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Anticonvulsant and sedative effects of ethanol whole plant extract of *Viscum album* in mice

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Abstract
Epilepsy is one of the world's oldest recognized conditions. Fear, discrimination and social stigma have surrounded epilepsy for centuries. Various drug treatments available are not readily accessible, affordable and may have associated side effects. This research focuses on the anticonvulsant and sedative effects of ethanol whole plant extract of *Viscum album* L in mice commonly used in herbal medicine to treat insomnia and convulsion. The fresh whole plant extract of *Viscum album* collected was air-dried for one week in the herbarium away from sunlight, and then grounded into powder form using a clean pestle and mortar. About 300 g of the powdered sample were extracted using 95% ethanol. Phytochemical screening and acute toxicity study was carried out using standard method. The extract was evaluated for anticonvulsant activity in pentylenetetrazole (PTZ) induced convulsion and sedative effect in pentobarbital induced sleeping time in mice. The phytochemical screening of the plant extract of *Viscum album* revealed the presence of alkaloids, carbohydrates, glycosides, flavonoids, saponins, triterpenes and tannins. However, Dragendorff's test for alkaloid gave a negative result. The LD_{50} obtained was greater than 2900 mg/kg intraperitoneally in mice. The plant extract of *Viscum album* produced a dose dependent inhibition of PTZ induced convulsion and death. The extract significantly ($p<0.05$) delay onset and prolong duration of sleep in pentobarbital induced sleep. The plant extract of *Viscum album* was found to contain phytochemical constituents that may be responsible for its anticonvulsant and sedative activity, justifying the traditional use in the treatment of convulsions and insomnia.

Keywords: *Viscum album;* Pentylenetetrazole; Anticonvulsant; Sedative

INTRODUCTION

Mental disorders such as anxiety, depression, stress, seizures and schizophrenia are serious problems in health management. People suffering with these disorders are often subjected to social isolation, poor quality of life and increased mortality [1]. Antiepileptic drugs currently available do not provide cure nor prevent relapse and they are often associated with serious side effects, including teratogenicity, chronic toxicity, and adverse effects on cognition and behavior [1]. Although several synthetic drugs are available, attention is currently being focused on the use of plants and plant products in the treatment of specific diseases because of

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several side effects associated with the use of synthetic drugs [2]. There is a wide spread use of traditional medicine alone or in combination with orthodox drugs in developing countries for treatments. Several medicinal plants and their active constituents are used as anticonvulsants in traditional medicine but mostly, have not been explored scientifically [3]. *Viscum album* mistletoe was reported to exhibit anticonvulsant, antihypertensive as well as its use for various bone and joint disorders including spondylitis, and arthritis in the rural areas [4,5]. This study therefore focuses on the anticonvulsant and sedative hypnotic screening of the ethanol whole extract of *Viscum album.*

EXPERIMENTAL

Collection and preparation of plant materials. The *Viscum album* were freshly collected from Hawul Local Government Area of Borno State on the 20th December 2016 and identified by a taxonomist Professor S.S Sanusi of Biological Science Department, University of Maiduguri. The whole plant collected (*Viscum album*) was dried at room temperature in a shade for weeks. The dried plant was ground to powder using wooden pestle and mortar.

Extraction of the plant material. About 300 g of the powdered plant materials was weighed and subjected to maceration using 3000 ml of 95% ethanol. The mixture was stirred and kept for 72 hours and then filtered. The filtrate was evaporated under reduced pressure using rotary vacuum evaporator at 40^{o}C and the content was air-dried. The dried extract was then coded VAEE (*Viscum album* ethanol extract). The percentage (%) yield was calculated.

Phytochemical analysis. The VAEE was screened for presence of phytochemical constituents using standard methods [6-8].

Acute toxicity studies (LD_{50} Determination). The acute toxicity of VAEE was determined using modified Lorke's method [9]. The experiment was performed using the intraperitoneal route. Mice of both sexes weighing 18-25 g were randomly selected and divided into three groups (A, B and C) of 2 mice each. The groups were then treated respectively with the extract at doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg. The animals were then observed for 24 hours for signs of toxicity and mortality. In the second phase, another three groups of two mice each were administered doses of 1600, 2900, and 5000 mg/kg of the extract. They were observed for signs of toxicity and deaths at which the LD_{50} was calculated as the square root of the least dose that kill and highest dose that did not kill.

Animal model. Mice of either sex (18-25g) were collected from the animal house of Faculty of Pharmacy University of Maiduguri. Housed in a standard wire meshed cages and fed with standard diet and water. All the animals were handled according to the International Guiding Principles for Biomedical Research Involving Animals as certified by the Animal Ethics Committee of the Faculty of Pharmacy, University of Maiduguri.

Anticonvulsant screening of the VAEE. The anticonvulsant screening was carried out according to the method described by Kulkarni [10]. Five groups of five (5) mice were groups I was pretreated with *IP* normal saline 10 ml/kg and groups II, III and IV pretreated with 200, 400 and 800 mg/kg extract of VAEE respectively. Group V was pretreated with the standard drug sodium valproic acid 200 mg/kg *IP*. Forty-five (45) minutes following pretreatment, PTZ at a dose of 60 mg/kg was administered to all five groups *IP* and observed for the onset of clonic tonic seizures as previously described by Kulkarni [10].

Pentobarbital induced sleeping time in mice. Adult mice of either sex were divided into 5 groups of 5 mice each. Group I received normal saline *IP* 10 ml/kg. Groups II, III and IV were pretreated with the extract VAEE at 125, 250 and 500 mg/kg *IP* respectively. Group V was given the standard drug Diazepam 4 mg/kg. Forty-five minutes following pretreatment, pentobarbital sodium (20 mg/kg, *IP*) was administered to all the groups. Each mouse was then observed for the loss of righting reflex, in which the mice cannot roll back when turned over. The interval between loss and recovery of the righting reflex was used as the index of hypnotic effect as outlined by Danjuma *et al* [11].

Statistical analysis. Results were expressed as mean ± standard error of the mean (S.E.M) and analyzed using Computer software GraphPadlnStat® @ USA, 2003. The significant difference between mean was determined using Student's t-test. Values of $p<0.05$ were considered significant.

RESULTS

Preliminary phytochemical screening. The preliminary phytochemical screening of the ethanol whole plant extract of *Viscum album* showed the presence of alkaloids, carbohydrates, flavonoids, glycosides, saponins, tannins, and triterpenes. However Dragendorff's reagent test for alkaloids was tested negative (Table 1).

Acute toxicity study. The acute toxicity study of the ethanol whole plant extract of *Viscum album* in mice was found to be greater than 2900 mg/kg intraperitoneally. However, neurobehavioral effects and death were not observed among the studied animals (Table 2).

Effect of ethanol whole plant extract of *Viscum album* L in pentylenetetrazole induced convulsion in mice. The ethanol extract of *Viscum album* showed a significant ($p<0.001$) dose dependent increase onset of convulsion and percentage protection in PTZ induced convulsion in mice. However, valproic acid used as positive control and 800 mg/kg of *Viscum album* was found to prevent convulsion in the study animals, even though the highest tested dose of the extract only protected 50% of the animal from death. Valproic acid and the extract at 200 and 400 mg/kg was able to protect the animals from death (Table 3).

Sedative hypnotic effect of *Viscum album* L ethanol extract in pentobarbital. The extract significantly ($p<0.001$) decrease the onset and prolong the duration of sleep dose dependently among the mice. However there was no statistically significant difference in the activity of the extract at 125 mg/kg or the onset of sleep when compared with control ($p<0.05$) (Table 4).

DISCUSSION

The presence of some phytochemical components detected in the present study agrees with several reports in which alkaloids, carbohydrates, flavonoids, glycosides, saponins and tannins active constituents were detected [12-15]. Flavonoids and tannins are reported in many neuro-pharmacological activities and in different experimental seizure models as having positive therapeutic outcome in the disorder [15,16]. There was no sign of convulsion, lethargy and death observed for the extract at the dose of 2900 mg/kg, suggesting that the extract is relatively none toxic and is safe in mice. This is in agreement with the findings of Gupta *et al* [16].

Table 1: Qualitative phytochemistry of ethanol whole plant extract of *Viscum album*

Phytoconstituents	Tests	Results
Alkaloid	Dragendorff's reagent	-
	Mayer's reagent	+
Carbohydrates	Molisch test	+
	Fehling's test	+
Flavonoid	Shinoda's test	+
	$FeCl_3$ test	+
	Pb acetate tests	+
Glycosides	(a) free anthraquinones	+
	(b) combined anthraquinones	+
Saponins	(a) frothing test	+
	(b) Fehling's test	+
Tannins	(a) FeCl3	+
	(b) Pb acetate	+
Triterpenes		+

- = absent, + = present

Table 2: Acute toxicity study of ethanol whole plant extract of *Viscum album*

Phases	Dose level (mg/kg)	Observation
Phase I	10	-
	100	-
	1000	-
Phase 2	1600	-
	2900	-
	5000	X
LD_{50} = >2900 mg/kg		

- = no death, X = not administered

Table 3: Effect of ethanol whole plant extract of *Viscum album* L in pentylenetetrazole induced convulsion in mice

Treatment	Onset of clonic convulsion (s)	Percentage protection from death (%)
Control (2 ml/kg)	120.80 ± 23.78	0%
VAEE (200 mg/kg)	445.60 ± 51.09*	100%
VAEE (400 mg/kg)	960.80 ± 44.12**	100%
VAEE (800 mg/kg)	-***	50%
Valproic acid (200 mg/kg)	-***	100%

Result is expressed as mean ± SEM, n=5, - = no convulsion, VAEE = Ethanol extract of *Viscum album*,* = p<0.001 (significant)

Table 4: Sedative-hypnotic effect of *Viscum album* L ethanol extract in mice

Treatment	Onset of sleep (min)	Duration of sleep (min)
Normal saline (2 ml/kg)	26.39 ± 0.57	71.23 ± 1.73
VAEE (125 mg/kg)	25.79 ± 0.48	116.06 ± 4.77*
VAEE (250 mg/kg)	11.50 ± 0.51*	169.38 ± 4.94**
VAEE (500 mg/kg)	7.32 ± 0.39**	178.27 ± 2.60**
Diazepam (4 mg/kg)	5.24 ± 0.28***	205.46 ± 4.36***

Result is expressed as mean ± SEM, n=5, - = no convulsion, VAEE = Ethanol extract of *Viscum album*,* = p<0.001 (significant)

Prolonged onset of convulsion and percentage protection from death in a PTZ induced convulsion is an indication of the extract effectiveness in the management of seizures. PTZ is a GABA receptor agonist that produces myoclonic seizures [17]. The VAEE might possibly be producing anti-epileptic action by increasing the level of GABA, an

inhibitory neurotransmitter in the central nervous system to ameliorate seizure activity. This is in accordance with the pharmacological effects of benzodiazepine and highlights the relevance of the putative anti-epileptic effects of VAEE [18]. The extract in a dose dependent manner significantly ($p<0.001$) decrease onset and prolong the duration of sleep in extract and diazepam treated mice. This confirms the action of the extract to be via GABAnergic transmission like the benzodiazepines positive allosteric modulators of GABA type a receptor. The GABAA receptors are ligand-gated chloride-selective ions channels that are activated by GABA, the major inhibitory neurotransmitter in the brain.

Conclusion. The whole plant extract of *Viscum album* contain phytochemical constituents which may be responsible for the observed anticonvulsant and sedative activities via GABA transmission, which may justify the traditional use of this plant in the treatment of convulsions and insomnia.

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