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Evaluation of Anticonvulsant Activity of *Kochia Scoparia* L. Schrad (Amaranthaceae) Volatile Oil

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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: Epilepsy is one of the most common serious neurological disorders. Most antiepileptic or anticonvulsant drugs do not prevent or reverse the pathological process that underlies epilepsy, hence the continuous search for new therapeutic agents with minimal side effects and greater efficacy.
Objective: The objectives of this study were to determine the acute toxicity profile and investigate the anticonvulsant activity of volatile oil of *Kochia scoparia* (Amaranthaceae).
Method: Volatile oil was extracted from fresh leaves of *K. scoparia* through hydrodistillation process, using a Clavenger-type apparatus. Acute toxicity testing was done using Lorke's method. The anticonvulsant models used were pentylenetetrazol, strychnine and maximal electroshock. Albino mice were randomly divided into five groups (n=5). Group I (control group) was given 0.2 ml each of water orally while groups II, III and IV received 75, 150 and 300 mg/kg of the volatile oil. Group V received the standard drug solution; 30 mg/kg phenobarbitone for Maximal electroshock and 2 mg/kg diazepam for pentylenetetrazol and strychnine models. The onset of tonic leg extension, duration and protection from mortality were noted.
Results: Sub acute toxicity test revealed that doses above 1000mg/kg of the volatile oil is toxic. Doses of 75, 150 and 300 mg/kg significantly ($P<0.05$) protected the mice against seizures with scores of 20, 20 and 40 % respectively in both Maximal electroshock and pentylenetetrazol induced convulsion models. No protection was offered in strychnine induced convulsion model; $P > 0.05$.
Conclusion: The volatile oil of *K. scoparia* could be useful in the management of epilepsy.
Keywords: Anticonvulsant; *Kochia scoparia*; Pentylenetetrazol; Strychnine; Phenobarbitone

INTRODUCTION

Approximately 450 million people in the entire world have suffered mental, neurological, or behavioral problems at some time in their life. Extensive research on plants and their derivatives has taken place in recent years that could provide some new alternative treatments and therapeutic uses for diseases of the central nervous system (CNS). Despite the ground laying progress made in the treatment of neurological disorders, epilepsy remains significantly resistant to therapy. Although a large number of western anticonvulsant drugs are available for the treatment of epilepsy in patients worldwide, seizures remain refractory in up to 30 – 40 % of the cases (Shetty *et. al.*, 2016). In most cases in developing countries, traditional healers are often the first line of contact in the search of therapy because of its link to supernatural powers (Al-Asmi *et. al*., 2013), unavailability and high cost of conventional antiepileptic drugs (AEDs) (Sucher and Charles, 2015). Herbal medicine plays a very important role in meeting the primary health care

requirements of the population, with Africa and Asia being the continents with most of the users (Barnes *et. al*., 2007). Extensive research on plants and their derivatives has taken place in recent years that could provide some new alternative treatments and therapeutic uses for diseases of the central nervous system (CNS). Some medicinal plants have shown potential as new, safe treatment options (Kakooza, 2015). Several species of aromatic plants are used medicinally because of their essential oil components. Although many of them have traditionally been used as sedative and antiepileptic agents, there is still lack of controlled experimental reports on therapeutic use (Sahus *et. al*., 2012).

Kochia scoparia L. Schrad is an erect, annual herbaceous plant that forms rounded bushes up to 7 ft. [2.1 m] tall, belonging to the grass root family; Amaranthaceae. It is a weed native to Asia and Central Europe and has been introduced to Canada as an ornamental plant. It has been reported to have antibacterial (Joung *et al*., 2012), antiparasitic (Chi *et al*., 2006), anti-inflammatory (Kim *et. al*., 2016), analgesic, anti-allergy (Matsuda *et. al*., 1997), anti-cancer, dermatological (Jeon *et. al*., 2016), antioxidant (Wang *et al*., 2014), and antidiabetic effects (Yoshikawa *et al*., 1997). The seeds are used in traditional Chinese medicine to help regulate disorders, such as hyperlipidemia, hypertension, obesity and atherosclerosis. (Han *et. al*., 2006).

The objectives of this study was to determine the acute toxicity profile and evaluate the anticonvulsant effect of the volatile oil from the leaves of *K. scoparia*.

METHODOLOGY

Plant collection

The leaves of *K. scoparia* were collected from within the University of Benin, Edo state, Nigeria in September, 2019. Botanical identification was carried out by a botanist; Dr Akinnibosun Henry from the department of plant biology and biotechnology, faculty of life science, university of Benin Benin city, Edo state. Voucher specimens were deposited in the laboratory under the code number: UBH-K465.

Extraction of volatile oil

The fresh leaves of *K. scoparia* (484.47 g) separated carefully from the stem was weighed and extracted using a Clavenger-type apparatus by hydrodistillation method. The essential oil was collected in a vial, weighed and the percentage yield determined. The oil was stored in a refrigerator at a temperature of 4ºc till time of use.

Experimental animals

Male and female albino mice weighing between 20-30 g were acclimatized in the animal house of the department of pharmacology and toxicology, faculty of pharmacy, university of Benin, Benin City, Nigeria. The animals were kept in separate plastic cages and housed at room temperature and humidity and allowed free access to dry rodent pellet feeds (grower) and water. Ethical approval was sought from the Ethics committee, faculty of pharmacy, with ethical approval number EC/FP/021/08. All experiments were carried out in accordance with Institute for Laboratory Animal Research Guidelines for the Care and Use of laboratory Animals.

Acute toxicity test

Oral median lethal dose (LD_{50}) of *K. scoparia* volatile oil was determined using the Lorkes method (Lorke, 1983). In the first phase, three groups of three mice each received oral doses of 10, 100 and 1000 mg/kg doses of the volatile oil respectively. The second phase involved three groups of one mouse each, administered oral doses of 1600, 2900 and 5000 mg/kg of the volatile oil respectively. In both phases, the animals were observed for signs of writhing, diarrhea, tremor, and mortality within a 24 hr period. At the end of this phase, the LD_{50} of the volatile oil was determined.

Anticonvulsant studies

Anticonvulsant activity of the volatile oil was evaluated, using maximal electroshock (MES), pentylentetrazol (PTZ) and strychnine (STN) induced seizure models.

Maximal electroshock model

The method described by Swinyard (1969) was followed. Twenty-five mice were weighed and divided into five groups comprising 5 mice each. The first group received 0.2 ml of water as the control, the second, third and fourth groups received oral doses of 75, 150 and 300 mg/kg volatile oils respectively, while the fifth group received 30 mg/kg phenobarbitone intaperitoneally, as standard. 1 hour later, animals in all groups were subjected to electroshock at a frequency of 100 Hz, current of 50 amp and duration of 0.2 sec. Normal saline was applied to the ears of the mice prior to shock, to increase conductivity before attachment of ear-clip electrodes. The number of animals protected from convulsion was noted.

Pentylenetetrazol induced convulsion

Another twenty-five mice were weighed and randomly allotted to five groups, comprising five mice each. Group 1 served as the control group and each mouse was given 0.2 ml of water orally. Groups 2, 3 and 4

received increasing oral doses of the volatile oil; 75, 150 and 300 mg/kg. Group 5 served as the standard and was administered 2 mg/kg dose of diazepam intraperitoneally. After a period of 1 hr, each animal received 70 mg/kg doses of pentylenetetrazol intraperitoneally. The onset of CNS stimulation, onset of tonic-clonic seizures, duration of convulsion as well as protection against mortality were recorded (Merit and Putman, 1938).

Strychnine induced seizure model

This was carried out based on the procedure described by McAllister in 1992. Twenty-five mice were weighed and allotted into five groups, comprising five mice each. Group 1 served as the control group, each receiving 0.2 ml of water. Group 2, 3 and 4 received 75, 150 and 300 mg/kg of *K. scoparia* volatile oil respectively. Diazepam (2 mg/kg) was administered to group five intraperitoneally as the standard. After a period of 30 minutes, all mice received 2 mg/kg doses of strychnine. The animals were observed and time recorded for onset of CNS stimulation, onset of seizures, duration of seizures as well as protection against mortality.

Statistical analysis

Data were expressed as mean ± standard error of mean (S.E.M). Sstatistical analysis was done using one way analysis of variance followed by Dunnet's post hoc test (Graph pad Prism version 7, San Diego, USA). $P<0.05$ was considered statistically significant.

RESULTS

Result of acute toxicity test for *K. scoparia* volatile oil

During phase 1 acute toxicity testing, there was neither evidence of toxicity or mortality in 10 and 100 mg/kg doses, however, there was evidence of death in sparse population with 1000 mg/kg dose; (Table 1), hence procession to phase 2. Phase 2 testing showed evidence of toxicity and mortality in all animals, as none survived (Table 1). The lethal median dose of *K. scoparia* was estimated to be 316.23 mg/kg.

Table 1: Acute toxicity testing of *K. scoparia* volatile oil in phase 1 and 2

Dose (mg/kg)	No of deaths/No of animals	Percentage mortality (%)
Phase 1		
10	0/3	0
100	0/3	0
1000	1/3	33.3
Phase 2		
1600	1/1	100
2900	1/1	100
5000	1/1	100

Phase 1: n=3, phase 2: n=1

Effect of volatile oil of *K. scoparia* on maximal electroshock-induced convulsion in mice.

Animals in the control group gave no protection. At doses of 75 and 150 mg/kg the volatile oil gave 20 % protection each, while 300 mg/kg gave 40 % protection. There was a significant difference ($P<0.05$) when compared with the control group. Phenobarbitone which served as the standard completely protected the mice against convulsion (Table 2).

Table 2: Effect of volatile oil of *K. scoparia* on maximal electroshock induced convulsion in mice

Treatment groups	No of mice protected /No of animals used	Percentage protection against convulsion (%)
Control (water)	0/5	0
75mg/kg	1/5	20
150mg/kg	1/5	20
300mg/kg	2/5	40
Phenobarbitone 30 mg/kg	5/5	100

Results are expressed as ratio and percentage, n=5.

Effect of *K. scoparia* volatile oil on pentylenetetrazol induced convulsion in mice.

At all doses, the volatile oil significantly delayed onset of seizures and reduced seizure duration (P<0.05) compared to the control, but offered no full protection against pentylenetetrazol induced convulsion. At both 75 and 150 mg/kg, 20 % protection against mortality was observed, while 40 % protection was observed with 300 mg/kg group. Diazepam completely protected the mice against convulsion while normal saline offered no protection against PTZ-induced convulsion in mice (Table 3).

Effect of *K. scoparia* volatile oil on strychnine induced convulsion in mice

At all doses of the volatile oil, there was no significant difference in time for onset of seizure from the control (P>0.05). There was a slight decrease in duration of seizures however, but it was not significant from the result of the statistical analysis. There was mortality in all treatment groups, however, the positive control (diazepam) significantly delayed seizure onset and reduced seizure duration compared to other treatment groups (Table 4).

Table 3: Effect of volatile oil of *K. scoparia* on pentylenetetrazol induced convulsion in mice

Treatment groups	Onset of CNS Stimulation (secs)	Onset of seizures (secs)	Duration of seizures (secs)	% Reduction in duration of seizures (%)	Protection against mortality (%)
Control	110.00 ±17.69	144.40 ± 13.62	44.80 ± 4.04	-	0
75 mg/kg	65.20 ±6.97	360.00 ± 56.64	26.33 ± 2.75	41.23	20 *
150 mg/kg	67.20 ± 2.66	365.80 ± 51.55	26.00 ± 2.30	41.96	20 *
300 mg/kg	81.60± 4.93	375.75 ± 90.05	22.25 ± 1.85	50.33	40 **
Diazepam (2 mg/kg)	-	-	-	100	100***

The results are expressed as mean ± SEM and percentage, n=5 mice/group, * indicates significance compared to negative control. *P < 0.05, **P < 0.01, ***P < 0.001.

Table 4: Effect of volatile oil of *K. scoparia* on strychnine induced convulsion in mice

Treatment groups	Onset of CNS stimulation (secs)	Onset of seizures (secs)	Duration of seizures (secs)	Protection against mortality (%)
Control	128 ± 5.93	140.4 ± 1.72	14.6 ± 1.29	0
0	54 ± 9.62	86 ± 16.18	9.2 ± 1.66	0
150 mg/kg	65 ± 6.24	138.4 ± 7.23	11.8 ± 0.71	0
300 mg/kg	82.2 ± 8.80	148.4± 12.82	11.6± 0.83	0
Diazepam (2 mg/kg)	105 ± 9.34	196 ± 11.56	6.2 ± 0.58	

Values are expressed as mean ± SEM, n=5, P > 0.05 compared to negative control.

DISCUSSION

Oral acute toxicity test shows that the volatile oil of *K. scoparia* is safe at low doses and might be potentially lethal at high doses as the chemical labeling and classification of acute systemic toxicity states that any substance with LD50 greater than 5000 mg/kg by oral route is assigned a class 5 status which is the least toxicity class (OECD, 2001).

The present study indicates that the volatile oils of *K. scoparia* possess anticonvulsant activity against MES and PTZ induced convulsive models. The anticonvulsant activity of this volatile oil might be attributable to its antioxidant propensity (Wang *et al*, 2014) as well as chemical constituents like terpenes (Abdelaamed *et al*., 2013) and, flavonoids (Asl *et al*., 2007)which are reported to possess anticonvulsant activity in some experimental seizure models such as MES and PTZ (Chauhan *et al*., 1988; Kasture *et al*., 2002). It is also found that many flavonoids could act as benzodiazepine- like molecules in the central nervous system and modulate GABA-generated

chloride currents in animal models of anxiety, sedation and convulsion (Asl *et al*., 2007).

Generally, anti-convulsant medications that block voltage-gated sodium channels e.g. phenytoin, carbamazepine, valproate, lamotrigine, topiramate e.t.c are effective for maximal electroshock induced seizures. It is therefore believed that the volatile oil of *K. scoparia* might be exerting its anticonvulsant effect via blockade of voltage gated sodium channels. . Elucidation of the particular mechanism could be considered in future studies.

In the pentylenetetrazol induced convulsive model, protective effect of the volatile oils against lethality was observed in varying degrees. PTZ is a drug formerly used as a circulatory and respiratory stimulant. High doses cause convulsions, as discovered by the Hungarian-American neurologist and psychiatrist Ladislas J. Meduna in 1934. It has been used in convulsive therapy, and was found to be effective—primarily for depression—but side-effects such as uncontrolled seizures were difficult to avoid (Charles, 1940) hence its use as a convulsive agent in anticonvulsant drug testing. Pentylenetetrazol-induced seizures are categorized as a model of generalized seizure (versus partial or focal seizure). It produces a myoclonic seizure that models absence (petit mal) seizures (Wolfgang, 2000).

The mechanism of pentylenetetrazol is not well understood, and it may have multiple mechanisms of action. Many GABA-A ligands are effective anticonvulsants, such as the sedatives diazepam and phenobarbitone, but presumably pentylenetetrazol has the opposite effect when it binds to the GABA-A receptor (Squires *et al*, 1984). The enhancement of the GABAergic neurotransmission is reported to antagonize seizures, while the inhibition of the neurotransmission promotes seizures. Activation of the N-methyl-d-aspartate (NMDA) receptors is also involved in the initiation and propagation of PTZ-induced seizures (Yudkoff *et al*., 2006). In this regard, drugs that block glutamatergic excitation mediated by NMDA receptors are useful anticonvulsants. The anticonvulsant activities of volatile oils of *K. scoparia* against PTZ seizures might therefore be due to an enhancement on the release of the inhibitory neurotransmitter GABA in the central nervous system, inhibiting T-type Ca2+ currents or blocking the glutamatergic neurotransmission mediated by NMDA receptors. The multiple mechanisms of the oil might be due to the presence of different active components as explained above.

The convulsive action of strychnine on the other hand, is due to interference with postsynaptic inhibition mediated by glycine, an important inhibitory transmitter to motor neurons and interneurons in the spinal cord (Kuno and Weakly, 1972). In this study, it was observed that *K. scoparia* volatile oil, produced no significant anticonvulsant effect against strychnine-induced seizures as compared to control, suggesting their inability to interact with the glycine-mediated inhibitory pathway.

The onset of CNS stimulation observed in both pentylentetrazol and strychnine models, were not actual markers for anticonvulsant activity, but an indication that a centrally active agent (convulsive agent in this case) was administered and hence, behavioural manifestations in animals in response to this stimulus portrayed as jerky and irregular movements etc.

CONCLUSION

K. scoparia volatile oil possess anticonvulsant activity against MES and PTZ induced convulsive models and can be potentially useful in the management of seizures.

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