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Anticonvulsant Effects of the Methanol Stem Bark Extract of *Pseudocedrela Kotschyi* (Meliaceae) in Mice and Chicks

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Original Research Article

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Abstract: The present study examined the anticonvulsant activity of the methanol stem bark extract of PK in mice and day old chicks against maximal electroshock (MES), pentylenetetrazole (PTZ), picrotoxin (PIC), and strychnine (STR) induced seizures. The CNS depressant ability of the extract was also investigated using diazepam-induced sleep test, and it was observed to significantly and dose dependently increase the sleep duration in the diazepam sleep test. The extract and fractions significantly and dose dependently increase the latency to onset of seizure in the PTZ and picrotoxin tests. In the strychnine test, the extract significantly increased the latency to seizure onset at the highest dose of 200 mg/kg, though none of the mice was protected from seizure as was the case with PTZ and PIC induced seizures. Considering the overall effect of PK, it may be concluded that the extract contains bioactive principles acting via enhancement of GABA inhibitory activity, and a second possibility via antagonism of the glutamate-NMDA receptors.
Keywords: Anticonvulsant, *Pseudocedrela kotschyi*, Seizure, epilepsy, Mice.

INTRODUCTION

Throughout our evolution, the importance of natural products for medicine and health has been enormous and in many developing countries, a large proportion of the population (80%) relies on traditional practitioners and their armamentarium of medicinal plants in order to meet health care needs [1,2]. Although modern medicine may co-exist side-by-side with such traditional practice, herbal medicines have often maintained their popularity for historical and cultural reasons [2]. Information about the actual or potential use of a plant is obtained from the traditional medical practitioners, and such information is evaluated before it is accepted as a scientific fact; this takes about 10 to 12 years [3].

The mainstay of epilepsy management is the use of anticonvulsants agents, the group consist of a large group of chemically unrelated compounds which could only treat but not prevent the initiation of seizures in an epileptic [4]. There still exists a need to search for newer agents with better seizure control and less side effects.

The currently available antiepileptic drugs are able to resolve the clinical symptoms in about 70% of patients but could not prevent the occurrence of another seizure [5]. The search for more promising agent with effective seizure control is imperative. Plant based anticonvulsant agents have been reported by many researchers [6, 7] .The current trend in anticonvulsant research is to isolate compounds with antiepileptogenic activity, since almost all of the currently available drugs are anticonvulsants rather than antiepileptogenic. The present research seeks to explore the possibility of isolating bioactive compounds with anticonvulsant activity from the methanol stem bark extract of *Pseudocedrela kotschyi* through activity guided procedure.

Pseudocedrela kotschyi. is a medium sized plant distributed across Senegal, Chad and Nigeria. The plant belongs to the meliaceae family and has various traditional uses ranging from anti-malarial [8], anti-rheumatic [9], antimicrobial [10], anti- diabetic [11] and analgesic among others. The plant is also enriched with a lot of compounds among which are pseudocedrelin and Limonoids [12].The current research reports for the

first time the isolation of Lupeol from the chloroform extract of the methanol stem bark of the plant.

MATERIALS AND METHODS

The stem bark of *Pseudocedrela kotschyi* was collected in the month of July 2013 from Zuru local government area, Kebbi state, Nigeria. It was identified by Mr. U.S Gallah of the Herbarium of the Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria, and a voucher specimen (900243) was deposited for future reference.

Preparation of the extract

The stem bark was chopped, cleaned, air dried for 12 days and milled into a coarse powder using a pestle and mortar. Five hundred grams (500 g) of the coarse powder was exhaustively extracted with 5 L of 95% methanol in 500 ml aliquots using a Soxhlet extractor for 12 h daily for three days. The extract was concentrated using Büchi RE121 rotary evaporator (Büchi Labor technik AG, Switzerland) and subsequently dried in a Hetovac VR-1 freeze dryer (Heto Lab. Equipment AS, Denmark).

Preparation of the Fractions

About 60 g of freeze dried extract was dissolved in 200 ml distilled water, and then placed in a sonicator at 45^{o}c for 20 min to facilitate dissolution, after which the solution was transferred into a 1000 ml separating funnel and extracted with n-hexane (200ml portions) for 48 hours. Thereafter, the n-hexane soluble portion was collected and labelled as n-hexane extract.

The same procedure was repeated with chloroform and n-butanol solvents while considering their relative polarity. During the process of fractionation similar fractions were pooled together, evaporated and the residue kept and labelled until when needed.

Animals

Swiss albino mice (20-30 g) were obtained from the Animal Research and Services Centre, University Sains Malaysia. The animals were acclimatized to laboratory conditions for seven days prior to the experiments. During acclimatization, 12 mice were housed in separate polycarbonate cages, with free access to normal diet (48% carbohydrate, 23% crude protein, 3% crude fat, 8% crude ash, 5% crude fibre and 13% moisture) and water *ad libitum*. The food pellets for the experimental animals were purchased from Gold Coin Holdings Sdn. Bhd. Malaysia. All procedures were performed according to the guidelines of care and use of Laboratory animals as approved by the Animals Health and Wellness Unit, University Sains Malaysia.

Two day old ranger cockerels weighing 20-30g were obtained from Ojuanu Agricultural Enterprises Sokoto, Nigeria. They were allowed to acclimatize to the laboratory conditions prior to the study

Determination of LD_{50} of Extracts of *Pseudocedrela kotschyi* in mice and Chicks

The method described by [13] was used; 3 groups of three mice or chicks were treated with the methanol stem bark extract of *Pseudocedrela kotschyi* at doses of 10, 100, and 1000 mg/kg body weight orally and observed for signs of toxicity and death for 24 hours. In the second phase, 3 groups of one mouse or chick was treated with 1600, 2900 and 5000 mg/kg body weight respectively and also observed for signs of toxicity and death. The LD_{50} value was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived (0/1 and 1/1) respectively.

Maximal electroshock seizure (MES) test in chicks

The method described by Swinyard and Kufferberg [14] and modified by Sayya *et al.* [15] was employed. Two day old Ranger cockerels were divided into ten chicks per group. The test groups (III, IV and V) were treated with 50, 100 and 200 mg/kg doses of the extract. Group 1 received normal saline (10 ml/kg), while group II was administered Phenytoin (20 mg/kg, *i.p*). Thirty minutes later, MES was induced in the chicks using the Ugo Basile electroconvulsive therapy (ECT) machine (Model 57800- 001) with corneal electrodes placed on the upper eyelids of the chicks. The current, shock duration, frequency and pulse width were set and maintained at 90 mA, 0.80 s, 200 pulse per second and 0.8 ms, respectively. Seizure was manifested as tonic hind limb extension (THLE) [16]. Ability to prevent this feature or decrease the recovery time post seizure was an indication of anticonvulsant activity [15]. The same procedure described above was employed to test the fractions at doses of 50, 100 and 200 mg/kg.

Pentylenetetrazole-induced seizure (PTZ) test in mice

Thirty mice were divided into 5 groups of 6 each. Groups I and II received 0.9% w/v of normal saline (10 ml/kg) and diazepam (5 mg/kg) *i.p*, respectively. Groups III, IV and V received *P. kotschyi* methanol extract 50, 100 and 200 mg/kg, *p.o* respectively. Seizure was induced by administering 90 mg/kg of PTZ, *sc* [17] as modified by [18]. Methanol stem bark extract of *P. kotschyi* was administered orally, one hour before the PTZ. Absence of an episode of clonic spasm of at least 5 seconds duration, hind limb extension or death indicated the extract‘s ability to abolish the effect of PTZ on seizure threshold. The same procedure was repeated for the fractions.

Strychnine (STN) induced seizure test in mice

Five groups of 6 mice each were employed in this study. Group I served as control and received 0.9%

w/v of normal saline (10 ml/kg), Group II received Phenobarbitone (30mg/kg) i.p, as positive control while Groups III, IV and V received *P. kotschyi* methanol extract 50, 100 and 200 mg/kg respectively. All drugs were administered orally, 60 min prior to the administration of strychnine nitrate (2.5 mg/kg) *i.p*. The animals were observed for 30 minutes by placing them in separate cages. The onset of seizures (tonic-clonic convulsions) and time of death were recorded [19]. The same procedure was repeated for the fractions.

Picrotoxin induced seizure test in mice

The animals in this study were randomly divided into five groups of 6 mice each. Group I served as the control that received normal saline 10 ml/kg orally, group II was administered diazepam 5 mg/kg i.p. Groups III, IV and V received the methanol extract of *P. kotschyi* orally at a dose of 50, 100 and 200 mg/kg respectively. Sixty minutes after extract administration and 30 min after the administration of diazepam, picrotoxin (10 mg/kg, i.p) was administered and observed for 30 min. Abolition of hind limb tonic extension indicates protection against picrotoxin induced seizure [20]. The same procedure was repeated for the fractions.

STATISTICAL ANALYSIS

Results were expressed as Mean ± Standard Error of the Mean (SEM) and Percentages. Data analysis was performed using Graph Pad Prism statistical software (version 6.0). Comparison between groups was made using analysis of variance (ANOVA). When a statistically significant difference was obtained, a *post hoc* Dunnets' test was performed for multiple comparisons, values of $P< 0.05$ were considered significant.

RESULTS

Percentage yield of *P. kotschyi* methanol stem bark extract and Fractions

The percentage yield of methanol stem bark extract of *P. kotschyi* was 14.8 % w/w. Fractionation of the methanol stem bark extract of *P. kotschyi* resulted into four fractions. The residual aqueous fraction had the highest yield (43.5%) while the n- hexane fraction had the lowest yield (3.3%). The n-butanol and chloroform fractions yielded 16.2 and 22.2% respectively (table 1).

Table-1: Percentage Yield of Fractions obtained from the Methanol Stem Bark Extract of P. kotschyi

Fractions	Yield (g)	Yield (%
Hexane	1.98	3.3
Chloroform	9.72	16.2
N Butanol	13.32	23.2
Residual Aqueous Fraction	26.10	43.5

Effects of *P. kotschyi* Methanol Stem Bark Extract and Fractions in the Diazepam Induced Sleep Test in Mice. The methanol stem bark extract of *P. kotschyi* significantly ($p< 0.05$) and dose dependently increased the duration of sleep, though the latency to sleep was not reduced (Figure 4.1). The chloroform fraction of *P. kotschyi* significantly ($p<0.001$) decreased the latency to sleep and increased the total sleep duration in the diazepam induced sleep test (Figure 2).

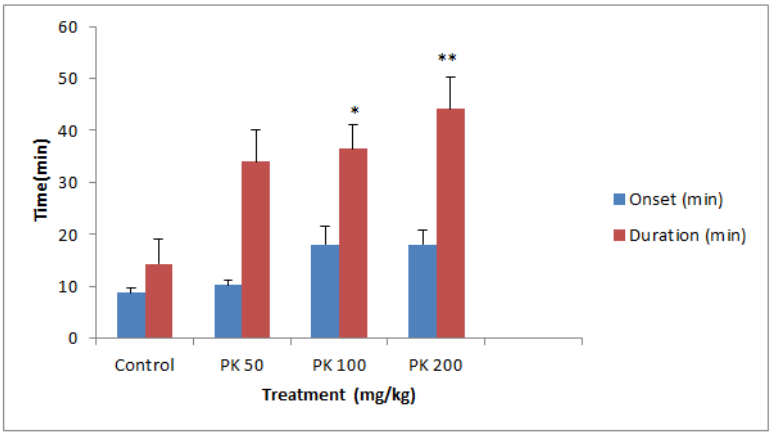

Fig-4.1: Effect of *P. kotschyi* methanol stem bark extract (MSBE) in the diazepam induced sleep in mice; Onset and duration of sleep presented as mean ± SEM;). PK: *Pseudocedrela kotschyi* (n=6). *P<0.05; ** P< 0.01; compared to control

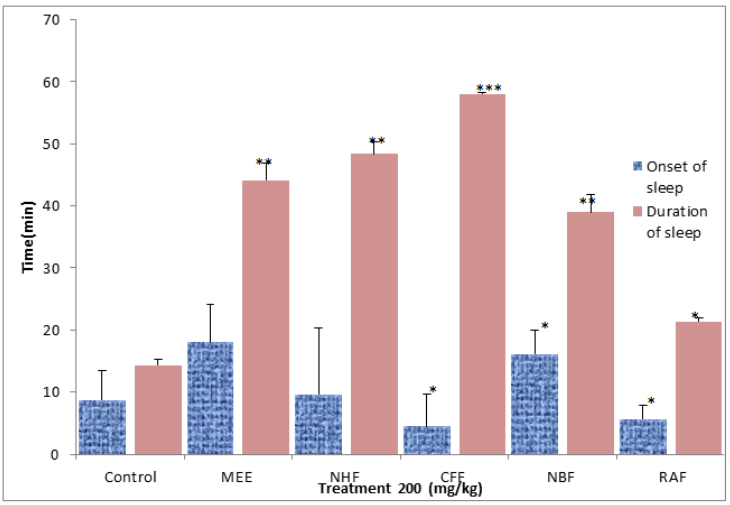

Fig-2: Comparative Effect of *P. kotschyi* MSBE and fractions on diazepam induced sleep in mice; Onset and duration of sleep presented as mean ± SEM; MEE: methanol extract; NHF: N hexane fraction; CFF: chloroform fraction; NBF: N-butanol fraction; RAF: residual aqueous fraction, (n=6). *P<0.05; ** P< 0.01, ***P< 0.001; compared to control

Anticonvulsant Effects of *P. Kotschyi* on Maximal Electroshock Seizure (MES) test in Chicks

Methanol stem bark extract of *P. kotschyi* did not significantly (P> 0.05) protect chicks against MES (Table 2), although there was a slight reduction in the recovery time after hind limb tonic extension (HLTE). Phenytoin 20 mg/kg, a known anticonvulsant agent showed significant (P< 0.01) reduction in the recovery time, and provided (80%) protection against HLTE.

Similarly, *P. kotschyi* fractions did not significant protect the chicks against MES (Table 3), although there was a decrease in the recovery time post convulsion with the entire fractions, the values were insignificant (P> 0.05). Phenytoin a known anticonvulsant offered 90% protection against hind limb tonic extension (HLTE).

Table-2: Anticonvulsant activity of methanol extract of *Pseudocedrela kotschyi* on maximal electroshock induced seizure test (MEST) in chicks

Treatment (mg/kg)	Recovery time (min)	Quantal protection	% protection	% mortality
N Saline 10	5.13 ± 1.91	0/10	0	100
PK 50	3.13 ± 1.60	0/10	0	100
PK 100	2.19 ± 1.61	0/10	0	100
PK 200	2.32 ± 1.23	0/10	0	100
Phenytoin 20	1.25 ± 0.34**	8/10	80	20

Values are expressed as Mean ± S.E.M., n = 6, ** P< 0.01 statistically significant as compared with the negative control.PK, *Pseudocedrela kotschyi*

Table-3: Anticonvulsant Activity of Fractions of *P. kotschyi* (200 mg/kg) on Maximal Electroshock Seizure (MES) test in chicks

Treatment (mg/kg)	Recovery time (min)	Quantal protection	% protection	% mortality
N Saline 10	8.82 ± 5.23	0/10	0	0
N Hexane	6.32 ± 2.45	0/10	0	0
Chloroform	5.67 ± 3.32	0/10	0	0
N-butanol	5.16 ± 1.18	0/10	0	0
R aqueous	7.05 ± 3.54	0/10	0	0
Phenytoin 20	-	9/10	90	0

Values are expressed as Mean ± S.E.M., n = 6, Statistical tool: ANOVA (one way). Dennett's *post hoc* test

Anticonvulsant Effects of *P. kotschyi* on Pentylenetetrazole- induced Seizures in Mice

Pseudocedrela kotschyi methanol extract at doses of 100 and 200 mg/kg significantly (P<0.05 and P< 0.01) and dose dependently increased the latency to onset of pentylenetetrazole-induced seizure, although the extract treated groups were not protected against seizure, the diazepam (5 mg/kg) group offered 100% protection against PTZ induced seizure (Figure 3).

The fractions of *P. kotschyi* at a dose of 200 mg/kg significantly (p<0.05-p<0.001) increased the onset of pentylenetetrazole induced seizure. The chloroform fraction offered 66.7% protection against tonic seizures induced by pentylenetetrazole. Diazepam (5 mg/kg) antagonized the seizure produced by PTZ and provided 100% protection from tonic seizures (Table 4).

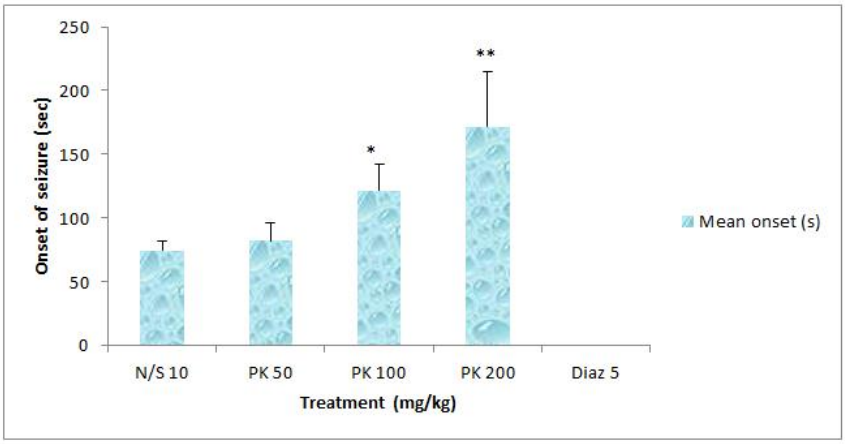

Fig-3: Effect of methanol extract of *P. kotschyi* on the onset of Pentylenetetrazole-induced seizures in mice; Onset of seizure presented as mean ± SEM; N/S: normal saline; PK: *Pseudocedrela kotschyi*; Diaz: Diazepam, (n=6). *P<0.05; ** P< 0.01; compared to control

Table-7: Anticonvulsant Effect of *P. kotschyi* Fractions on Pentylenetetrazole Induced Seizure in Mice

Treatment (mg/kg)	Mean onset (sec)	Protection (%)	Quantal protection
N Saline10	72.83 ± 6.23	0	0/6
Hexane 200	118.2 ± 15.13*	16.67	1/6
Chloroform 200	160 ± 10.09***	66.67	4/6
N-butanol 200	128.25 ± 10.13**	33.33	2/6
R aqueous 200	104.17 ± 7.34*	0	0/6
Diazepam 5	0	100	6/6

Values are expressed as Mean ± S.E.M., n = 6,* statistically significant P< 0.05 as compared with the negative control, ** statistically significant P< 0.01 as compared with the negative control. *** Statistically significant P< 0.001 as compared with the negative control. Statistical tool: ANOVA (one way). Dunnett's multiple comparison test.

Anticonvulsant Effects of *P. kotschyi* on Picrotoxin-induced Seizures in Mice

The methanol extract of *P. kotschyi* produced a dose dependent increase in the latency of picrotoxin-induced seizure. The extract at a dose of 100 and 200 mg/kg significantly (p<0.05 and 0.01) increased seizure latency and provided 16.7% protection against seizure (Figure 4). There was 100% protection against seizure in the group treated with Diazepam 5 mg/kg (Figure 5).

The chloroform and n-butanol fractions at a dose of 200 mg/kg offered 16.7% protection against seizure induced by picrotoxin while the hexane and residual aqueous fractions did not (Table 9).

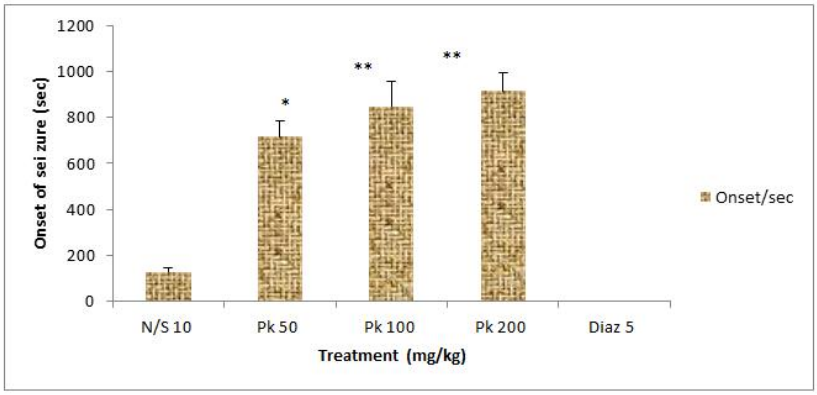

Fig-4.4: Effect of methanol extract of *P. kotschyi* on the onset of Picrotoxin-induced seizures in mice; Onset of seizure presented as mean ± SEM; N/S: normal saline; PK: *Pseudocedrela kotschyi*; Diaz: Diazepam, (n=6). *P<0.05; ** P< 0.01; compared to control

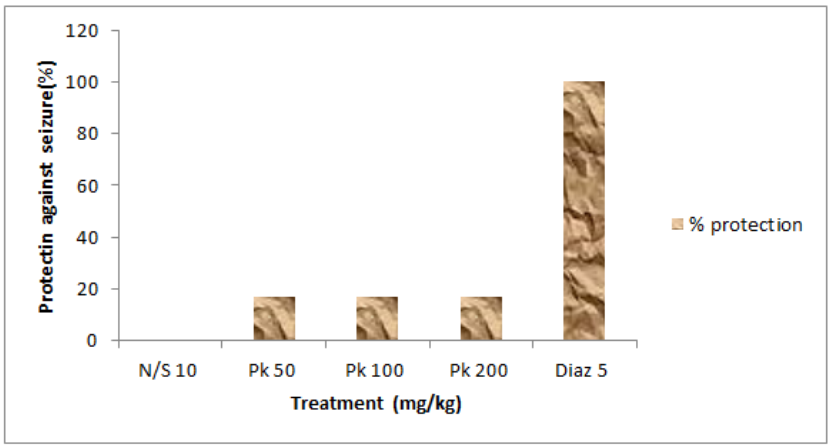

Fig-4.5: Effect of methanol extract of *P. kotschyi* on Picrotoxin-induced seizures in mice; N/S: normal saline; Pk: *Pseudocedrela kotschyi*; Diaz: Diazepam, (n=6)

Table-4.9: Anticonvulsant effect of *P. Kotschyi* fractions on Picrotoxin induced seizure in Mice

Treatment (mg/kg)	Mean onset (min)	% Protection	Quantal protection
N saline10	4.55 ± 0.2	0	0/6
Diazepam 5	0	100	6/6
Hexane 200	10.28 ± 0.31*	0	0/6
Chloroform 200	16.11 ± 0.54***	16.67	1/6
N-butanol 200	12.86 ± 0.36**	16.67	1/6
R aqueous 200	12.28 ± 0.53**	0	0/6

Values are expressed as Mean ± S.E.M., n = 6,* statistically significant P< 0.05,** statistically significant P< 0.01 and ***P< 0.001 as compared with the negative control. Statistical tool: ANOVA (one way). Dunnett's multiple comparison test

Anticonvulsant Effects of *P. kotschyi* on Strychnine-induced Seizures test in Mice

The highest dose of the methanol stem bark of *P. kotschyi* (200 mg/kg) administered significantly (P<0.01) increased the latency to strychnine induced seizure. None of the treatments offered protection against strychnine induced seizures; the positive control group diazepam (5 mg/kg) significantly increased seizure latency and protected the mice from death (Figure 6).

From the results in (Table 10), 50, 100 and 200 mg/kg of the n-butanol and chloroform fractions at a dose of (200 mg/kg) significantly P<0.05 and P<0.01, increased the latency to strychnine induced seizure in mice, however none of the fractions offered protection against seizure. There was a significant (P<0.05 and P<0.01) prolongation in the time of death at all the doses tested (50,100 and 200 mg/kg). All the Mice in the extract treated groups did not survive for more than 3 minutes post strychnine challenge except the diazepam group in which 33.3% of the mice survived.

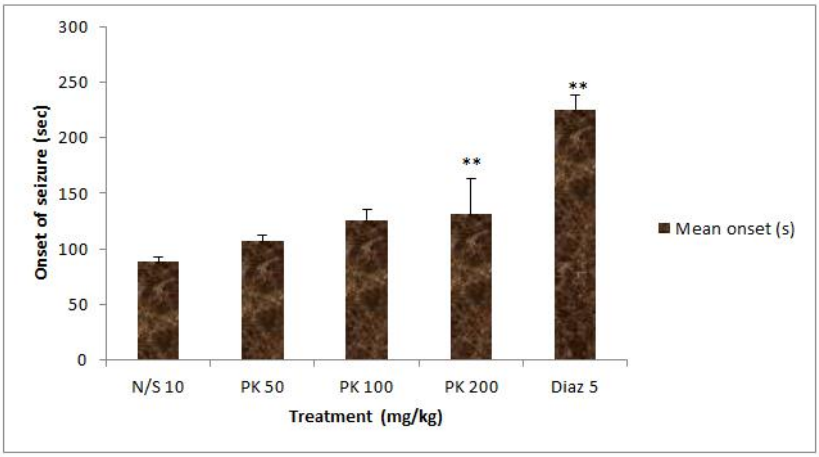

Fig-4.6: Effect of methanol extract of *P. kotschyi* on Strychnine-induced seizures in mice; Onset of seizure presented as mean ± SEM; N/S: normal saline; PK: *Pseudocedrela kotschyi*; Diaz: Diazepam, (n=6). ** P< 0.01; compared to control

Table-4.10: Anticonvulsant effect of *P. kotschyi* fractions on strychnine induced seizure in mice

Treatment (mg/kg)	Onset(s)	Time of death(s)	% protection
N Saline	104.33 ± 5.31	69.25 ± 3.42	0
Diazepam 5	219.17 ± 7.90	458.75 ± 7.95	33.33
Hexane 200	119.33 ± 6.86	140.17 ± 9.77**	0
Chloroform 200	132.33 ± 7.30*	115.83 ± 5.77*	0
N-butanol 200	154.17 ± 6.09**	146.17 ± 8.88**	0
R aqueous 200	100.83 ± 7.88	111.83 ± 5.70*	0

Values are expressed as Mean ± S.E.M., n = 6,* statistically significant P< 0.05 compared to the negative control, ** statistically significant P< 0.01 as compared with the negative control. Statistical tool: ANOVA (one way). Dunnett's multiple comparison test.

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DISCUSSION

Several studies reporting the anticonvulsant activity of plant phytochemicals have been documented some examples include the anticonvulsant activity of saponin rich portion of *Ficus platyphylla* stem bark [21, 22]. Similarly steroids saponins and triterpenoidal saponins are reported to possess anticonvulsant activity in some experimental seizure models such as MES and PTZ [23].

P. kotschyi stem bark methanol extract yielded 14.4% w/w in this study, fractionation of the methanolic extract yielded four fractions namely: n-hexane, chloroform, n-butanol and residual aqueous fraction. The high yield of the chloroform, n-butanol and residual aqueous fractions suggests that the bioactive constituents may be polar, because of the opinion that, "like dissolves like".

Pseudocedrela kotschyi extract and fractions significantly reduced the onset and prolonged the duration of sleep induced by diazepam. This action suggests that the extract of *P. kotschyi* may possess sedating properties [24, 25]. Agents with sedative properties also have anticonvulsant activities; examples include the barbiturates and benzodiazepines. This sedative-hypnotic property could be related to the presence of triterpenes in the fraction activating the benzodiazepine, barbiturate and/or GABA receptors in the $GABA_A$ receptor complex [26-28]. Diazepam is known to potentiate GABA-mediated inhibition via increase in the affinity of this inhibitory neurotransmitter to its recognition sites within the GABAA receptor complex; it increase the opening frequency of the chloride ion channel which leads to the enhancement of influx of chloride anions into the neuron and subsequent hyperpolarisation [29].

P. kotschyi crude MSBE did not protect chicks against tonic hind limb extension (THLE) a common feature of maximal electroshock. The inability of the crude methanol extract to protect against Maximal electroshock test suggests that it may not be beneficial in the management of generalized tonic-clonic seizures (GTCS). Antiepileptic drugs that are effective against GTCS such as phenytoin and Lamotrigine have been observed to block THLE in rodents [30]. Apart from protection against THLE, other features indicating anticonvulsant activity of a substance includes; increase

in latency to onset of seizure, decrease in recovery time and protection against mortality.

The maximal electroshock seizure test is the best validated preclinical test that predicts drug effectiveness against generalized tonic clonic seizure [31, 32]. The popularity of the MES test is attributed the ease with which it is used to determine anticonvulsant activity in rodents and the high correlation established between the ability of a drug to inhibit MES in rodents and its effectiveness in generalized tonic–clonic seizures in humans [33]. Several standard and newly developed AEDs are effective in the MES test, thus making it possible to quantify their anticonvulsant potency after both single and combined application [31, 34]. Although it has been stated that the MES test restricts the testing of drugs acting on Na+ channels such as carbamazepine, and phenytoin [35], the majority of standard and newly developed anticonvulsant drugs are effective in the MES model, despite the fact that these drugs interact with other drug targets [36]. The inability of the MSBE of *P. kotschyi* to inhibit THLE in mice suggests that it may not be effective in generalized tonic-clonic seizures in humans.

Pentylenetetrazole is a most frequently used model in the preliminary screening of potential anticonvulsant drugs. Pentylenetetrazole (PTZ) induced seizure model is employed for the evaluation of drugs activity against myoclonic or absence seizure [37]. Anticonvulsant effect against PTZ identifies compounds that can raise seizure threshold in the brain [15]. The mechanism by which PTZ elicits its action is not very well understood; it is believed to exert its action by acting as an antagonist at the gamma-aminobutyric acid $GABA_A$ receptor complex [38]. Therefore agents that enhance $GABA_A$ receptor mediated inhibitory transmission such as benzodiazepines and barbiturates prevent PTZ induced seizures. Similarly the activity of PTZ is shown to be as a result of reduction in the chloride conductance and to a negligible degree the sodium and potassium conductance [39]. In addition, a variety of other systems are affected by PTZ administration, i.e., other transmitters such as acetylcholine, dopamine and norepinephrine and several brain metabolic enzymes [40]. Taken together, multiple changes in transmitter systems occur as a consequence of PTZ administration and how these changes contribute to the mechanism is not clear [41].

The moderate activity of the methanol extract and fractions against PTZ-induced seizure suggested the presence of bioactive compounds that may be beneficial in the management of absence or myoclonic seizures. It is therefore reasonable to suggest that the anticonvulsant effect of the extract and fractions may be due to either interaction with GABA neurotransmission, central noradrenergic activity and/or blockage of glutamatergic neurotransmission mediated by N-methyl D-aspartate (NMDA) receptors [42].

Picrotoxin is known to elicit seizures, by antagonizing the effect of GABA via blockage of the chloride channels linked to GABAA-receptor [28]. In this study, diazepam was shown to antagonize the effect of picrotoxin while the extract was also shown to delay the latency of picrotoxin–induced seizures, suggesting that the extract may be affecting GABAergic mechanisms, probably by opening the chloride channels associated with GABAA receptors. Agents such as barbiturates, benzodiazepines, valproic acid, gabapentin and tiagabine which protecet against picrotoxin induced seizure interfere with the GABAergic pathway thereby enhancing GABA mediated neurotransmission [43, 28].

The methanol extract and fractions of the *P. kotschyi* did not protect the mice against strychnine (STN) induced seizures, though it significantly increased the latency of myoclonic jerks. The convulsing action of strychnine is due to interference with postsynaptic inhibition mediated by glycine, an important inhibitory transmitter to motor neurons and interneurons in the spinal cord [44]. Strychnine sensitive postsynaptic inhibition in higher centers of the CNS is also mediated by glycine [45]. The effects of the extract against strychnine-induced seizures suggest additional mechanism of action via interference with glycine- sensitive channels.

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