EXPLORING THE EFFECTS OF *BYROPHYLLUM PINNATUM* EXTRACT ON THE CEREBRUM OF ADULT WISTAR RATS

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BEING A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF ANATOMY, SCHOOL OF BASIC MEDICAL SCIENCES, UNIVERSITY OF BENIN, BENIN CITY.

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DEDICATION

I dedicate this project to God Almighty, who has been my rock and guiding light throughout this journey. I also extend my deepest gratitude to my parents, Elder Matthew Apologun and Mrs. Rachael Apologun whose love, support and sacrifices have enabled me to achieve this milestone.

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ABSTRACT

Herbal extracts which can serve as medicinal plants have been used since ancient times and even considered the source of modern medicine. *Bryphullum pinnatum* extracts have been shown to possess neuroprotective properties potentially useful in treating neurodegenerative diseases. *Bryophyllum pinnatum* is a plant used to treat inflammations, infections, and also have anti cancer properties. This study was carried out to explore the effects of *Bryophyllum pinnatum* extract on the cerebrum of Adult Wistar rats.A total of thirty (30) rats weighing between 140g-200g were randomly assigned into six (6) groups (A,B,C,D,E and F),with five rats per group. Group A was control group while Group B,C,D,E and F were administered with *Bryophyllum pinnatum* extract in doses of 200,400,600,800 and 1000mg/kg respectively.The rats were acclimatized for a period of two weeks and administered for a period of four weeks using oral route by the use of orogastric tube. The rats were anesthesized with chloroform and then sacrificed. The cerebrum was harvested and immediately fixed for antioxidant stress test and for tissue processing. H&E stains were used for histological test. The result of the study shows that there was no statistically significant difference ($P<0.05$) in the final body weight of rats in entire group compared to it's initial body weigh except group B where there is a significant change in weight .There was no statistically significant difference ($P<0.05$) in cerebrum weight and organo-somatic index. In the chart showing the antioxidant results, it shows that it is not statistically significant. Histological slides in control group show: molecular, external granular, external pyramidal, internal granular, internal pyramidal and multi-form layers. Group B show: vasodilatation normal pyramidal neurons with conspicuous nucleoles oligodendrocytes and neuropil. Group C show: normal neurons with conspicuous nucleoles, oligodendrocytes, neuropril cerebral vasodilatation. Group D show: normal granular cell neurons with conspicuous nucleoli, normal oligodendrocytes and marked vasodilatation and active congestion. Group E and F marked show vasodilatation and active congestion, normal granular cells with conspicuous nucleolus and oligodendrocytes. In conclusion, graded concentration of *Bryophyllum pinnatum* induced vasogenic effects and increased protein synthesis in a dose dependent fashion and it had no adverse effect on it.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND STUDY

Herbal extracts which can serve as medicinal plants have been used since ancient times and even considered the source of modern medicine (Gurib *et al.,* 2006).

Bryophyllum pinnatum extracts have been shown to possess neuroprotective properties potentially useful in treating neurodegenerative diseases (Ogidigo *et al.,* 2022).

Another study investigated the effects of *Bryophyllum pinnatum* on cognitive function in rats and found improved memory and learning performance (Bakre *et al.,* 2022).

The cerebrum is the largest part of the brain, responsible for processing sensory information, controlling movement, and facilitating thought, emotion, and memory (Cotteril *et al .,* 2001). The cerebrum is divided into two hemispheres (left and right), connected by the corpus callosum (Witelson *et al.,* 1985). Each hemisphere is further divided into four lobes (frontal, parietal, temporal, and occipital), each with distinct functions (Donnelly *et al.,* 2011).The cerebrum plays a critical role in processing sensory information from the environment, including visual, auditory, tactile, olfactory, and gustatory inputs (Thesen *et al .,* 2004). It also coordinates voluntary movements and regulates reflexes (Waterhouse *et al.,* 2014). Higher-level cognitive functions, such as thought, emotion, and memory, are also mediated by the cerebrum (Drevets *et al.,* 1998).

1.2 AIM OF THE STUDY

The aim of this study is to explore effects of extract *Bryophyllum pinnatum* on the cerebrum of Adult Wistar rats.

1.3 SPECIFIC OBJECTIVES OF THE STUDY

The specific objectives of this study includes exploring the effects of *Bryophyllum pinnatum* extract on;

- Body weight of experimental animals across all groups.
- Weight of the cerebrum of experimental animals across all groups.
- Organo-somatic index of experimental animals across all groups.
- Oxidative stress and Lipid peroxidation of experimental animals across all groups.
- Histology of the cerebrum of experimental animals across all groups.

1.4 SIGNIFICANCE OF THE STUDY

By exploring medicinal plants, we can unlock the potential to justify human health, converse biodiversity, support sustainable development. Medicinal plants are considered an important source of human health because of their therapeutic capabilities in treating various diseases. Sometimes during research, the organ is damaged by inducing a chemical and later the extract of the plant of study is administered to cure the damaged organ, but it's not so in this case.

The significance of this study is to explore the effects of *Bryophyllum pinnatum* extract on the cerebrum. This study will indicate if it's toxic or beneficial to the organ. If it's toxic, this study would monitor the dosage at which the extract is administered that makes it toxic.

1.5 JUSTIFICATION OF STUDY

Bryophyllum pinnatum, a plant widely utilized in traditional medicine, has demonstrated a variety of biological activities, including anti-inflammatory, antioxidant, and neuroprotective effects (Sharma *et al.,* 2024). The cerebrum, as the most intricate and sensitive part of the brain, is particularly vulnerable to damage from factors such as oxidative stress, inflammation, and neurodegeneration (Leszek *et al.,* 2016).

Natural products derived from plants, including *Bryophyllum pinnatum,* have recently gained attention for their potential therapeutic effects on neurodegenerative diseases (Ogidigo et al*.,* 2022). Traditionally, *Bryophyllum pinnatum* has been used to treat conditions such as epilepsy, anxiety, and insomnia (Elufioye *et al.,* 2022).

However, despite its widespread traditional use, scientific research on the effects of *Bryophyllum pinnatum* on the cerebrum, particularly in adult Wistar rats, remains limited. This study aims to explore the neuroprotective and antioxidant properties of *Bryophyllum pinnatum,* focusing on its potential effects on the cerebrum of adult Wistar rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 PLANT OF STUDY (*Bryophyllum pinnatum*)

Figure 1: *Bryophyllum pinnatum*(Nagaratna *et al.,* 2015).

Bryophyllum pinnatum comes from the Greek word "*Byron*" meaning "sprout" and "*Phyllon*" meaning "*leaf*" which is a group of plant species in the Crassulaceae family.

Bryophyllum pinnatum, a succulent plant native to Madagascar and tropical Africa, has a rich history of traditional use and modern research. (Füre *et al.,* 2016).

The plant has been used in folk medicine for centuries to treat various ailments, including fever, rheumatism, and respiratory issues. (Dailah *et al.,* 2022).

Bryophyllum pinnatum was introduced to India and Southeast Asia by African traders and travelers and to the Caribbean and Central America by European colonizers in the 19th century (Berry *et al.,* 1999).

Recent studies have confirmed the plant's antioxidant, anti-inflammatory, and antimicrobial properties (Zhang *et al.,* 2011). It's popularly called Miracle leaf, life plant , resurrection plant.

2.1.1 TAXONOMY OF *Bryophyllum pinnatum*

Kingdom: Plantae

Division: Spermatophyta

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Rosales

Family: Crassulaceae

Genus: *Bryophyllum*

Species: *Bryophyllum pinnatum*

2.1.2 BOTANICAL DESCRIPTION

Bryophyllum pinnatum is a perennial herbaceous plant that belongs to the family Crassulaceae.It is characterized by its thick,fleshy leaves and stems which are adapted for water storage(Dilip *et al .,* 2024) **.** The leaves have a waxy coating surface with green colour and reddish-brown tints which is 5-15cm long,2-5cm wide with its margins serrated.Its inflorescence is terminal cyme and the pendulous bell shaped flowers about 10cm long are arranged in clusters of branches at the end of the stems.The fruits are in form of follicles of about 5-10mm long with its ellipsoidal seeds about 1-2mm long.

Figure 2: *Bryophyllum pinnatum* plant (Ingole R, *et al.,* 2020) (A) Leaves, (B) Inflorescence

2.1.3 ORIGIN AND DISTRIBUTION

Bryophyllum pinnatum, commonly known as the "*life plant"* or "*miracle leaf*," is native to Madagascar. The plant belongs to the Crassulaceae family, which includes other succulents. Its origin in Madagascar is significant because the unique climatic conditions there, particularly the island's arid and semi-arid zones, have influenced the development of such succulent plants that can store water in their leaves(Hannan *et al*. 2002).Although *Bryophyllum pinnatum* is native to Madagascar, it has since spread to various tropical and subtropical regions of the world. Due to its hardiness and water-storing capabilities, it has become widespread in places with warm climates, such as tropical Asia, Africa, the Caribbean, and parts of South America.Bryophyllum pinnatum thrives in regions that experience mild temperatures and regular rainfall, but it can also survive in arid conditions. The plant has been introduced and naturalized in various parts of the world, especially in the tropical and subtropical zones of Africa, Asia, and the Caribbean. Its ability to propagate through leaf cuttings has facilitated its spread (Deb, D,*et al.,* 2005). In some regions, *Bryophyllum pinnatum* is considered invasive due to its rapid growth and ability to form dense colonies, especially in areas with a favorable climate. This has led to its spread in non-native areas such as the Pacific Islands and parts of Southeast Asia . It is used in traditional medicine for a variety of ailments and also known for its wound healing and hemostatic properties.

2.1.4 PHYTOCHEMICAL ANALYSIS

Phytochemical analysis of *Bryophyllum pinnatum* involves identifying and characterizing the active chemical constituents present in the plant, which are often linked to its pharmacological activities. Several studies have explored its phytochemical properties, and these studies reveal a variety of bioactive compounds in the plant. Below is a detailed overview of the phytochemical analysis with references for each group of compounds.

Alkaloids

Bryophyllum pinnatum contains alkaloids such as bryophylline and other nitrogen-containing compounds that have shown potential pharmacological effects, including anti-inflammatory and antimicrobial properties. Alkaloids are one of the primary bioactive groups found in this plant (Selvakuma *et al.,* 2022).

Flavonoids

Flavonoids are another major class of compounds in *Bryophyllum pinnatum*. These include quercetin, kaempferol, and other polyphenolic compounds. Flavonoids are known for their antioxidant, anti-inflammatory, and anticancer activities. (Elufioye *et al.,* 2022).

Tannins

Tannins are polyphenolic compounds that have been shown to possess astringent, antimicrobial, and antioxidant properties. Tannins in *Bryophyllum pinnatum* contribute to the plant's wound-healing abilities and general therapeutic effects (Kamboj *et al.,* 2009)

Saponins

Saponins are glycosides that have been reported to have immune-boosting and cholesterol-lowering effects. They are also known for their antimicrobial and antifungal activities. *Bryophyllum pinnatum* contains saponins that are likely responsible for some of its therapeutic effects. (Jolly *et al.,* 2024).

Phenolic compounds

Phenolic compounds are abundant in *Bryophyllum pinnatum* and play a major role in its antioxidant activity. They also have anti-inflammatory, anticancer, and antimicrobial properties(Daniel *et al.,* 2020).

Glycosides

Glycosides in *Bryophyllum pinnatum* are linked to the plant's anti-inflammatory and antimicrobial activities. These compounds consist of a sugar part and an aglycone part (the non-sugar component) that contribute to the plant's pharmacological properties. (Das *et al* 2024).

Steroids

Steroids, particularly steroidal saponins, are also present in *Bryophyllum pinnatum*. These compounds have shown anti-inflammatory, antidiabetic, and anticancer properties in various studies. (Kamboj *et al.,* 2009).

Organic Acids

Organic acids such as oxalates are also found in *Bryophyllum pinnatum*.These compounds can have physiological effects and contribute to the plant's medicinal properties, including antimicrobial actions.(Bashir *et al.,* 2023).

Bryophyllum pinnatum has a rich array of bioactive compounds, including alkaloids, flavonoids, saponins, tannins, phenolics, glycosides, and others. These phytochemicals contribute to its wide range of pharmacological activities, such as antimicrobial, anti-inflammatory, and antioxidant effects.

Methods used for bioactive compound extraction

Scientists commonly use dried plant powders to extract bioactive compound while eliminating interference from water.The solvent used to extract bio molecules is selected based on the polarity of the solute of interest. Solvent with similar polarity to the solute will desolve the solute appropriately.

The polarity from least to most common;

Hexane > Chloroform> Ethyl > acetate > Acetone > Methanol Water.

Two extraction method were used;

Microwave assisted extraction

Ultrasound assisted extraction

Various spectroscopic techniques such as UV - Visible, Infrared and mass spectroscopy, were used to identify purified materials.

Purification

Numerous bioactive compounds have been extracted and refined using paper chromatography, thin-layer chromatography, column chromatography, and pure-layer chromatography. These techniques employ silica, aluminum, cellulose, and polyamine, which are highly effective in separating phytochemicals. Since plant materials contain a vast array of complex phytochemicals, achieving proper separation can be challenging. Thus, utilizing multiple mobile phases to enhance polarity aids in achieving superior separations.

Thin-layer chromatography was utilized to examine the composition of compounds through column chromatography. Both silica gel column chromatography and thin-layer chromatography were employed to isolate bioactive molecules (Kumar *et al .,* 2013)

Determination of Isolate

Various spectroscopic techniques, including ultraviolet (UV)-Visible, infrared, magnetic resonance, and mass spectrometry, were used for analysis. Spectroscopy operates on the fundamental principle of transmitting electromagnetic radiation through organic molecules, which selectively absorb certain wavelengths. By measuring this absorption, a spectrum is produced, which is distinctive to the molecular structure and can identify specific chemical bonds. Spectra derived from different techniques, including UV, Visible, radar, and electron beam analysis, were assessed for structural elucidation using nuclear magnetic resonance and mass spectrometry.Plants serve as sources of antioxidants, which can be categorized as either natural or synthetic.

2.1.5 MEDICINAL AND THERAPEUTIC BENEFITS Of bryophyllum *pinnatum*

Bryophyllum pinnatum is well-regarded in traditional medicine and has been utilized to manage various health conditions, including rheumatism, cough, asthma, tonsillitis, diarrhea, body pain, arthritis, heartburn, skin and peptic ulcers, diabetes mellitus, and microbial infections. Additionally, it is effective in treating boils and insect bites. In Nigeria, this plant is particularly valued for its wound-healing properties. When heated, the leaves of *Bryophyllum pinnatum* are applied to tumors and abscesses in Jamaica. The plant is also known for its blood-purifying abilities and is widely recognized as a natural remedy for kidney stones (Taran *et al.,* 2025).

Tea prepared from the leaves and stems of *Bryophyllum pinnatum* is consumed to relieve menstrual cramps. Furthermore, the plant helps cleanse the bladder and eliminate harmful toxins from the intestines (Pal *et al.,* 2020).

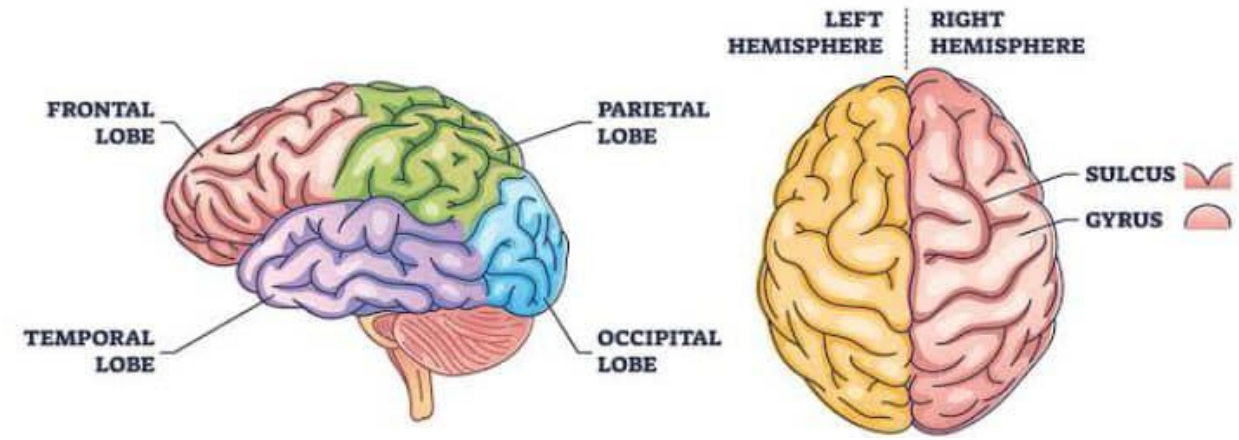
Toxicity of Bryophyllum *pinnatum*

Commonly referred to as the "life plant," *Bryophyllum pinnatum* is a succulent perennial species containing bufadienolide cardiac glycosides and phenanthrene, which can induce cardiotoxicity in grazing animals (Fürer *et al.,* 2016).

Also known as the "Mother of Millions," this plant is toxic to livestock if ingested. The harmful effects are mainly attributed to bufadienolides, which can lead to heart failure. Toxins are present in all parts of the plant, with the flowers being approximately five times more toxic than the leaves and stems.

2.2 ORGAN OF STUDY

2.2.1 GROSS ANATOMY OF THE CEREBRUM



Lateral view **Superior view**

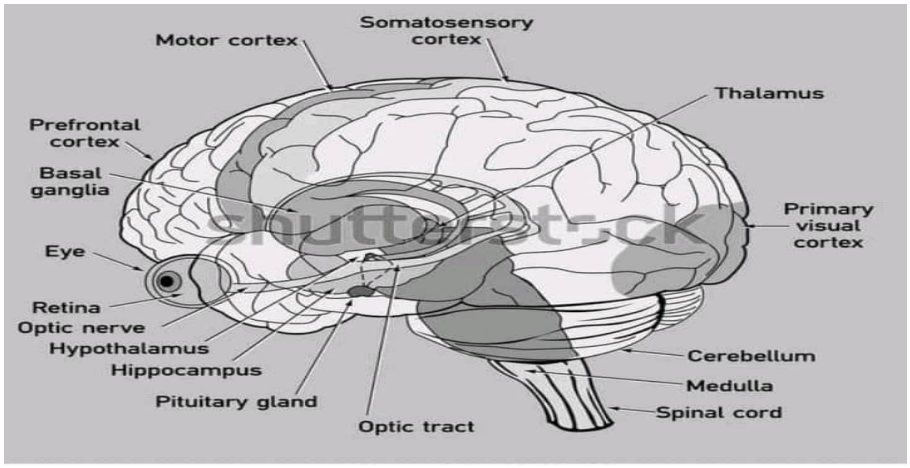


Figure 3: Anatomy of the cerebrum

The cerebrum, the brain's largest and most intricate structure, governs a variety of functions such as sensory interpretation, movement coordination, problem-solving, emotional regulation, and higher cognitive processes. It consists of two hemispheres left and right each subdivided into specialized regions.

The cerebrum's exterior is marked by gyri (elevated folds) and sulci (depressions), which expand its surface area to accommodate a higher density of neurons. Prominent surface divisions include the frontal, parietal, temporal, and occipital lobes, each linked to specific

roles in sensory integration, motor activity, and advanced cognition. The cerebral cortex, its outermost layer, manages sophisticated tasks like perception, decision-making, and conscious movement (Rolls et *al.,* 2016).

The two cerebral hemispheres govern opposite sides of the body. The left hemisphere dominates language, logic, and analytical tasks, whereas the right hemisphere specializes in spatial reasoning, creativity, and emotional interpretation. Communication between the hemispheres occurs via the corpus callosum, a fibrous bundle bridging the two (Harris *et al.,* 2020).

Key Cerebral Lobes:

Frontal Lobe: Situated at the brain's front, it oversees voluntary movement, complex cognition (e.g*.,* decision-making, problem-solving), and emotional control. It also contains Broca's area, critical for speech generation.

Parietal Lobe: Positioned posterior to the frontal lobe, it integrates sensory data (touch, temperature, pain) and supports spatial navigation and motor coordination.

Temporal Lobe: Located laterally, it processes sound, aids memory formation, and houses Wernicke's area, vital for language understanding.

Occipital Lobe: At the brain's rear, it primarily manages visual input interpretation.

Functional Regions:

Primary Motor Cortex: In the frontal lobe's precentral gyrus, it directs voluntary muscle activation.

Primary Somatosensory Cortex: Within the parietal lobe's postcentral gyrus, it deciphers tactile, pain, and temperature signals.

Broca's Area: Typically in the left frontal lobe, it enables speech articulation.

Wernicke's Area: Often in the left temporal lobe, it underpins language comprehension.

Beneath the cortex, subcortical structures like the basal ganglia (movement regulation), thalamus (sensory relay), and hypothalamus (homeostasis and autonomic control) play critical roles (Herrero *et al.,* 2002).

Blood reaches the cerebrum via the internal carotid and vertebral arteries. The Circle of Willis, a vascular network at the brain's base, ensures redundant blood flow to cerebral regions (Vrselja *et al.,* 2014).

The cerebrum comprises gray matter (neuron-rich cortex) and white matter (myelinated axons connecting brain regions for rapid signaling). The ventricles four fluid-filled cavities produce and circulate cerebrospinal fluid (CSF), which cushions the brain, removes waste, and transports nutrients (Rasmussen et al*.,* 2022).

2.2.2 EMBRYOLOGY OF THE CEREBRUM

During the 3rd–4th week of gestation, the neural plate emerges and folds inward to form the neural tube. By day 25, closure of the anterior (rostral) neuropore establishes the prosencephalon (forebrain), the precursor to the cerebrum.By the 5th week, the prosencephalon subdivides into Telencephalon which develops into the cerebral hemispheres (including the cortex, basal ganglia, and white matter)and diencephalon which gives rise to the thalamus, hypothalamus, and epithalamus.The telencephalon's lateral growth forms the two cerebral hemispheres, while cavities within them evolve into the lateral ventricles.

Cortical Organization

-Neurogenesis (Weeks 8–20): Neural stem cells produce neurons and glial cells.

- Neuronal Migration (Weeks 12–24): Neurons travel along radial glial fibers to assemble the cortex's layered structure (inside-out pattern).

- Gyrification (Weeks 24–38): The initially smooth cortical surface folds into gyri and sulci, maximizing surface area.

- Synaptogenesis & Myelination (Late gestation through childhood): Synaptic networks develop, and white matter tracts mature.

After birth, synaptic pruning streamlines neural pathways, while myelination persists into adulthood, particularly in regions governing complex cognition.

2.2.3 HISTOLOGY OF THE CEREBRUM

The cerebrum, the largest and most complex region of the human brain, serves as the epicenter of sensory integration, motor coordination, and higher cognitive functions. Its intricate cellular architecture, studied through the lens of histology, reveals a meticulously organized structure divided into distinct regions and layers, each contributing uniquely to its overall function. At the core of this organization lies the interplay between neurons, glial cells, and vascular networks, all working in concert to sustain the brain's dynamic activities.

The cerebral cortex, a folded mantle of gray matter, forms the outermost layer of the cerebrum. Composed of six histologically distinct layers, the cortex is a mosaic of neuronal diversity. The outermost molecular layer, sparsely populated by neurons, is a dense neuropil of interwoven dendrites and axons that facilitates synaptic communication. Beneath this layer lies the external granular layer, dominated by small pyramidal neurons and stellate cells, which are inhibitory interneurons critical for modulating local cortical activity. Progressing

inward, the external pyramidal layer houses medium-sized pyramidal neurons that establish connections with other cortical regions, enabling complex intracortical communication.

The internal granular layer, a hallmark of sensory cortical areas, is densely packed with stellate cell.Deeper still, the internal pyramidal layer contains large pyramidal neurons, such as the Betz cells of the motor cortex, whose long axons project to the spinal cord and brainstem, orchestrating voluntary movement. Beneath the cortex lies the white matter, a vast network of myelinated axons . These axons form three functional tracts: commissural fibers, which unite the hemispheres through structures like the corpus callosum; projection fibers, which link the cortex to distant regions such as the spinal cord; and association fibers, which interconnect cortical areas within the same hemisphere.

Supporting this neuronal network are glial cells, the unsung heroes of cerebral histology. Astrocytes, with their star-shaped morphology, regulate the blood-brain barrier, maintain ion balance, and provide metabolic support to neurons. Oligodendrocytes ensheathe axons in myelin, accelerating signal transmission, while microglia act as vigilant immune sentinels, pruning damaged neurons and responding to inflammation. Together, these cells create a stable microenvironment crucial for neuronal survival and function.

The cerebrum’s vitality depends on a rich vascular supply delivered by the internal carotid and vertebral arteries. These vessels give rise to a capillary network lined with endothelial cells bound by tight junctions, forming the blood-brain barrier. This selective barrier, reinforced by astrocytic endfeet, shields the brain from toxins while permitting the passage of essential nutrients. Its integrity is vital for maintaining neural homeostasis and preventing neurotoxic insults.

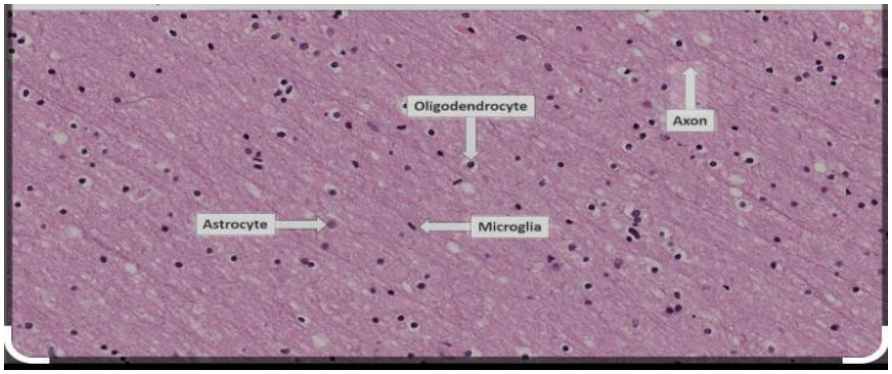


Figure 4: Histology of the cerebrum

2.2.4 FUNCTIONS OF THE CEREBRUM

- Perception and interpretation of sensory information (touch, sight, sound, taste, and smell).
- Coordination and execution of voluntary muscle movements.
- Thinking, reasoning, problem-solving, and memory.
- Regulation of emotions and behaviors.
- Processing of language, including speech production and comprehension.
- Encoding, storage, and recall of information and experiences.
- Maintenance of consciousness, self-awareness, and integration of sensory information.
- Coordination of sensory input and motor output for adaptive responses.

2.2.5 BLOOD SUPPLY TO THE CEREBRUM

The cerebrum, which governs various vital functions including sensory interpretation, motor coordination, and cognitive processes, depends on an intricate and effective vascular network to operate optimally. This blood supply is predominantly delivered by two primary arterial pathways: the internal carotid arteries and the vertebral arteries, which converge to create the circle of Willis at the brain's base.

The internal carotid arteries, originating from the common carotid arteries, deliver blood to the anterior and central regions of the cerebrum. These vessels branch into the anterior cerebral artery (ACA), chiefly irrigating the frontal lobes and portions of the parietal lobes, and the middle cerebral artery (MCA), which nourishes the lateral areas of the cerebral hemispheres. These regions encompass structures essential for motor and sensory activities, as well as language comprehension.

The vertebral arteries, emerging from the subclavian arteries, travel upward through the spinal column before joining to form the basilar artery. This artery feeds the brainstem and posterior cerebral areas. Extending from the basilar artery, the posterior cerebral arteries (PCA) distribute blood to the occipital lobes (critical for visual interpretation) and sections of the temporal lobes.

The circle of Willis, a crucial arterial structure, interlinks the internal carotid and vertebral systems, guaranteeing uninterrupted blood flow to the cerebrum. This anatomical arrangement establishes alternative pathways for circulation if an artery is obstructed, thereby safeguarding against ischemic injury.

Furthermore, the cerebrum's venous drainage comprises a system of veins that drain into the dural venous sinuses. These sinuses eventually transport deoxygenated blood back to the cardiovascular system via the internal jugular veins (Agarwal *et al.,* 2021).

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

Animals: 30 Adult Wistar Rats

Extract: Aqueous extract of *Bryophyllum pinnatum*

Feed:Growers Mash

Instruments: Cotton wool, disposable gloves, specimen bottles,forceps, surgical blade,orogastric tube,5ml syringe, plastic cages, masking tape, weighing balance,microtome,slide tray, tissue embedding station, microscope.

Reagent: 10% formal saline, chloroform, distilled water,eoisin, hematoxylin, paraffin wax, xylene.

3.2 EXPERIMENTAL ANIMALS

Thirty (30) Adult Wistar rats weighing 140-200g were used as experimental animals in this study.The animals were purchased and maintained at the animal house of the Department of Anatomy, University of Benin, Benin city. The cages used to house the rats for the experiment were cleaned and disinfected before the rats were transferred. The rats were allowed to acclimatize for a period of 2 weeks in their cages ;they were fed with livestock growers mash manufactured by Bendel Feed and Flour Mills Limited, Ewu, Edo State , Nigeria.

Acclimatization and the experimental period lasted for a total of 6 weeks (2 weeks of acclimatization and 4 weeks of experimental period).The cages were made of plastic and wire gauzed at the top to allow proper ventilation. The cages were cleaned after 4 days and their beddings changed at intervals.

3.3 PROCUREMENT AND PREPARATION OF PLANT EXTRACT

Procurement of plant

The leaves of *Bryophyllum pinnatum* were harvested in Uhunmwonde LGA, Ehor, Edo State.

Preparation of plant extract

A stainless steel tubular filter with high filtration precision was used. After maceration, extraction was performed using basic extraction and fractionation procedures to determine the quality and quantity of bioactive compounds. Methanol was used for solvent extraction.

Purification of phytochemicals was achieved using various chromatographic techniques such as paper chromatography and tin layer chromatography. Finally, UV spectroscopy was used to characterise the compound. The plant were collected accurately and timely, and there authenticity was confirmed true expert drying and proper grinding.

Bioactive compounds were isolated through extraction and fractionation and there quantity and quality was determined. This various test were used to identify bioactive substances.

1. Dragenforf test for alkalodis and wagners test.

2 Bontragers for glycosides.

3. Lieberman burcherdic's test for steroids and triterpenoids.

4. Gold becter's test for tannins.

5. Biuret test for protein.

6. Ferric chloride test for phenolic compounds.

7. Shynodas test flavonoids.

The various separation techniques used included fractional distillation, fractionational crystalization, fractional liberation and sublimation. Separation was performed using separate funnels and appropriate reagents.

3.4 EXPERIMENTAL DESIGN

In this study, thirty (30) Adult Wistar rats were divided into six groups: A, B, C, D, E, F, with five rats in each group. The experimental period lasted for 6weeks .The rats were administered aqueous *Bryophyllum pinnatum* as shown in a table below:

Table 1: Showing the experimental design of the study.

GROUPS	DOSAGE
GROUP A	Control
GROUP B	200mg/kg of *Bryophyllum pinnatum*
GROUP C	400mg/kg of *Bryophyllum pinnatum*
GROUP D	600mg/kg of *Bryophyllum pinnatum*
GROUP E	800mg/kg of *Bryophyllum pinnatum*
GROUP F	1000mg/kg of *Bryophyllum pinnatum*

3.5 METHODS OF SACRIFICE AND TISSUE COLLECTION

At the end of the experimental period, the final weight of the rats were taken using compact electric weighing scale calibrated in grams. Cotton wool was soaked in chloroform of about 50ml in an enclosed container and the rat was placed in the enclosed container containing the chloroform for about 30-60 sec to anaesthetize it. After anaesthetization, the rat was placed in a supine position on the dissection table to be dissected. After that the cerebrum was harvested, weighed and fixed in 10% formal saline in a universal bottle for histological analysis.

3.6 HISTOLOGICAL TECHNIQUE

1. The cerebrum was usually preserved using fixatives like formaldehyde or paraformaldehyde. Fixation stabilizes the tissue and prevents degradation.

2. The fixed tissue is then dehydrated using increasing concentrations of alcohol (70%, 90%, 96% and absolute alcohol 100%) to remove water.

3. After dehydration, the tissue is treated with a clearing agent like xylene to make it transparent, allowing for better infiltration of embedding materials.

4. The cleared tissue is embedded in a solid medium, usually paraffin wax, which provides support for thin slicing.

5. Once the tissue is embedded, it is sliced into very thin sections using a microtome. Typical section thickness is around 5 to 10 micrometers. These thin sections are crucial for allowing light to pass through during microscopic examination.

6. After sectioning,the tissues came out in ribbons and were placed in 20% alcohol for spreading of the tissue and it was allowed to float in a water bath at a temperature of 30°c.

7. The sectioned tissue was picked using a microscope slide from the water bath and placed on the hotplate to melt excess wax and dry the tissue on the slide.

8. The sectioned tissues were placed in xylene for 5 minutes to remove paraffin wax from the tissues.

9. Hydration was carried out by passing the tissues through descending grades of alcohol (100%, 96%, 90%, and 70%) and water for 5 minutes each.

10. Staining was done using hematoxylin and eosin dyes.The tissues were stained in hematoxylin for 10 minutes and rinsed in water.After that,they were differentiated in 1% acid-alcohol briefly to develop colour.

11. They were subsequently counterstained with eosin and rinsed in 90% alcohol. Dehydration was done in 90% alcohol and two changes of absolute alcohol at 5 minutes each.

12. The sections were thereafter cleared in xylene for 5 minutes.

13. Finally, a coverslip is applied to the medium usually with a mounting medium to protect the tissues and allow for microscopic examination.

3.7 STATISTICAL ANALYSIS

Results obtained was expressed in mean ± standard error of mean (SEM). Difference among the mean were determined by one way ANOVA. Values were considered to be statistically significant if P value were less than 0.05. ($P<0.05$). LSD post Hoc test was used to determine where the significance lay. Statistical package Graph pad prism version 9.0 for Windows was used to analyze the data obtained in this study.

CHAPTER FOUR

RESULT

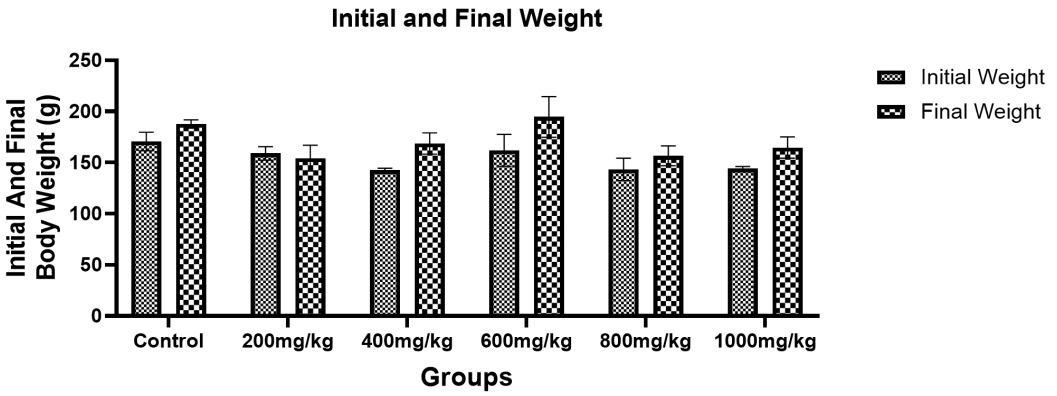


Chart 1: Initial and Final weight after administration Values are given as mean ± SEM.

There was change in the final body weight compared to the initial body weight but it is not statistically significant across the groups when compared with the control group.

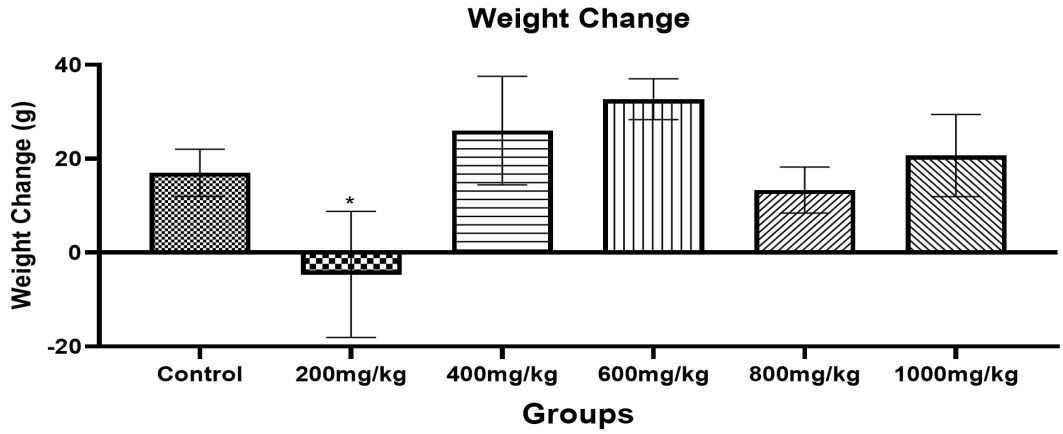


Chart 2: weight change after administration Values are given as mean ± SEM. $p< 0.05$ compared with the control group. There was a significance decrease in cerebrum weight change across group B when compared with control.

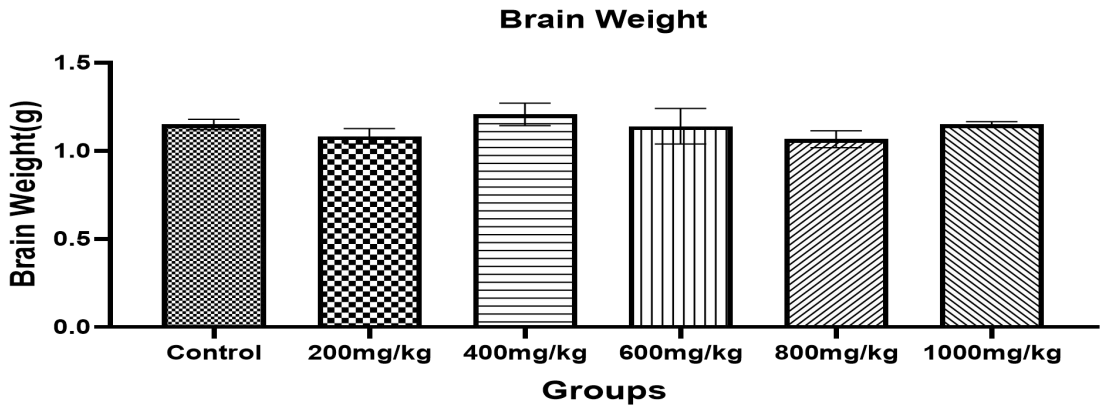


Chart 3: Cerebrum weight after administration Values are given as mean ± SEM.

There was no statistically significant difference (P<0.05) in cerebrum weight for rats across the group when compared with control.

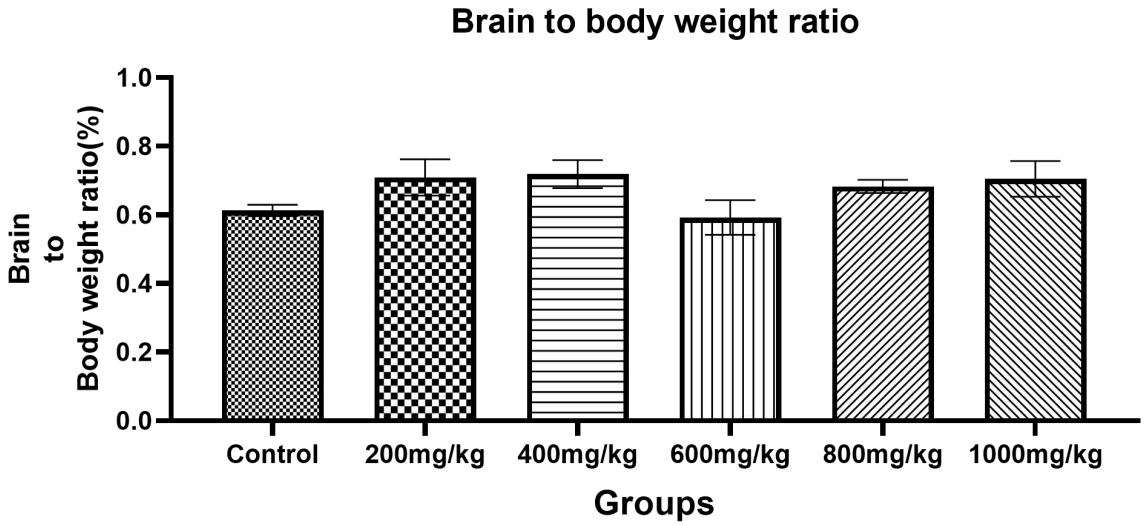


Chart 4: Brain to Body weight ratio after administration Values are given as mean ± SEM. There was no statistically significant difference (P<0.05) in cerebrum Organo-somatic index for rats treated across each group compared to the control.

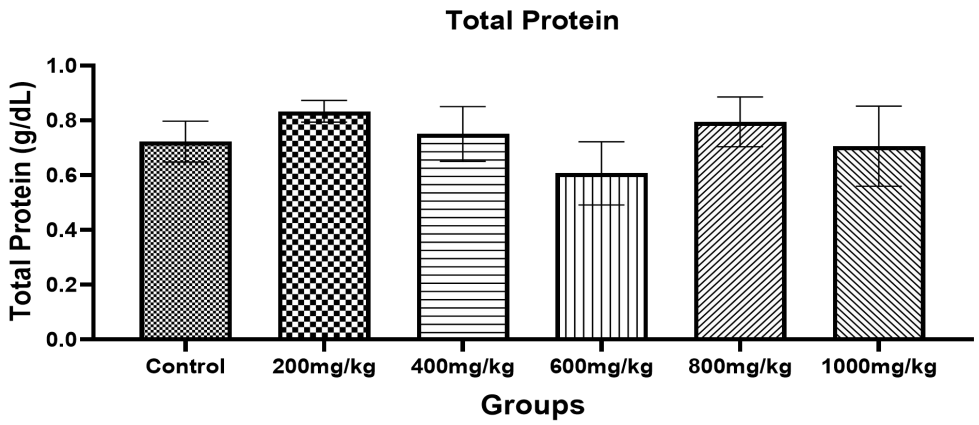


Chart 5: Activity of total protein in the cerebrum of control and treatment groups after administration. Values are given as mean ± SEM.

There was no statistically significant difference (P<0.05) in total protein for rats across each group compared to the control

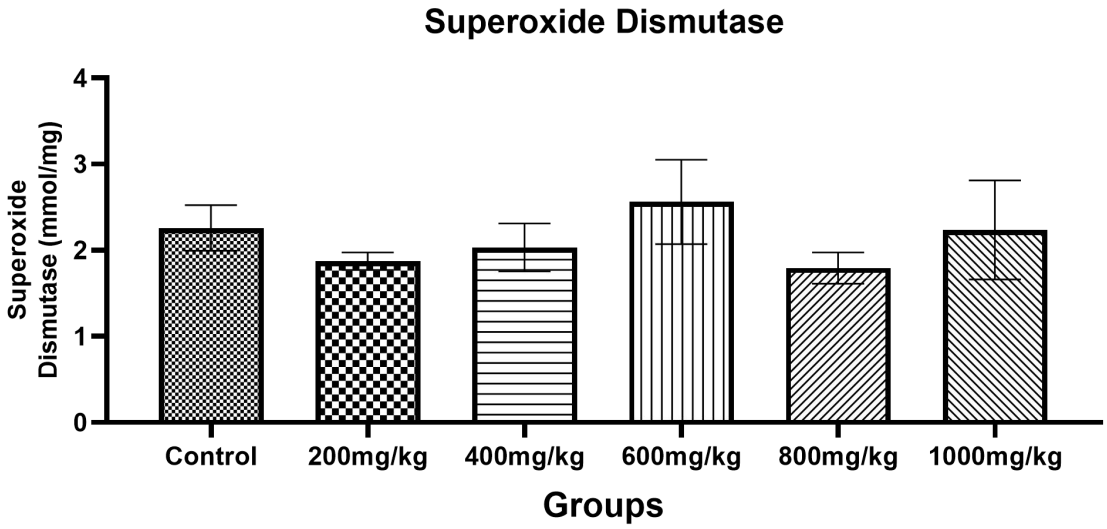


Chart 6: Superoxide dismutase activity in the cerebrum of control and treatment groups after administration. Values are given as mean ± SEM.

There was no statistically significant difference (P<0.05) in the superoxide dismutase for rats treated across the group when compared with control.

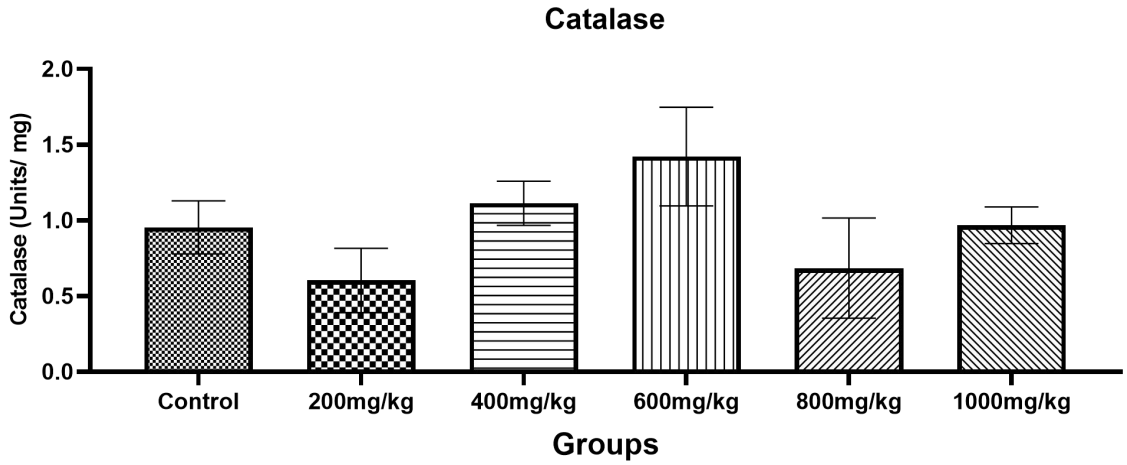


Chart 7: Catalase activity in the cerebrum of control and treatment groups after administration. Values are given as mean ± SEM.

There was no statistically significant difference (P<0.05) in the catalase for rats treated across the group when compared with control

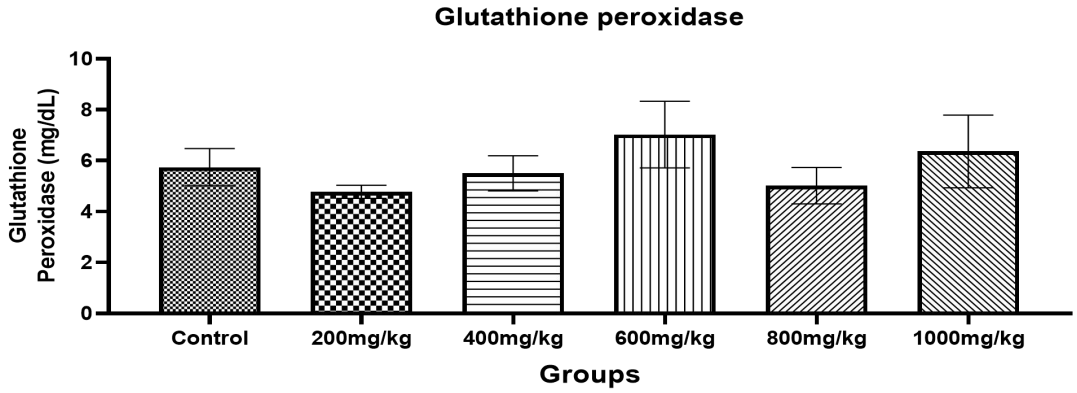


Chart 8: Glutathione Peroxidase activity in the cerebrum of control and treatment groups after administration. Values are given as mean ± SEM. There was no statistically significant difference (P<0.05) for rats treated across the group when compared to control.

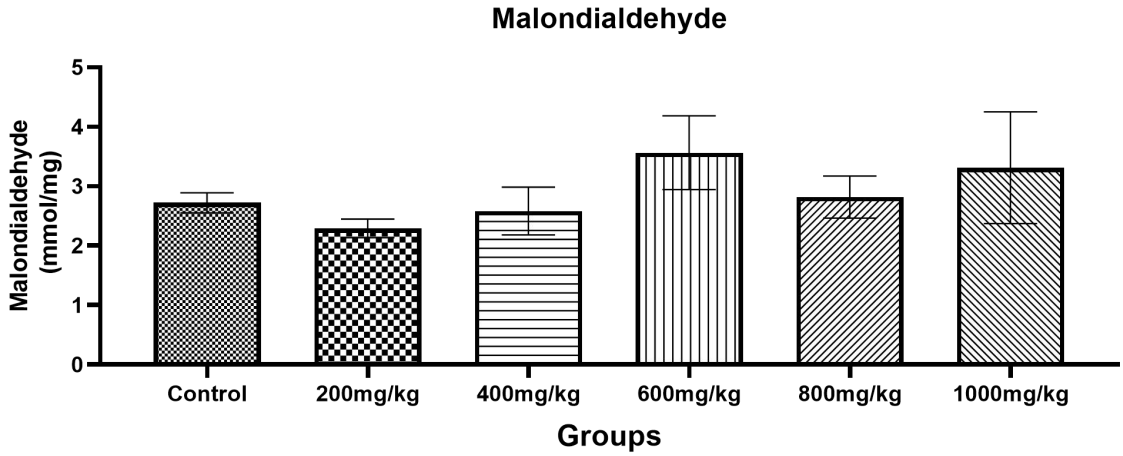


Chart 9: Lipid peroxidation activity in the cerebrum of control and treatment groups after administration. Values are given as mean ± SEM. There was no statistically significant difference ($P<0.05$) in malondialdehyde for rats treated across the group when compared to control.

HISTOLOGICAL RESULTS

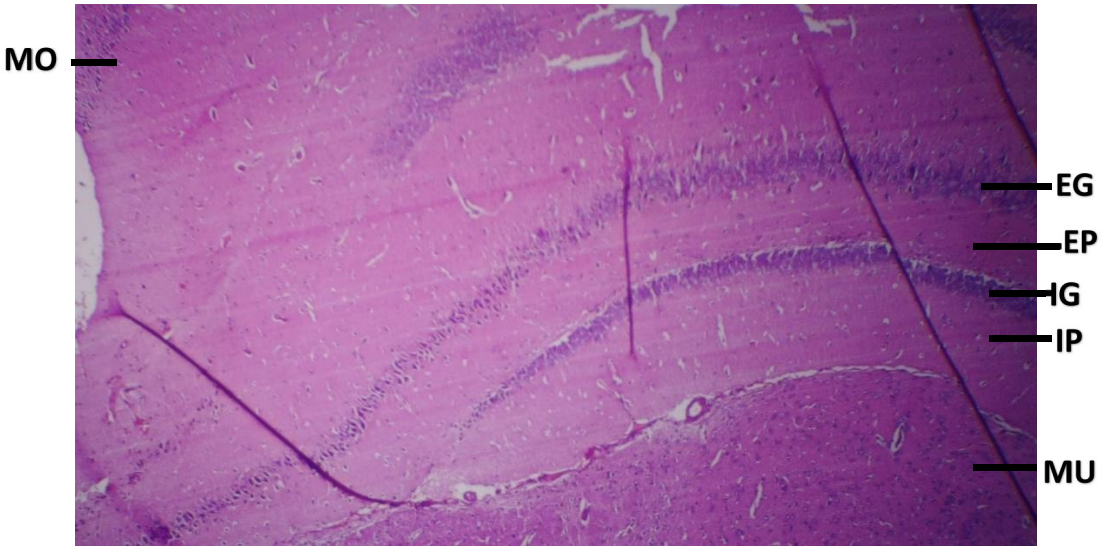


Plate 1. Rat prefrontal cortex, control, show: molecular (MO), external granular (EG), external pyramidal (EP), internal granular (IG), internal pyramidal (IP) and multi-form layers (MU): H&E 40 X

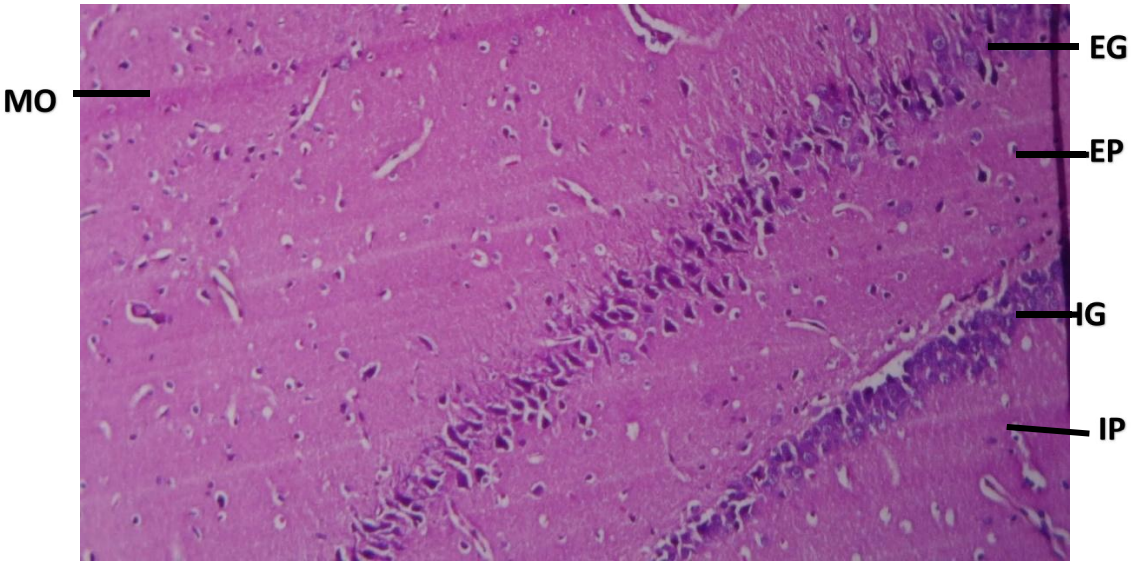


Plate 2. Rat prefrontal cortex, control, show: molecular (MO), external granular (EG), external pyramidal (EP), internal granular (IG), internal pyramidal (IP) and multi-form layers (MU) : H&E 100 X

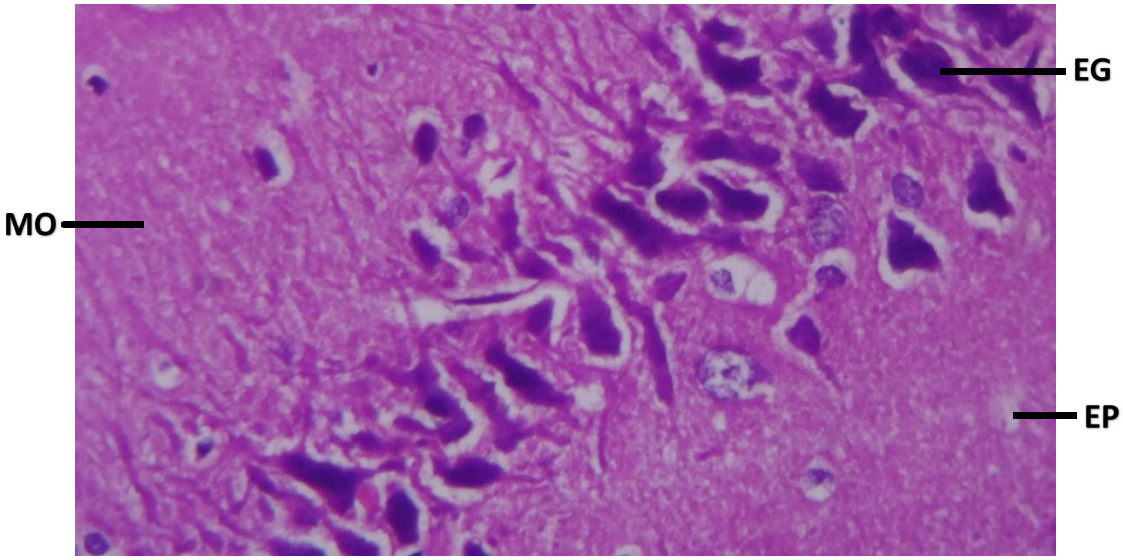


Plate 3. Rat prefrontal cortex, control, show: molecular (MO), external granular (EG), external pyramidal (EP), internal granular (IG), internal pyramidal (IP) and multi-form layers (MU): H&E 400 X

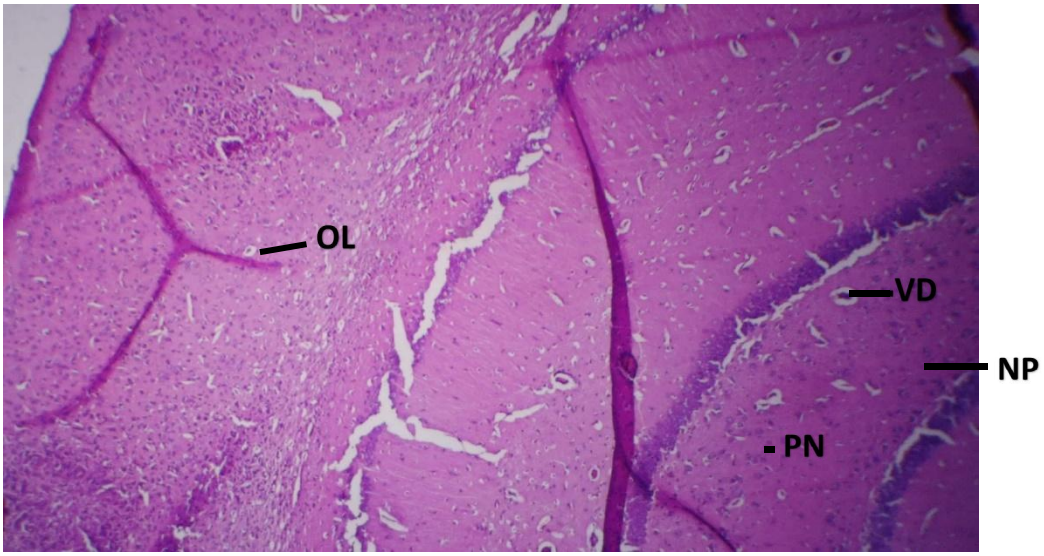


Plate 4. Rat cerebral cortex given 200mg Extract show: vasodilatation (VD), normal pyramidal neurons with conspicuous nucleoles (PN), oligodendrocytes (OL) and neuropil (NP): H&E 40 X

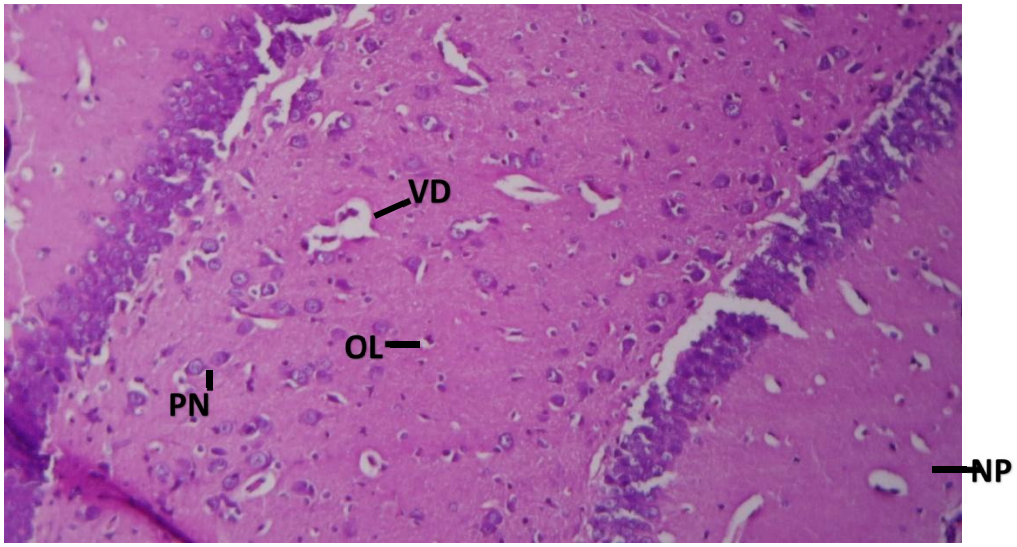


Plate 5. Rat cerebral cortex given 200mg Extract show: vasodilatation (VD), normal pyramidal neurons with conspicuous nucleoles (PN), oligodendrocytes (OL) and neuropil (NP) : H&E 100 X

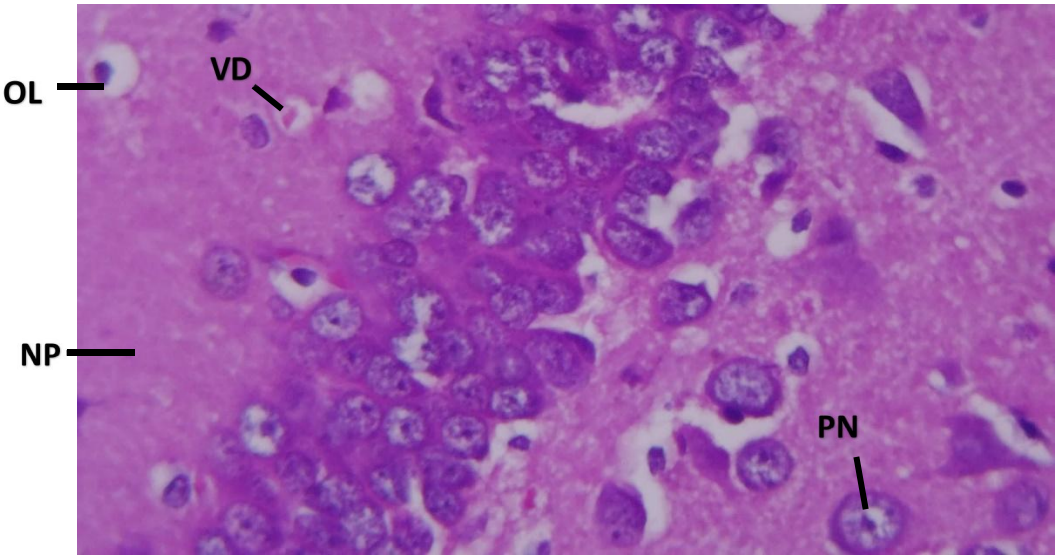


Plate 6. Rat cerebral cortex given 200mg Extract show: vasodilatation (VD), normal pyramidal neurons with conspicuous nucleoles (PN), oligodendrocytes (OL) and neuropil (NP) : H&E 400 X

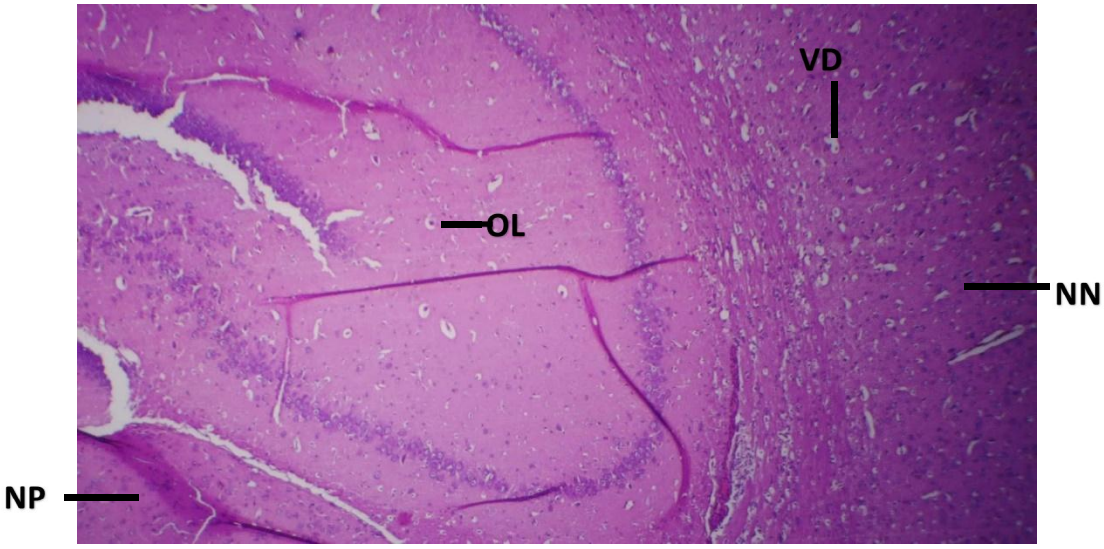


Plate 7. Rat prefrontal cortex given 400mg extract show: normal neurons with conspicuous nucleoles (NN), oligodendrocytes (OL), neuropril (NP), cerebral vasodilatation (VD): H&E 40 X

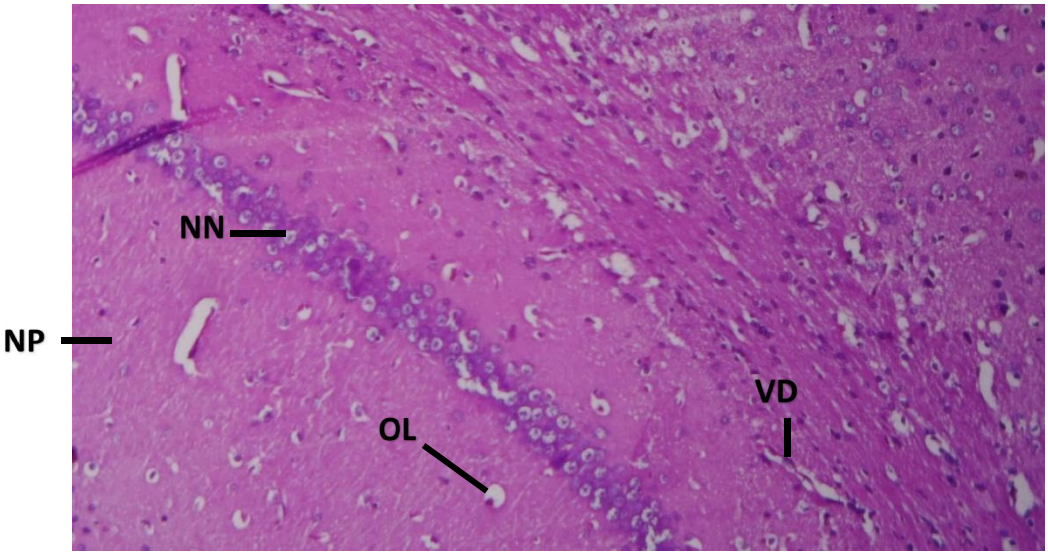


Plate 8. Rat prefrontal cortex given 400mg extract show: normal neurons with conspicuous nucleoles (NN), oligodendrocytes (OL), neuropril (NP), cerebral vasodilatation (VD) : H&E 100 X

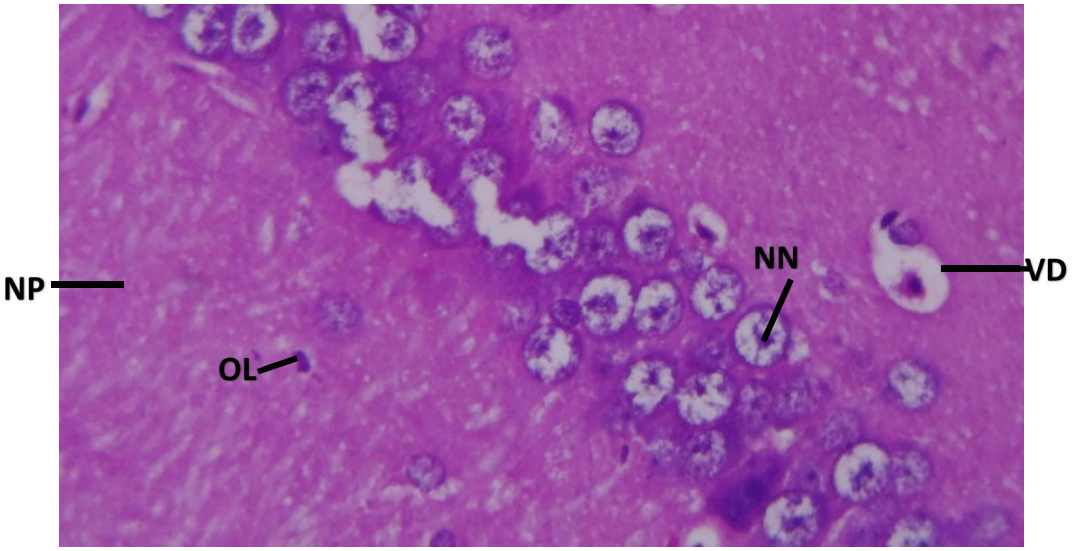


Plate 9. Rat prefrontal cortex given 400mg extract show: normal neurons with conspicuous nucleoles (NN), oligodendrocytes (OL), neuropril (NP), cerebral vasodilatation (VD) : H&E 400 X

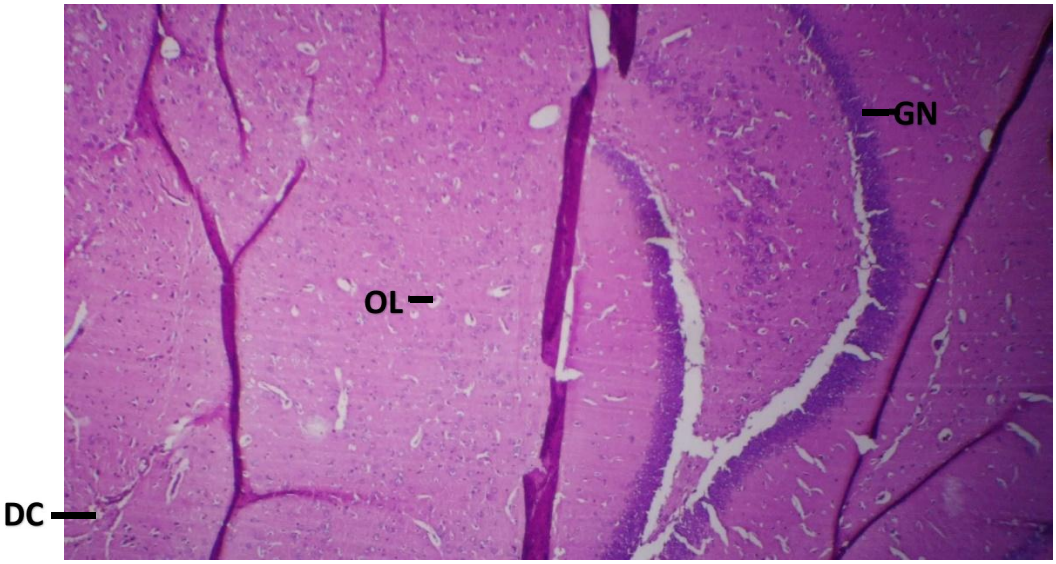


Plate 10. Rat cerebral cortex given 600mg extract show: normal granular cell neurons with conspicuous nucleoli (GN), normal oligodendrocytes (OL) and marked vasodilatation and active congestion (DC): H&E 40 X

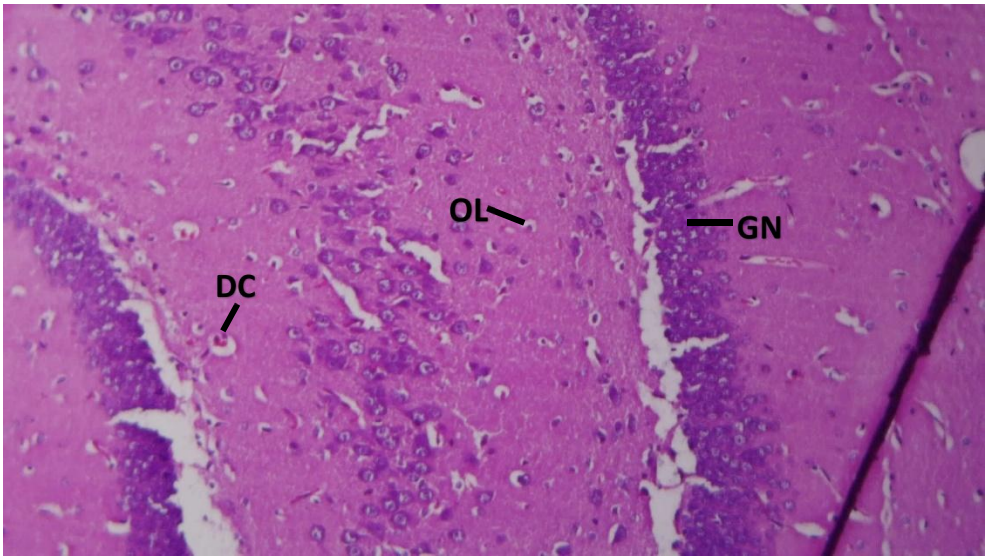


Plate 11. Rat cerebral cortex given 600mg extract show: normal granular All neurons with conspicuous nucleoli (GN), normal oligodendrocytes (OL) and marked vasodilatation and active congestion (DC): H&E 100 X

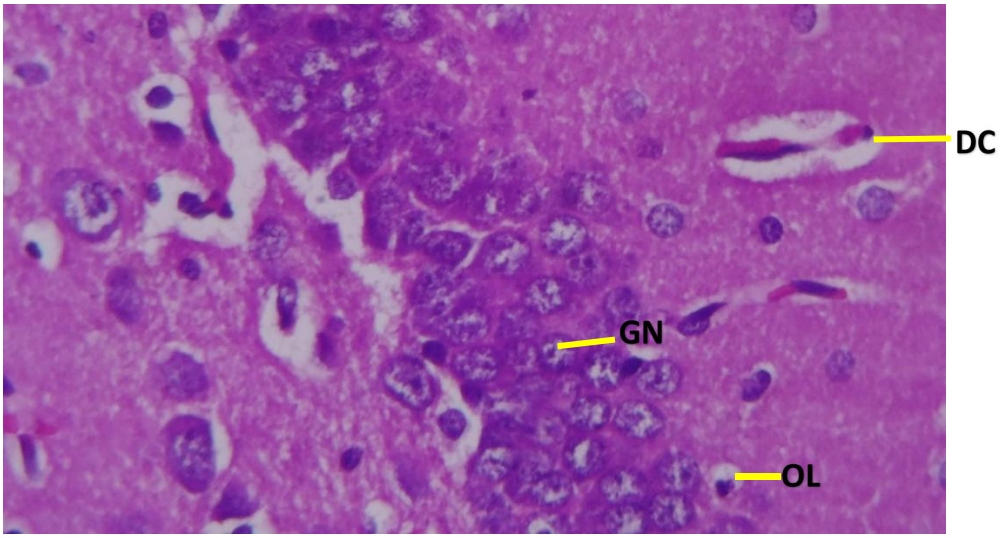


Plate 12. Rat cerebral cortex given 600mg extract show: normal granular All neurons with conspicuous nucleoli (GN), normal oligodendrocytes (OL) and marked vasodilatation and active congestion (DC) : H&E 400 X

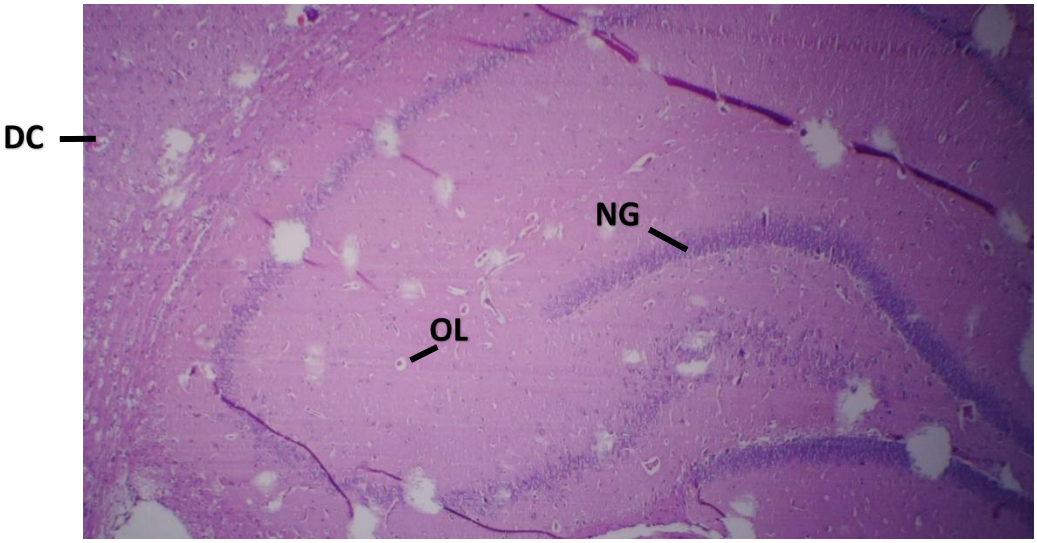


Plate 13. Rat cerebral cortex given 800mg extract show: marked vasodilatation and active congestion (DC), normal granular cells with conspicuous nucleolus (NG) and oligodendrocytes (OL): H&E 40 X

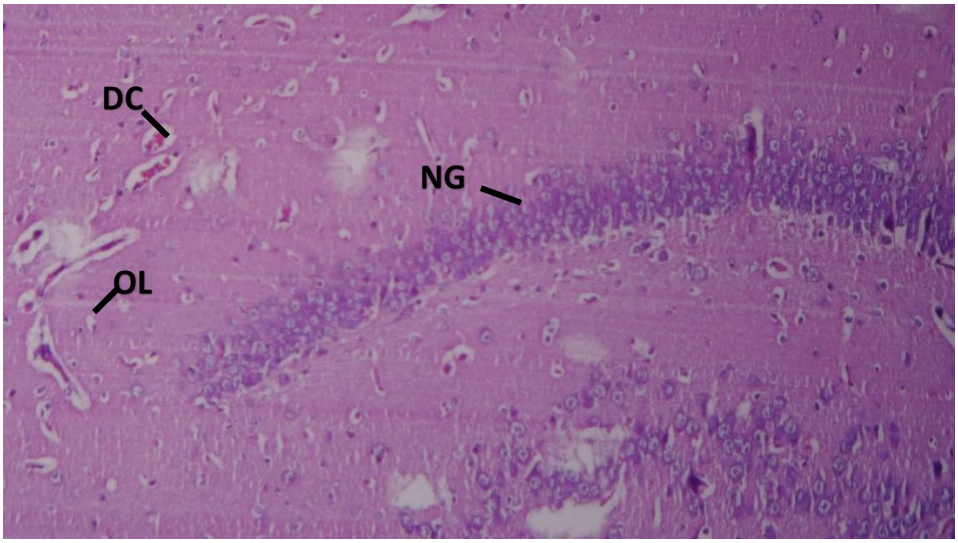


Plate 14. Rat cerebral cortex given 800mg extract show: marked vasodilatation and active congestion (DC), normal granular cells with conspicuous nucleolus (NG) and oligodendrocytes (OL) : H&E 100 X

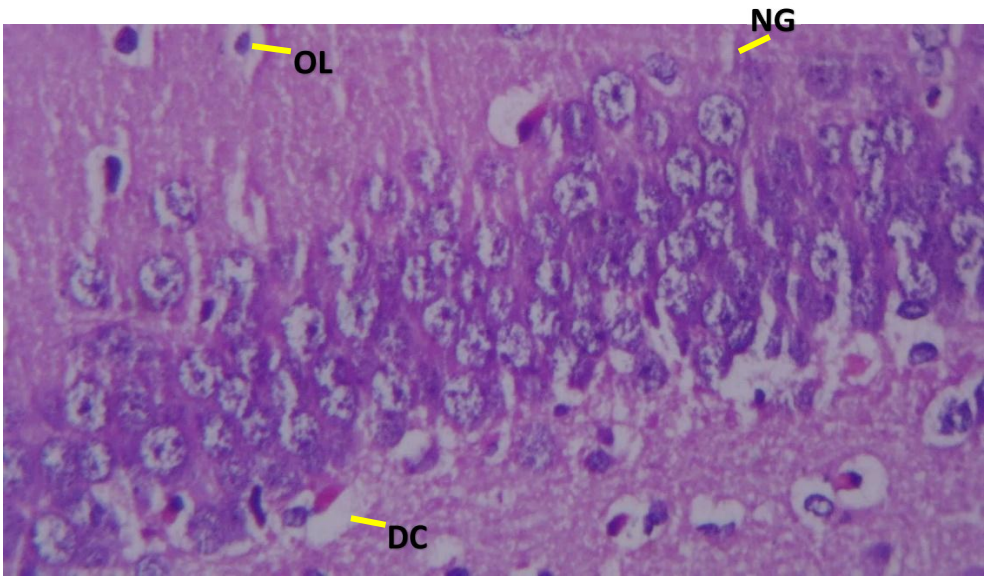


Plate 15. Rat cerebral cortex given 800mg extract show: marked vasodilatation and active congestion (DC), normal granular cells with conspicuous nucleolus (NG) and oligodendrocytes (OL): H&E 400 X

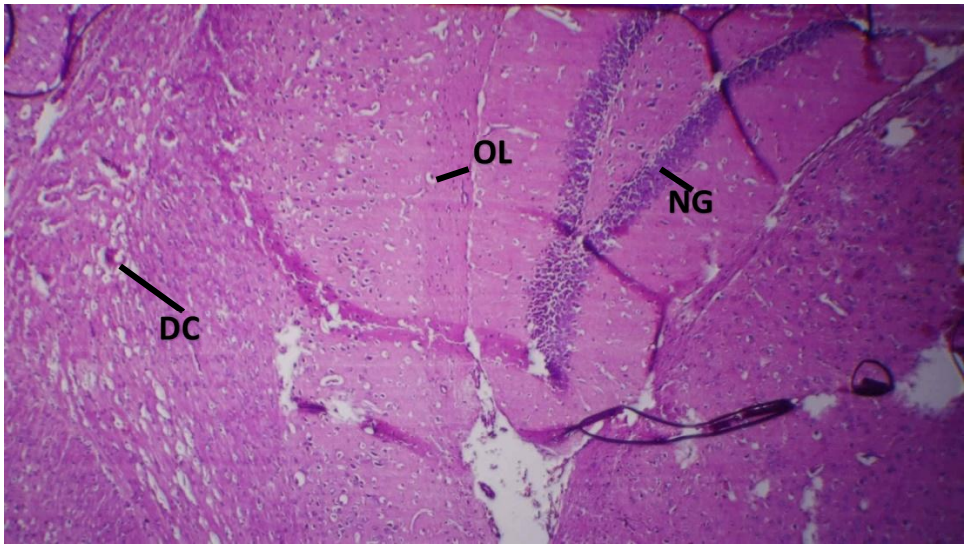


Plate 16. Rat cerebral cortex given 1000mg Extract show: marked vasodilatation and active congestion (DC), normal oligodendrocytes (OL) and granular cell with conspicuous nucleolus (NG): H&E 40 X

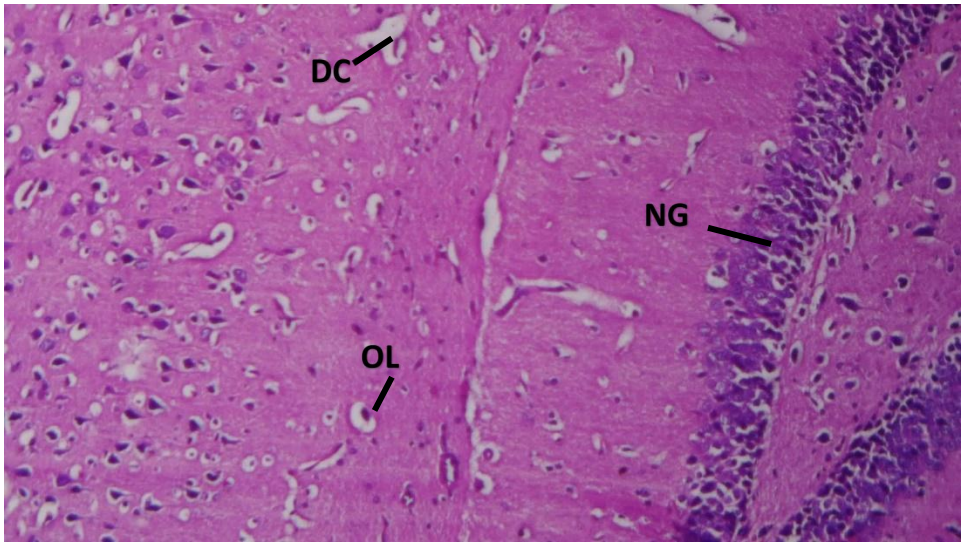


Plate 17. Rat cerebral cortex given 1000mg Extract show: marked vasodilatation and active congestion (DC), normal oligodendrocytes (OL) and granular cell with conspicuous nucleolus (NG): H&E 100 X

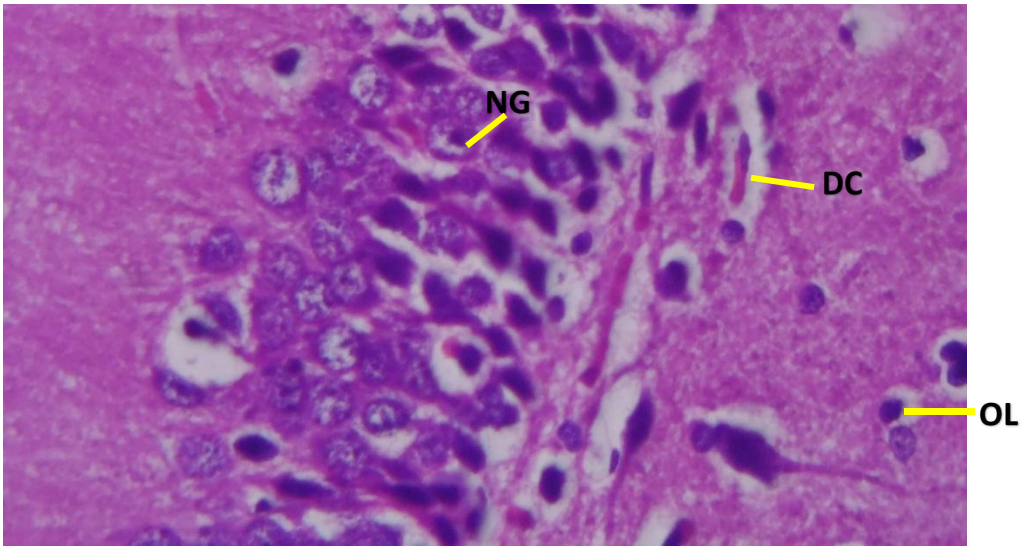


Plate 18. Rat cerebral cortex given 1000mg Extract show: marked vasodilatation and active congestion (DC), normal oligodendrocytes (OL) and granular cell with conspicuous nucleolus (NG) : H&E 400 X

CHAPTER FIVE

DISCUSSION

When the initial and final body weight of the rats were compared, there was a change in the initial and final body weight but it was not statistically significant ($P<0.05$) across the groups when compared to the control group. In charts showing the weight of the cerebrum and Organo somatic index, there was no statistically significant difference ($P<0.05$) across the groups when compared with the control group.

In the charts showing the antioxidant results;

There was no statistically significant difference ($P<0.05$) in the ***Total Protein*** for rats across the groups when compared with the control group. ***Superoxide Dismutase*** showed no statistically significant difference ($P<0.05$) across the groups when compared with the control group. ***Catalase*** showed no statistically significant difference ($P<0.05$) across the groups when compared with the control group. ***Glutathione Peroxidase*** showed no statistically significant difference ($P<0.05$) across the groups when compared with the control group. ***Malondialdehyde*** showed no statistically significant difference ($P<0.05$) across the groups when compared with the control group. This might mean that the *Bryophyllum pinnatum* aqueous extract has minimal or no effect on the antioxidant activity on the cerebrum which correlates to (Daniel *et al.,* 2020) used *Bryophyllum pinnatum* on a project titled "**Evaluation of the antioxidant potential of *Bryophyllum pinnatum*"** evaluated the study of the antioxidant properties of *Bryophyllum pinnatum* leaf extract. It found some antioxidant activity in vitro, but the effects in vivo, particularly on the brain, were not conclusively demonstrated to be significant, also a study looked at the neuroprotective effects of *Bryophyllum pinnatum*, particularly focusing on its ability to reduce oxidative stress. While the results were promising, the effects on antioxidant activity in the cerebrum specifically

were less pronounced than expected on a project titled **"Neuroprotective and antioxidant effects of *Bryophyllum pinnatum* in oxidative stress model"** (Ogidigo *et al.,* 2022)

The histological analysis of the cerebrum (prefrontal cortex) of the Adult Wistar Rat shows normal histological appearance in control Group A showing molecular, external granular, external pyramidal, internal granular, internal pyramidal and multi-form layers.

Group B which was administered 200mg/kg of *Bryophyllum pinnatum* extract showed vasodilation, normal pyramidal neurons with conspicuous nucleoles, oligodendrocytes and neutrophils.

Group C which was administered 400mg/kg body weight of *Bryophyllum pinnnatum* extract showed normal neurons with conspicuous nuecleoles, oligodendrocytes, neutrophil and cerebral vasodilation.

Group D which was administered 600mg/kg body weight of *Bryophyllum pinnatum* show normal granular cell neurons with conspicuous nucloeles, normal oligodendrocytes and marked vasodilation and active congestion.

Group E which was administered 800mg/kg body weight of *Bryopyllum pinnatum* extract showed marked vasodilation and active congestion, normal granular cell with conspicuous nucleolus and oligodendrocyte.

Group F which was administered 1000mg/kg body weight of *Bryopyllum pinnatum* extract showed marked vasodilation and active congestion, normal oligodendrocyte and granular cells with conspicuous nucleoles.

Adult Wistar rats were treated with graded concentration of *Bryophyllum pinnatum* extracts (200mg, 400mg, 600mg, 800mg and 1000mg/kg body weight respectively) for six weeks,

after which they were sacrificed and the cerebrum harvested. The cerebral tissue was subjected to histological analysis for possible reactions.

Sections of the cerebrum taken from the rats in the normal control group show normal tissue microscopic architecture, with well-defined cerebral layers and their constituent cells (pyramidal, granular cell neurons and oligodendrocytes) including: molecular, outer granular, outer pyramidal, internal granular, internal pyramidal and multiform layers. Also constitutes the neutrophil and cerebral blood vessels.

Sections taken from rats treated with graded concentration of *Bryophyllum pinnatum* show normal cerebral microscopic architecture, with well-defined layers and cellular components like pyramidal, granular cell neurons and oligodendrocytes. There were also observable activities such as, vasoactive changes of vasodilatation and increased cerebral blood circulation (active congestion), and conspicuous nucleolus in the neuronal nuclei. Conspicuous nucleolus signifies increased activity in protein synthesis, since nucleoli are factories for protein storage in the nucleus of the cell.

The increase in the beneficial activities outlined above is along an upward concentration gradient. Thus the higher the concentration of *Bryophyllum pinnatum* extract, the more intense the cerebral vasodilatation, increased blood circulation and protein synthesis.

In the phytochemical analysis of *Byrophyllum pinnatum* extract, flavonoids and phenolic compound was in high quantity, this is responsible for the anti inflammatory, anti microbial properties and have been linked to various health benefits.

CONCLUSION

Byrophyllum pinnatum extract in higher concentration increased blood circulation, protein synthesis and induced vasogenic effect in a dose of dependent fashion in the cerebrum of Adult Wistar Rat. Hence it has no adverse effect but rather reduce vascular permeability and edema in the cerebrum of the Adult Wistar Rat.

RECOMMENDATION *Bryophyllum pinnatum* can be used for treating various conditions related to inflammation, edema and so on. Further research is necessary to explore the potential of *Bryophyllum pinnatum* extract.

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