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Neuropharmacological activities of ethanol leaf extract of *Cussonia barteri* Seeman (Araliaceae) in laboratory animals

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Abstract
The anticonvulsant studies on *Cussonia barteri* Seeman (Araliaceae) were carried out using maximal electroshock test (MEST), pentylenetetrazole and strychnine-induced seizures model in chicks and mice. In addition, sedative and anxiolytic effect of the extract was evaluated using diazepam-induced sleeping time, hole-board, beam walk assay and open field test in mice. The extract was also evaluated for acute toxicity. The oral and intraperitoneal LD_{50} of the extract was estimated to be greater than 5,000 mg/kg and 2, 154.1 mg/kg body weight respectively. The extract did not protect the chicks against maximal electroshock seizure; neither did it shorten the mean recovery time. The extract produced 66.67% and 83.33% protection against strychnine and pentylenetetrazole induced seizures respectively at the highest dose (400 mg/kg) tested. The extract decreases the number of head dips in hole-board test, suggesting its sedative property, which was confirmed by the ability of extract to prolonged diazepam sleeping time. The extract did not significantly increase the time spent on the beam but at the highest dose tested significantly increased the number of foot slips, an index of motor coordination deficit. The extract insignificantly decreased number of rearing, Total Square and Central Square crossed in an open field test. These results suggest that the extract may contain compound(s) that may be beneficial in the management of absence or myoclonic seizures.

Keywords: *Cussonia barteri*; Epilepsy; Seizure; Sedative; Anxiolytic

INTRODUCTION

Epilepsy is a chronic brain disorder that is characterized by recurrent seizure [1]. It is estimated that epilepsy affect 65 million people worldwide, making it one of the most common chronic diseases affecting human beings [2]. Sadly, almost 80% of people with epilepsy reside in resource-limited and poor countries; about 90% of these people receive no treatment at all due to lack of access to antiepileptic drugs thus, there exist a treatment gap in epilepsy treatment [3]. The treatment of epilepsy is also associated with the challenges of chronic adverse effects of antiepileptic drugs, high cost of treatment, and pharmacoresistance. Consequently, there has been a shift in focus to the use of natural medicinal plants in the treatment of epilepsy, probably because is easily accessible to people and are not usually as expensive as the orthodox drugs [4].

Some medicinal plants used in the treatment of epilepsy in traditional medicine have been shown to possess promising

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anticonvulsant activities and can be invaluable source of new antiepileptic drugs [5]. *Cussonia barteri* is one of the plants found in savanna from Guinea to Nigeria, and in East Africa, which is reportedly used, in traditional medicine for the management of convulsion and epilepsy, especially in children in Ghana and Nigeria [6]. This study was therefore designed to evaluate the anticonvulsant, sedative and anxiolytic activities of *Cussonia barteri* in order to scientifically justify its use in traditional medicine to treat epilepsy.

EXPERIMENTAL

Collection and identification of plant material. *Cussonia barteri* was collected in May 2013 from Basawa, Sabon-gari Local Government Area, Kaduna state and authenticated by a taxonomist, Mallam U. S. Gallah at the Herbarium Section of the Department of Biological Sciences, Ahmadu Bello University (ABU), Zaria-Nigeria by comparing with an existing voucher number (193).

Experimental animals. Swiss albino mice (18-25 g) of either sex used in the studies were procured and housed in the animal house of Pharmacology and Therapeutics department, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria-Nigeria. Day old rangers' cockerels used in the studies were obtained from National Animal Production Research Institute (NAPRI), Zaria. The animals were kept in standard cages and well maintained under standard hygienic conditions, at 27 ± 2^0c, humidity (60 ± 10 %) with 12 hours day and night cycle, with food and water *ad libitum* and handled in compliances with the ARRIVE guidelines [7] and the experiments were conducted in accordance with the [8].

Equipment and reagents/drugs used. Pentylenetetrazole (Sigma chemical Co. USA) and Strychnine hydrochloride (Sigma Chemical Co. USA) were the chemical agents used to induce seizures in the experimental animals. The standard drugs used for the experiments were phenytoin sodium, phenobarbitone (Lab. Renaudin, France) and sodium valproate (Sanofi, France).

Preparation of the extract. The plant material was air dried, crushed into coarse powder with pestle and mortar. The powdered plant material was then subjected to extraction with 70% v/v ethanol using cold maceration for 72 hours. The solvent was evaporated on a water bath at a low temperature to give a dry extract. The extract was then stored in a desiccator and was reconstituted into a fresh aqueous solution prior to each experiment.

Phytochemical screening. Phytochemical screening of the ethanolic leaf extract of *Cussonia barteri* was carried out according to the methods described by [9].

Acute toxicity study. The median lethal dose of *Cussonia barteri* was determined using the method of [10]. Briefly, the method was divided into two phases. In the initial phase, 3 groups of three mice each were treated with the ethanolic leaf extract of the plant at doses of 10,100 and 1,000 mg/kg body weight orally and intraperitoneally. They were observed for signs of toxicity and death for 24 hours. In the second phase, three groups each containing one mouse was injected with three more specific doses of the extract based on the result of the initial phase. The LD_{50} value was determined by calculating the geometric mean of the lowest dose that caused death (minimum toxic dose) and the highest dose for which the animal survived (maximum tolerated dose).

Maximum electroshock-induced convulsion in chicks. The method described by [11] as modified by [12] was employed in this study. Fifty (50) day old white cockerels were randomly divided into five groups each containing ten chicks. The first group

received normal saline (10 ml/kg) *i.p.*; second, third and fourth groups were treated with different doses (100, 200 and 400 mg/kg) of the plant extract respectively. The last group was administered with phenytoin (20 mg/kg), *i.p.* Thirty minutes after pre-treatment, maximal electroshock was administered to induce seizure in the chicks using Ugo Basile electroconvulsive machine (Model 7801) connected to Claude Lyons stabilizer with corneal electrodes placed on the upper eyelids of the chicks. The shock duration, frequency and pulse width were set and maintained at 0.80 s, 200 pulse per second and 0.8ms respectively. A current of 90 mA, which produced tonic seizures in 90% of the control chicks, was used throughout the study. Seizures were manifested as tonic hind-limb extension (THLE). The ability to prevent tonic hind-limb extension (THLE) or prolong the latency and or onset of the THLE was considered as an indication of anticonvulsant activity [12].

Pentylenetetrazole-induced seizure in mice. The method of [13] was employed. Thirty mice were divided into five groups each containing six mice. The first group received normal saline (10 ml/kg) *i.p.* Second, third and fourth groups were treated with different doses (100, 200 and 400 mg/kg) of the plant extract, *i.p.* The last group was treated with sodium valproate 200 mg/kg, *i.p.* (positive control). Thirty minutes post treatment, the mice in all the groups received pentylenetetrazole 85 mg/kg *s.c.* and were observed over a period of 30 minutes. The ability of the extract to prevent or delay an episode of clonic spasm or tonic-clonic seizure is an indication of its anticonvulsant activity.

Strychnine- induced convulsions in mice. The method of [14] was employed. A total of thirty (30) mice divided into five groups of six mice each. The first group received 10 ml normal saline per kg body weight *i.p.* The second group (positive control) was given 200 mg valproic acid per kg body weight *i.p.*, while the third, fourth and fifth groups received 100, 200 and 400 mg/kg respective doses of the plant extract *i.p.* Thirty minutes later, mice in all the groups received 1.0 mg strychnine per kg, *s.c.* Any mouse that did not convulse within 30 min after strychnine administration was considered protected.

The effect of the extract on diazepam-induced sleeping time. The method described by [15] and modified by [16] was used in this study. Four groups of each containing six mice were used for the study. The first group was treated with normal saline 10ml/kg body weight. The remaining groups (2, 3 and 4) were treated with 100, 200, and 400 mg/kg of the plant extract respectively. Thirty (30) minutes after pretreatment with different doses (100, 200 and 400 mg/kg body weight) of the plant extract and normal saline, all the mice were administered with diazepam at a dose of 20 mg/kg. The onset and duration of sleep was recorded for each mouse. The loss of straightening reflex was regarded as the onset of sleep while the time difference between the disappearing and the recovery of the righting reflex was taken as the duration of sleep (sleeping time).

Test for exploratory behaviour in mice. The method for the hole-board test in mice was similar to those described previously by [17]. The apparatus consists of a wooden board (60 cm x 30 cm) with 16 evenly spaced holes (1cm x 2 cm depth). Thirty mice were randomly divided into five groups of six mice each. The first group was treated with normal saline (10ml/kg, *i.p.*); the fifth group was treated with 0.5 mg/kg diazepam while the remaining groups were treated with different doses of the extract; 100, 200, and 400 mg/kg body weight *i.p.* respectively. Thirty (30) minutes post-treatment, each mouse was placed at a corner of the board and the number head dips on the hole was counted. A head dip was considered when the mouse dipped its head into the hole to the level of the

eyes.

Beam walking assay in mice. The method used for this study was similar to that previously described by [18]. Adult mice were trained to walk from a start platform along a ruler (80 cm long, 3 cm wide) elevated 30 cm above the bench by metal supports to a goal box. The successful mice after three trials were randomly grouped into five groups (n=6). The first group received normal saline (10ml/kg), intraperitoneally. The second, third and the fourth groups were treated intraperitoneally with 100, 200 and 400 mg/kg body weight of the plant extract respectively while the fifth group received diazepam (1 mg/kg body weight). Thirty minutes post-treatment, each mouse was placed on the beam at one end and allowed to walk to the goal box. Mice that fell were returned to the position they fell from, with a maximum time of 60 s allowed on the beam. The number of foot slips (one or both hind limb slipped from the beam) was recorded with the aid of a tally counter. The time taken to complete the task was also recorded. The number of foot slip is a measure of motor coordination deficit [18].

Open field test in mice. The study was conducted according to method previously described by [19] with some modifications. The apparatus was made up of plywood measuring 72 cm x 72 cm x 36 cm. One of the walls was made of transparent Perspex glass to ensure that the mouse under investigation was visible to the observer. The floor, made of cardboard was divided into 16 equal squares (18 cm x 18 cm) with blue marker and a central square drawn with black marker. The cardboard was covered with a transparent Plexiglas. 30 mice divided into five groups (n=6). The groups were treated with normal saline (10ml/kg), the plant extract (100, 200 and 400 mg/kg) or diazepam (0.25 mg/kg). Thirty (30) minute post-treatment, each mouse was placed individually at the corner of the arena and its behaviour monitored for a period of 5 minutes with the aid of a video camera hung 2 m above the apparatus and connected to a monitor. The number of rearing, number of squares and number of Central Square crossed by each mouse was recorded. The apparatus was wiped between observations with 70% ethyl alcohol and allowed to dry to remove any olfactory cue.

Statistical analysis. Results were expressed as mean ± standard error of mean (mean ± SEM) and percentage protection. The difference between the control and the test groups were analyzed for statistical difference using One Way ANOVA followed by Dunnett's post hoc t-test for multiple comparisons. Values of $p < 0.05$ or lower were considered significant.

RESULTS AND DISCUSSION

Phytochemical constituents. Preliminary phytochemical screening of ethanolic leaf extract of *Cussonia barteri* revealed the presence of cardiac glycosides, flavonoids, saponins, steroids, tannins and glycosides.

Median lethal dose (LD_{50}). The oral and intraperitoneal administration of the ethanolic leaf extract of *C. barteri* (10-1,000 mg/kg) in mice did not produce any visible sign of toxicity or mortality over a period of 24 h for the first phase of the Lorke's method. In the second phase, there was mortality from a dose of 2,900 mg/kg to 5,000 mg/kg *i.p*. The oral median lethal dose (LD_{50}) of the ethanolic leaf extract of *Cussonia barteri* for mice was estimated to be greater than 5,000 mg/kg while the *i.p* median lethal dose (LD_{50}) of the extract was estimated to be 2,154.1 mg/kg in mice.

Effect of the extract on maximal electroshock-induced seizures in chicks. The extract did not protect the chicks against tonic hind limb extension induced by maximal electroshock. There was a significant increase ($p<0.05$) in the mean recovery time at a dose of 100 mg/kg and at

200 and 400 mg/kg of the extract, but with no significant reduction in the mean recovery time. Phenytoin protected 80% of the chicks at the dose of 20 mg/kg.

Effect of the extract on Pentylenetetrazole-induced Seizure in mice. The extract protected mice against PTZ seizures with the highest dose (400mg/kg) producing 83.33% protection, while at the doses of 200 and 100 mg/kg, the extract produced 33.33% protection. The standard agent, sodium valproate at the dose of 200 mg/kg produced 100% protection against PTZ-induced seizure in mice. The extract had no significant effect on the mean onset of seizure.

Effect of the extract of on Strychnine-induced seizure in mice. The extract at the highest dose (400 mg/kg) produced 66.67% protection against seizure, while at the doses of 100 and 200 mg/kg produced 50% protection against this seizure. Similarly, at the highest dose tested, the extract offered 66.67% protections against mortality while there was 50% protection at the dose of 100 mg/kg and 83.33% protection at the dose of 200 mg/kg against mortality induced by strychnine at the dose of 1.2 mg/kg. However, there was no significant effect on the mean onset of seizure. Phenobarbitone (20 mg/kg) produced 100% protection against strychnine-induced seizure.

Effect of the extract on Diazepam induced-sleep in Mice. The extract significantly (p<0.01) at 200 mg/kg and (p<0.001) at 400 mg/kg increased the mean onset of sleep. Also, the extract significantly (p<0.05) at 200 mg/kg and (p<0.01) at 400 mg/kg increased the duration of sleep in mice.

Effect of the extract on exploratory activity of mice in hole -board test. The extract significantly (P<0.001) decreased the number of head dips in the hole board test. Diazepam at the dose of 0.5 mg/kg, also, significantly (P<0.001) decreased the number of head dips.

Effect of the extract on motor co-ordination in mice (Beam Walk Assay). The extract did not significantly affect the time taken to complete the task on the beam but at the dose of 400 mg/kg it significantly (p<0.05) increased the number of foot slips. Diazepam at the dose of 1 mg/kg significantly (p<0.05) increased the number of foot slips but did not significantly affect the time taken to complete the task.

Effect of the extract on behaviour of mice in Open Field Test. The extract did not significantly affect the number of rearing, number of total square crossed and the number of Central Square crossed at all the doses tested. Diazepam at the dose of 0.25 mg/kg did not significantly affect the number of total square crossed and the number of Central Square crossed but significantly decreased the number of rearing.

Generally, the data presented here suggest that the ethanolic leaf extract of *Cussonia barteri* may contain psychoactive substances with potential anticonvulsant properties. The preliminary phytochemical screening of the ethanolic leaf extract of *Cussonia barteri* revealed the presence of flavonoids, saponins, cardiac glycosides, steroids/terpenoids and tannins, which might be responsible for the observed anticonvulsant activity of the extract. Based on the results obtained from the acute toxicity study, the extract may be said to be slightly toxic according to [10]. The absence of protection against hind limb tonic-clonic extension (HLTE) in the maximal electroshock test (MEST) indicates that the extract is not effective against tonic-clonic seizure and cannot inhibit or prevent seizure spread within the brain stem seizure substrate [20]. This suggests that the ethanolic leaf extract of *Cussonia barteri* may not be useful in the treatment of generalized tonic-clonic seizure. Pentylenetetrazole (PTZ) is a known convulsant that act by inhibiting the activity of GABA at $GABA_A$ receptors [21], and is

used to identify compounds that can raise the seizure threshold in the brain [22]. This inhibition of GABA neurotransmission is said to be an underlying factor in epilepsy [23]. The moderate activity of the extract against PTZ-induced seizure in this study indicates that the plant may have the potential of raising seizure threshold could be beneficial in the treatment of myoclonic and absence seizure [24].

Antiepileptic drugs like diazepam, sodium valproate and Phenobarbital that modulate $GABA_A$ receptor-mediated inhibitory neurotransmission can prevent PTZ-induced seizures [25]. Sodium valproate, which was used in this study as a reference anticonvulsant agent, produced 100% protection against PTZ-induced seizures. The inhibition of PTZ-induced seizures by the extract at the highest dose treated in this study is an indication that the extract may exert its anticonvulsant effect by enhancing GABAergic neurotransmission. Strychnine (STN) is a competitive glycine receptor antagonist that induces convulsions by competitively antagonizes the postsynaptic inhibitory effects of glycine [26]. The inhibition of strychnine-induced convulsion by ethanol leaf extract of *Cussonia barteri* in this study indicates that the plant may contain bioactive compound(s) that interact with the glycine receptors probably as agonists or enhancing the binding of glycine to its receptors. Diazepam is a CNS depressant of benzodiazepine group, used in the management of insomnia. Diazepam act by binding to $GABA_A$ receptors and enhances the activation of GABA. This enhancement of neuronal inhibition by GABA produces sedation, which is mediated via α1 $GABA_A$ receptors [27]. Many medicinal plants or herbal preparations like chamomile tea and valerian have been shown to enhance the positive allosteric modulating effects of benzodiazepines on $GABA_A$ receptors [28]. In the present study, ethanol leaf extract of *Cussonia barteri* significantly prolonged the duration of sleep induced by diazepam. The ability of the extract to prolong the duration of sleep induced by diazepam suggests that it may possibly act by interacting with GABA-mediated synaptic transmission. Phytoconstituents like flavonoids, terpenes and saponins have been found to have sedative effect, and flavonoids with anxiolytic activities have been obtained from many herbs or plants used in traditional medicine [29]. In addition, the anticonvulsant and sedative properties of flavonoids have been reported in some previous studies [28,30,31]. Therefore, it is possible that the observed anticonvulsant and sedative activities of ethanol leaf extract of *Cussonia barteri* might be due to presence of flavonoids.

The Hole-board test is a measure of exploratory behaviour in animals [32]. The head dipping behaviour is sensitive to changes in the emotional state of the animals and increase in head dipping behaviour is a reflection of anxiolytic activity [33] while a decrease in the head dipping reveals sedative behavior [34]. In this test, the extract at the doses of 200 and 400 mg/kg significantly (p<0.01 and P<0.001 respectively) produced a decrease in exploratory behaviour as indicated by decrease in the number of head dip. This finding supports the sedative property of the extract. The mouse beam walking assay was employed to evaluate the effect of the plant extract on the motor coordination behaviour of the animals. It is a good predicator of drug producing clinical sedation and offers improved sensitivity over the mouse rota-rod in motor coordination deficit determination [18]. The increase in the number of foot slips produced by the extract in this study is an index of motor coordination deficit and supports other findings in this study that the extract might cause clinical sedation at the highest dose tested. The open field test provides simultaneous measures of locomotion, exploration and anxiety [35]. The

open field model examines anxiety related behavior of rodents based on the natural aversion of the animal to an open, brightly light novel environment. When animals are removed from their acclimatized cage and placed in a new environment, they are expected to express anxiety and fear, by showing alteration in all or some parameters. Drugs with anxiolytic effects reduce such fearful behavior of animals in open field [36]. This is demonstrated with significant increase in number of rearing and crossing by the animals in an open field. In this study, the ethanol leaf extract of *Cussonia barteri* did not significantly increase the number of rearing and number of square crossing and the animals were seen spending more time in the corners and the periphery than in the centre, which suggests that the extract did not produce anxiolytic effect at the doses tested.

Table 1: Effects of the extract on Maximal Electroshock-induced Seizures in chicks

Treatment	Dose (mg/kg)	Mean Recovery Time(min) ± SEM	Quantal protection against seizure	% Protection against Seizure
N/S		6.2 ± 0.79	0/10	0
ELCB	100	9.2 ± 0.67*	0/10	0
ELCB	200	6.3 ± 0.72	0/10	0
ELCB	400	6.1 ± 0.69	0/10	0
Phenytoin	20	5.9 ± 2.83	8/10	80

Data presented as; Mean ± SEM=Standard Error of Mean, N/S= Normal saline 10ml/kg, ELCB = Ethanol Leaf extract of *Cussonia barteri*, * (p<0.05), n=10.

Table 2: Effect of the extract on mice behaviour in Open field Test

Treatment	Dose (mg/kg)	Number of rearing ± SEM	Number of total square crossed ± SEM	Number of central square crossed ± SEM
Normal saline	10ml/kg	11.0 ± 0.58	97.00 ± 13.00	3.0 ± 2.52
ELCB	100	14.3 ± 1.86	84.0 ± 11.27	2.3 ± 0.88
ELCB	200	5.7 ± 1.86	6.7 ± 1.20	0.67 ± 0.33
ELCB	400	7.33 ± 2.96	85.0 ± 30.39	2.33 ± 1.86
Diazepam	0.25	4.0 ± 0.00*	157.67 ± 1.45	0.67 ± 0.33

Data presented as; Mean ± SEM= Standard Error of Mean, ELCB = Ethanol Leaf extract of *Cussonia barteri*. *p<0.05, n= 6.

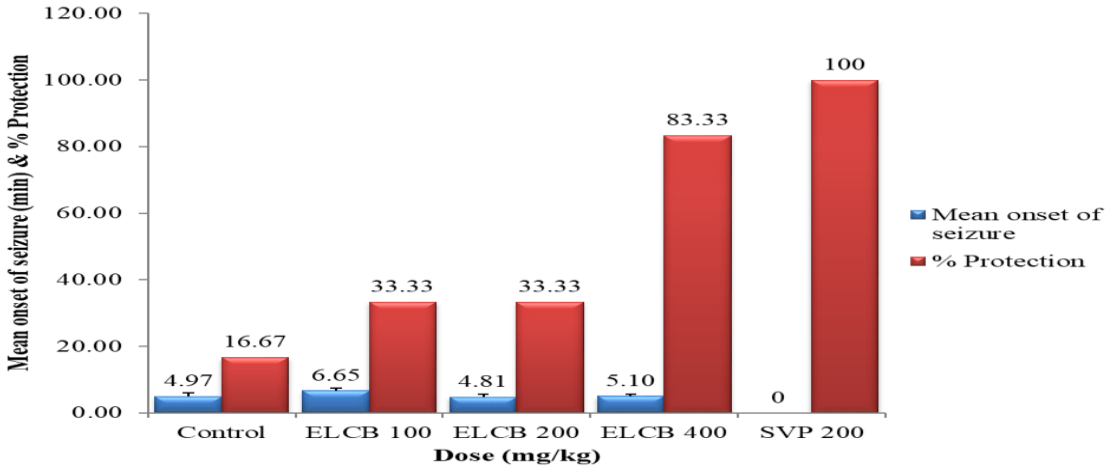

Fig.1: Effects of ethanol leaf extract of *Cussonia barteri* on PTZ-induced seizure in mice
ELCB= ethanol extract of *Cussonia barteri* leaf, PTZ= Pentylenetetrazole, SVP= Sodium valproate, n=6

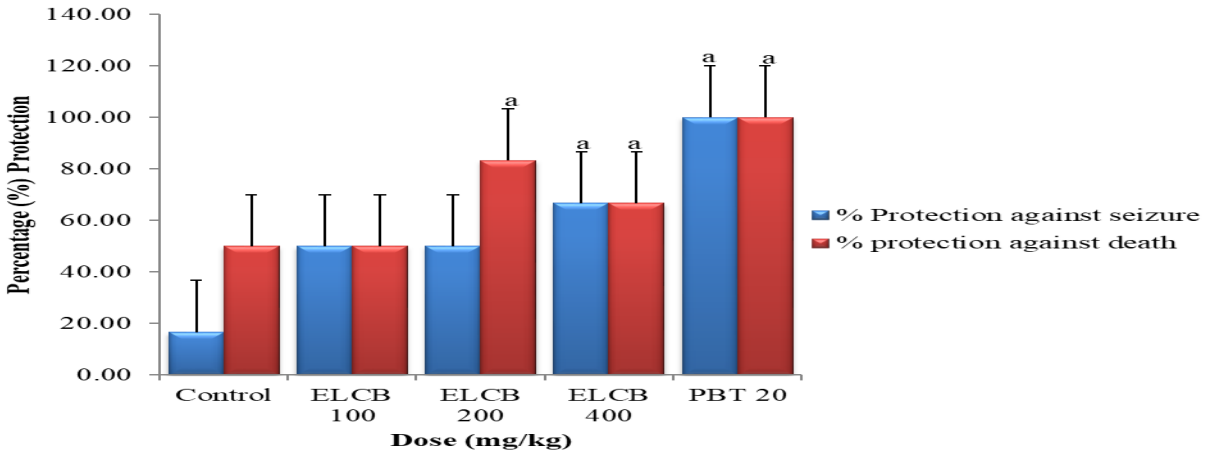

Fig.2: Effect of ethanol leaf extract of *C. barteri* on Strychnine-induced seizure in mice Control=Normal saline 10 ml/kg, ELCB= Ethanol Leaf extract of *Cussonia barteri*, PBT= Phenobarbitone, n=6, a= $p< 0.05$.

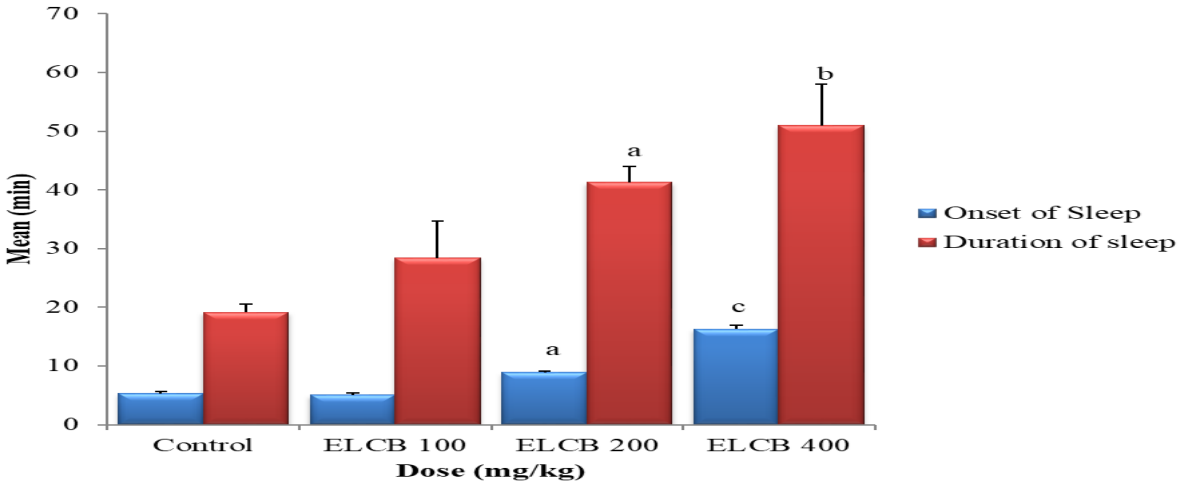

Fig.3: Effect of the extract on Diazepam-induced sleep in mice; onset and duration of sleep presented as mean ± SEM= Standard Error of Mean, Control= Normal saline 10ml/kg; ELCB= Ethanol Leaf extract of *Cussonia barteri*. a= $p<0.05$, b= $p<0.01$, c= $p<0.001$.

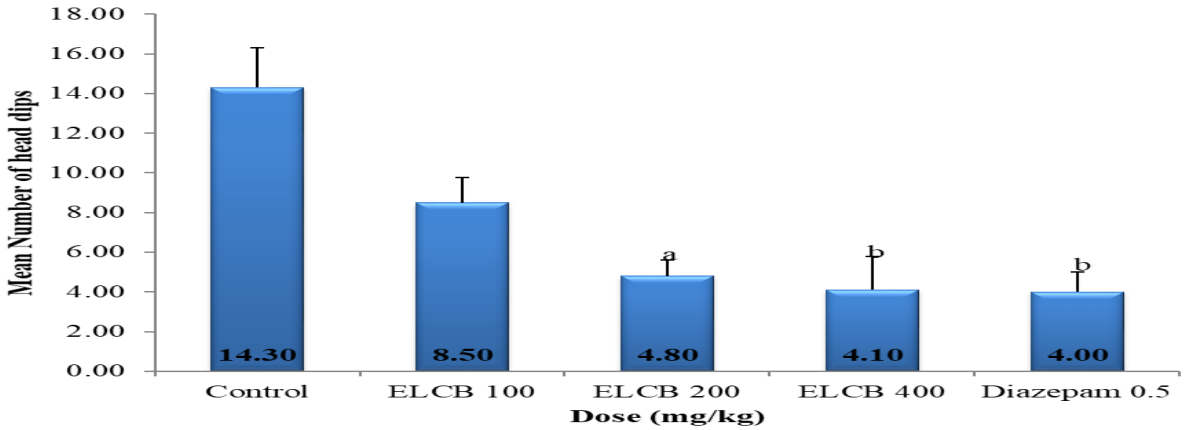

Fig.4: Effect of the extract on exploratory activity of mice in hole-board test; control= Normal saline 10ml/kg, ELCB= Ethanol Leaf extract of *Cussonia barteri*, a= $p< 0.01$, b=$p<0.001$.

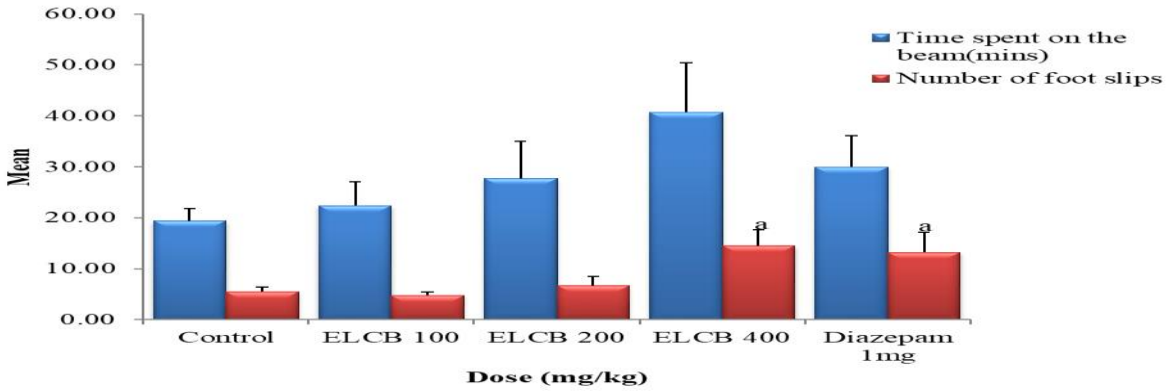

Fig.5: Effect of the extract on motor co-ordination in mice (Beam Walk Assay), Control= Normal saline 10ml/kg, ELCB= Ethanol Leaf extract of *Cussonia barteri*, a= $p< 0.05$

Findings of these studies suggest that the ethanol leaf extract of *Cussonia barteri* may contain bioactive compounds which possess significant anticonvulsant activity against pentylenetetrazole and strychnine-induced seizures therefore may be beneficial for the management of myoclonic and absence (petit mal) seizures. Thus, the use of *Cussonia barteri* in traditional medicine for the management of epilepsy in Nigeria and Ghana may be justifiable.

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REFERENCES

1. World Health Organization, (2018). Fact sheets on Epilepsy.Available:http://www.who.int/news-oom/fact-sheets/detail/epilepsy: accessed on 6th September, 2018.

2. Katsarou, A.M., Galanopoulou, A.S. and Mosh, S.L., (2017). Epileptogenesis in neonatal brain. *Seminars in Fetal & Neonatal Medicine*, 1-9.

3. Caraballo, R., and Fejerman, N., (2015). Management of epilepsy in resource-limited settings. *Epileptic disorders*, 17 (1): 13-18.

4. Balamurugan, G., Muralidharan, P., and Selvarajan, S., (2009). Antiepileptic activity of Polyherbal extract from Indian medicinal plants. Journal of Scientific Research, 1(1): 153-159.

5. Senthil, N. D., (2013). Effect of *Fagonia cretica (L)* extracts on biogenic amines concentrations in rat brain after induction of seizure. *International Journal of Preclinical & Pharmaceutical Research*, 4(2) (2013) 81-84.

6. Burkill, H.M., (1985). The useful plants of West Tropical Africa, 1: 211-212.

7. Kikenny, C., Browne, W.J., Cuthill, I.N., Emerson, M., and Altman, D.S., (2010). The ARRIVE Guidelines. Animal Research: Reporting of In Vivo Experiments, National Centre for the Replacement Refinement and Reduction of Animals Research

8. National Institute of Health, (1978). Guidelines for the care and use of laboratory animals. 8th ed., Institute for Laboratory Animal Research, National Academics Press, No. 8023, rev Washington D.C.

9. Evans, W.C. (2002). Phytochemistry In: Trease and Evans' Pharmacognosy. Fourteenth Edition, WB Sanders Company Ltd. London, UK, 224-343.

10. Lorke, D.A., (1983). A new approach to practical acute toxicity testing. Archives of Toxicology, 54: 275-287.

11. Swinyard, E. A., and Kupferber, H.J., (1985). Antiepileptic drugs: detection, quantification and toxicity and phytochemical studies of the stem bark aqueous extract of *Musanga cecropioides* in rats. *Journal of Ethnopharmacology*, 105: 374-379.

12. Sayyah, M., Valizadeh, J., Kamalinejad, M., (2002). Anticonvulsant activity of the leaf essential oil of *Laurusnobilis* against pentylenetetrazole and maximal electroshock-induced seizures. *Phytomedicine*, 9: 212-216

13. Swinyard, E.A., Woodhead, J.H., White, H.S., and Franklin, M.R., (1989). General principles; experimental selection, quantification and evaluation of anticonvulsants In: R. Levy, R. Mattson, B. Meldium, J.K. Penry and F.E. Dreifuss (Eds.), Anti-

epileptic Drugs, 3rd ed., New York, NY: Raven Press Ltd, 85-102.

14. Porter, R.J., Cereghino, J.J., and Gladding, G.D., (1984). Antiepileptic drug development program. *Cleveland Clinic Journal of Medicine* Quarterly, 51: 293-305

15. Beretz, A., Haag-Berrurie, R. M., Anton, R., (1978). Choice of pharmacological methods for studying the activities of aubepine. *Medicinal plants and herbal medicine*, 4: 305-314.

16. Rakotonirina, S.V., Ngo Bum, E., Rakotonirina, A., Bopelet, M., (2001). Sedative properties of the decoction of the rhizome of *Cyperusarticularis fitoterapia*, 72: 22–29.

17. File, S. E., (1973). Effects of chlorpromazine on exploration and habitation in the rat. *British Journal of Pharmacology,* 49: 303-310.

18. Stanley, J.L. Lincoln, R.J., Brown, T.A., McDonald, L.M., Dawson, G.R., and Reynolds, D.S., (2005). The mouse beam walking assay offers improved sensitivity over the mouse Rota-rod on determining motor coordination deficits induced by benzodiazepine. *Journal of Psychopharmacology*, 19: 221 – 227.

19. Brown, R.E., Corey, S.C., and Moore, A.K., (1999). Differences in the measure of exploration and fear MHC-congenic C57BL/6J and B6-H-2K Mice. Behaviour Genetics, 26:263-271.

20. Browing, R. (1992). The electroshock model, neuronal network and antiepileptic drugs. In: Faingold, C.L. and Fromn, G.H. (Eds). Drugs for control of Epilepsy*:* Actions on Neuronal Networks in seizure Disorders, CRC press. Boca Raton, FL. Pp.195-211.

21. Vasconcelos, S.M., Lima, N.M., Silveira, E.R. *et al*., (2007). Anticonvulsant activity of hydroalcoholic extracts from *Erythrina velutina* and *Erythrina mulungu. Journal of Ethnopharmacology 110*: 271-274.

22. White, H. S., Wolf, H. H., Woodhead, J. H., Kupferberg, J. H., (1998). The National Institute of Health anticonvulsant drug development program: Screening for efficacy. In: French J, Leppik IE, Dichter MA, eds. Antiepileptic Drug Development: Advances in Neurology. Philadelphia: Lippincott-Raven Publishers, 29–39.

23. Silambujanaki, P., Chitra, V., Kumari, S., Sankari, M., Raju, D., Chandra, C. H., (2010). Anticonvulsant activity of aqueous extract of *Butea monosperma* leaves. *RJPBCS 1*: 431-435.

24. Loscher, W., Fassbender, C. P., Noting, B., (1991). The role of technical, biological and pharmacological evaluation of anticonvulsant drugs in maximal electroshock seizure models. Epilepsy Res 8:79–94.

25. Jain, P.D., Tambe, R.M., Sancheti, J.S., Nahire, M.S., Bhardwaj, A.K., and Sathaye, S., (2015). Screening of Pistacia integerrima extracts for their anticonvulsant activity in acute zebrafish and rodent models of epilepsy. *International Journal of Nutrition, Pharmacology, Neurological Diseases* 5: 56-62.

26. Rajendra, S., Lynch, J.W., and Schofield, P.R., (1997). The glycine receptor. *Pharmacological Therapeutics,* 73:121-146.

27. Tobler, I., Kopp, C. Deboer, T. and Rudolph, U., (2001). Diazepam-induced changes in sleep, Role of α1GABA$_A$receptor subtypes. Proceedings of the National Academy of Sciences, 98(11): 6464-6469. [28] Johnston, G.A.R., (2005). GABA$_A$ Receptor Channel Pharmacology. *Current Pharmaceutical Design*, 11:1867-1885.

29. Aziz, A., and Khan, I.A., (2013). Pharmacological evaluation of Sedative and Hypnotic activities of methanolic extract of *Lycopus europaeus* in mice. *The Journal of Phytopharmacology*, 2(4): 8-12.

30. Yao, Y., Jia, M., Wu, J.G., Zhang, H., *et al*., (2010). Anxiolytic and Sedative-hypnotic activities of Polygalasaponins from *Polygala tenuifolia* in mice. *Pharmaceutical Biology*, 48 (7): 801-807.

31. Hanrahan, J.R., Chebib, M., Johnston, G.A.R., (2011). Flavonoid modulation of GABA$_A$ receptors. British Journal of Pharmacology, 163: 234–245.

32. File, S. and Wardil, A. G., (1975). Validity of head-dipping as a measure of explorating a modified hole-board. Psychopharmacology, 44: 53-59.

33. Takede, N. Tanji, M., and Matsumiya, T., (1998). Changes in head-dipping behavior in the hole- board test reflect the anxiogenic and/or anxiolytic state in mice. European Journal of Pharmacology, 350: 21-29.

34. File, S., and Pellow, S., (1985). The effect of Triazolobenzodiazepines in two animal tests of anxiety and on the hole-board. *British Journal of Pharmacology*, 86: 729-735.

35. Walsh, R.N., and Cummins, R.A., (1976). The open-field test: a critical review. Psychological Bulletin, 83: 482-504.

36. Mechan, A.O., Moran, P.M., Elliott, M., Young, A.J., Joseph, M.H. Green, R., (2002). A comparison between dark agouti and Sprague-Dawely rats in their behaviour on the elevated plus-maze, open field apparatus and activity meters and their response to diazepam. *Psychopharmacology* (Berl), 159: 188–95.