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A Study of Some Ameliorative Effects of Aqueous Extract of *Bryophyllum pinnatum* in Lead Acetate Induced Neurotoxicity in the Cerebellum of Adult Wistar Rats (*Rattus norvegicus*)

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Abstract

Bryophyllum pinnatum (Lam.), is a widely distributed perennial medicinal herb. In Nigeria, the plant is particularly known for its effective wound healing properties and detachment of the umbilicus of infants. The present study was thus aimed at investigating the effects of administration of aqueous *B. pinnatum* leaf extract on the histological function of the cerebellum of adult wistar rats. Fresh leaves of *Bryophyllum pinnatum* were harvested from Herbal Centre, Ikenne, Ogun-state, Nigeria, were authenticated in the pharmaceutical department of Olabisi Onabanjo university Sagamu. Sixteen (16) adult wistar rats of both sexes weighing 180g -320g was purchased from the animal holding of the faculty of pharmacy, Olabisi Onabanjo university, Sagamu, Ogun-state. The animals were acclimatized for a period of three weeks in the animal holding of the Department of Anatomy, Olabisi Onabanjo University. At the end of the acclimatization period, the rats were randomly aligned into four groups (n=4 of control, treatment group; T1, T2 and T3. Control (C) rats received normal feed and water; T1 rats received 2mls of Bryophyllum pinnatum extract; T2 rats receive 0.075g\kg of lead acetate; T3 rats received 2mls of B. pinnatum extract with 75mg/kg of lead acetate. About 0.075g of lead acetate was dissolved in 20mls of distilled water to make a solution and were administered to the rat at 0.2mls per 100g of rat body weight. About 2g of Bryophylum pinnatum extract was dissolved in 20mls of distilled water and were administered to the rat at 2mls to the treatment groups. The body weight of the rats was closely monitored with aid of a digital weighing balance. The group T2 and T3 were noticed to be having severe itching of the body throughout the period of administration. The group T1 and T3 were noticed to develop cystosis / boil at some parts of their body such as chest, back and legs but the group T3 shows a form of quick healing to this cystosis growth but were more chronic in the group T1. Administration of Pb to the studied animals has led to morphological alteration in the neuronal cells in the cerebellar cortex, while the co-administration of Pb and B. pinnatum resulted in amelioration of these effects to a great extent due to its anti-oxidant activity. We recommend more investigations on the neurotoxic effects of lead on laboratory animals and human, with regular administration of B. pinnatum where lead exposure could not be avoided.

Keywords: Bryophyllum pinnatum, Lead, Acetate, Neurotoxicity, Cerebellum, Wistar Rats

1. Introduction

Bryophyllum pinnatum (Lam.), syn. *Kalanchoe pinnat*a (Lam.), and *B. calycinum* (Salisb.) is a widely distributed perennial medicinal herb. It is native to Madagascar, but has been naturalized in several other regions, including the temperate regions of Asia, Australia, and New Zealand. *B. pinnatum* is known by some common names including, life plant, air plant, maternity plant, love plant, miracle leaf, cathedral bells, mother of thousands, leaf of resurrection plant, and Lao di Sheng gen. The plant is locally called "Never Die" in Nigeria, and is very popular in folklore medicine. It has been used for the treatment of a variety of conditions in tropical America, India, China, Australia and Africa, including, rheumatism, body pain, arthritis, heartburn, skin ulcers, peptic ulcer, diabetes mellitus, microbial infections, and hypertension (Ghasi *et al.*, 2011).

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1.1 Introduce the Problem

In Nigeria, the plant is particularly known for its effective wound healing properties and detachment of the umbilicus of infants (Aprioku & Igbe, 2017). Pharmacological studies on *B. pinnatum* reported several biological activities some of which could authenticate the plant's traditional uses including, immunomodulatory, CNS depressant, analgesic, anti-inflammatory, antimicrobial, antitumor, antiulcer, insecticidal, antidiabetic, anticonvulsant, antioxidant, and antihypertensive properties (Okwu & Josiah, 2006). Studies have also reported a wide range of active phytochemicals such as alkaloids, triterpenes, glycosides, flavonoids, steroids, bufadienolides, lipids and organic acids (Marriage & Wilson, 1971). These compounds have been considered to be responsible for the plant's diverse pharmacological activities. Although, folklore claims of many herbal remedies are yet to be authenticated scientifically, *B. pinnatum* has been reasonably studied with justification of most of the claims. Furthermore, Pal and Nag (1999) provided evidence for the neuropsychopharmacologic activities of the plant. The present study is therefore designed to further investigate the effects of the aqueous leaf extract of *B. pinnatum* on some central nervous.

Lead (Pb) is a highly toxic heavy metal that persists in the environment and the human body and can disrupt neurological & other biological body functions (Bauchi *et al.*, 2016) chronic poisoning by it is one of the major public health hazards especially in developing countries (Flora *et al.*, 2012; Iyevhobu *et al.*, 2022). Small amount of lead is excreted in urine and the rest accumulates in various body tissues, mainly the (CNS) which may result in structural changes that can persist even after lowering of its blood level (Sidhu & Nehru, 2004; Taib *et al.*, 2004; Flora *et al.*, 2012; Ibrahim *et al.*, 2012). Lead was reported to produce oxidative stress by generating release of reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals, hydrogen peroxide and lipid peroxides which increase oxidative damage of cellular materials (Ercal *et al.*, 2001; El-Nekeety *et al.*, 2009; Usiobeigbe *et al.*, 2024). Depending on the observation that free radicals were generated during the pathogenesis processes induced by lead, it was presumed that supplementation of antioxidants will interrupt or minimize the damaging effects of lead and improve the effects of chelating agents (Flora *et al.*, 2012; Usiobeigbe *et al.*, 2012; Usiobeigbe *et al.*, 2025).

1.2 Explore Importance of the Problem

Biological interests in lead have been centered principally on its properties as a highly toxic commutative poison in humans and animals. Despite several decades of research in the neurotoxicology of lead and its continued prominence as a major environmental and occupational health hazard, the mechanism of its toxic action in the nervous system is still unknown (Silbergeld,1992; Iyevhobu *et al.*, 2022). Lead can cause immediate effects by altering chemistry, physiology, or histology of the brain. Collins et al., (1982) reported that lead levels in the brain do not decrease as rapidly as blood levels since the washout time of lead from some regions of rat brain receiving chronic treatment is relatively slow (Collins *et al.*, 1982). Once in the brain, lead-induced damage occurs primarily the prefrontal cortex, cerebellum, and

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hippocampus, affecting many biological activities at the molecular, cellular, and intracellular levels, which may result in morphological alterations in brain that can remain even after lead levels have fallen (Chen *et al.*, 1998; Usiobeigbe *et al.*, 2024; Usiobeigbe *et al.*, 2025).

1.3 Describe Relevant Scholarship

The cerebellum ("little brain") is a structure that is located at the back of the brain, underlying the occipital and temporal lobes of the cerebral cortex (Hodos, 2009). Although the cerebellum accounts for approximately 10% of the brain's volume, it contains over 50% of the total number of neurons in the brain. Historically, the cerebellum has been considered a motor structure, because cerebellar damage leads to impairments in motor control and posture and because the majority of the cerebellum's outputs are to parts of the motor system (Usiobeigbe *et al.*, 2024). Motor commands are not initiated in the cerebellum; rather, the cerebellum modifies the motor commands of the descending pathways to make movements more adaptive and accurate (Wolf et al., 2009). Recently, human neuroimaging and animal behaviour studies have implicated the cerebellum in the processing of signals for perception, cognition, and emotion (D'Angelo & Casali, 2012), particularly in circumstances involving predictions or timing. Participation of the cerebellum in higher order brain function is likely mediated by extensive connections with cortical and sub-cortical centers. These anatomical connections raise the intriguing possibility that cerebellar dysfunction may lead not only to motor impairments, but also to non-motor deficits in complex neurological conditions. Furthermore, the implication that cerebellar circuits malfunction in certain neurodevelopmental disorders suggests that cerebellar processing could be required during development for proper wiring in other brain areas (Kuemerle et al., 2007). The present study was thus aimed at investigating the effects of administration of aqueous B. pinnatum leaf extract on the histological function of the cerebellum of adult wistar rats.

2. Method

2.1 Materials

Laboratory mortar and pestle, separating funnel, filter paper, water bath, evaporating dish, spatula, weighing scale, plastic tubes, syringes, canula, timer, chloroform chamber, scalpels, scissors, forceps, blades, icebox, ice, centrifuge (HERMLE MR-2 BY Labnet), Pasteur pipettes, Apetman Micropipettes, test tubes, curvettes, spectrophotometer, beakers, measuring cylinders, eppendorfmicrocentrifuge tubes, plastic homogenizer, incubator (Thermolyne Type 17600 Dribath), microwave oven (Binatone MWO2519EG), gel electrophoresis machine, gel documenting machine, couplin jars, funnel, rotary microtome(RM2125RTS, Leica Biosystems, Germany), glass slides, cover slips, drying racks, light microscope (Olympus BX43), Fluorescence microscope (Olympus BX51), Armscope MD900 and Scoptek 2.0 M digital camera, Sysmex xn350 haematological analyzer.

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2.1.1 Reagents used includes;

Ethanol, 10% formal saline solution, Paraffin wax, Xylene, Chloroform water, Olive oil, Methylated spirit, Water, Buffer solution, Hematoxylin stain, Eosin stain, Schiff reagent, 1% light green solution, Hydrochloric acid, Cresyl violet solution.

2.1.2 Constituent of Animal Feed:

Corn, Rice polishing, Canola meal, Guar meal, Soybeans meal, Fish meal, Limestone, Dicalcium phosphate.

2.2 Experimental Animals

Sixteen adult wistar rats of both sexes weighing 180g -320g was purchased from the animal holding of the faculty of pharmacy, Olabisi Onabanjo university, Sagamu, Ogun-state. The animals were acclimatized for a period of three weeks in the animal holding of the Department of Anatomy, Olabisi Onabanjo University.

Animals were fed with standard laboratory mouse chow purchased from Joyful feeds Sagamu, Ogun-State and were given water ad-libitum. At the end of the acclimatization period, the rats were randomly alligned into four groups (n=4 of control, treatment group; T1, T2 and T3.

2.3 Ethical Approval

Ethical considerations were ensured in consonance with the guiding rules and regulations for the use and care of rats, approval was obtained from the Ethical committee of Research of the Faculty of Basic Medical Sciences (FBMS) of Olabisi Onabanjo University.

2.4 Acclimatization of Animals

The rats were acclimatized for three weeks before the commencement of treatment. Their environment was daily cleaned to avoid any form of infection on the animals. Pelletized feeds from (Joyful feed Nig. Ltd) was given to the rats during acclimatization alongside, water was also given and libitum, with their beddings changed daily.

2.5 Collection and Authentication

Fresh leaves of *Bryophyllum pinnatum* were harvested from herbal centre, Ikenne, Ogun State, Nigeria, were authenticated in the pharmaceutical department of Olabisi Onabanjo university Sagamu.

2.6 Method of Extraction

2.6.1 Preparation of aqueous Extract of Bryophyllum pinnatum

Maximum quantity (2kg) of the fresh specimens of *Bryophyllum pinnatum* were wash free of soil and debris. The fresh leaves were blended and extracted. Weighed portion(600g) of the pulverized specimens were macerated with distilled water (1:4 at./vol.) For 72 hours at room

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temperature (26-28°C). The resulting solution was then filtered using a sieve with minimal filtering pores (0.25 mm). The distilled water then evaporated using steam water bath to give a percentage yield of 16.5% of the starting material.

2.6.2 Administration of Lead Acetate:

About 0.075g of lead acetate was dissolved in 20mls of distilled water to make a solution and were administered to the rat at 0.2mls per 100g of rat body weight.

2.6.3 Administration of Bryophyllum Pinnatum:

About 2g of *Bryophylum pinnatum* extract was dissolved in 20mls of distilled water and were administered to the rat at 2mls to the treatment groups.

2.6.4 Determination of their Body Weight:

The body weight of the rats was closely monitored with aid of a digital weighing balance. The record was used to analysed the state of the health as well as monitor the lead acetate induced and treatment with administered oral solution (extract of *Bryophyllum pinnatum*) on the body weight of the treated groups as well as the control group. Each rat in a group was weighed using digital weighing balance.

2.7 Experimental Design

Sixteen animals were randomly divided into four groups with each group consisting of four rats. The four groups of rats were subjected to the following oral treatments once a day for 21 days:

C (control) rats received normal feed and water

T1 rats received 2mls of Bryophyllum pinnatum extract

T2 rats receive 0.075g\kg of lead acetate

T3 rats received 2mls of *B. pinnatum* extract with 75mg/kg of lead acetate.

2.8 Animal Sacrifice and Organ Collection

At the end of the three weeks of the exposure, the animals were sacrificed via cervical dislocation. The brain was removed from the skull and the cerebellum was comfortably dissected out via stereotaxis coordinate method of Paxinos (2018)

2.9 Preparation of Tissues for Histological Examination

The brain tissues were processed for histological protocols at Tissue-Tek Specialist Diagnostics laboratory, Sagamu, Ogun State, Nigeria. As followings;

2.9.1 Fixation:

The tissues were fixed in 10% formal saline solution (from 0.85g of NaCl, 90ml of water, 10ml formaldehyde) for about 24 hours after which the process, dehydration was emleavesed upon.

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2.9.2 Dehydration:

The tissues were dehydrated in the following solutions at different stages; 60% alcohol, 70% alcohol, 80%, 90% alcohol, 100 % alcohol, first absolute alcohol and second absolute alcohol (all at 1 hour interval each).

2.9.3 Clearing:

Clearing was done using xylene (a hydrophilic clearing agent) to remove the alcohol from the prostate glandular tissue which was changed at 1hour interval first xylene and second xylene each.

2.9.4 Infiltration:

The tissues were infiltrated with paraffin wax at a temperature between the ranges of 50-60°c for I hour, the tissues were then embedded in paraffin wax with the proper orientation.

2.9.5 Embedding:

The tissues were then embedded in paraffin wax with the proper orientation. The embedding took place in a Lukat embedding mold coated with glycerol. The paraffin was allowed to solidify forming a visible scum before cooling with at a temperature of about 10-15°C. The block obtained appeared clear and contiguous. The block obtained, were formed into a cassette for sectioning

2.9.6 Sectioning:

The cassette containing the embedded tissue was mounted on the microtome. The microtome was Set to 5μ m thickness. During sectioning cubes of ice block were placed on the block (so as to reduce the heat generated). The first set of sections was discarded due to trimming of the blocks.

While sectioning, both thick and thins sections were gotten but only the thin sections were used. The thin sections were placed immediately in 5%% alcohol for 5 minutes later they were transferred into warm bath for 5 minutes (this ensures the sections spread out, in other to be visible when viewed under the microscope). Fresh slides were smeared with egg albumin. They were later dipped in the warm water to pick the sectioned tissues. The tissues haven glued on the slides were then dried on the hot plate.

2.10 H&E Staining Method and Protocol

2.10.1 Preparation of Harris Alum Haematoxylin:

The alum was dissolved in hot water; the haematoxylin (1g) powder was also dissolved in absolute alcohol (10 ml) and was added to the alum (20 g) solution, this was brought quickly to boil and mercury oxide (0.5 g) was added to the mixture. It was cooled rapidly under tap water.

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The addition of about 8.0ml of glacial acetic acid to the stain was recommended to sharpen nuclear staining and filtered before use. Staining time usually 5-10 minutes.

2.10.2 Preparation of Eosin: 2% solution:

About 350ml of 70% Acid alcohol was added to 1500ml of distilled water and 10g of Eosin powder was added, shaken and the stock solution was filtered before use.

Preparation of acid alcohol: About 99ml of 70% alcohol was mixed with 1ml of concentrated hydrochloric acid (HCL)

2.11 H&E Staining

The labeled slides were dewaxed in xylene for I5 minutes. Hydration was done in descending grades of alcohol (100%, 90%, 80%, 70%, 60%,50%). Slides were stained in Harris Hematoxylin solution for 5 minutes. Slides were rinsed in running tap water for few minutes. Penetration in 1 acid alcohol (differentiation solution) for one to two dips. Checking was done under the microscope for a satisfactory effect of the stain. Sides were rinsed in running tap water. Differentiation was repeated on some for best result, which were checked microscopically. Sides were then immersed in the bluing solution for I minute. Rinsing done in running tap water. Immersion of slides in 95% alcohol for 30 seconds. Sections were stained with Eosin Y solution for 30 seconds to 3 minutes. Rinsing done in running tap water done for 30 seconds. Stained sections were then dehydrated in 80% alcohol, 95% alcohol for 1 minute each and changed to 100%% alcohol for 3 minutes. Clearing was done in two changes of xylene for 5 minutes. Mounting done with mounting medium -DPX and then air dried. Result: nuclei stained blue black and cytoplasm pink.

2.12 Photomicrography

Image acquisition and analysis: a bright-field digital microscope (10-40x magnification objective used), was used to take the photomicrographs.

2.13 Statistical Analysis

For statistical analysis, data were analyzed by one-way analysis of variance (ANOVA) using SPSS (statistical package for social sciences) and GraphPad Prism (version 5) software. The results were expressed as mean standard deviation and Statistical significance was considered at a 95% confidence interval (P<0.05)

3. Results

3.1 Physical Observations

The group T2 and T3 were noticed to be having severe itching of the body throughout the period of administration. The group T1 and T3 were noticed to develop cystosis / boil at some parts of

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their body such as chest, back and legs but the group T3 shows a form of quick healing to this cystosis growth but were more chronic in the group T1.

Fig. 1 showing the bar chart of the weight analysis.

^{3.2} Histological Demonstrations

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Plate 1: Photomicrograph of cerebellum(control section C) showing the distinct cortical layers of ML(Molecular layer), PL (Purkinje layer)(Green Arrow); GL (Grannular layer) and WM (White matter)(White Mater) H&E stain x100.



Plate 2: Photomicrograph of cerebellum(control section C) showing the distinct cortical layers with normal cells of purkinje(Blue arrows), Golgi (Red arrows) and Stellate(Green arrow) respectively. *H&E stain* x400.

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Plate 3: Photomicrograph of cerebellum(Treatment section T1) showing the distinct cortical layers of ML(Molecular layer), PL (Purkinje layer)(Green Arrow); GL (Grannular layer) and WM (White matter)(White Mater) *H&E stain* x100.



Plate 4: Photomicrograph of cerebellum(Treatment section T1) showing the distinct cortical layers with distorted and scanty cells of purkinje(Blue arrows), Golgi (Red arrows)and Stellate(Green arrow) respectively. *H&E stain* x400.

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Plate 5: Photomicrograph of cerebellum(Treatment section T2) showing the distinct cortical layers of **ML**(Molecular layer), **PL** (Purkinje layer)(Green Arrow); **GL** (Grannular layer) and **WM** (White Mater) *H&E stain* x100.



Plate 6: Photomicrograph of cerebellum(Treatment section T2) showing the distinct cortical layers with normal cells of purkinje(Blue arrows), Golgi (Red arrows) and Stellate(Green arrow) respectively. *H&E stain* x400.

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Plate 7: Photomicrograph of cerebellum(Treatment section T3) showing the distinct cortical layers of **ML**(Molecular layer), **PL** (Purkinje layer)(Green Arrow); **GL** (Grannular layer) and **WM** (White matter)(White Mater) *H&E stain* x100.



Plate 8: Photomicrograph of cerebellum(Treatment section T3) showing the distinct cortical layers with scanty distorted (Blue arrows) and normal (Brown arrows) purkinje cells with Golgi (White arrows) and Stellate(Green arrow) cells respectively. *H&E stain* x400.

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4. Discussion

The neurotoxic effects of high-level lead exposure has been proved in both animals and human, where the damage involves peripheral and central nervous systems (Brenet, 2006; Usiobeigbe *et al.*, 2024). Many factors account for the neurotoxic effects of lead; they include integrity of blood-brain barrier, lead-binding proteins, cellular scavengers (e.g. glutathione) and interactions with other micro-nutrients (Sidhu & Nehru, 2004).

In the present study, lead induced damage and disorganization in Purkinje cells was evident. By light microscope they were shrunken with distorted shape and their nuclei appear irregular. Ultrastructural examination proved the Purkinje cells damage in the form of irregular euchromatic nucleus, ratified cytoplasm, the mitochondria are small dense with destroyed or dilated cristae and the cells are surrounded by empty spaces. Alterations of Granular cells in the form of increased condensation of nuclear chromatin, the nuclei are surrounded by a shell of vacuolated cytoplasm, and the mitochondria appeared with destroyed cristae were also detected. These findings are in agreement with (Engin, 2006), who reported that when rats received lead acetate in their drinking water for 60 days degeneration in the neuron cells was evident. The histological findings were also similar to those reported by Villeda Hernandez et al., (2006), Macauley et al., (2008), Amal and Mona (2009), Sohair et al. (2010), Musa et al. (2012) and Fakunle et al. (2013). Changes in neuronal cells observed in this study could be explained by the generation of reactive oxygen species, and depletion of antioxidant reserves. Lead exposure inactivates glutathione molecule (important endogenous antioxidant) by binding of its sulfhydryl group directly (Pajović et al., 2003) and (Sanders et al., 2009). Zhu et al. (2006) and Mattson et al. (2008) reported that Mitochondrial dysfunction and distortion have been recorded in many neurodegenerative diseases which associated with oxidative damage.

On the other hand, the animals treated with Pb and *B. pinnatum* revealed marked improvement in the altered histological architecture of cerebellar cortex, whereby light microscope the purkinje, molecular and the granular cell layers appeared almost normal, while by electron microscope the purkinje cells appeared with euchromatic nucleus, normal prominent nucleolus, strands of RER in the cytoplasm and some dilated Golgi cisternae around nucleus.

There was an increase in the body weight of animals at Group C and T1 as the weeks increases in both male and female rats but a diminishing decrease in the Group T1who are exposed to lead only. Group T3 has a fluctuation weight as the weigh first decrease at the first week of administration of lead and *B. pinnatum* but increases towards the third week of treatment with both lead and *B. pinnatum* showing the ameliorative effect of *B. pinnatum* on the rats as seen.

5. Conclusion

Administration of Pb to the studied animals has led to morphological alteration in the neuronal cells in the cerebellar cortex, while the co-administration of Pb and *B. pinnatum* resulted in amelioration of these effects to a great extent due to its anti-oxidant activity. We recommend

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more investigations on the neurotoxic effects of lead on laboratory animals and human, with regular administration of *B. pinnatum* where lead exposure could not be avoided.

Conflict of Interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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