* leaves.

The intraperitoneal median lethal dose of methanolic extract of *Olax subscorpioidea* leaves was estimated to be 3800 mg/kg in mice**.** This showed that the extract is moderately toxic in mice following intraperitoneal administration according to Lu, (1996) classification of LD_{50} values.

The methanolic extract of *Olax subscorpioidea* leaves provided 30 and 70% protection against HLTE induced by MES in chicks at doses of 100 and 200 mg/kg respectively (Fig. 1a). The extract also significantly ($p < 0.05$) increased the latency to seizures at doses 100, 200, and 400 mg/kg compared to the normal saline control group. However, there was no significant difference ($p > 0.05$) in the mean recovery period (Figure 1b).

Table 1: Phytochemical Constituents of Methanolic Extract of *Olax subscorpioidea* Leaves

Plant constituents	Inference
Alkaloids	+
Flavonoids	+
Saponins	+
Cardiac glycosides	+
Tannins	+
Anthraquinones	-
Carbohydrates	+

+ = present; - = absent

Table 2: Effect of Methanolic Extract of *Olax subscorpioidea* Leaves on PTZ-Induced Seizures in Mice

Treatment	Dose (mg/kg)	Onset of seizures (min.)	Quantal Protection	% Protection
NS	10 ml/kg	6.50 ± 0.76	0/6	0.00
MEOS	100	9.00 ± 1.00	0/6	0.00
MEOS	200	12.33 ± 2.91	0/6	0.00
MEOS	400	11.80 ± 1.69	0/6	0.00
SV	200	-	6/6	100.00

Values are presented as Mean ± S.E.M., No significant difference from NS - One way ANOVA, n = 6, MEOS=Methanolic extract of *Olax subscorpioidea*, NS = Normal saline, SV= Sodium valproate

Table 3: Effect of Methanolic Extract of *Olax subscorpioidea* Leaves on Strychnine-Induced Seizures in Mice

Treatment	Dose (mg/kg)	Onset of seizures (min.)	Quantal Protection	% Protection
NS	10 ml/kg	6.50 ± 0.76	0/6	0.00
MEOS	100	9.00 ± 1.79	3/6	50.00
MEOS	200	17.33 ± 4.10**	0/6	0.00
MEOS	400	13.33 ± 1.20*	1/6	16.67
PBT	20	-	6/6	100.00

Values are presented as Mean ± S.E.M., *= $p < 0.05$, **= $p < 0.01$ from NS-One way ANOVA followed by Dunnett's t-test, n = 6, NS=Normal Saline, MEOS=Methanolic extract of *Olax subscorpioidea*, PBT=Phenobarbitone

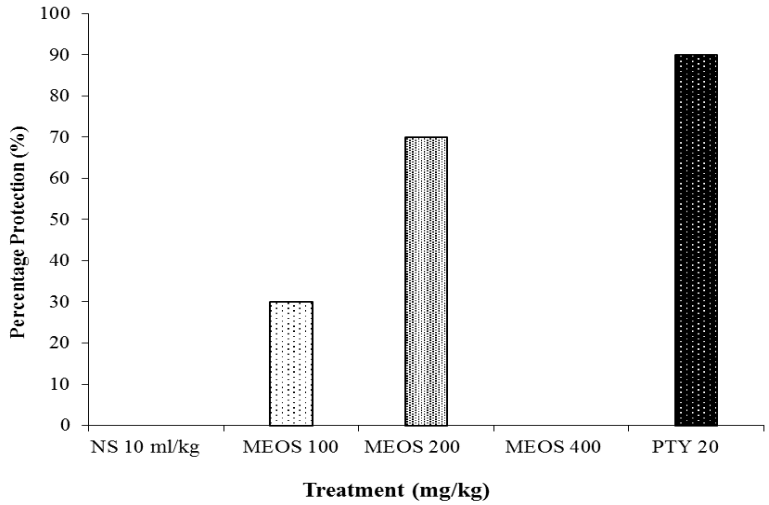

Figure 1a: Effect of Methanolic Extract of *Olax subscorpioidea* (MOES) Leaves on Hind Limb Tonic Extension Phase in Chicks using Maximal Electroshock test, n = 10, NS = Normal saline, PTY = Phenytoin

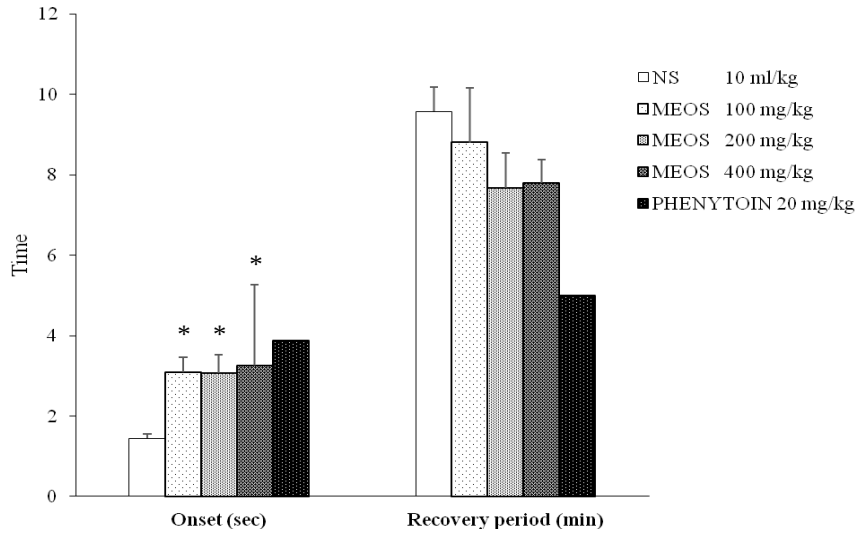

Figure 1b: Effect of Methanolic Extract of *Olax subscorpioidea* (MOES) Leaves on Onset and Recovery period of Hind Limb Tonic Extension Phase in Chicks using Maximal Electroshock test. * = $p < 0.05$ from NS - One way ANOVA followed by Dunnett's t-test, n = 10, NS = Normal saline

Protection against HLTE predicts anticonvulsant activity of antiepileptic drugs that prevent the spread of the epileptic seizure discharges from an epileptic focus during seizures (Raza *et al*., 2001). Furthermore, antiepileptic drugs that are clinically effective in the management of generalized tonic-clonic and partial seizures such as carbamazepine, phenytoin and lamotrigine also suppress HLTE in MEST (Browning, 1992). The significant inhibitory activity of *Olax* subscorpioidea leaf extract against HLTE suggests that it possesses anticonvulsant activity and therefore, it may be of value in the treatment of generalized tonic-clonic and partial seizures.

MEOS offered no protection against PTZ-induced seizures at all the doses tested. There was no significant difference ($p > 0.05$) in the mean onset of seizures either when compared to the normal saline control group (Table 2). PTZ test identifies compounds that

can raise the seizure threshold in the brain (White *et al.*, 1998) and it has been shown to interfere with gamma amino butyric acid (GABA) neurotransmitter and the GABA receptor complex (DeDeyn *et al*, 1992; Bum *et al*., 2001). PTZ-induced seizures are similar to the symptoms observed in the absence seizures and drugs such as sodium valproate and ethosuximide which are useful in the management of absence seizures inhibit PTZ-induced seizures (McNamara, 2006). Antagonism of PTZ induced seizure suggests potentiating effect on GABAergic neurotransmission and therefore, the absence of anticonvulsant activity of MEOS against PTZ-induced seizures suggested that compounds of *Olax subscorpioidea* may not interact with GABA receptor complex or GABA neurotransmission.

MEOS provided 50% protection against strychnine-induced seizures in mice at dose of 100 mg/kg. The extract also prolonged the mean onset of seizures which was significant ($p < 0.01$) and ($p< 0.05$) at doses of 200 and 400 mg/kg respectively when compared to the normal saline control group (Table 3). The increase in latency was biphasic and could probably be due to interaction between the phytochemical constituents of the crude extract. The convulsive action of strychnine is due to its ability to inhibit spinal reflexes of glycine (Sayin *et al.*, 1993) which is an important inhibitory transmitter to motor neurons and interneurons in the spinal cord. Strychnine sensitive postsynaptic inhibition in higher centers of the central nervous system is also mediated by glycine (Parmar and Prakash, 2006). Therefore, the anticonvulsant effect produced by MEOS against strychnine–induced seizures shows that it contains compound(s) that interact with glycine.

Conclusion. The results obtained from this study provided scientific evidence that methanolic extract of *Olax subscorpioidea* leaves possess anticonvulsant activity and therefore supports the ethnomedicinal use of the plant in management of convulsions.

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