EFFECTS OF HYDRO-METHANOL LEAF EXTRACT OF *BRYOPHYLLUM PINNATUM* ON DIAZEPAM-INDUCED SLEEPING TIME AND PENTYLENETETRAZOLE-INDUCED SEIZURE TEST IN MICE.

Momoh, Ibrahim Joseph*[1], Abba, Sunday[2]Jonah, Achile Caleb[1] Omotoso, Olusegun Dare[2] Ukanu Paul Idoko[2]

[1] Department of Physiology, Prince Abubakar Audu University, Anyigba, Nigeria
[2] Department of Anatomy,Prince Abubakar AuduUniversity, Anyigba, Nigeria
**Correspondence Author's email: momoh.ij@ksu.edu.ng*

Abstract

Thousands of plants are used traditionally all over the world in the management of convulsion and sleep disorders. However, only a few have received scientific scrutiny. This study was carried out to evaluate the effect of the hydro-methanol leaf extract of Bryophyllum pinnatum (family: Crassulaceae) in some neurobehaviours in mice weighing between 25-30g at doses of 25mg/kg 50mg/kg, and 100mg/kg. The parameters evaluated were the diazepam-induced sleeping time and Pentylenetetrazole (PTZ)-induced convulsion. The extract at 25mg/kg, 50mg/kg and 100mg/kg showed the duration of sleep to be 269.60 ± 53.07,218.20 ± 60.24 and 298.20 ± 54.37 respectively. The results revealed a statistical significant (P<0.05) decrease in the onset of sleep and a statistical significant (P<0.05) prolongation of sleep time in a dose dependent manner. The extract also showed a biphasic activity by delaying the onset of convulsion and increasing protection at the doses of 25mg/kg and 100mg/kg in mice. These results showed that the extract possesses some central nervous system (CNS) depressant activity which may be due to the effect or activity of one or more of the phytochemicals present in the plant.

Keywords: *Bryophyllum pinnatum*, Diazepam, Sleeping time, Seizure, Pentylenetetrazole (PTZ)

Introduction

Sleep can be defined as unconsciousness from which the person can be aroused by sensory or other stimuli. It is a physiological state characterised by decreased motility and decreased responsiveness to sensory stimuli and its reversible (Stenberg, 2007; Guyton and Hall 2006). The British Epilepsy Association in 2012 defined epilepsy as a tendency to have recurrent seizures (sometimes called fits). A seizure occurs as a result of a sudden burst of excess electrical activity in the brain, leading to a temporary disruption in the normal information passing between brain cells. The World Health Organization (2019) stated that epilepsy as a neurological disorder make upthe second most common condition treated by neurologists. About 50 million people worldwide have epilepsy (WHO, 2019).It is

Estimated that its prevalence in Nigeria is about 8 to 13 per thousand people (Azubuike and Nkanginieme, 1996).People in Africa and other continents still depend largely on traditional healing practices despite the advancement in modern medicine (Ojewole, 2005). It is estimated that about 80% of the world population is dependent (wholly or partially) on plant-based drugs (WHO, 2022).

Bryophyllum pinnatum(*Crassulaceae*) or life plant (Priyanka *et al.,* 2020) is a fleshy shrub, which grows 2-4 feet tall, it is widely grown in the tropical region for ornamental and medicinal uses (Chaithra *et al.,* 2020). It is a perennial herbgrowing widely and used in folkloric medicine in tropical Africa, India, China, Australia and tropical America (Balzer, 1949). Though classified as a weed, the plant flourishes throughout the Southern part of Nigeria, it is popularly known as "ewe abamoda or odundun" by the Yoruba tribe of Southwestern Nigeria, "odaa opue" among the Igbos, "da bu si" in Chinese (Ghasi *et al.*, 2011) and "sutura" by the Hausa peoples of Nigeria.The leaves of *Bryophyllum pinnatum* have been reported to possess antimicrobia, antifungal, antiulcer, anti-inflammatory and analgesic, antihypertensive and CNS depressant (Momoh *et al*, 2016; Ojewole, 2002) activities.

Materials And Methods

Animals

A total of forty-five (45) adult mice of both sexes weighing between 25g to 30g were used. They were obtained from the Animal House laboratory of the Department of Pharmacology and Clinical Pharmacy, ABU, Zaria, and were kept in the laboratory for 7 days before use. They were placed on standard feed and allowed free access to food and water. (Momoh *et al*., 2016).

Plant Material Identification

Fresh leaves of *Bryophyllum pinnatum* were collected around Zaria, northern Nigeria. The identification and authentication of the plant was carried out at the Herbarium unit of the Department of Biological Science, Ahmadu Bello University, Zaria, where a specimen was deposited with a voucher number, 1834 as reported in our previous work.

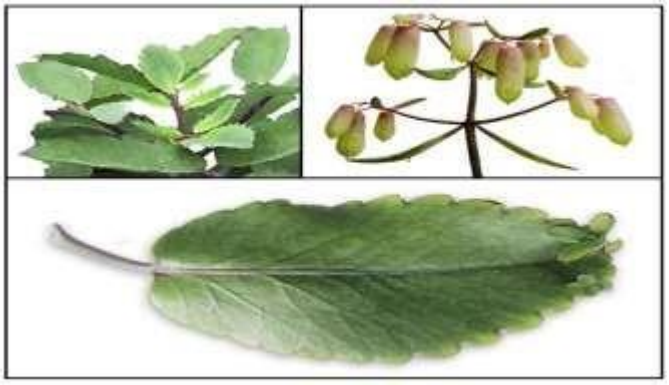

Figure1.0. Picture of the leaves and flowers of *Bryophyllum pinnatum* (Chaithra *et al.,* 2020)

Preparation of the Plant Extract.

The extract was prepared as reported in a previous work published earlier (Momoh *et al*, 2016)

Chemicals and Drugs Used

All chemicals and drugs used were of analytical grade. Diazepam was purchased from La Roche Ltd. Basel, Switzerland.

Experimental design

The extract of *Bryophyllum pinnatum* was administered interperitoneally at 25mg/kg, 50mg/mg and 100mg/kg (Momoh *et al*, 2016).

Diazepam-induced Sleep in Mice

The method described by Rakotonirina *et al.* (2001) was adopted in this study. 20 Swiss adult albino mice of either sex were divided into 4 groups of five mice in each group. The first group served as the control, second, third and fourth groups were administered the extract of *Bryophyllum pinnatum* at the doses of 25mg/kg, 50mg/kg and 100 mg/kg intraperitoneally respectively. Thirty minutes later, diazepam at a dose of (3 mg/kg) was administered to all the mice intraperitoneally. Each mouse was then observed for the onset and duration of sleep. The criterion for sleep is the loss of rightening reflex, in which the mice cannot roll back when turned over (Miya *et al.*, 1973).The interval between loss and recovery of rightening reflex was used as the index of hypnotic effect (Soulimani *et al.*, 2001).

Pentylenetetrazole (PTZ) Test

The method as described by Swinyard *et al.* (1989) was employed. Clonic seizures were induced in male mice by the intrapreitoneal injection of 8.5 mg/kg Pentylenetetrazole (PTZ). 25 Swiss albino adult mice were divided into 5 groups of 5 mice each. Group 1 received normal saline intraperitonealy. Groups 2, 3 and 4 received the extract at the doses of 25, 50, and 100 mg/kg intraperitonealy respectively. Group 5 received valproic acid 20 mg/kg. The protective effect of the plant was recorded in mice treated 30 minutes before with the extract. The time of onset of seizures in non-protected mice was also recorded. The general clonus was

characterized by forelimb clonus followed by full clonus of the body. The time taken before the onset of clonic convulsions, the duration of clonic convulsions, and the percentage of seizure and mortality protection were recorded (Vogel *et al*., 1997).

Statistical analysis

The data obtained from the experiment were expressed as Mean ± SEM. The data was statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey's Post-Hoc test.The values of $p < 0.05$ were considered as significant (Duncan *et al.,* 1977).

RESULTS

Diazepam-induced sleeping time

Table 1: Effect of hydro-methanol leaf extract of *Bryophyllum pinnatum* on diazepam-induced sleep in mice

Treatment / Dose		Onset of sleep (min)	Duration of sleep (min)
Control	1ml/kg	3.60 ± 0.68	11.80 ± 1.71
Extract	25mg/kg	3.00 ± 1.05	218.20 ± 60.24*
Extract	50mg/kg	2.60 ± 0.24	269.60 ± 53.07*
Extract	100mg/kg	2.8 ± 0.37	298.20 ± 54.37*

Values are given as mean ± SEM; experimental groups were compared with control. Values are statistically significant at *= $p < 0.05$

The extract at the different doses decreased the onset of sleep in mice but statistically and significantly ($P<0.05$) prolonged the duration of sleep in mice as demonstrated in 25mg/kg, 50mg/kg and 100mg/kg of the extract with corresponding duration of 218.20 ± 60.24, 269.60 ± 53.07 and 298.20±54.37 minutes respectively. The duration of sleep was dose dependent, with the 100mg/kg producing the highest duration of 298.20 ±54.37 minutes.

Pentylenetetrazole-induced Seizures Test

Table 2: Effect ofhydro-methanol leaf extract of *Bryophyllum pinnatum* on PTZ-induced seizure in mice.

Treatment / Dose	Onsetof clonic seizure	Protection (%)	Mortality (%)
Control 1ml/kg	6.80 ± 2.74	0.0	100
Valproic acid 200mg/kg	0.00 ± 0.00	100	0.0
Extract 25mg/kg	19.00 ± 1.00	80	20

Extract 50mg/kg	6.75 ± 2.43	20	80
Extract 100mg/kg	12.00 ± 3.89	60	40

Values are given as mean ± SEM; experimental groups were compared withcontrol. Values are statistically significant at *=p < 0.05

The extract at doses 25 g/kg and 100mg/kg prolonged the onset of convulsion in the mice when compared with the control. These doses also protected the mice from death when compared with the control. The extract showed a biphasic activity by prolonging the onset of convulsion and increasing protection at the doses of 25 and 100mg/kg but less effective at 50mg/kg.

DISCUSSION

The extract was observed to cause a decrease in the onset of sleep and significantly prolonged the duration of sleep in mice when statistically compared with the control. This effect was found to be dose dependent. Substances that promote sleep in mice are said to possess sleep inducing properties (McKillop*etal.*, 2021). This agrees with the study conducted by Okoye *et al.* (2023) who reported that the methanol stem extract of Bryopphyllum pinnatum increased significantly the quality and duration of sleep. It may be suggested that the extract possesses sleep inducing properties since it potentiated diazepam-induced sleep in the mice. Sedative-hypnotics act by increasing GABA mediated synapticinhibition either by directly activating the GABA receptors or more usually by enhancing the action of $GABA_A$ receptors. Benzodiazepines and barbiturates are examples of widely used therapeutic agents that act as positive allosteric modulators at GABA receptors (Johnston, 2005). The ability of the extract to potentiate the sedative property of diazepam suggests that it may possibly act by interacting with GABA-mediated synaptic transmission.Diazepam act selectively on $GABA_A$ receptor which mediates fast inhibitory synaptic transmission throughout the CNS (Argyropoulos *et al*., 2000).

The extract delayed the onset of convulsion in mice treated with 25mg/kg and 100mg/kg of the extract when compared with the control. It also increased the protection in the mice by 60% and 80% in 100mg/kg and 25mg/kg respectively, while in 50mg/kg, the protection was 20%. The delayed onset of convulsion and increased protection was not dose dependent. The ability of the extract to inhibit clonic seizures suggests anticonvulsant activity of could be due to the presence of alkaloids, flavonoids, saponins and tannins (Momoh *et al.,* 2016) as suggested by Mishra *et al.* (2011). The anticonvulsant activity of PTZ test identifies compounds that can raise seizure threshold in the brain (White *et al.,* 1998). PTZ interact with GABA receptor complex (DeDeyn *et al.,* 1992).

Ability of *Bryophyllum pinnatum* to inhibit clonic seizure in the PTZ test suggests that it may have the ability to raise seizure threshold. The effectiveness of a drug

against PTZ-induced seizures indicate its probable effectiveness against seizures (McNamara, 1989). PTZ inhibit chloride conductance by binding to picrotoxin sites of $GABA_A$ receptor complex (Krall *et al.,* 1978). GABA is the major inhibitory neurotransmitter which is implicated in epilepsy (Gale, 1992). PTZ may be exerting its convulsive effect by inhibiting the activity of GABA at $GABA_A$ receptors. The promotion and inhibition of the neurotransmission of GABA will promote and attenuate convulsion respectively (Westmorland *et al*., 1994). Phenobarbitone and Diazepam have been shown to exert their antiepileptic effects by enhancing the GABA-mediated inhibition in the brain (Porter and Meldrum, 2001). Ahmed *et al*. (2009) reported that the antagonism of PTZ-induced seizures suggests effects on GABA-ergic neurotransmission

Conclusion

Based on these results, the hydro-methanol leaf extract of *Bryophyllum pinnatum* has shown to possess considerable sedative and anticonvulsant actions that might involve the presence of some phytochemicals.

References

Ahmed A., Yaro A. H., Magaji M. G., Ibrahim H., Musa K. Y. and Abubakar L.Y. (2009). Anticonvulsant activity of Tacazzea apiculata oliv. (Periplocaceae). *Nigerian Journal of Pharmaceutical Sciences*. 8: 41- 46

Akinpelu, D.A. (2000). Anti-microbial activity of Bryophyllum pinnatum leaves. *Fitoterapia*; 71(2):193-194

Argyropoulos, S.V., J.J. Standford and D.J. Nutt, (2000). The Psychobiology of anxiolytic drugs. Part 2: pharmacological treatments of anxiolytic. *Pharmacology and Therapeutics.* 83: 213-227.

Azubuike, J.C., and Nkanginieme, K.E.O. (1996). *Paediatrics and Child Health in a Tropical*

Region. Fourth Dimension Publishers Enugu, pp. 388

Balzer, G. (1949). Bryophyllum. Ein Beitrag zu seina Pflanzen-morphologie. Berlin-leinmachnow: Gartenverlag GmbH,

Chaithra, S.R., Bijesh, V and Siju, E. N. (2020). A Review On Pharmacological activities of Bryophyllum pinnatum. Linn. *American Journal of Pharmacy & Health Research,* 8(2)1-12.

De Deyn, P.P., D'Hoope, R., Marescau, B., and Pei,Y.Q (1992). Chemical model for epilepsy with some references to their applicability in the development of anticonvulsants. *Epilepsy Research,* 12: 87-110.

Duncan. R.C., Knapp. R.G., and Miller. M.C. (1977). Test of hypothesis in Population Means. *In Introductory Biostatistics for the Health Sciences*. John Wiley and Sons Incorporation, New York, 71-96 pp.

Gale, M.K.(1992). GABA and epilepsy basic concepts from preclinical research. *Epilepsia*; 33 Suppl: S3-12.

Ghasi, S., Egwuibe, C., Achukwu, P.U. and Onyeanusi, J.C. (2011). Assessment of the medical benefit in the folkloric use of *Bryophyllum Pinnatum* leafamong the Igbos of Nigeria for the treatment of hypertension. *African Journal of Pharmacy and Pharmacology*5(1): 83-92.

Gill, L.S. (1992). Ethno-medical uses of plants in Nigeria, University of Benin Press, Benin City, Nigeria, pp.46-100.

Guyton, A.C. and Hall, J.E. (2021). Textbook of Medical Physiology, 11th Ed. Elsevier Saunders, Philadelphia. Pp 739.

Johnston, G.A.R. (2005). $GABA_A$ Receptor channel pharmacology. *Current pharmacological Design*, 11: 1867-1885.

Krall, R. L., Penry, J. K., Kuperberg, H.J. and Swinyard, E.A. (1978). *Epilepsia*, , 19, 409-428.

McKillop, E. L., Fisher, S. P., Milinski, L., Krone, L. B. and Vyazovskiy, V. V. (2021). Diazepam effects on local cortical neural activity during sleep in mice. *Biochemical Pharmacology,* 191: 114515

McNamara, J.O. (1989) *Epilepsia*, 30: 13-18.

Mishra, G., Singh, P., Garg, V. K., Parvez, N., Yadav, S. and Hwisa, S. (2011). Phytochemical screening and anticonvulsant activity of Wedeliachinensis. *International Journal of Pharmaceutical Sciences and Research,* 2(1): 39-43

Miya, T.S., H.G.L. Holck, G.K.W. Yu, and G .R Spratto (1973). Laboratory guide in pharmacology, Burgess Publishing Company, Minneapolis MN, pp: 44-46.

Momoh, I. J., Suleiman, J. B., Mabrouk, M. A., and Tanko, Y. (2016). Actions of hydro-methanol leaf extract of bryophyllum pinnatum (crassulaceae) on motor coordination and exploratory activities in mice.*International Journal of Current Research in Life Sciences* Vol. 05, No. 03, pp. 562-565.

Ojewole, J.A.O. (2005). Antinociceptive, anti-inflammatory and antidiabetic effects of *Bryophyllum pinnatum*(Crassulaceae) leaf aqueous extract. *Journal of Ethnopharmacology*. 99:13–19

Ojewole, J.A.O.(2002). Antihypertensive properties of Bryophyllum pinnatum leaf extracts. *American Journal of Hypertension*; 15(4); A34-A39.

Okoye, K. F., Anowi, F. C., Ejiofor, I. I. and Ololade, O. M. (2023). Pharmacognostic study of and sedative activity of Bryophyllum pinnatum stem methanol extract and fractions. *Sciences of Phytochemistry,* 2(2): 127-138

Perez, G.R.M., Perez, L.J.A., Garcia, D.I.M. and Sossa, M.H. (1998). Neuropharmocological activity of Solanum nigrum fruit. *Journal of Ethnopharmacology*. 62:43 – 48.

Priyanka, M., Mohini, U., Poonam, T. and Shashikant Dhole. (2020). A Review of Bryophyllum pinnatum (Lam.) Oken. *Research Journal of Pharmacognosy and Phytochemistry,*12(2) 111-113.

Porter, R.J., and Meldrum, B.S. (2001). Antiseizure drugs. In: Basic and clinical pharmacology. Katzung BG (ed). New York, Lange Medical Books/McGraw-Hill, pp 403-17.

Rakotonirina, S.V., Ngo Bum, E., Rakotonirina, A and Bopelet, M. (2001). Sedative Properties of the decoction of the rhizome of *Cyperus articularis*. *Fitoerapia*, 72: 22-29.

Region. Fourth Dimension Publishers Enugu, 388 pp.

Soulimani, R., C. Younos, S. Jarmouni-Idrissi, D. BousiaF. Khalouki and A. Laila (2001). Behavioral and pharmaco-toxicological study of Papaver rhoeas L. in mice. Journal of Ethnopharmacology. 74: 265-274.

Sridharan, R. (2002). Epidemiology of epilepsy. *Current Science*, 82 (6): 664-670

Swinyard, E.A., Woodhead, J.H., White, H.S. and Franklin, M.R. (1989). General Principles: *Experimental selection, quantification, and evaluation of anticonvulsants*. *In*:Levy, R.H., Mattson, B., Melrum, J.K. and Dreifuss, F.E. (Eds) *Antiepileptic Drugs*, 3rd edition. Raven Press New York, pp 85-103.

Vogel, G.W., Thurmond, A., Gibbons, P., Sloan, K., Boyd, M. and Walker, M., (1975). "REM sleep reduction effects on depression syndromes," *Archieves of General Psychiatry,* **32**,765-776.

Westmoreland, B.F., Benarroch, E.E., Dube, J.R., Regan, T.J. and Sandok, B.A. (1994). *Medical neurosciences.* Mayo Foundation Rochester, pp 307-12.

White, H.S., Wolf, H.H., Woodhead, J.H. and Kupferberg, H.J. (1998). The National Institute of Health anticonvulsant drug development program: Screening for efficacy. *In*: French, J., Leppik, I.E. and Dichter, M.A. (Eds.) *Antiepileptic Drug Development: Advances in Neurology*, Vol. 76, Lippincott-Raven Publishers Philadelphia, 29-39.

World Health Organization (2022):African traditional medicine Day 2022. Message of WHO Regional Director for Africa

World Health Organization (2019). *Epilepsy: Aetiology, Epidemiology and Prognosis*. Fact sheet No 165.