ANTICONVULSANT ACTIVITY AND SAFETY PROFILE OF METHANOL STEM BARK EXTRACT OF *BLIGHIA SAPIDA* KOENIG (SAPINDACEAE) IN LABORATORY ANIMALS

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Abstract: *Blighia sapida* is used locally for the treatment of fever, diabetes, epilepsy and psychosis. This study was conducted to assess the anticonvulsant potential and toxic effect of the methanol extract (MEBS) of the plant. Pentylenetetrazole (PTZ), 4-aminopyridine, picrotoxin and maximal electroshock (MES) induced seizures were used to evaluate the anticonvulsant activities in mice and chicks. The oral LD_{50} was found to be greater than 5000mg/kg. Tannins and alkaloids make up the major secondary metabolites. In (PTZ) seizure test, the extracts significantly ($P < 0.01$) increase the mean onset of seizure. The same doses conferred 16.67 to 50.0% protection against PTZ-induced convulsion in mice. The extract produced a weak activity against 4-aminopyridine induced convulsion, the effect was only significant ($p<0.05$) at higher dose of 1000 mg/kg. The doses of 250 mg/kg and 500 mg/kg protected 33.3% , while 1000 mg/kg protected 66.7% of the animals against clonic-tonic convulsions induced by picrotoxin and prolonged the latency of convulsed animals with significant difference ($p<0.05$) at 500mg/kg and $p<0.01$ at 1000 mg/kg. Treatment with the MEBS did not produce significant changes in hematological parameters and liver function indices in all cases as compared to the distilled water groups. These findings suggest that the (MEBS) possess anticonvulsant activity and is worthy of further investigation.

Keywords: Anticonvulsant, *Blighia sapida*, Epilepsy, Pentylenetetrazole, Picrotoxin, Strychnine

Introduction

Epilepsy is a global public health issue affecting the world population requiring a global response with the prevalence of 10 per 1000 in low income countries and 5-8 per 1000 in high income countries. There are an estimated 50 million people with epilepsy globally, of which 75-80% of such people live in developing countries with little or no access to medical services or treatment (WHO, 2017). Epilepsy is a neurological disorder with severe morbidity (Fisher *et al.*, 2014). It is a non-communicable disorder that affects people worldwide, characterized by recurrent (two or more), spontaneous brain seizures or convulsions (WHO, 2019). Epilepsy is the most prevalent neurologic disorder in sub-Saharan Africa (Eisenberg, 1997; Leonardi and Ustun, 2002). The prevalence of epilepsy in Nigeria is about 6.2 per 1000 (Banerjee *et al.*, 2009). It is one of the most common neurological disorders that affect people of all ages, race and social class; and causes important economic implications in terms of health care needs, premature death and loss of work productivity (WHO, 2017). The world health organization (WHO) estimates that 10 million people in Africa live with epilepsy, and 8 million (80%) are not adequately treated (WHO, 2004).The rate of epilepsy varies greatly with age, with the highest rate in children and elderly (Pevarello *et al*., 1998). Epilepsy is the most common non-infectious neurologic disease in developing African countries, including Nigeria and it is a major medical and social problem. In many African countries, people with epilepsy are out-cast due to the believes that the disease results from visitation of the evil, effect of witch-craft, the revenge of an aggrieved ancestral spirit or consumption of harmful substances. The epileptic patient is likely to drop out of school, loses job, finds it difficult to marry, loses wife or husband, and may attempt to commit suicide due to discrimination and stigmatization (Ogunrin, 2006). Epilepsy may lead to impaired intellectual function or death if left untreated and is typically accompanied with psychosocial prejudices and other psycho pathological consequences such as loss of self-esteem and poor quality of life (Idris *et al*., 2008). Many available anticonvulsants are used in the management of epilepsy and 30% of epileptic patients do not have seizure control even with the best anticonvulsants (Yemitan and Adeyemi, 2013). The use of the anticonvulsants are also associated with drug interactions and debilitating adverse reactions including allergies, sedation, blood dyscrasias, teratogenesis, changes in mood and memory problems (Loscher, 2002). Therefore, the development of new, affordable and accessible pharmacological agents with better safety and efficacy profiles has become important in epilepsy research (Yaro *et al.*, 2015). Similarly, the use of herbal medicine in the management of epilepsy is widely accepted in most communities in Nigeria and their efficacies are well acclaimed. Some of these plants remain to be investigated for their value as sources of antiepileptic drugs and therefore, research is needed to validate the traditional claims of these medicinal plants so as to provide scientific evidence of their safety and efficacy (Yaro *et al*., 2015).

Despite the successful development of various new antiepileptic drugs in recent decades, the search for new therapies with better efficacy and tolerability remains an important goal (Bialer and White, 2010). Synthetic anticonvulsants (such as Sodium valproate, Diazepam, Carbamazepine, Gabapentine, Phenytoin, Topiramate e.t.c) are regarded as the main category of compounds prescribed for the treatment of Epilepsy. Current therapy with antiepileptic drugs only suppresses seizures but do not alter the underlying epileptogenic process (Shinner and Berg, 1996). Many people in the developing countries may not receive basic treatment due to high cost, unavailability

and unwanted effects associated with the available antiepileptic drugs (WHO, 2018). WHO encourages the inclusion of herbal medicines of proven safety and efficacy in the healthcare programs of developing countries. Traditional medicines enjoy a wide patronage and acceptance (Stanley, 2005). The use of available medicinal plants in a locality to treat diseases will continue to play important roles in medical health care implementation in the developing countries of the world (Akharaiyi and Boboye, 2010). Therefore it has becomes relevant to evaluate the folkloric claims of medicinal plants used in traditional healing practices so as to provide a scientific basis for their use and also to optimize their safety and efficacy. These ethnomedicinal plants could serve as sources of effective medication that may be more readily accessible and less expensive and thus would be helpful in improving the present predicament (Kumar *et al*., 2012). The main constituents of *B. sapida* include Alkaloids, Saponins, Flavonoids, Tannins and Glycosides. To the best of my knowledge and literature review, the anticonvulsant properties of any parts of the plant *(B. sapida*) have not been reported.

Materials and Methods

Plant material collection and authentication

The stem bark of *Blighia sapida* was freshly collected from Gbutiyi village, Paikoro Local Government Area of Niger State in March 2018. The plant parts were taken to a Taxonomist for identification and authentication in the Herbarium and Ethnobotany Unit, National Institute for Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria. The plant was identified and authenticated by Mr Abdulhakeem Adeniyi.

Animals

Swiss albino mice (16g to 26g) and Wistar strain rats (165-190 g) of either sex were obtained from the animal house facility, of Department of Pharmacology and Therapeutics, Bayero University Kano. In addition, one-day old cockerels were obtained from National Animal Production and Research Institute (NAPRI) Shika, Zaria. Mice were housed and allowed to acclimatize with free access to food and water in the animal house of the Department of Pharmacology and Therapeutics, Bayero University Kano and maintained under normal laboratory conditions.

Test drugs, chemicals and equipments

Pentylenetetrazole, Picrotoxin and strychnine (Sigma chemical co. St Louis USA), 4-aminopyridine (Merck-Schuchardt, Germany) were the chemical agents used to induce seizure in the experimental animals. The standard drugs used for the experiment were phenytoin sodium (Hospira, UK Limited), phenobarbitone (Lab Renavudin, France), Diazepam and sodium valproate (Sanofi Aventis). Solvents used include methanol, (Sigma chemical co. St Louis USA) and distilled water, other reagents were ferric chloride, dragendorf reagent, wagner reagent, sulphuric acid, sodium hydroxide, hydrochloric acid, magnesium chips, syringes, needles, spatula, beaker, funnel, rota-vapour machine, electrical weighing balance, electroshock convulsive machine etc. All chemicals/drugs were of analytical grade and were procured locally.

Preparation of plant extracts

Fresh plant material (stem bark) of *Blighia sapida* was dried under shade until attainment of constant weight after which they were blended using mortar and pestle and sieved until a fine powder was produced. The powdered plant materials were macerated with 70% v/v aqueous methanol (70% methanol: 30% water) solution, with occasional shaking for 7 days and then filtered. The filtrate was evaporated to dryness in an oven at 45oC and then stored in a dessicator until required for the study.

Preliminary phytochemical analysis of plant extracts

The phytochemical screening was carried out on the dried extracts of Blighia sapida Koenig using the standard procedures described by Prashant *et al*., (2011) and Abdullahi *et al*., (2023).

Preparation of Drug solutions

The methanol stem bark extract of *Blighia sapida*, pentylenetetrazole, sodium valproate, strychnine, 4-aminopyridine, picrotoxin, diazepam, phenytoin and phenobarbitone were prepared by dissolving in distilled water prior to administration to produce required stock concentrations. The drug solutions were always prepared fresh after every 24 hours.

Acute toxicity studies

A single oral dose of the extract was administered at 5000mg /kg body weight to three mice each with an oral gavage needle in accordance with the guidelines stipulated by Organization for Economic Cooperation Development (OECD 407). Mortality and general behaviour of the animals were observed over a 48-hour period. Surviving animals were observed for further period of 14 days for toxic symptoms.

Anticonvulsant Studies

Pentylenetetrazole (PTZ)-induced convulsion test in mice

The method of Swinyard *et al*., 1989) was employed. Thirty mice were divided randomly into five groups of six mice each. The first group was pre-treated with distilled water 10 ml/kg orally. The second, third and fourth groups were pre-treated with the methanol stem bark extract of *B. sapida* at graded doses of 250mg/kg, 500mg/kg and 1000mg/kg respectively, while the fifth group was treated with 200 mg/kg of sodium valproate orally. One hour later, mice in all the groups were injected with a convulsive dose CD90 of PTZ (90 mg/kg) subcutaneously and observed for a period of thirty minutes for the presence or absence of clonic spasm. The time of onset of convulsion, the number of animals protected (including percentage protection) per group were recorded. The ability of the extract to prevent clonic spasm or prolong the latency and or onset of the seizure was considered as an indication of anticonvulsant activity of the extracts against PTZ on seizure threshold.

4-aminopyridine (4-AP)-induced convulsion test in mice

The method described by Yamaguchi and Rogawski, (1992) was adopted for this study. Thirty mice were randomly divided into 5 groups each containing six mice. The first group served as negative control and was pre-treated with distilled water 10 ml/kg. The second, third and fourth groups were pre-treated with the methanol stem bark extract of *B. sapida* at the doses of 250, 500 and 1000mg/kg respectively, while the fifth group was pre-treated with 20 mg/kg of phenobarbitone all through the oral route. One-hour post treatment 4-AP was administered

at a dose of 14 mg/kg subcutaneously to each mouse. The mice were then observed for 30 min for characteristic behavioural signs, such as hyperactivity, trembling, intermittent forelimb extension, tonic seizures and death. Ability of the extract to prolong the onset of seizure or protect the mice from lethality within the 30 min observation period was considered as an indication of anticonvulsant activity (Yamagachi and Rogawski, 1992).

Picrotoxin-induced convulsion test in mice

The method described by Vogel, (2008) was adopted in this study. Thirty mice were randomly divided into five groups containing six mice in each group. The first group served as negative control and was pre-treated with distilled water 10 ml/kg. The second, third and fourth groups were pretreated with the methanol stem bark extract of *B. sapida* at the doses 250, 500 and 1000mg/kg, while the fifth group served as a positive control and was pretreated with 3mg/kg diazepam all through the oral route. Sixty minutes' post treatment all mice were then treated with 3.5 mg/kg picrotoxin subcutaneously. Immediately after picrotoxin injection, the mice were then observed for the following symptoms during the next 30 min: clonic seizures, tonic seizures. The ability to prevent this feature or prolong the latency and or onset of the seizure was considered as an indication of anticonvulsant activity of the extract.

Maximal electroshock induced convulsion test in chicks

The methods of Swinyard and Kupferberg, (1985) and of Browning, (1992), were employed. Fifty day-old cockerels were randomly divided into five groups of 10 chicks each. The first group was pretreated with distilled water 10 ml/kg, the second, third and fourth groups were pretreated with the methanol stem bark extract of *B. sapida* orally at the doses of 250, 500 and 1000mg/kg, and the fifth group was then pretreated with phenytoin 20 mg/kg all through the oral route. One hour later, the chicks were subjected to maximum electroshock using Ugo Basile Electroconvulsive Machine (Model 7801, Italy) connected to a stabilizer with corneal electrodes placed on the upper eyelids of the chicks. A day-old chick has an underdeveloped blood brain barrier, thereby facilitating easy passage of drugs and current into the brain. A current (80 mA) which induced tonic seizures in 90% of the control group of chicks was selected. The shock duration, frequency and pulse width was then set at 0.8 sec, 100 pulse/sec and 0.6 ms respectively, and was used throughout the study. Seizures which manifested as hind limb tonic extension (HLTE) were observed. The ability to prevent this feature or prolong the latency and or onset of the HLTE was considered as an indication of anticonvulsant activity (Swinyard, 1969).

Strychnine-induced convulsion test in mice

The method described by Porter *et al.*, (1984) was used for this study. Thirty mice were randomly divided into five groups of six mice each; the first group was pre-treated with distilled water 10 ml/kg. The second, third and fourth groups were pre-treated with the methanol stem bark extract of *B. sapida* at three different doses (250mg/kg, 500mg/kg, 1000mg/kg), while the fifth group was treated with 20 mg/kg of phenobarbitone all through the oral route. One hour later, mice in all the groups were injected with a convulsive dose of strychnine (1.0 mg/kg) subcutaneously. Mice were then observed for tonic extensor jerks of the hind limbs for 30 minutes. Abolition of tonic extensor jerks of the hind limbs or ability to prolong the onset of seizure was considered an indicator that the extract could prevent strychnine-induced seizures (Raza *et al*., 2001)

Sub-Chronic Toxicity Studies

The study was carried out in accordance to WHO (1992) and OECD 407(1995) guidelines. Twenty-four rats of either sex were deprived of food for 24 hours, and divided into four groups of six rats each. Group 1, which served as control received distilled water 1 ml/kg, while rats in groups 2, 3 and
4 were given doses of the extract (250mg/kg, 500 mg/kg and 1000 mg/kg) body weight respectively daily for 28 days. The rats were allowed free access to food and water throughout the duration of the experiment and were observed daily. Rats were weighed twice weekly and the average change in weight calculated. Rats were then sacrificed on the 29th day of the experiment, blood samples were collected for estimation of biochemical and haematological parameter. Organs such as heart, liver, lung and kidney were removed for determination of organ weight ratio using the formula:

$$\text{ROW} = \frac{\text{Absolute weight (g) of the organ}}{\text{Body weight of rat (g)}}$$

where ROW stands for relative organ weight

Biochemical studies

Blood samples were collected into plain bottles, allowed to clot and centrifuged at 3500rpm for 10 minutes. The sera were separated, stored at -4°C, and used for evaluation of biochemical parameters which include, alanine aminotranferase (ALT), aspartate aminotransferase (AST) levels and alkaline phosphatase (ALP) levels, and serum bilirubin, serum urea nitrogen, creatinine, chloride, sodium, potassium, and bicarbonate using commercial kits obtained from Reckon Diagnostics P. Ltd, India.

Haematological studies

Blood samples were collected into ethylene diamine tetra acetic acid (EDTA) bottles for estimation of haemoglobin concentration (Hb), platelets (PLT), red blood cell count (RBC), white blood cell count (WBC) and differentials, mean corpuscular haemoglobin concentration (MCHC), using an automated haematological machine (Cell-DynTM Abbott, US).

Statistical analysis

Results were expressed as mean ± standard error of mean (S.E.M), and presented as tables, charts and graph. Data were analysed using one-way analysis of variance (ANOVA) followed by Dunnett t test and repeated measures ANOVA followed by Bonferroni test for multiple comparison. Results were considered significant at $p< 0.05$.

Results and Discussion

Phytochemical screening of the methanol stem bark extract of *Blighia sapida* revealed the presence of various phytochemicals such as alkaloids, flavonoids, cardiac glycosides.

Acute toxicity study (LD50 determination)

The mice and rats that received the methanol stem bark extract of *Blighia sapida* orally did not show any sign of toxicity after the first four hours and no mortality were recorded 48hours after and up to twelve days of extract administration. The median oral lethal dose for both mice and rats was found to be greater than 5000mg/kg using OECD method.

Anticonvulsant Studies

Effect of Methanol Stem Bark Extracts of B. sapida on Pentylenetetrazole - Induced Convulsion in Mice

The stem bark extract of *B. Sapida* showed a significant increase ($P < 0.01$) in the mean onset of seizure at 250, 500 and 1000 mg/kg doses. The same doses conferred 16.67 and 50.0% protection against PTZ-induced convulsion in mice respectively while standard anticonvulsant Sodium valproate gave 100% protection against PTZ-induced convulsion (Table 1).

Table 1: Effect of Methanol Stem Bark Extracts of *B. sapida* on Pentylenetetrazole - Induced Convulsion in Mice

Treatment (mg/kg)	Mean onset of seizure (min)	Quantal Protection	%Protection against seizure
D.W 10ml/kg	3.93±0.07	0/6	0.00
MEBS 250	7.50±0.39*	1/6	16.67
MEBS 500	10.83±0.34*	3/6	50.00
MEBS 1000	13.30±0.33*	3/6	50.00
SV 200	>30	6/6	100.00

Data presented as Mean ± SEM. *$P < 0.01$ compared to control group using One-way ANOVA followed by Dunnett's post hoc, n=6, D.W- distilled water, MEBS- Methanol Extract of *B. sapida*, SV- Sodium Valproate.

Effect of Methanol Stem Bark Extracts of B. sapida on 4 aminopyridine-Induced Convulsion in Mice

The extract at 1000 mg/kg protected 16.7% of mice against 4-aminopyridine induced convulsion and mortality. This effect was dose dependent. The extract at 1000 mg/kg significantly ($p<0.05$) increased the mean onset of seizure compared to distilled water group. The standard drug phenobarbitone provided 66.7% protection and significant ($p<0.001$).increase in the mean onset of seizure. The mortality recorded for the extract (250 mg/kg) and (500 mg/ kg) where 100% respectively (Table 2)

Table 2: Effect of Methanol Stem Bark Extracts of *B. sapida* on 4-aminopyridine Induced Convulsion in Mice

Treatment (Mg/kg)	Mean onset of seizure (min)	Mortality	%protection against Mortality
D.W 10ml/kg	9.56±0.75	6/6	0.0
MEBS 250	11.15±0.68	6/6	0.0
MEBS 500	14.68±2.35	6/6	0.0
MEBS 1000	16.87±0.69 *	5/6	16.7
PBS 20	24.14±0.52**	2/6	66.7

Data presented as Mean ± SEM. *P <0.05, **P < 0.001 compared to control group using One way ANOVA followed by Dunnett's post hoc, n=6, DW- distilled water, MEBS- Methanol stem bark Extract of *Blighia sapida*, PBS- Phenobarbitone.

Effect of methanol stem bark extract of Bligiah sapida on picrotoxin-induced seizures in mice

The extract at 250 mg/kg, 500 mg/kg and 1000 mg/kg protected 33.3%, 33.3% and 66.7% of the mice against picrotoxin induced convulsion and the standard drug, diazepam produced 83% protection. The extract also produced a dose dependent increase in latency to convulsions with significant difference ($p<0.05$) at 500mg/kg and 1000 mg/kg while diazepam significantly ($p<0.01$) compared to the control group (Figure 1).

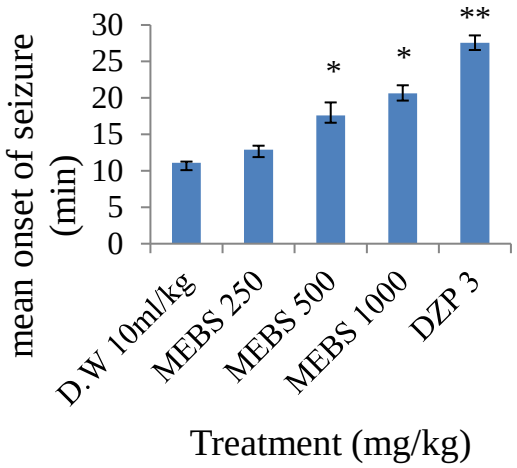

Fig 1: Effect of methanol stem bark extract of *Bligiah sapida* on picrotoxin-induced seizures in mice

Data were analysed using One-way ANOVA followed by Dunnett's test *p< 0.05 and **p< 0.01, level of significance respectively. Values are Mean ± SEM, n= 6, DW=distilled water, MEBS=Methanol extract of *Blighia sapida*, DZP= Diazepam.

Effect of methanol stem bark extract of Blighia sapida on maximal electroshock-induced convulsions in chicks

The methanol stem bark extract of *Bligiha sapida* produced significant (*P<0.05) increase in the mean onset of seizures induced by maximal electroshock in chicks in the doses of 250 mg/kg and 500 mg/kg and (**P<0.01) at 1000 mg/kg dose respectively. However, the extracts did not produced

significant effect on the mean recovery time and did not protect the chicks against convulsion. The standard drug phenobarbitone gave 70% protection to the chicks (Tale 3).

Table 3: Effect of methanol stem bark extract of *Blighia sapida* on maximal electroshock-induced convulsions in chicks

Treatment (Mg/kg)	Mean onset of seizure (s)	Mean time of recovery (min)	Quantal protection
D.W 10ml/kg	2.15±0.21	12.51±0.96	0/10
MEBS 250	3.71±0.31*	10.38±1.23	0/10
MEBS 500	3.7±0.80*	9.44±0.49	0/10
MEBS 1000	5.94±1.27**	8.69±0.57	0/10
PHN 20	7±1.41**	5.35±1.75*	7/10

Values are mean± SEM, Data were analyzed using One-way ANOVA followed by Dunnett's post hoc test with p< 0.05 and p< 0.01, level of significance respectively. n=10, DW- distilled water, MEBS- Methanol Extract of *Blighia sapida*, PHN- Phenytoin.

Effect of methanol stem bark extract of B. sapida on strychnine-induced seizures in mice

Blighia sapida extract provided 16.67 % protection against strychnine-induced seizure at 250 mg/kg and 500 mg/kg while at 1000 mg/kg, 50 % protection was provided. The extract also significantly (p< 0.05) increased the latency of convulsion at 250 and 1000 mg/kg compared to control group (figure 2)

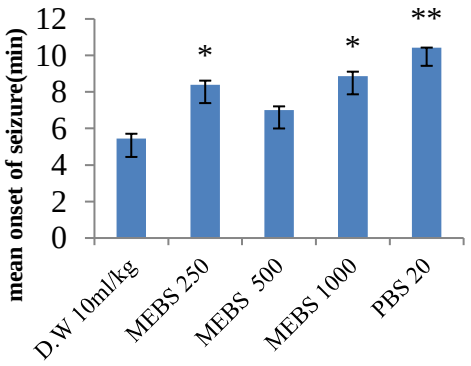

Fig 2: Effect of Methanol Stem bark extract of *B. sapida* on Strychnine-induced convulsion in mice

Values are Mean ± S.E.M., n=6, * denotes significant difference from the control group at p<0.05, **P≤ 0.01- One way ANOVA followed by Dunnett's test, n=6, DW- distilled water, MEBS- Methanol stem bark extract of *Blighia sapida*, PBS- Phenobarbitone

Sub-chronic toxicity studies

Effect of the methanol stem bark extract of B. sapida on average body weight (g)

The effect of sub-chronic oral administration of the methanol stem bark extract of *B. sapida* for 28 days on animal body weights is shown in table 4. There was no significant increase in the animal weights in all the groups compared to the control group. Although there is a slight increase in the animal weight within the same group across the weeks compared to week 0.

Table 4: Effect of the methanol stem bark extract of *B. sapida* on average body weight (g)

TREATMENT (mg/kg)	WEEKO	WEEK1	WEEK2	WEEK3 WEEK4
D.W1ML	113.67 ±13.91	114.50 ±13.97	116.50 ±13.03	126.33 ±11.91 128.33±12.56
MEBS250	115.50±12.78	117.17±11.97	120.20±15.28	128.80±13.93 134.60±15.58
MEBS500	114.33±13.26	119.17±13.78	121.17±13.97	130.50±14.13 135.67±15.31
MEBS1000	113.71±7.32	118.83±8.27	120.00±7.75	129.83±7.38 134.67±7.51

Data were analyzed using repeated measures of ANOVA followed by Bonferroni test and expressed as mean ± SEM, n = 6, no statistical significant differences noted in all groups compared to the control group. Although there is a slight increase in the animal weight within the same group across the weeks compared to week 0. D.W=distilled water. MEBS= methanol extract of *blighia sapida*.

Effect of methanol stem bark extract of B. sapida on haematological indices

The effects of sub-chronic oral administration of the methanol extract of *B. sapida* Stem bark on haematological indices are shown in fig 3. There were no significant changes observed in the concentrations of all the haematological parameters except in the white blood cell (WBC) with slight decrease in their concentrations in the group of 500 and 1000 mg/kg and slight increase in platelet were observed at all doses of the extract.

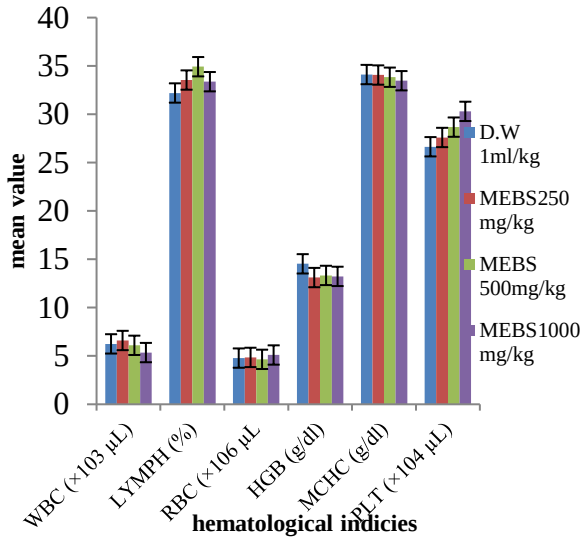

Fig. 3: Effect of methanol stem bark extract of *B. sapida* on haematological indices

Data were analyzed using one-way ANOVA followed by Dunnett *t-test*. t. Values are mean ± SEM, n=6, D.W=Distilled water, MEBS= methanol extract of *blighia sapida*

Effect of methanol extract of *B. sapida* on liver function indices

The effects of sub-chronic administration of methanol extract of *B. sapida* stem bark on liver function indices are shown in figure 4. There were no significant changes observed in any parameters in all the groups as compared to the control group.

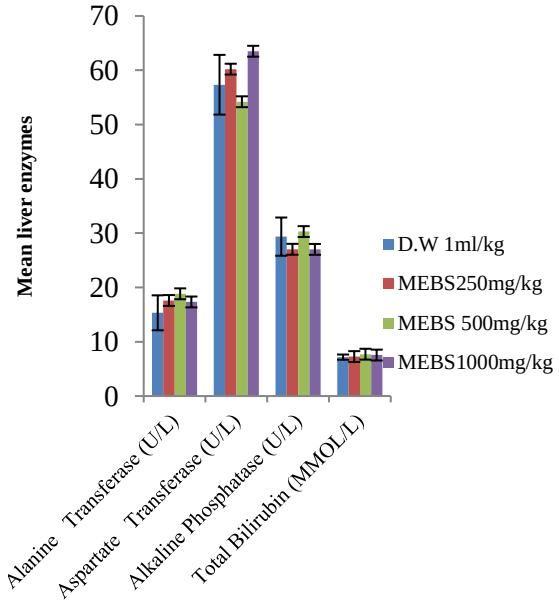

Fig 4: Effect of methanol extract of *B. sapida* on liver function indices

Data were analyzed using one-way ANOVA followed by Dunnett *t-test*. Values are mean ± SEM, n= 6, D.W =Distilled water, MEBS= methanol extract of *Blighia sapida*, no significant differences noted in all groups

Effect of methanol stem bark extract of *B. sapida* on renal indices

The effects of sub-chronic administration of methanol extract of *B. sapida* on renal indices are presented in figure 5. A significant ($p < 0.05$) reduction in serum concentrations of potassium was observed at 250 mg/kg, 500 mg/kg and 1000mg/kg groups of the extract respectively.

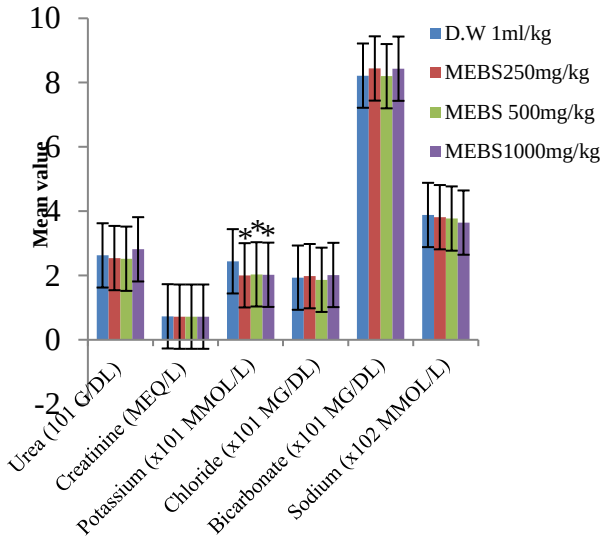

Fig 5: Effect of methanol Stem extract of *B. sapida* on renal indices

Values are Mean ±, Data were analysed using one-way ANOVA followed by Dunnett *t-test*. Significant *$P < 0.05$ reduction in serum concentrations of potassium. SEM, n =6, D.W=distilled water, MEBS=methanol extract of *Blighia sapida*.

Effect of the methanol Stem bark extract of B. sapida on relative organ weight

The effect of methanol stem bark extracts of *B. sapida* on relative organ weight is shown in table 5. No significance difference was observed in all the relative organ weights and at all doses of the extract. But the weights in the 1000 mg/kg group were comparable to the control group. The weights in the 250 mg/kg and 500 mg/kg groups were similar and slightly increased compared to the control.

Table 5: Effect of the methanol stem bark extract of *B. sapida* on relative organ weight

Organ	D.W 1ml	MEBS250	MEBS500	MEBS1000
Liver	4.42±0.34	5.09±0.64	5.13±0.33	4.45±0.26
Kidney	0.78±0.05	0.86±0.06	0.88±0.08	0.76±0.41
Heart	0.48±0.05	0.47±0.09	0.60±0.04	0.46±0.04
Lungs	1.96±0.34	2.55±0.39	1.86±0.22	1.82±0.28

Data were expressed as mean ± SEM, n = 6, no statistical significant differences noted in all organs.

Discussion

Blighia sapida is an important medicinal plant whose leaves and bark are well known all over the world but most predominantly in Africa especially in the treatment of epilepsy (Kean and Hare. 1980) and psychosis (Okogun, 1996 and Owonubi, 2006). Preliminary phytochemical screening carried out on the methanol stem bark extracts revealed the presence of tannins, alkaloids, saponins, glycosides, flavonoids, steroids and carbohydrates. These phytochemical constituents are known to be responsible for different pharmacologic activities. Flavonoids and saponins have been reported to possess anticonvulsant activity (Kavvadias *et al.,* 2004). Thus, the anticonvulsant activities of the methanol stem bark extract of *Blighia sapida* (MEBS) may be due to the presence of flavonoids and saponins among other phytoconstituents. Other plants with anticonvulsant activity that contain saponins and flavonoids include; *Acacia albida* (Danjuma *et al*., 2011) and *Holoptelea integrifolia* (Ravindra *et al.,* 2014). The use of medicinal plants in the treatment of some diseases have been accepted as one of the major source for drug discovery and development, but just few have been investigated scientifically for their toxicity. The indices of acute toxicity studies that give an idea of substance toxicity is the median lethal dose (LD_{50}) (Van Brummelen, 2000). Median lethal dose (LD_{50}) is the dose that will kill fifty percent of the population. In this experiment the oral median lethal dose (LD_{50}) value for the methanol stem bark extracts of *Blighia sapida* obtained in both mice and rats was found to be above 5000mg/kg. This suggests that the plant extract is non-toxic as no death was recorded even after twelve days of observation.

In the screening of new antiepileptic drugs, there are different animal models that can be used, Pentalinetetrazole-induced and Maximal electroshock convulsion models have been used mostly in the early stages of drug testing (Yamagachi and Rogawaski, 1992). Pentylenetetrazole (PTZ) is a known convulsant and anticonvulsant activity in PTZ test identifies compounds that can raise the seizure threshold in the Brain (White *et al*, 1999). PTZ has been shown to interfere with GABA neurotransmitter and the GABA receptor complex. Antagonism of PTZ induced seizure suggests potentiating effect on GABAergic neurotransmission in the Brain. Standard antiepileptic drugs like phenobarbitone, sodium valproate and benzodiazepines produce a dose dependent suppression of PTZ-induced seizure. Similarly, the symptoms of PTZ induced seizure are similar to those in petit mal seizure (McNamara, 2006). Administration of 250, 500 and 1000mg/kg methanol stem bark extract of *Blighia sapida* produced significant ($P < 0.01$) increase in the mean onset of seizure compared to control group (table:1). The same doses conferred 16.67 and 50.0% protection against PTZ-induced convulsion in mice respectively and no mortality was recorded. The dose dependent increase in the onset of seizures by the extract against seizure threshold induced by PTZ suggests the presence of bioactive compounds that could be effective in the therapy of absence or myoclonic seizures.

4-aminopyridine is a known potassium channel blocker that interferes with all aspect of neuronal excitability, including resting membrane potential, responsiveness to synaptic inputs, frequency adaptation and neurotransmitters release (Wickenden, 2002). Drugs like phenytoin, which block seizure spread are effective antagonists of seizures induced by K+ channel blockade, while those with specific actions on other cellular targets may be weak or inactive, probably because they are unable to prevent the spread of intense (non-NMDA receptor mediated) excitation evoked by 4-aminopyridine (Yamaguchi and Rogawski, 1992). The extract produced a weak activity against 4-aminopyridine induced convulsion. This effect was observed at higher dose of 1000 mg/kg which prolong the mean onset of the seizure significantly ($p<0.05$) (table: 2).The weak anticonvulsant activity against 4-aminopyridine-induced seizures suggests methanol stem bark extract of *Blighia sapida* may not be interacting with potassium channels in producing its anticonvulsant activity.

Picrotoxin is used to determine mechanism of action of sedative-hypnotic and anticonvulsants (Vogel, 2008). Picrotoxin induces convulsions by blocking the inhibitory synaptic action of GABA on $GABA_A$ receptor chloride channels, but not competitively (Rang *et al*., 2007). As an antagonist of GABA inhibitory action in different areas of the central nervous system, picrotoxin produces generalize clonic-tonic convulsions which can lead to death in most cases. Methanol stem bark extract of *Blighia sapida* at 250 mg/kg, 500 mg/kg and 1000 mg/kg protected 33.3%, 33.3% and 66.7% of the animals against clonic-tonic convulsions induced by picrotoxin and also significantly prolonged the latency of convulsed animals with significant difference ($p<0.05$) at 500mg/kg and $p<0.01$ at 1000 mg/kg compared to the control group (Fig. 1). Therefore, the methanol stem bark extract may act to enhance GABA-mediated inhibitory neurotransmission by interacting with $GABA_A$ – receptor activated chloride channel to produce the anticonvulsant action. This is further supported by the result of PTZ-induced convulsion test.

Maximal electroshock test (MEST) is a standard anti-epileptic drug (AED) test that evaluates the test material's ability to protect against hind limb tonic extension (HLTE). Such protection indicates anticonvulsant activity of AEDs that prevent the spread of the epileptic seizure discharge from an epileptic focus during seizure activity (Raza *et al.,* 2001). Compounds, such as phenytoin, carbamazepine, oxcarbazepine and lamotrigine suppress HLTE in MEST (Browning, 1992). The electrographic and behavioural seizures are similar to that presented in human grand mal

epilepsy; therefore, it is used primarily for compounds which are effective in grand mal epilepsy (Rang *et al.,* 1995). The methanol stem bark extract of *Blighia sapida* significantly (P<0.05) prolonged the mean onset of seizure at the doses of 250 mg/kg and 500 mg/kg and *(*P<0.01) at 1000 mg/kg respectively (table: 3).This further suggests that the extract may be of value in the treatment of generalized tonic clonic and partial seizures. However, the extracts did not produce significant effect on the mean recovery time and did not protect the chicks against convulsion.

Strychnine is a competitive glycine receptor antagonist (Rajendra *et al*., 1997). The methanol extract of *Blighia sapida* gave 16.67 % protection at doses of 250 and 500 mg/kg and 50% at a dose of 1000 mg/kg, against strychnine-induced seizure. The extract (MEBS) also significantly ($p< 0.05$) increased the latency to first tonic extensor jerk at 250 and 1000 mg/kg compared to control group suggesting that the extract may act by enhancing inhibitory neurotransmission mediated by glycine (Fig. 2).

The oral median lethal dose value obtained for the methanol stem bark extract of *Blighia sapida* in both mice and rats was found to be above 5000mg/kg. This shows that the extract is non-toxic since no death was recorded. Acute toxicity studies are normally carried out to determine the dose that will cause serious toxic manifestations or death when administered singly or severally at few doses so as to establish doses that can be used in subsequent studies. The Organization for Economic Cooperation and Development (OECD), Paris, France, recommended chemical labelling and classification of acute systemic toxicity based on oral median lethal dose values as: very toxic if ≤ 5 mg/kg, toxic if > 5 mg/kg but ≤ 50 mg/kg, harmful if > 50 mg/kg but ≤ 500 mg/kg, and non-toxic or not harmful if > 500 mg/kg or ≤ 2000 mg/kg (Walum, 1998). Based on this classification, the oral median lethal dose obtained was above 5000 mg/kg, and is relatively safe orally. But, the situation is not the same on repeat dose experiment in sub-chronic toxicity studies. Since the LD_{50} suggest the MEBS to be relatively safe, sub-chronic toxicity effect of the MEBS was evaluated.

Generally, reductions or increase in body weight and internal organ weights are considered to be simple and sensitive indices of toxicity after exposure to toxic substances (Teo *et al*., 2002). All rats used for the sub-chronic toxicity studies appeared normal before treatment; the results from the present study indicated that the extract did not significantly affect the body weight, suggesting that it does not hinder the animal growth. However, the extract slightly increases the animal body weight as shown in table 4 and relative weight of the liver and lungs compared to the control group table 4.8, which suggest that the extract might have caused hypertrophy of these organs.

The haematopoietic system is one of the most sensitive targets of toxic compounds and is an important index of physiological and pathological status in man and animals (Adeneye *et al*., 2006). After 28 days of treatment with stem bark extract of *B. sapida*, there were no treatment-related changes in haematological parameters between control and treated groups, indicating that the extract was not toxic to circulating red cells, nor interfered with their production and that of platelets. Although there was a slight increase in the platelet level compared to the control which play a vital role in regulating haemostasis and a relatively slight decrease in white blood cell as shown in Fig. 3. White blood cells are very important component of the blood. They defend the body against injury and offending organisms, and play a vital role in inflammation, infections and immune responses (Abramson and Melton, 2000). A reduction in WBC count is termed leukopenia resulting from decreased production of total white blood cells in the bone marrow or increased destruction of the cells. Leukopenia indicates bone marrow depression which occurs as a result of viral infection or toxic reactions (Goerge-Gay and Parker, 2003). Disease of the liver or spleen and autoimmune diseases can also cause reduction in white blood cell count. The data presented suggests that the slight reduction in white blood cells by the extract is not due to liver damage as evidenced in the findings of the liver function test. Fig.4

Liver and kidney play a key role in metabolic processes, while the liver detoxifies substances that are harmful to the body, the kidney help in maintenance of homeostasis by reabsorbing vital substances and excretion of waste product. The sub-chronic oral administration of methanol extract of *B. sapida* stem bark on liver function indices such as alanine transferase, aspartate transferase, alkaline phosphate, and total bilirubin shows no statistical significant increase in any parameters in all the groups as compared to the control group. The lack of significant alterations in the levels of ALT, AST, alkaline phosphatase and total bilirubin which are good indicators of liver functions (El Hilaly *et al.,* 2004), suggests that sub-chronic oral administration of methanol stem bark extract of *Blighia sapida* neither altered the liver functions nor the normal metabolism of the animals as shown in Fig. 4.

The sub-chronic studies of the methanol extract of *B. sapida* stem bark on renal indices shows a significant reduction in the concentrations of serum potassium at 250 mg/kg and 500 mg/kg and 1000 mg/kg of the extract, but there were no significant changes observed in other parameters as shown in Fig. 5.

Electrolytes are molecules that are electrically charged, which help move nutrients into and waste products out of the body's cells. They maintain healthy water balance and help stabilize the body's acid level. Potassium is regulated by the kidneys and adrenal glands; potassium has many biological functions. It is a co-factor for many enzymes and is required for insulin secretion, carbohydrate metabolism and protein synthesis. The ratio of intracellular to extracellular potassium is the major determinant of muscular and neuronal excitability, and if this balance is altered many pathological state (life-threatening cardiac conduction disturbances and neuromuscular dysfunction) can develop (Anthon *et al*., 2015). Low potassium levels are caused by kidney disease, adrenal disease vomiting and diuretics. The balance of potassium in the blood is an indicator of how well the kidneys and heart are working. Change in serum potassium concentrations indicates an alteration in muscular and neuronal excitability (Liamis *et al.*, 2013)

Conclusion

The study revealed that the methanol stem bark extracts of *Blighia sapida* is relatively safe and contain bioactive substances that possess anticonvulsant activity and may be useful as anti-epileptic agent thereby validating its traditional application in the management of the ailment.

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Conflict of Interest

The authors did not highlight any conflict of interest associated with the work or its publication.

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