ANTICONVULSANT ACTIVITY OF METHANOL LEAF EXTRACT OF *STRYCHNOS SPINOSA* (Lam.) IN MICE AND CHICKS

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Abstract

Strychnos spinosa is a pantropical plant with wide applications in the treatment of both communicable and non-communicable diseases. The objective of this study is to evaluate the anticonvulsant activities of methanol leaf extract of *Strychnos spinosa* at 150, 300 and 600mg/kg in experimental animals. Phytochemical screening and acute toxicity studies were conducted on the extract as well as evaluation of anticonvulsant activity against maximal electroshock induced convulsions in a day old chicks, pentylenetetrazole, 4-aminopyridine, strychnine, picritoxin and isoniazide induced seizures in mice. The median lethal dose of *Strycnos spinosa* was estimated to be 2000mg/kg in mice and >5000 mg/kg in chicks. Phytochemicals screening revealed the presence of alkaloids, flavonoids, cardiac glycosides, saponins, tannins, streroids, anthraquinones and triterpines. The extract provided 30% seizure protection at 300mg/kg against maximal electroshock test. Protection of 66.67% was offered at 300 and 600 mg/kg against pentylenetetrazole induced convulsions. There was significant($p<0.001$) increase in the mean onset of 4-aminopyridine induced convulsions at 300 and 600 mg/kg. Protections was provided in 50% of the mice at 300mg/kg in strychnine induced convulsions model. Upto 16.67% seizure protection was observed at both 150 and 300 mg/kg against picritoxin induced convulsions. On isoniazide induced convulsions the plant extract (150 mg/kg), diazeparm(5 mg) and pyridoxin(300 mg/kg) each provided 83.33% seizure protection, similarly, there was significant increase in the mean onset of seizures by the extract (150 mg/kg)($p<0.01$) and diazeparm(5 mg)($p<0.001$). The result revealed that the methanol leaf extract of *Strychnos spinosa* possesses anticonvulsant activity against different models of epilepsy. This provides scientific credence for the ethno-medical use of the plant in the management of various types of epilepsy.

Keywords: Anticonvulsant, Epilepsy, Isoniazide, Pentylenetetrazole, *Strychnos spinosa* , 4-aminopyridine.

Introduction

Strychnos spinosa Lam. is one of the genus of *Strychnos* and it belongs to the family of Loganiaceae which is the largest of the family. Its commonly known as Monkey Orange in English, *Kokiya* (Hausa), *Angboroko* (Igbo), *Narbatanaje* (Fulde), *Ata oro or Atako* (Yoruba) and *Toliya* (Kanuri). It is a pantropical plant and the genus comprises of about 200 species and they are splited across four geographical regions, namely American (75 species), African (73 species), Asian and Australian (44 species) (Ohiri *et al*., 1983; Leeuwenberg, 1969). In sub-Saharan Africa, *S. spinosa* Lam. is the commonest specie, native to the area and found in Northeast tropical Africa (from Somalia to Sudan), in East tropical Africa (from Kenya to Uganda), West-central tropical Africa (from Rwanda to Cameroon), West tropical Africa (from Nigeria to Senegal), South tropical Africa (from Angola to South Africa) and in Western Indian ocean (Madagascar and Mauritius) (USDA 2020). *Strychnos spinosa* is a thorny shrub or small tree 1-9cm in height. The bark is grey and rough while the leaves are eleptical light to dark green in colour. It produces creamy green flowers and spherical friuts deep yellow to yellow brown when mature and contains many flat seeds (Orwa *et al*., 2009). Majority of the species found within this genus are considered to be poisonous, due to the presence of alkaloids, strychnine and brucine. These toxic compounds are typically restricted to the seeds, while the fruit pulp remains edible (Dausmann *et al*., 2008).

Juice obtained from the fruit and roots of *Strycnos spinosa* is used as ear drop for earache; the roots, leaves and bark are used in the treatment of male organs disorders. A decoction of the roots is taken orally for colds or is drunk with milk to cure dropsy. The roots or green fruits are used by the Zulu of South Africa as an antidote for snakebite. The roots alone are used as an emetic and in the treatment of fever and inflammation of the eyes (Orwa *et al.*, 2009). Other medicinal uses of the plant as according to Tittikpina *et al*., (2020) include treatment of both infectious and non-infectious ailments such as malaria (Asase *et al*., 2005), trypanosomiasis (Bizimana *et al.*, 2006), tuberculosis (Molander *et al*., 2014); female sexual disorders, gastrointestinal disorders (Tchacondo *et al*., 2011), hypertension (Avakoudjo *et al*., 2019), and epilepsy (Bukill, 1985). Considering the various ethnomedicinal importance of *Strychnos spinosa*, the aim of this study is to evaluate the anticonvulsant activities of the plant in laboratory animals, and based on our investigations studies of similar nature has not been previously conducted

Epilepsy is among the commonest chronic and seriously encountered neurological medical condition affecting people irrespective of their ages in the wold, with the peak of the incidence usually seen in children and adults over the age of 60 years. The disease is characterized by

abnormal electrical activity in the brain, leading to seizures or unusual behaviour, sensations and sometimes loss of awareness. The etiology include events that lead to prenatal or perinatal injuries, congenital abnormalities or brain malformations, head injuries, stroke, neurological infections such as meningitis, encephalitis and neurocysticercosis, and brain tumours. In some ocasions it may be attributed to underlying genetic abnormalities; whearas close to a half of the cases have no identifiable causes (WHO, 2019)[a]. Approximately 50 million people have epilepsy world wide of which about 80% are from low and middle income nations. It has an annual incidence of more than 50 million with likelyhood of it been increased. Epilepsy accounts for more than 0.5% of the total burden of diseases worldwide (WHO, 2019)[b].

Anticonvulsant, antiepileptic or antiseizure drugs are used to control the convulsive episodes by inhibiting the electrical discharge and then produce hypnosis. The drugs suppress seizures without correcting the underlying cause that lead to the generation of seizures, and their effectiveness is upto 60–70% (Beghi, 2020). Despite the fact that several synthetic anticonvulsant drugs are available for the management of convulsions, the treatment of epilepsy is still not far fetched because of their cost, unavoidable and intolerable side effects (neurotoxicity, dose related and chronic toxicity and teratogenic tendencies), drug interactions and poor patient compliance. This necessitate the need for discovery of newer and more effective anticonvulsant drugs for intractable convulsion (Asif, 2013).

The use of herbal products are preferred over synthetic drugs for neurological disorders such as Alzheimer disease, Parkinson disease, depression, epilepsy, schizophrenia, anxiety, and neuropathy due to their affordability, accessibility, lesser and tolerable side effects, and better therapeutic effects. (Balkrishna and Misra, 2017). In developing countries about 70% of people still rely on complimentary and alternative medicines despite the improvement of conventional medications (Shaheen and Kamran, 2017). The herbs serve as a substitute for orthodox Western scientific medicine and supplimentary treatments (Schachter, 2009). Although herbal medicine is accepted worldwide and extensively used in managing epilepsy in many countries due to their wide applicability and therapeutic efficacy with low adverse effects, there is lack of robust evidence for efficacy and toxicity (Liu *et al*., 2017).

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Materials and methods

Drugs, chemicals and equipments:

Methanol, strychinine, pentylenetetrazole and picrotoxin (Sigma chemical Co., St. Louis, USA) and 4-amino pyridine (Merch-schuchardt, Germany), sodium valproate (Sanofi Aventis,UK), phenobarbitone (Lab Renaudin, France),phenytoin (Parker-Davis and Co. Ltd), diazepam (RocheLtd,France), Electroconvulsive machine (Orchid International EC01), analytical balance (Mettler Instrument Corporation, U.S.A.) .

Animals

Swiss albino mice (18-25g) of either sex were obtained from Animal Facility, Department of Pharmacology and Therapeutics, Bayero University, Kano. A day old Ranger cockerels (30-40 g) were obtained from Yammfy chicks Illemona, Kwara State, Nigeria. The animals were kept in a well-ventilated condition at ambient temperature and were fed with a standard animal feed with adequate access to water *ad libitum*. The experimental animals used were handled in accordance with the Bayero University guidelines for the Care and Use of Laboratory Animals.

Collection and identification of plant material

Fresh leaves of *S. spinosa* were collected from Kiru Local Government area, Kano state, Nigeria, in the month of September, 2021. It was identified and authenticated by Dr Yusuf Nuhu, a taxonomist with the Department of Botany, Faculty of Life Sciences, Bayero University, Kano, Nigeria by comparing with an already deposited voucher specimen number, (BUKHAN127) as reference at the Herbarium Section of the department.

Plant preparation and extraction

The leaves of the plant were shade dried at room temperature for two weeks until constant weight was attained. The dried leaves were then grinded into fine powdered form using pestle and mortar. The powdered leaf (2000g) was extracted with 8 liters of 70% v/v methanol (70% Methanol: 30% water) for 1 week using cold maceration extraction method. The extract obtained was then evaporated in a thermostat oven at 50 ºC . The dried extract was weighed and then stored in a desiccator until when its needed.

Phytochemical screening

Phytochemical screening was conducted on the methanol leaf extract of *S. spinosa* in oder to validate the presence or otherwise of secondary metabolites such as alkaloids, cardiac glycosides, saponins, tannins, streroids and anthraquinones (Sofowora, 1993 ; Evans, 2009).

Acute toxicity study

Median lethal dose (LD_{50}) of methanol leaf extract of *S. spinosa* was determined via intraperitoneal route (*i.p*) in mice using the method described by Lorke (1983). The study was carried out in two phases. In the first phase, nine mice of either sex were randomly selected and divided into three groups of three mice per group, which were treated with 10, 100, 1000 mg/kg of the methanol leaf extract of *S. spinosa*. The treated animals were then observed for signs of toxicity including death over a period of 24 hours. In the second phase, three mice were treated with more specific doses of the extract *i.p* (based on the outcome of first phase study) and also observed for signs of toxicity and death in 24 hours. The LD_{50} was calculated as the geometric mean of the lowest dose that caused death and the highest dosage for which the animal survived.

Anticonvulsant studies

Maximal electroshock (MEST)-induced convulsion test in chicks

Maximal electroshock (MEST) induced convulsion test was conducted using a method described by Swinyard and Kupferberg (1985). Fifty day old cockerels were randomly divided into five groups each containing ten chicks. The 1st group received distilled water (10 mL/kg) *i.p* and served as a negative control. Groups 2-4 received the graded doses of the extract (150, 300 and 600 mg/kg *i.p* respectively) while the 5th group received phenytoin (20mg/kg, *i.p*) and served as positive control. Thirty minutes later, maximum

electroshock was administered to induce seizure in the chicks using Orchid international electroconvulsiometer EC01 connected to a corneal electrodes, placed on the upper eyelids of the chicks after dipping them in normal saline. The current, shock duration, frequency and pulse width were set and maintained at 150 mA, 0.2s, 50 Hz and 0.6 ms-1 respectively throughout the study. Tonic hind limb extension was considered as the convulsion. Anticonvulsant activity was considered as the ability of the extract to prevent features of tonic hind limb extension.

Pentylenetetrazole-induced convulsion test in mice

The method described by Swinyard *et al*., (1989) was employed. Thirty mice were randomly divided into five groups of six mice each. The 1st group received distilled water (10 mL/kg *i.p.*); the 2nd, 3rd and 4th groups were treated with 150, 300 and 600 mg/kg (*i.p*) of *S. spinosa* extract respectively and the 5th group received 200 mg/kg of sodium valproate. Thirty minutes post treatment, mice in all the groups were administered 80 mg/kg body weight of freshly prepared pentylenetetrazole (PTZ) subcutaneously (*s.c.*). Thirty minutes later each mouse was observed for for onset of seizures. Episodes of clonic spasm (loss of righting reflex) was considered as convulsions. The absence of loss of righting reflex during the 30 minutes of observation was regarded as protection against PTZ induced convulsions.

Strychnine-induced convulsion test in mice

The study was conducted using method described by (Lehmann *et al*.,1988).Thirty mice were divided into five groups of six mice each, the 1st group received distilled water (10 mL/kg body weight *i.p.*); the 2nd, 3rd and 4th groups were treated with 150,300 and 600 mg/kg (*i.p*) of *S.spinosaa* extract respectively. The 5th group received 30mg/kg of phenobarbitone *i.p.* Thirty minutes later, mice in all the groups were treated with 1mg/kg (*s.c.*) of freshly prepared strychnine. Abolition of tonic extensor jerks of the hind limbs and/or latency of death was considered as protection against strychnine induced convulsion.

4-aminopyridine-induced convulsion test in mice

The study was performed as described by Yamaguchi and Rogawski (1992). Thirty (30) mice were divided into five groups each containing six mice each. The 1st group served as negative control and was pretreated with normal saline 10mL/kg (*i.p.*) body weight. The 2nd, 3rd and 4th groups were treated with 150, 300 and 600 mg/kg (*i.p*) of *S. spinosa* extract respectively where as the 5th group were pretreated with 30 mg/kg body weight phenobarbitone (*i.p*). Thirty minutes after pretreatment; 4-aminopyridine was freshly prepared and administered at a dose of 14 mg/kg body weight (*s.c.*) to each mouse in all the groups. The mice were observed for 30 minutes for presence or absence of tonic extension as well as onset of seizures.

Picrotoxin-induced convulsion test in mice

The method described by Ogbonnia (2003) was employed. Thirty mice were randomly divided into five groups containing six mice in each group. The first group served as control and was pretreated with normal saline 10 mL/kg body weight *i.p.* The 2nd, 3rd and 4th groups were pretreated were treated with 150, 300 and 600 mg/kg (*i.p*) of *S. spinosa* extract respectively while the 5th group were pretreated with 30 mg/kg (*i.p.*) phenobarbitone. Thirty minutes after pretreatments; mice in all groups were treated with freshly prepared picrotoxin (1.2 mg/kg, *s.c*). The mice were then observed for presence or absence of convulsion for 30 minutes.

Isoniazide-induced convulsion test in mice

Isoniazid-induced convulsion was assessed using the method described by Asehinde *et al*. (2018). The mice were divided into different treatment groups (n=6) and were given distilled water (control, 10 mL/kg), diazepam (5 mg/kg), pyridoxine (300 mg/kg) or *S. Spinosa* extract (150, 300, 600 mg/kg *ip*) . Thirty minutes later, the mice were given isoniazid (300 mg/kg, *i.p*) and were observed for 1 hour for latency to convulsion and death.

Data analysis

Data obtained were analyzed by one way analysis of variance (ANOVA) followed by Dunnett's post hoc test using Statistical Package for Social Sciences (SPSS) software. Values of $p<0.05$ were considered statistically significant. The data were then presented as mean ± the standard error of the mean (S.E.M.).

Results and Discussion

The major aim of this study is to provide the scientific rationale for the fockloric claim for the ethnomedicinal use of the leaves of *Strychnos spinosa* in the treatment of epilepsy. The methanol leaf extract of the plant showed the presence of alkaloids, cardiac glycosides, saponins, triterpenes, tannins and flavanoids (Table 1). These constituents are thought to be responsible for the anticonvulsant activities observed in the extract. Several previous studies on medicinal plants have established anticonvulsant activities and the phytochemical constituents were attributed to such activitie. These plants include; *Boswellia dalzielii* (Nazifi *et al*., 2017), *Aspilia africana* (Kemelayefa and Kagbo, 2018) and *Securidaca longipedinculata* (Abubakar *et al*., 2019). Phytoconstituents such as flavonoids,tannins and saponins have been shown to be modulators of central nervous system activities (Muhammed *et al*., 2017). In the same note studies have shown that alkaloid derivatives were effective inhibitors of both maximal electroshock and pentylenetetrazole induced seizure (Ribeiro and Leite, 2003). Therefore the observed anticonvulsant activities of *Strychnos spinosa* might be attributed to the presence of its phytochemicals constituents.

Table 1: Phytochemical Constituents of Methanol Leaf Extract of *Strychnos spinosa*

Constituents	Inference
Cardiac glycosides	+
Saponins	+
Triterpenes	+
Tannins	+
Steroids	+
Flavonoids	+
Alkaloids	+
Anthraqiunones	-

Key: Present= (+), Absent = (-)

The intraperitoneal LD_{50} of the methanol leaf extract of *Strychnos spinosa* in mice was found to be 2000 mg/kg and >5000 mg/kg in chicks. Some chemical are considered to be toxic (or posonous) if they cause death with minute concentration in microgram, while others may be

relatively harmless following doses in excess of several grams. Base on that, categories of toxicity have been devised on the basis of amounts of the chemicals necessary to produce harm. Such categorization, along side the respective lethal doses ranges from extremely toxic (1 mg/kg or less), highly toxic (1 to 50 mg/kg), moderately toxic (50 to 500 mg/kg), shghtly toxic (0.5 to 5 g/kg), practically nontoxic (5 to 15 g/kg) to relatively harmless (more than 15 g/kg) (Loomis and Hayes, 1996). Therefore the acute toxicity studies on the methanoloic leaf extract of *Strychnos spinosa* has shown that it is slightly toxic in mice.

The methanol leaf extract of *Strychnos spinosa* at 300 mg/kg body weight provided 30% seizure protection against tonic hind limb extension (THLE) in the maximal electroshock induced convulsion test.While the standard drug phenytoin (20mg/kg) provided 100% seizure protection against THLE. None of the tested doses demonstrated any significant (p>0.05) decrease in the mean recorvery period (Table 2). The protection against maximal electroshock test provided by the methanol leaf extract of *Strychnos spinosa* was aweak one. This result is consistant with the findings of Wapa *et al*., (2018) and in contrast with Muhammad *et al*., (2017). The contrast might be due to differences in the pathways throughn which the palnts produced their anticonvulsant activity. MEST model is perhaps among the best validated preclinical test that is used to predict the effectiviness of a drug against generalized tonic clonic convulsions (grandmal seizures)(White, 2003; Mares and Kubova, 2006; Holmes and Brown, 2008). The test predict the anticonvulsant activity of antiepileptic drugs that limit or prevent the spread of the seizure discharge from the epileptic focus during seizural events (Raza *et al*., 2001). Antiepileptic drugs that act through this pathway are able to decrease the repetitive firing of action potentials by slowing the rate of recovery of voltage-activated sodium channels from inactivation and suppress hind limb tonic extension in maximal electroshock seizures(Rho and Sankar, 1999). The results obtained from this study suggest that *Strychnos spinosa* contains some compounds with the ability to limit seizure spread and may be useful in the treatment of generalized tonic clonic and partial seizures.

Table 2 : Effect of Methanol Leaf Extract of *Strychnos spinosus* on maximal electroshock test Induced Convulsion in Chicks

TREATMENT (mg/kg)	MEAN RECORVERY PERIOD (Min)	QUANTAL PROTECTION	% SEIZURE PROTECTION
D/W10 mL/kg	8.20±0.99	0/10	0.00
MLSS 600	10.14±2.26	2/10	20.00
MLSS 300	10.86±1.68	3/10	30.00
MLSS 150	6.75±2.11	2/10	20.00
PTY 20	-	10/10	100.00

Values are presented as Mean ± SEM, no significant difference compared to distilled water control group – One-way ANOVA followed by Dunnets *post hoc* test, n=10, DW = Distilled water, MLSS = Methanol leaf extract of *Strychnos spinosus*, PTY = Phenytoin

Strychnos spinosa extract confered 66.67% protection against tonic clonic seizures at 600 and 300 mg/kg. While the standard anticonvulsant, sodium valproate, showed 100% seizure protection against PTZ-induced convulsion (Table 3). The findings in this study is in conformity to the findings of the study by Muhammad *et al*., (2017), but slightly different with Yaro *et al*., 2015. Pentylenetetrazole is a known chemoconvulsant and it has the ability to interfere or decrease the GABAergic neurotransmission or tone by blocking the benzodiazepine binding site of the gama amino butyric acid (GABA) receptor complex (Bum *et al*., 2001). Anticonvulsant activity in PTZ test describe compounds that can elevate the seizure threshold in the brain tissues (White *et al*., 1998). Anti epileptic drugs with proven efficacy in the treatment of generalized seizures (absence or myoclonic) include sodium valproate, phenobarbitone, benzodiazepines and ethosuximimide (McNamara, 2006). The ability of *Strychnos spinosa* to confer protection against PTZ induced seizures suggests the plants is likely to contain some bioactive constituents that could be effective in the pharmacotherapy of abscence (petitmal) or myoclonic seizures.

Table 3 : Effect of Methanol Leaf Extract f *Strychnos spinosa* on Pentylenetetrazole Induced Convulsion in Mice

TREATMENT (mg/kg)	MEAN ONSET OF SEIZURES (Min)	QUANTAL PROTECTION	% SEIZURE PROTECTION	% MORTALITY PROTECTION
D/W 10mL/kg	11.20±0.66	0/6	0.00	33.33
MLSS 600	7.50±0.50	4/6	66.67	16.67
MLSS 300	7.00±1.00	4/6	66.67	0.00
MLSS 150	8.33±0.33	1/6	16.67	16.67
SVP 200	-	6/6	100.00	0.00

Onset of seizures presented as Mean ± SEM, No significant difference compared to distilled water control group – One-way ANOVA followed by Dunnetts *post hoc* test n=6, DW – Distilled water, MLSS = Methanol leaf extract of *strychnos spiunosa*, SVP = Sodium valproate

Strychnos spinosa extract produced a significant (p<0.001) increase in the mean onset of seizures induced by 4-aminopyridine at 600 and 300 mg/kg when compared to distilled water control group. While only 16.67% of the animals were protected against seizures at 600mg/kg. While phenobarbitone, the standard anticonvulsant produced 100% protection against convulsion and mortality (Table 4). This is consistant with the findings of Nazifi *et al.*, 2017 and Muhammad *et al.*, (2017). 4-aminopyridine induces tonic-clonic type of seizures via antagonistic effect on potasium channels (Yamaguchi and Rogawski, 1992). It interferes with all aspect of neuronal excitability, including resting membrane potential, responsiveness to synaptic inputs, frequency adaptation and neurotransmitter release (Wickenden, 2003). The ability of the methanol leaf extract of *Strychnos spinosa* to significantly prolong the latency to convulsion by 4-aminopyridine suggests the likely hood of its interactions with potassium channel to produce anticonvulsant activity

Table 4 : Effect of Methanol Leaf Extract of *Strychnos spinosa* on 4-aminopyridine Induced Convulsion in Mice

TREATMENT (mg/kg)	MEAN ONSET OF SEIZURES (Min)	QUANTAL PROTECTION	% SEIZURE PROTECTION	% MORTALITY PROTECTION
D/W10 mL/kg	12.40±0.60	0/6	0.00	100.00
MLSS 600	24.75±1.65*	1/6	16.67	83.33
MLSS 300	21.25±0.63*	0/6	0.00	100.00
MLSS 150	10.33±0.67	0/6	0.00	100.00
PHB 30	-	6/6	100.00	0.00

Onset of seizures presented as Mean ± SEM, * = p<0.001 compared to distilled water control group – One-way ANOVA followed by Dunnetts *post hoc* test, n=6, DW =Distilled water, MLSS = Methanol leaf extract of *strychnos spiunosa*, PHB = Phenobarbitone

The methanolic extract of *Strychnos spinosa* exhibited 50, 33.33 and 16.67 % protection against strychnine-induced seizures at 300, 600 and 150 mg/kg respectively. The plant extract did not produce significant (p>0.05) increase in the mean onset of seizures induced by strychnine at all the tested doses when compared to distilled water control group. The standard drug (phenobarbitone, 30 mg/kg) and the extract at 150mg/kg provided 100% protection against mortality (Table 5). Strychnine is a competitive glycine receptor antagonist (Rajendra *et al*., 1997) . Together with some of its related alkaloids such as brucine and thebaine, they induce generalized convulsion by selectively and competitively blocking postsynaptic neurotransmission mediated by glycine, an important inhibitory neurotransmitter to motor neurones and interneurones in the spinal cord (Vogel, 2007) . Studies have shown that chemical agents that reverse the action of strychnine by antagonism of glycine is likely to have antiepileptic effects (Raza *et al*., 2010). The protection offered by the methanol leaf extract of *Strychnos spinosa* against tonic extensor jerk and death suggests that the leaf extract may likely act by enhancing inhibitory neurotransmission mediated by glycine.

Table 5: Effect of Methanol Leaf Extract of *Strychnos spinosa* on Strychnine Induced Convulsion in Mice

TREATMENT (mg/kg)	MEAN ONSET OF SEIZURES (Min)	QUANTAL PROTECTION	% SEIZURE PROTECTION	% MORTALITY PROTECTION
D/W 10mL/kg	10.20±1.03	0/6	0.00	33.33
MLSS 600	10.50±1.50	2/6	33.33	16.67
MLSS 300	8.33±0.33	3/6	50.00	33.33
MLSS 150	10.80±0.73	1/6	16.67	0.00
PHB 30	-	6/6	100.00	0.00

Onset of seizures presented as Mean ± SEM, No significant difference compared to distilled water control group – One-way ANOVA followed by Dunnetts *post hoc* test n=6, DW – Distilled water, MLSS = Methanol leaf extract of *strychnos spiunosa*, PHB = Phenobarbitone

The extract offered 16.67% protection against picrotoxin-induced seizures at both 300 and 150 mg/kg. The standard drug (diazepam, 10 mg/kg) and all the doses of the extract tested provided 100% protection against mortality (Table 6). Similar result were seen in Wapa *et al*., 2018 and Muhammad *et al*., (2017). Picrotoxin is a chemoconvulsant agent derived from plants products, it acts by blocking the GABAA receptor chloride channel, thus blocking the postsynaptic inhibitory effect of GABA. It also blocks the glycine receptors. It has no known therapeutic application (Rang *et al*., 2016). It is used in determining mechanism of action of agents with sedative-hypnotic and anticonvulsant activities (Vogel, 2008). Standard drugs used as antiepileptics such as phenobarbitone, sodium valproate, benzodiazepines and the newer antiepileptics such as tiagabine and gabapentin were effective in suppressing seizure induced by picrotoxin (Porter *et al*., 1984; Taylor, 1995 ; Wapa *et al*., 2018). The inability of the methanol leaf exttact of *Strychnos spinosa* to offer protection against picrotoxin-induced seizure suggests that its anti-convulsant action may not involve interaction with picrotoxin sites on $GABA_A$-chloride ion channel complex.

Table 6 : Effect of Methanol Leaf Extract of *Strychnos spinosa* on Picritoxin Induced Convulsion in Mice

TREATMENT (mg/kg)	MEAN ONSET OF SEIZURES (Min)	QUANTAL PROTECTION	% SEIZURE PROTECTION	% MORTALITY PROTECTION
D/W 10mL/kg	13.00±0.41	0/6	0.00	0.00
MLSS 600	14.60±1.08	0/6	0.00	0.00
MLSS 300	13.00±0.77	1/6	16.67	0.00
MLSS 150	15.60±0.81	1/6	16.67	0.00
PHB 30	-	6/6	100.00	0.00

Onset of seizures presented as Mean ± SEM, No significant difference compared to distilled water control group – One-way ANOVA followed by Dunnetts *post hoc* test n=6, DW = Distilled water, MLSS = Methanol leaf extract of *strychnos spinosa*, PHB = Phenobarbitone

Strychnos spinosa extract (150mg/kg) and diazeparm (5mg/kg) produce significant ($p<0.01$)($p<0.001$) increase in the mean onset of seizures induced by isoniazide. While the extract (150 mg/kg), diazeparm (5 mg/kg) and pyridoxine each provided 83.33% protection against isoniazide induced seizures and offered 100% protection against mortality (Table 7). Similar findings were seen in previous studies by Ishola *et al*., 2013 and Asehinde *et al*., (2018). Isoniazide (INH) induce convulsions as a result of pyridoxine (vitamin B6) induced deficiency which leads to decrease in inhibitory neurotransmitter Gamma Amino Butyric Acid (GABA) resulting into the decrea in threshold for convulsions (Okutur *et al*., 2006). Pyridoxal 5 phosphate is an active form of pyridoxine and it is a co-factor for glutamic acid decarboxylase which is required for GABA synthesis. Isoniazid inhibits glutamic acid decarboxylase by binding to pyridoxal 5-phosphate and reduces synthesis of GABA (Tsubouchi *et al*., 2014; Sridhar *et al*., 2012 ; Okutur *et al*., 2006; Sinan *et al*., 2013; Vasu and Saluja, 2006). Deficiency of GABA can therefore manifest itself in form of seizures especialy in acute toxicity (Kukuia *et al*., 2016). Methanol leaf extract prolonged the latency to seizures as well as offered protection against INH-induced seizures and mortality. Previous studies however revealed that standard anticonvulsant agents do not depend on the prevention of convulsion but on their ability to prolong the latency to seizures (Kendall and Enna, 1981). Furthermore, compound s that only delay the latency to convulsions block the the spread of seizure in epileptic brain (Corda *et al*., 1982).

Table 7 : Effect of Methanol Leaf Extract of *Strychnos Spinosa* on Isoniazide Induced Convulsion in Mice

TREATMENT (mg/kg)	MEAN ONSET OF SEIZURES (Min)	QUANTAL PROTECTION	% SEIZURE PROTECTION	LATENCY TO DEATH (min)	% MORTALITY PROTECTION
D/W 10mL/kg	27.17±1.64	0/6	0.00	34.67±1.89	100.00
MLSS 600	28.83±1.38	0/6	0.00	34.17±0.79	100.00
MLSS 300	30.83±3.58	0/6	0.00	30.83±3.58	100.00
MLSS 150	47.00±0.00*	5/6	83.33	-	0.00
DZP 5	60.00±0.00**	5/6	83.33	-	0.00
PDZ 300	39.00±0.00	5/6	83.33	-	0.00

Onset of seizures presented as Mean ± SEM, * p = <0.01, ** p = <0.001 compared to distilled water control group – One-way ANOVA followed by Dunnetts *post hoc* test, n=6, DW – Distilled water, MLSS = Methanol leaf extract *Strychnos spinosa*, DZP = Diazeparm, PDZ= Pyridoxine

Conclusion:
The findings from this study revealed that the methanol leaf extract of *Strychnos spinosa* possesses anticonvulsant activity in maximal electroshock test, pentylenetetrazole, strychnine and isoniazide induce seizure models. The mechanism of anticonvulsant activity may likely involved multiple pathways.This provide scientific credence for the ethnomedicinal use of the plant in the management of different types of epilepsy.

Conflict of interest
The authors declare no conflict of interest

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