THE PROTECTIVE EFFECTS OF THE ETHANOLIC LEAF EXTRACT OF *CENTELLA ASIATICA* ON MERCURIC CHLORIDE INDUCED CEREBELLAR DAMAGE ON ADULTWistarRATS

BY

AMORIGHOYE SUCCESS OMAYULI

BMS1802238.

BEING A PROJECT SUBMITTED TO THE DEPARTMENT OF ANATOMY,

SCHOOL OF BASIC MEDICAL SCIENCES,

UNIVERSITY OF BENIN, BENIN CITY,

EDO STATE, NIGERIA.

IN PARTIAL FULFILMENT OF REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc) IN ANATOMY

SUPERVISOR:

MRS. EJEGUO AKPOROBO

SEPTEMBER, 2023.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Primarily, the nervous system consists of the brain. Being the body's central command center, it is an intricate organ. It regulates respiration, touch, temperature, emotions, memory, and mental processes. According to Hopkin (2023), the central nervous system is comprised of the brain and the spinal cord that emanates from it. The cerebellum and cerebrum make up the two main divisions of the brain. The term "cerebrum" refers to the biggest and highest area of the brain. It comprises the cerebral hemispheres and accounts for roughly two thirds of the brain's total weight. Temperature regulation, speech, cognition, learning, hearing, touch, and other processes are all made easier by it. Movement is also initiated and organized by it (Zimbardo et al., 2003).

The cerebellum, often known as the "little brain" in Latin, is situated immediately behind the brain stem at the base of the brain (Roostaei et al., 2014). While comprising just approximately 10% of the brain's mass, it is home to 50% to 80% of all neurons, or brain cells. Coordinating and adjusting voluntary movement is one of its primary duties. Maintaining balance, tone in the muscles, and posture depend on this. Other processes including language acquisition, reflexes, eye movement, and cognition also depend on it..

Brain injury is another name for brain damage. Trauma, such as an accident, or illnesses, such as infections, diseases, or exposure to heavy, poisonous substances like lead and mercury, can all cause it (Jaishankar et.al., 2014). Lack of oxygen to the brain can also result in brain injury.

Owing to its location, the cerebellum is shielded from external pressures; yet, anoxic brain injury, infections, neurodegenerative diseases, and many other conditions can cause harm to

it.(Andrzejewska and others, 2021). Damage to it results in weakened or sometimes stopped messages being sent throughout the body. Among its side effects are apraxia, nystagmus, reduced muscular tone, altered speech, and cognitive impairments. Calomel, commonly known as mercury chloride (Hgcl2), is a particularly poisonous form of the metal that affects the brain. One of the harmful heavy metal pollutants that can harm a person's health, especially the brain, is mercury. Because of industrial activities such as increasing mining, increased use of fossil fuels, and widespread use of raw materials containing mercury, it is present in the environment (Boylan et al., 2003).

Because of their everyday mobility, human and animal populations are exposed to a variety of heavy metals through their interactions with the environment through food, air, and water (Burger et al., 2011). Awareness of the potential consequences of exposure to heavy metals like mercury is developing on the body particularly the brain and nervous system and this is because some of these metals can cross the blood brain barrier and accumulate in the brain and tissues and cause damage (Langford and Fernar 1999, Valko *et. al*., 2005).

Mercuric chloride may have a highly negative, extremely mild, or no effect at all on the brain. In severe situations, it may cause renal and respiratory failure as well as death. Other symptoms include tremor, sleeplessness, mood abnormalities, and neuromuscular alterations such as weakness and muscle atrophy. According to Teixera et al. (2018), mercuric chloride still has the ability to create motor deficits since it induces apoptosis and reduces the amount of neurons and astrocytes in the motor cortex.

Additionally, mercury has been found to be a highly toxic chemical in the environment, which can lead to changes in energy metabolism, structural degeneracy of cells, imbalance in the antioxidant system, disruption of calcium homeostasis, and induction of genotoxicity (Berg et al., 2010; Berntssen et al., 2003; Yadav and Trivedi, 2009a, 2009b). All chemical form of mercury has the potential to disrupt proteins, inactivate enzymes and cause critical changes in the physiological activities of any tissue which it comes in contact with in adequate concentration (Crump and Trudeau, 2009b; Gonzalez et al., 2005; K. V. Sastry and Sharma, 1980; K. V. Sastry and Rao, 1981; Suhendrayatna et.al., 2019;Vasanthi et al., 2019a).

Plant based natural products in the form of fresh plants, herbs,extract or their phytochemicals generally have wide range of pharmacological effects since ancient times) and one of such plant is *CENTALLA ASIATICA.* Its potentials as neuroprotective agents were also evident from many previous literatures (Moore et al., 2013).

Growing in or next to water, Centella asiatica (L.) Urban (C. asiatica) is a brown and green leafy plant belonging to the parsley family. (Godu K., 2015). Flowers of C. asiatica are light purple and bloom in August and September. The scent of tobacco leaves permeates the plant. The leafstalk branches into individual leaf clusters that resemble roses as it develops from a base to a height of 5 to 15 cm. The edible leaves have a greenish-yellow hue. There are few, dispersed hairs on the very short leafstalk (Brinkhaus et al., 2000). It is a significant medicinal plant that is extensively used in Ayurvedic and traditional Chinese medicine. It was estimated that Centella asiatica was first used medicinally in 1700 A.D. (Hamidpour et al., 2015). Triterpene, its primary component, is thought to be responsible for its medicinal properties (Gohil *et.al*., 2010). Its traditional use is mainly related to central nervous system management such as improvement of memory and learning, treatment of mental illness, and epilepsy and as sedatives. It is also used to treat skin disease, fever, jaundice,

diarrhea, ulcer, urinary infection and asthma (Gohil *et.al*.,2010,Roy *et.al*., 2013). In U.S and Europe, it is used to treat varicose vein and chronic venous insufficiency. Also it can be consumed as a cooked vegetable, fresh salad or as drink (Hashim *et.al*., 2011).

According to studies, the extract from Centella asiatica can lessen the harm that mercury does to the brain and protect against mercuric chloride toxicity. Studies have demonstrated the anti-inflammatory and antioxidant qualities of Centella asiatica ethanoic leaf extract, which aid in mitigating the oxidative stress and inflammation brought on by exposure to mercuric chloride.

1.2 AIM OF THE STUDY

The aim of the present study is to evaluate the protective potential of the extracts of *Centella asiatica* towards neurotoxic mercuric chloridein the cerebellum.

1.3 SPECIFIC OBJECTIVES OF THE STUDY

The following are the objectives of the study:

i. To investigate the effect of Mercuric chlorideon the body and organ weight of the cerebellum of experimental animals.

ii. To investigate the effect of ethanoic leaf extract of *Centella asiatica* on the body and organ weights (cerebellum) of experimental animals.

iii. To evaluate the antioxidant activity of the ethanoic leaf extract of *Centella asiatica.*

iv. To examine the histological changes mitigated by administration of the extract on the cerebellum.

1.4 JUSTIFICATION OF THE STUDY

A hazardous metal utilized extensively in manufacturing and other industries is mercury. Numerous body organs may be harmed, depending on the situation. For several neurological conditions, Centella asiatica has been used as a brain tonic. In order to determine whether plant extract may shield subjects from mercury chloride exposure, this study was conducted.

According to Teixeira et al. (2018), brain tissues have also been discovered to be more vulnerable to oxidative damage due to the high concentration of polyunsaturated fatty acids, which are primarily prone to lipid peroxidation, which is crucial for necrosis and cell death in the brain.

CHAPTER TWO

LITERATURE REVIEW

2.1 PLANT OF STUDY

Centella asciatica, which is a native to tropical parts of Africa, Asia, Australia, and islands in the Western Pacific Ocean is a perennial herbaceous creeper plant. It is a member of the Apiacaece family.Plantae is the kingdom, and Apiale is the order (Meena et al., 2012).The long, greenish-reddish stem and fan-shaped green leaves have a maximum growth radius of 15 centimeters. According to Arora et al. (2002), the plant is widely distributed in damp regions. Indian pennywort, Icudwane, Gotu kola, Pegaga, Indian water navelwort, Asciatic pennywort, wild violet, and tiger plant are other names for Centella asciatica (Orhan 2012).While Centella asciatica includes a variety of active chemicals, the most significant component of numerous other active components is triterpenoid saponins. Other active ingredients include genin triterpenoids, essential oils, flavonoids, and phytosterols.

Fig 2.1 Diagram Showing the Stem and Leaves Of *Centella Asciatica*

SOURCE: BONNY ISLAND, PORT HARCOURT

2.1.1 BIOLOGICAL ACTIVITIES OF *CENTELLA ASIATICA*

Numerous biological properties of *Centella asiatica* are well documented, some of which include wound healing, cytotoxic anti-tumor, immunostimulant, antidiabetic, antifungal, antiviral, and antibacterial actions (Orhan, 2012). Centella asiatica has medicinal potential that can be investigated in a variety of in vivo and in vitro settings using extracts or isolated single compounds**.**

Anti-inflammatory Properties

According to Chippada and Vangalapati (2011), an excess of free radicals raises the possibility of oxidative damage to proteins, lipids, DNA, and biomolecules.Chronic illnesses like atherosclerosis, diabetes mellitus, cancer, and degenerative disorders can all be brought on by oxidative damage. According to Chippada and Vangalapati (2011), natural antioxidants and the consumption of them, which may be found in fruits, vegetables, and medicinal herbs, contain molecules that scavenge free radicals and can help lower the risk of degenerative diseases, cardiovascular disease, and diabetes mellitus. According to George et al. (2009), centella asiatica extracts, both alcoholic and aqueous, have been shown to exhibit in vivo anti-inflammatory effect against carrageenan-induced paw odema rats that is comparable to that of a conventional ibuprofen dose.

Anxiolytic Properties:

Centella asiatica has long been known to alleviate anxiety. Centella asiatica was tested by Waijeweera et al. on rats' anxiety levels using the elevated plus maze, open field, social interaction, locomotor activity, Vogel, and novel environment tests. The results indicated that extracts from Centella asiatica may contain heavy triterpene components that contribute to anxiolytic activity. Asiaticoside is the most active and prevalent triterpene, even if other active substances may also

play a significant role in plant activity influenced by or associated with asiaticoside (Wijeweera et al., 2006).

Diuretics (Water Pills)

C. asiatica can serve a diuretic and individuals should be aware of the dangers of losing large amounts of fluids if diuretic symptoms occur (Gotu k 2015)

Antioxidant Activity:

Physical function is impacted by oxidative stress as we age. According to Mateo et al. (2009), antioxidants prevent age-related deteriorations in cognitive function and physical function and also have a good impact on psychological capacity. In order to ascertain the benefits and anti-oxidant properties of C. asiatica, Mato et al. examined the effects of the plant on aged, healthy volunteers' physical capacity, physical satisfaction, mental well-being, and outlook (Mato et al., 2009). A "dynamic condition of well-being characterized by a physical, mental, and social potential altered by "health" is how Mato et al. (2009) described health. Because of its antioxidant components, C. asiatica was considered a potential herbal therapy for better physically in healthy older people. C. asiatica was identified by Mato et al. as a potential herbal treatment for improved physicality in healthy elderly because of its antioxidant compounds.

Venous Insufficiency and Varicose Veins

When blood in the veins in the legs stagnates, the blood vessels lose their elasticity and start to leak fluid, which is known as venous insufficiency. According to a few studies, C. asiatica can help minimize abscesses and improve blood flow to lessen venous insufficiency symptoms and conditions (Godu k 2015). Arpaia et al. administered carefully regulated dosages of C. asiatica to twenty patients suffering from varicose veins in their legs, monitoring the patients' progress over a

period of three months in order to assess the biochemical components of the plant. Arpaia et al. established a higher baseline serum of uric acid and elevated lysosomal enzymes as the result of probing the relationship between mucopolysaccharide metabolism and uric acid lysosomal enzyme activity. After *C. asiatica* treatment, Arpaia *et al*. found the reduced uric acid serum levels and lysosomal enzymes (Brinkhaus *et al.*, 2000)

Chronic Venous Insufficiency

The disorder known as chronic venous insufficiency (CVI) impairs the ability of the veins in the lower limbs to return blood to the heart. Variations in the venous wall and valves can result in CVI (Chong and Aziz Z 2013). The degree of effectiveness of medications like aminaftone and calcium dobesilate, which have been used as part of therapy treatments for CVI, is not well established. C. asiatica contains flavonoids, saponosides, and diosmin, which have all been investigated for potential treatment of venous microangiopathy and CVI onset symptoms (Chong and Aziz 2013). Due to the presence of triterpenes, C.asiatica may be able to lessen microangiopathy and CVI symptoms in the lower extremities. Triterpenes have anti-inflammatory properties, as demonstrated by (Chong J, Aziz Z 2013). Chong *et al.* conducted a comprehensive scientific literature review to determine the ways in which *C. asiatica* may improve CVI and CVI symptoms.

While asiaticoside, a compound derived from Centella asciatica, showed good antioxidant activity and helped the wounded rats heal, Centella asciatica also increases collagen synthesis and cellular proliferation at the wound site in rats (Sunilkumar et al., 1998). (Shukla et al., 1999). According to George et al. (2009), centella asciatica extracts, both alcoholic and aqueous, have been shown to exhibit in vivo anti-inflammatory efficacy against carrageenan-induced paw oedema rats. This action is comparable to that of conventional Ibuprofen.

Wound Healing and Skin Lesions

In rats, Centella asciatica promotes collagen production and cellular proliferation at the site of wound healing (Sunilkumar et al., 1998). The most active class of chemicals found in C. asiatica are called terpenes. Asiaticoside, the largest triterpene glycoside, is known to accelerate wound healing and raise antioxidant levels in the early stages of the healing process (Wijeweera et al., 2006). Triterpenouds, a substance present in C. asiatica, have been shown in numerous animal and laboratory tests to promote wound healing. Triterpenoids promote wound antioxidant levels, improve skin suppleness, and improve blood flow to the injured area. Topical skin creams and ointments containing C. asiatica may be beneficial in treating small burns and psoriasis, as well as in preventing or lessening the look of stretch marks, and prevent or reduce the appearance of surgery scars (Gotu k 2015) *Centella asiatica* demonstrates healing of wounds by increasing the collagen synthesis and cellular proliferation at wound sites in rats (Sunilkumar *et al*., 1998).

Reduce Skin Aging

Polyacetylenes, sapoogenins, and triterpenoid saponins (madecassoside andasiaticoside) are well-known bioactive compounds of C. asiatica (Antognoni et al., 2011). Researchers discovered that when these compounds were being produced in vitro, there were low concentrations of these agents and significant concentrations of caffeoyl derivatives, notably 3,5-O-dicaffeoyl4-omalonilquinic acid. This kind of acid is important because it inhibits the breakdown of collagen, reduces the rate at which skin ages, shields skin from UV damage, and absorbs UV light between 300 and 330 nm (Antognoni et al., 2011). According to a 2010 study by Gohil et al., C. asiatica affected the vascular wall's connective components. C. asiatica can lower capillary filtration and enhance microcirculatory parameters in hypertensive microangiopathy and venous insufficiency. This makes C. asiatica a potential choice for reducing the appearance of skin ageing (Gohil *et al*., 2010).

Sedatives

As a natural sedative, C. asiatica may conflict with other prescription drugs or therapies intended to treat anxiety or insomnia (Gotu 2015). A standard dosage of 600 mg of either dried or infused C. assigmatica in a single dose capsule is thought to be appropriate. According to Gohil et al. (2010), a single-dose capsule contains between 300 mg and 680 mg and can be taken up to three times per day.

Anti-cancer Activities

According to Babykutty et al. (2009), *centella asciatica* has anti-cancer properties. A methanolic extract of the plant caused human breast MFC-7 cancer cells to undergo apotosis. The antioxidant properties of *centella asciatica* were also studied by Jayashress et al. (2003), who found that giving mice with lymphoma an oral methanolic extract of the plant enhanced the amount of antioxidants and antioxidant enzymes in the mice. According to Emran et al. (2015), *centella asciatica* extract has the ability to lower blood glucose levels in rats that have diabetes brought on by alloxan. According to a recent study by Idriz and Nadriz (2017), gram-positive bacteria Bcillus subtilis and the fungus Aspergillus niger are both inhibited in their growth by centella asciatica extracts. *Centella asciatica* water and methanolic extracts also showed antiviralproperty against alpha-herpesvirus in in vitro model (Hanisa *et al.*, 2014).

Nervous Activity

In addition to the benefits listed above, centella asciatica is also well-known in traditional medicine for its neuro-protective, sedative, depressive, rejuvenating, memory-enhancing, and anticonvulsant properties, which make it a brain tonic (Chong et al., 2009). In a research on centella asciatica's ability to enhance memory, Sari et al. (2014) found that rats underwent chronic stress and that memory function was enhanced by an ethanolic extract of centella asciatica. Additionally, the extract demonstrated antiepileptic and depressive properties in an in vivo model (Visweswari et al., 2010; Goola and Tirupathi, 2016). The acetylcholinesterase enzyme, which causes AD, is inhibited by the hydroalcoholic extract of centella asciatica (Mukherjee et al., 2007). In an invitro model, centella asciatica's asiatic acid also has neuroprotective effects against renetone in in vitro model by reducing the production of ROS, increasing mitochondrial membrane property andtherefore inhibiting apoptosis (Nataraj *et al.,* 2017).

2.1.2 PHYTOCHEMICALS

It is also called plant-chemicals. They are naturally occurring bioactivie compounds found in plants i.e fruits, vegetables and herbs (Kumar and Khanum, 2012). Examples of Phytochemicals includes:

Triterpene; Triterpenes are a diverse group of natural compounds having molecular formula $C_{30}H_{48}$ (Grace-Lynn *et al.,* 2012). They are synthesized from an isoprene unit, which is derived naturally from the acetate/mevalonate (MVA) pathway.They act as an important physiological molecule in the cell. Triterpenes protects against pathogen by these molecules (Thimmappa *et al.,* 2014). Moreover, simple and conjugated triterpenes also have a number of roles in the health, food, and industrial biotechnology sectors. Oxygen derivatives of triterpenes are known as triterpenoids.

Glycosides: A glucose molecule enclosed by an aglycone makes up a glycoside. Glycosides are represented by the type of glycosidic bond, aglycone, and glycone. They are essential to living things in a number of ways. Glycosides come in many different forms, such as cardiac glycosides, saponins, alcoholic glycosides, steviol glycosides, thioglycosides, and many more. They are quite effective in treating several heart conditions, such as arrhythmia and congestive heart failure. Additionally, they have laxative, purgative, antibacterial, allelopathic, and analgesic properties. Steviol glycosides function as a sweetener in nature.

Flavonoids : Secondary metabolites called flavonoids are mostly made up of a benzopyrone ring with phenolic or polyphenolic groups attached to it at various locations (Cavalcante et al., 2018). According to Shan X et al. (2017) and Feliciano et al. (2015), they are most frequently found in fruits, herbs, stems, cereals, nuts, vegetables, flowers, and seeds. Flavonoids have been utilized in skin care products and cosmetics (Lanzendorfer et al., 1996), natural dyes (Villela et al., 2019), anti-wrinkle skin agents (Chuarienthong et al., 2010), and cosmetics and skin care products (Paramita et al., 2018). Nonetheless, the medical field is where these polyphenols are most prominently used. Numerous studies have employed flavonoids for their anticancer, antibacterial, antiviral, antiangiogenic, antioxidant, antimalarial, neuroprotective, antitumor, and anti-proliferative properties (Zhao et al., 2019; Camero et al., 2018), antimalarial, antioxidant, neuroprotective, antitumor, and anti-proliferative agents (Patel *et al.*, 2018).

Sterols: Tanins together with fatty acids (Kumar et al., 2015). These phytochemicals have important pharmacological qualities that make them promising treatments for a variety of illnesses.

Since phytochemicals have antioxidant properties, numerous scientific research have demonstrated their potential as an alternative natural therapy for brain damage and neurodegenerative illnesses (Kumar and Khanum, 2012). Additionally, Guo et al. (2007) demonstrated that while resveratrol, a

polyphenol primarily found in red grapes, protected the neurons from amyloid beta peptide toxicity and decreased the intracellular amyloid beta peptide formation, catechins shielded dopaminergic neurons from 6-hydroxydopamine (6-OHDA)-induced oxidative stress in rats (Marabaud et al., 2005).

2.2 MERCURIC CHLORIDE ($HgCl_2$)

Mercuric chloride (HgCl2) is the result of a chemical reaction between the elements mercury and chlorine.Dichlor Mercury or Mercury (II) Chloride are other names for it. This substance is extremely harmful and caustic to the mucosal membrane. Its main applications are as an antiseptic, fungicide, disinfectant, and wood preservative. Mercuric chloride has no smell and is white in color. This crystalline solid, known as a triatomic molecule since it is bordered by two chlorine atoms and dissolves in water, is made up of one mercury atom (Fisher, J. F., and World Health Organization 2003).

Mercuric chloride has a tendency to sublime because it is not a salt made of discrete ions but rather consists of linear triatomic molecules. Every mercury atom in the crystal is bound to two chloride ligands with Hg—Cl distance of 2.38 Å; six more chlorides are more distant at 3.38 Å. (Canty et.al 1982).

2.2.1 STRUCTURE OF MERCURIC CHLORIDE ($HgCl_2$)

The exact mass of Mercuric Chloride and its monoisotopic mass is 271.908 g/mol. The numbers of donors and acceptors of the number of Hydrogen bonds are equal to zero. This compound consists of only one covalently bonded unit and the compound is canonicalized.(Bolton et.al., 2008).

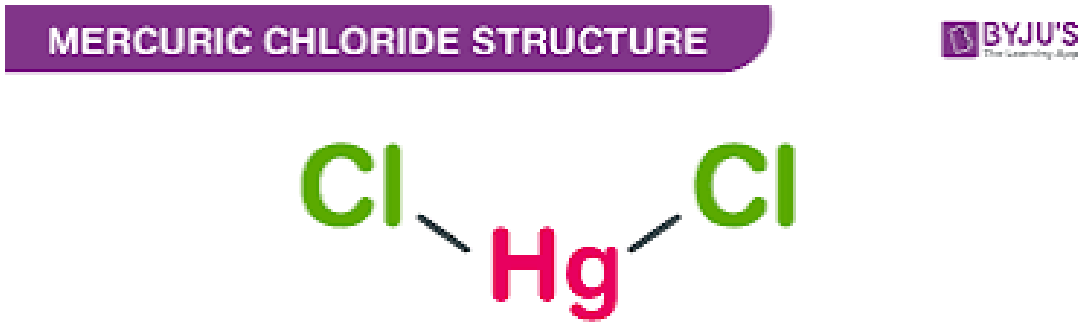


Fig 2.2 Diagram showing the Structure of Mercuric Chloride

SOURCE: BYJUS

2.2.2 PROPERTIES OF MERCURIC CHLORIDE ($HgCl_2$)

- The chemical formula of mercuric chloride is $HgCl_2$
- Its molecular weight is 271.52 g/mol and density of 5.43 g/cm2.
- It has a boiling point of 304c and melting point of 276C.
- At ordinary temperature, Mercuric chlorideis highly volatile.
- Mercuric Chloride can be sublime unchanged.
- The solubility of $HgCl_2$ is 5 to 10 my/ml.

When mercuric chloride is inhaled, absorbed by skin or ingested, it causes toxicity.(Barta, Č. (1970)

2.2.3 USES OF MERCURIC CHLORIDE ($HgCl_2$)

Mercuric chloride is a common reagent used in laboratories for analytical testing, cleaning, and preservation of biological specimens (Waheed et al., 2020). It has been used as a pesticide and fungicide to treat crops and wood items, despite the fact that its use in these applications has significantly declined due to its toxicity (Rocha et al., 2019; Ahmad and Mahmood, 2019).

Mercuric chloride is used as an electrolyte in some batteries and in electrochemical reactions, according to Mumtaz et al. (2019). Mercuric chloride was formerly used as an antiseptic and disinfectant, but its medical use has mostly been discontinued due to its toxicity and the availability of safer alternatives (Ali et al., 2020; Liu et al., 2021).

In rats and mice, mercuric chloride has increased the incidence of several tumor forms. Mercuric chloride has been identified by the EPA as a possible human carcinogen. It can cause brain damage, intellectual incapacity, coordination issues, convulsions, blindness, and speech abnormalities in the fetus, among other negative effects. In addition to kidney impairment, young children who have mercury poisoning may also experience neurological and digestive problems (Frills 2012).

The production of intracellular reactive oxygen species (ROS), which can cause cellular damage, is linked to the toxicity of mercury chloride (HgCl2) (Ahmad and Mahmood, 2019; Livingstone, 2001). According to Cappello et al. (2016), one important mechanism that starts fish mercury poisoning is oxidative stress.

Additionally, it has been found that elevated mercuric exposure is linked to carotid artery disease, hypertension, and coronary heart disease due to formation of reactive oxygen species (ROS) which are responsible for production of oxidized low density lipoprotein with subsequent atherosclerosis (Asgary et al., 2017; Rizzetti *et al*., 2016).

2.2.4 TOXICOKINETIC OF MERCURIC CHLORIDE

Absorption

ingestion, inhalation, and skin contact (Cappelletti et al., 2019). Inhaling mercury vapor or particulate matter containing mercuric chloride is a common way to be exposed, especially in work contexts (Caglayan et al., 2019; Cappelletti et al., 2019). Many occupational environments, such as

those involving the use of mercury, mining, dentistry, and manufacturing, pose a considerable risk of inhalation exposure for workers (Cappelletti et al., 2019). In certain home or environmental conditions, accidental exposures can also lead to the inhalation of mercuric chloride (Caglayan et al., 2019). Contaminated food or drink may potentially enter the body through the digestive tract and be absorbed (Almeer et al., 2020). Ingestion of contaminated food or water may cause inadvertent mercury chloride absorption. Where industrial activity or environmental pollution have contaminated water bodies with mercury, fish and shellfish may accumulate high levels of mercury (Almeer et al., 2020). After being consumed, mercuric chloride enters the gastrointestinal tract, where its destiny and potential toxicity are ascertained. The stomach's acidic environment can facilitate the release of mercury ions, which can increase the toxicity of mercuric chloride (Zhou et al., 2020). It exits the stomach and enters the bloodstream through the intestinal wall (Zhou et al., 2020).

Distribution

Once swallowed, mercuric chloride enters the bloodstream and travels throughout the body (Skdokur et al., 2020). When interacting with proteins, it strongly prefers sulfhydryl groups (-SH) found in enzymes and other biological components (Masoomi et al., 2020). This binding allows mercuric chloride to be distributed to a range of organs and tissues, including the kidneys, liver, brain, and nervous system (Zhou et al., 2020; Fan et al., 2020).

Metabolism

Mercuric chloride enters the body by multiple routes of exposure, where it undergoes transformations, is transported to different organs and tissues, and is then removed. Mercuric chloride is broken down by the body through a number of enzymatic processes (Gao et al., 2021;

Zhu et al., 2019). According to Fan et al. (2020), one of the primary metabolic processes is the enzymatic conversion of mercuric chloride to inorganic mercury (Hg2+) by enzymes such mercuric reductase. (Caglayan et al., 2019; Skdokur et al., 2020) Inorganic mercury can also change into organic forms, such methylmercury (MeHg), through enzyme activity or microbial action in the gut. Certain varieties of mercury, including methylmercury that is produced from mercuric chloride, can get into and accumulate inbrain tissues after crossing the blood-brain barrier (Gao *et al.,* 2021).

Excretion

Mercuric chloride and its metabolites are primarily excreted through urine and feces (Caglayan et al., 2019; Ali et al., 2020). Urine is the main method of excretion for inorganic mercury, as opposed to feces, which are mainly responsible for the removal of organic mercury molecules such methylmercury (Cappelletti et al., 2019; Caglayan et al., 2019; Zhu et al., 2019).To eliminate this hazardous material and minimize any possible side effects, the body must expel mercuric chloride. According to Caglayan et al. (2019), the kidneys have the ability to filter and remove inorganic mercury from mercuric chloride through the urine. Through this process, part of the ingested mercury might be eliminated by the body. A little amount of mercury can be excreted through sweat. Sweat helps the body eliminate mercury's trace amounts overall, despite being a small method of excretion (Zhu et al., 2019). Breastfeeding mothers may produce breast milk containing mercury and its derivatives. As a result, newborns who are breastfed could be exposed to mercury.

Zhu et al. (2019) state that the elimination half-life of mercury chloride can be impacted by a variety of factors, such as exposure routes, quantities, and individual characteristics. According to Mumtaz et al. (2019) and Gao et al. (2021), the elimination half-life of mercuric chloride in humans can range from a few days to a few weeks. It is possible to convert mercuric chloride to

methylmercury, which has a longer elimination half-life and can stay in the body for months to years (Masoomi *et al.*, 2020).

2.2.5 TOXICITY OF MERCURY

Since mercuric chloride can enter the body through eating, inhalation, or skin contact, it is extremely poisonous and poses major health hazards (Masoomi et al., 2020). Skin, eyes, kidneys, liver, respiratory system, and central nervous system can all be adversely affected by mercuric chloride (Almeer et al., 2020). Mercury poisoning can be fatal and result from high-level or prolonged exposure.

As a result of exposure, red blood cells (RBCs) undergo increased oxidative stress because of the generation of reactive species and changes to the endogenous antioxidant defense system (Almeer et al., 2020). Short-term contact to the chemical causes irritation of the respiratory system as well as deterioration of the skin and eyes (Anyanwu et al., 2020; Ogaly et al., 2022). Tissue damage, collapse, and even death are possible outcomes of the medication's effects on the kidneys and gastrointestinal tract. According to Khatun et al. (2022) cutaneous sensitization may result from prolonged or repeated exposure. According to Johnson-Arbor et al. (2021) and Bist and Choudhary (2022), the medicine may have an impact on the kidneys, peripheral nervous system, and central nervous system. as stated by Johnson-Arbor et al. (2021). Male fertility may be affected by the medicine.

2.2.6 EFFECT OF MERCURIC CHLORIDE ON VARIOUS SYSTEMS OF THE BODY

C.C. Bridges et al. (2010) asserted that oxidative stress has been connected to the development of neurological disorders such as amyotrophic lateral sclerosis, Parkinson's disease, and Alzheimer's disease. Mercury exposure during pregnancy has been linked to decreased neuronal and other

cellular components in pregnant women (P. Grandjean et al., 1997). Because methyl mercury has a great affinity for the sulfhydryl groups of tubulin, it prevents the formation of microtubules, which are essential for the development of the central nervous system. Inorganic mercury has the ability to induce neuronal hyperpolarization through enhancing the permeability of GABA A receptor chloride channels in the dorsal root ganglia (N. K. Mottet et al., 1997). Mercury exposure has also been associated with a higher incidence of hypertension, myocardial infarction, coronary dysfunction, and atherosclerosis, per research by S. Hussain et al. and H. M. Rhee et al. (1989 and 1997, respectively). Mercury's damaging effects were mostly directed towards the central nervous system for a very long period. According to studies by Yoshizawa et al. published in 2002, there is a connection between mercury exposure and the onset of cardiovascular disease and the progression of atherosclerosis. Levels of mercury were correlated with oxidized low-density lipoproteins (LDL). Oxidized LDL particles, which are commonly found in atherosclerotic lesions, have been related to the development of acute coronary insufficiency and atherosclerotic disease (M.C. Houston et al., 2007; JK Virtanen et al., 2005).

2.3 ORGAN OF STUDY.

The cerebellum, also referred to as the tiny brain, is the largest region of the hindbrain and is situated in the posterior cranial fossa, behind the medulla oblongata, pons, and fourth ventricle. Although it cannot start a muscular contraction, it plays a crucial role in synchronizing gait and maintains posture, voluntary muscle activation, and muscle tone. Loss of control over fine motor skills, maintaining posture, and motor learning are consequences of cerebellar damage. Afferent data regarding voluntary muscular movements is sent from the cerebral cortex to the cerebellum via the muscles, tendons, and joints. The vestibular nuclei also supply it with information related to balance. Tentorium cerebella, a dura matter extension, divides the cerebellum from the cerebrum. It

is composed of two hemispheres connected by the vermis, and it is further divided into anterior, posterior, and flocculonodular lobes, each of which is divided by a pair of transverse fissures. The anterior and posterior lobes are divided by the V-shaped primary fissure, while the posterior and flocculonodular lobes are divided by the posterolateral fissure. There is a deep horizontal gap between the superior and inferior surfaces of the cerebellum in the posterior lobe. According to Rootaei et al. (2014) and Van Essen et al. (2018), the cerebellum is home to 80% of the brain's neurons, which are arranged in a dense cellular layerThe cerebellar cortex is composed of accordion-like folds that are joined at the midline, and a single sheet that is less than 1 mm thick (Essen 2018). The inner white matter core of each fold is encased in a layer of gray matter. The three layers of the cortex's gray matter are the molecular layer on the outside, the Purkinje cell layer in the middle, and the granular layer on the inside. According to Van Essen et al. (2018), there are two different kinds of neurons in the molecular layer: inner basket cells and outer stellate cells.

Large Golgi type I neurons called Purkinje cells make up the Purkinje layer. Their dendrites have several branches and reach the molecular layer. The long axons end in the intracerebellar nuclei after passing through the granular layer, entering the white matter, and acquiring a myelin coating. Their collateral branches connect synaptically with the granular layer's stellate cells and basket. The cerebellar cortex receives its principal input from mossy and climbing fibers. The primary excitatory neurotransmitter used by ascending fibers is aspartate, whereas glutamate is used by mossy fibers to excite Purkinje cells. The climbing fibers get their name from the way they move across the cortex, resembling a tree's vine branches. They stand for the olivocerebellar tracts' terminal terminus. The terminal branches of every other cerebellar afferent tract are the mossy fibers. Via many branches, each mossy fiber has the potential to activate thousands of Purkinje cells (Van Essen et al., 2018; Yang et al., 2014).

2.3.1 Function: The trunk, which includes the neck, shoulders, thorax, belly, and hips, is coordinated by the cortex of the vermis. The intermediate zone of the cerebellar hemispheres, which is next to the vermis, is responsible for controlling the muscles of the distal extremity. The planning of successive movements of the whole body and the conscious evaluation of movement faults are functions of the remaining lateral portion of each cerebellar hemisphere (Manto et al., 2012).

Nuclei: The three pairs of deep cerebellar nuclei embedded in the central cerebellar white matter (corpus medullaris) are surrounded by a highly branched body of white matter known as the arbor vitae, which is Latin for "tree of life." The cerebellum is made up of an outer layer of highly convoluted gray matter called the cerebellar cortex. According to Roostaei et al. (2014), the largest nucleus in the deep nuclei are dentate, which is the fastigial, interposed (which includes globose and emboliform nuclei), and lateral nuclei. Through the superior cerebellar peduncle, fibers from the dentate, emboliform, and globose nuclei exit the cerebellum. According to Akakin et al. (2014) and Roostaei et al. (2014), the inferior cerebellar peduncle is where fibers from the fastigial nucleus leave.

2.3.2 EMBRYOLOGY OF THE CEREBELLUM

The vesicle of the hindbrain that forms the metencephalon's posterior alar plates is where the cerebellum develops. By the twelfth week, the cerebellar hemisphere and vermis form. Around the fourth month, accordion-like folds begin to progressively form. In the ventricular zone, neuroblasts generated from matrix cells form the neurons of the cerebellar cortex. Additional neuroblasts from the ventricular surface give rise to cerebellar nuclei, from which the superior cerebellar peduncle is formed by axons that extend toward the mesencephalon (midbrain). The middle cerebellar peduncle will eventually develop and link the cerebral cortex and cerebellum through projections of the axons of the corticopontine and the pontocerebellar fibers. Mainly, sensory axons from the

vestibular and olivary nuclei of the spinal cord will develop to produce the inferior cerebellar peduncle (Haldipur et.al., 2018).

2.3.3 HISTOLOGY OF THE CEREBELLUM

A region of the cerebellum resembles a cauliflower due to the white matter's stemmed appearance, which is covered in gray matter from the cortex's outer layer. The lobulations and folia of the cerebellum are visible when looking at the cerebellar tissue under a microscope. These folia are the cerebellar cortex's leaf-like gyri. Three layers make up the gray matter: the inner granular layer, the middle layer of purkinje fibers, and the outside molecular layer. The cerebellum's motor tasks are facilitated by the abundance of neurons, glial cells, and fibers found in the cortex.

Molecular layer: Due to its synaptic nature, the outer molecular layer has the lowest cell density and is home to numerous granule cell axons and Purkinje cell dendrites. This layer contains basket cells and stellate cells that are superficially positioned (William et al., 2012). (Ross M. H., Pawlina W).

Typically, stellate cells have short dendrites that come into contact with a tiny number of dendrites from Purkinje cells. Conversely, basket cells possess broad dendritic processes that enable them to establish contact with a significantly higher quantity of Purkinje cells. According to William et al. (2012), both cells show inhibitory influence on the Purkinje cells after receiving excitatory input from the parallel fibers (Ross M. H., Pawlina W).

The Middle Layer (Purkinje cell layer): Consists of a single layer of large pear-shaped Purkinje cells. Their cell bodies are largest in the cerebellum with unique and distinct appearance. The dendrites of these cells reside in the molecular layer, while their axons project deep through the granular layer and synapse into the deep nuclei of cerebellum. (William *et al.*, 2012), (Ross M. H., Pawlina W).

The Inner Layer (Granular cell layer): This layer contains many, tightly packed granule cells and Golgi type II cells. Granule cells, which are among the smallest neurons in the brain almost 5µm in diameter with round to oval in shape, usually represent the extensions of the mossy fibers. Their axons extend into the outer molecular layer where they branch in T shape forming parallel fibers and synapse with the dendrites of Purkinje, basket and stellate cells. The nuclei of these granule cells generally stain dark, giving the whole granular layer a darker appearance compared to the white matter and molecular layer of the cortex. Golgi cells are also scattered throughout the granular layer, with their dendrites branching out in the molecular layer, while their axons synapses with the granule cells.Since there are no cell bodies in the inner medulla of white matter, it will stain a lighter color than the cortex of grey matter. It is made up of nerve fibers that support small blood arteries and neuroglial cells. (Williams et al., 2012), (Pawlina W. and Ross M. H).

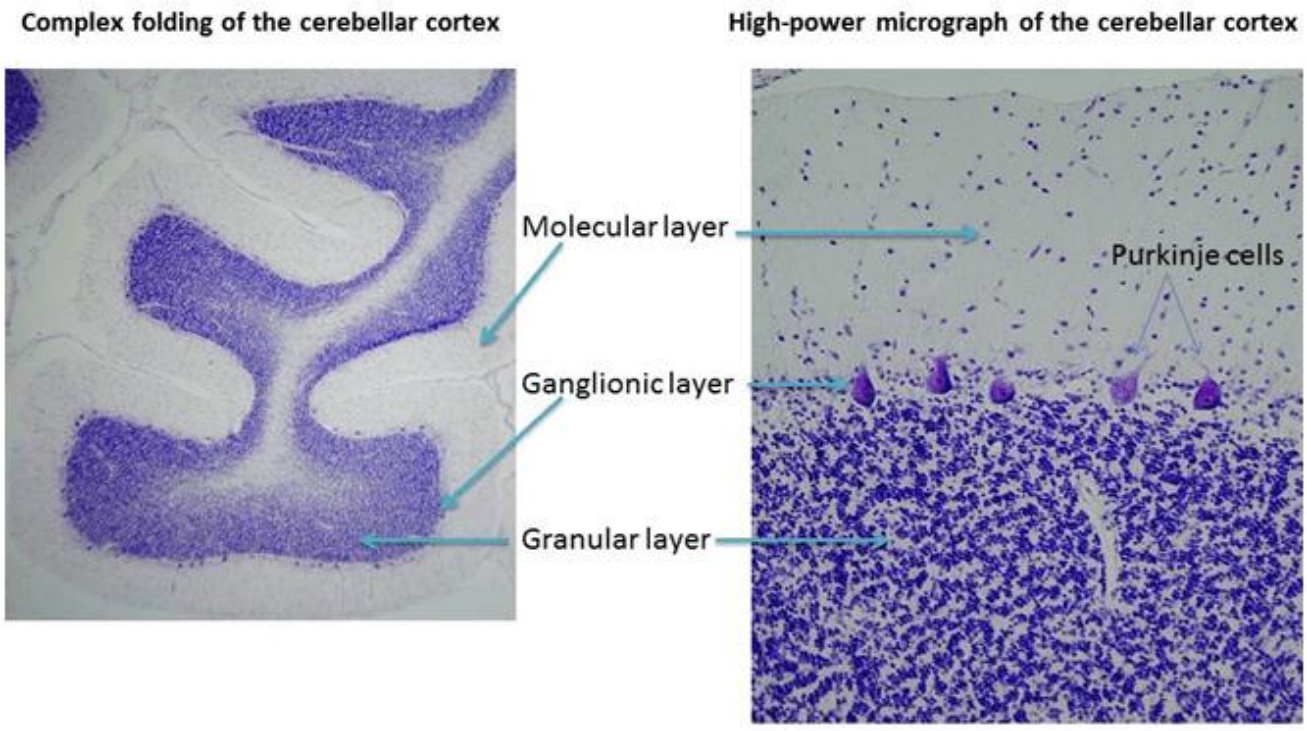


Fig 2.3 Showing The Different Histological Layers Of The Cerebellum.

SOURCE:SISU@UT

2.3.4 BLOOD SUPPLY TO THE CEREBELLUM

Three major arteries that emerge from the vertebrobasilar anterior system feed blood to the cerebellum: the posterior inferior cerebellar artery (PICA), the anterior inferior cerebellar artery (AICA), and the superior cerebellar artery (SCA).

Depending on the embryology, the SCA can branch either directly from the posterior cerebral artery and pass above the oculomotor nerve, or it can branch from the junction point of the basilar artery and posterior cerebral artery and pass below the oculomotor nerve. The SCA surrounds the brainstem above the trigeminal nerve and below the oculomotor nerve in most patients. The medial and lateral branches emerge from the SCA's divide. The superior part of the vermis and the superomedial cerebellar cortex are supplied by the second branch of the SCA, which further divides into two branches. The first branch supplies the mesencephalon and the inferior and superior colliculi. The superolateral cerebellar cortex receives its energy from the SCA's lateral branch. On fetal ultrasonography, blood vessels are more echogenic due to their deeper penetration in the vermis. 2017; Delion et al.; 2016; Matsushima et al.

In nearly every subject, the AICA splits out from the basilar stem. It crosses the abducens nerve and, at the cerebellopontine angle, it joins the facial and vestibulocochlear nerves. Then it splits into two branches, one of which nourishes the middle cerebellar peduncle, the flocculus, and the choroid plexus, and the other the anterior inferior cerebellum.

PICA is the biggest branch of the vertebral artery. It supplies the cerebellar nuclei, the inferior surface of the vermis, and the undersurface area of the cerebellar hemisphere as it travels between the cerebellum and the medulla. PICA supplies the medulla oblongata and the choroid plexus of the fourth ventricle; in certain anatomical alterations, this may result in the posterior spinal arteries. Veins draining the cerebellum empty into the nearby venous sinuses or the great cerebral vein (Delion et.al., 2017).

2.3.5 NERVE SUPPLY

Three sets of nerve fibers known as the superior, middle, and inferior cerebellar peduncles connect the cerebellum to the brainstem. Efferent and afferent fibers go through these groups to connect to the rest of the nervous system.According to (Roostaei et al., 2014) and (Manto et al., 2012).

2.3.6 CLINICAL CORRELATES

Each cerebellar hemisphere controls the same side of the body, thus if damaged the symptoms will occur ipsilaterally.

Hypotonia: The muscles become less resistant to palpation as a result of the cerebellum's gamma motor neurons having less of an impact.

Ataxia: Ataxia is a disorder characterized by tremor in voluntary motions, including writing and buttoning garments.

Dysdiadochokinesia: The inability to execute quick, alternating motions is known as dysdiadochokinesia. On the side of the cerebellar lesion, movements will be sluggish and imperfect.Manto et al. (2018), Marsden et al. (2018), and Javalkar et al. (2014)

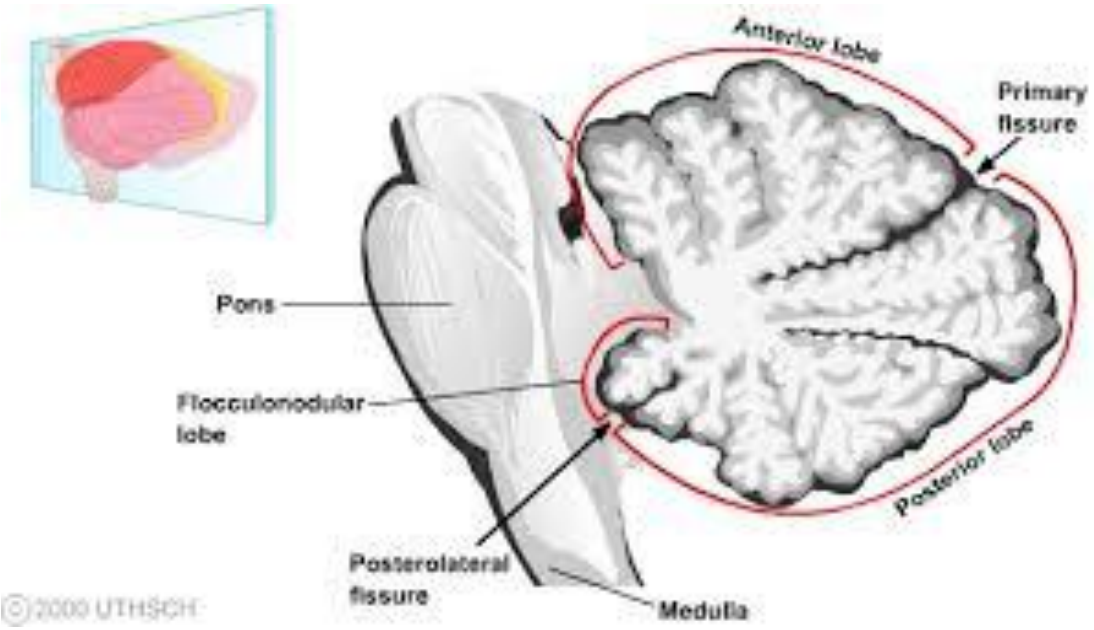


Fig 2.4 diagram showing the structure of the Cerebellum

SOURCE:James Knierim, Ph.D., Department of Neuroscience, The Johns Hopkins University

CHAPTER THREE

MATERIALS AND METHOD

3.1 MATERIALS

Ethanol, Mercury Chloride(toxicant), Orogastric tubes, cotton wool, dissecting scissors, universal bottle, plain bottles, surgical blade, beakers, sample bottles, distilled water,plastic cages, ceramic plates, disposable gloves, dissecting table, formalin, chloroform, sawdust,formal saline, weighing scale, animal feed and *Centella asciatica*(extract).

3.2 PLANT MATERIALS AND PREPARATION OF EXTRACT

Centella asciatica was sourced from a farm in Bonny Island, Rivers State. Authenticated at the Department of Plant Biology and Biotechnology University of Benin with herbarium number UBH-C601

3.2.1 EXTRACTION OF PLANT

10,600g of leaves were utilized in this investigation. The British milling machine Viking Exclusive Joncod (Type YL112M-2) was used to grind the leaves into a fine powder after they were allowed to air dry at ambient temperature. After soaking the powdered leaves in distilled water for a full day, the mixture was filtered using 110mm filter paper that was placed atop a funnel. A water bath was used to evaporate the filtrate at 40ºC, yielding 254g in the end. The remnant was kept refrigerated at the University of Benin, Benin City, at the Department of Anatomy. To be used in the experiment, an aliquot of the residue was dissolved in the proper amount of distilled water.

3.2.2 PREPARATION OF SAMPLE

Little changes were made to the procedure outlined by Aiyegoro and Oko (2010) in order to extract Centella asiatica. First, the Centella asiatica was dried for around four hours at 100 degrees Celsius in the oven. After that, Centella asiatica was dried and ground into a powder.

In distilled water (10 L) at room temperature on a shaker for 48 hours, the powdered Centella asiatica (1.00 kg) was extracted three times. A Buchner funnel and Whatman No. 1 filter paper were

used to filter the extract. After being rapidly frozen at -40°C, the resulting aqueous extract filtrate was dried for 48 hours in a freeze drier. To obtain the appropriate concentrations for this investigation, the resultant extract was reconstituted with distilled water.

Estimation of tannins content

Quantitative Determination of Tannin

Exactly 0.20 mL of sample was added to 20 mL of 50% methanol and placed in a water bath at 77˚C - 80˚C for

1 hr and shaken. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper and

20 mL of distilled water, 2.5 mL Folin-Denis reagent and 10 mL 17% Na2CO3 were added and mixed. The mixture was allowed to stand for 20 min. A series of standard tannic acids solutions were prepared in methanol and their absorbance as well as samples was read after colour development on a UV/ Visible spectrophotometer at a wavelength of 760 nm. Total tannin content was calculated from calibration curve.

Determination of total phenolic contents

Tannic acid was used as a reference to calculate the total phenolic content of the extract using the Folin-Ciocalteu reagent, slightly altering the Singleton and Rossi (1965) technique.

In a nutshell, 1.0 milliliter of the extract solution (250 Ug/ml) was put to a test tube. After that, 1.0 milliliter of Folin-Ciocalteu reagent was added, and the flask's contents were well combined. 15.0 ml of 20% Na2CO3 was added after 5 minutes, and the mixture was left to stand for two hours. The absorbance was determined at 760 nm with a Jenway 6100 UV-Vis spectrophotometer located in Dunmow, Essex, United Kingdom. Using an equation derived from the standard tannic acid calibration graph, the total phenolic content was calculated as Ug of tannic acid equivalent (TAE).

Determination of Alkaloids Content

The method outlined by Harborne (1973) was used to measure the total alkaloid content. A 250 mL beaker was filled with 5g of the extract, 100 mL of 20% acetic acid in ethanol, and the lid was closed. The mixture was let to stand for two hours. After filtering, the extract was reduced to a quarter of its initial volume by utilizing a water bath. Drop by drop, concentrated

ammonium hydroxide was added to the extract until the precipitate was fully formed. Following a period of time for the entire solution to settle, the precipitate was filtered, cleaned with 1% ammonia solution, dried, and weighed. Three duplicates of each sample were examined.

$$\text{Alkaloid (\%)} = \frac{\text{Weight of residue}}{\text{weight of sample}} \times 100$$

Flavonoid content determination

On triplicate aliquots of the homogeneous cabbage extract (1.5 g), the flavonoid concentration was measured (Ilahy et al., 2011). For the purpose of determining the flavonoids, thirty-microliter aliquots of the methanolic extract were utilized. The samples were diluted with 90 µL of methanol, followed by the addition of 6 µL of 10% aluminum chloride (AlCl3), 6 µL of 1mol/l sodium acetate (CH3CO2Na), and 170 µL of methanol. After 30 minutes, the absorbance was measured at 415 nm. Utilizing quercetin as a reference, the flavonoid concentration (Ug Qe/g) was determined.

Estimation of total saponins content

With few adjustments, the Makkar et al.-described approach based on the vanillin-sulphuric acid colorimetric reaction was used to estimate the total saponin content. 250 µL of distilled water were added along with about 50 µL of plant extract. About 250 µL of vanillin reagent—800 mg of vanillin in 10 milliliters of 99.5% ethanol—was added to this. The mixture was thoroughly stirred after adding 2.5 mL of 72% sulfuric acid. For ten minutes, this solution was maintained in a water bath at 60°C. Following a 10-minute cooling period in ice-cold water, the absorbance at 570 nm was measured. From the saponin stock solution, standard saponin solutions ranging from 0 to 25 ppm were made. The test samples were handled in the same way as the standard solutions. The values were given in PPM units.

PROXIMATE ANALYSIS

Ash Content

2g of the dried sample was placed into a porcelain crucible which initially was weigh and transform into a preheated muffle furnace set at the temperature of 900^0C. The furnace was left on for one hour after which the crucible and it content was transferred to a decicator and allowed to cool the crucible and it content was re-weigh and the weigh noted. The percentage ash content was then calculated from the relationship.

$$\text{Ash} = \frac{100W_{ash}(\%)}{W_0}$$

W_{ash} = content weight after final drying.

W_0 = the dried weight of the sample

Moisture Content

2g of the sample was weighed and dried in an oven continuously. The dried sample was constantly re-weighed at 10 minutes intervals until a constant weight was obtained. The ratio of the change in weight to the original weight expressed in percentage gives the moisture content given by

$$\frac{W_0 - W_{dry}(\%)}{W_0}$$

Crude fibre determination: This was completed in accordance with the AOAC (1980) method. In a nutshell, 4 g of each moisture-free sample were weighed into a 250 mL beaker, and then 50 mL of 4% H2SO4 and 200 mL of distilled water were added. Then, using a glass rod with a rubber tip, this was brought to a rolling boil and maintained there for precisely thirty minutes, stirring continuously to ensure that all of the particles were removed from the beaker's edges. Hot distilled water was added to maintain a constant volume. Following 30 minutes of boiling, the mixture was transferred into a butchner funnel that was lined with an ash-free what man no. 40 filter paper and attached to a vacuum pump. Many times, hot distilled water was used to wash the beaker then it was transferred

quantitatively with a jet of hot water. Washing continued on the funnel until the filtrate was acid-free as indicated by litmus paper. The acid-free residue was transferred quantitatively from the filter paper into the same beaker removing the last traces with 5% NaOH solution and hot water to a volume of 200 mL. The mixture was boiled for 30 min with constant stirring as earlier described, keeping the volume constant with hot water. The mixture was then filtered and washed as earlier described until its alkaline free. Finally, the resultant residue was washed with two portions of 2 mL 95% alcohol. Residues on filter paper were transferred to a pre-weighed porclain crucible. The content of the crucible was then dried in an oven maintained at 110°C to a constant weight after cooling in a desiccator. Crucible content was then ignited in a muffle furnace at 550°C for 8 h, cooled and weighed. A triplicate determination was carried out on each sample. The percentage crude fibre was therefore

Calculated as:

$$\% \text{ Crude Fibre} = \frac{100(y - a)}{x}$$

x = Weight of sample (g)

y = Weight of insoluble matter (g)

a = Weight of Ash (g)

Crude fat determination*:* The method of Pearson (1973) was employed; this method was based on the principle that non-polar components of samples are easily extracted into organic solvents.

Procedure: Each sample was weighed at three grams (moisture-free) and put into fat-free thimbles. Following that, these were weighed, glass wool-plugged, and added to soxhlet extractors holding 160 mL of petroleum ether (b.p. 60–80°C). Receiver flask, dry and clean, weighed and adjusted for the extractors. After that, the extraction unit was put together and the water bath was kept at 60°C with the flow of cold water enabled. The extraction process took eight hours. After this period of time, the thimble containing the sample was taken out and dried to constant weight in an oven set at 70°C for three hours. After that, a typical analytical balance was used to determine the weight of the Thimble and its contents. The unprocessed fat was acquired as the difference in weight before and after the exhaustive extraction.

Hence the percentage fat was therefore calculated as:

$$\%FAT = \frac{X - Y}{Z}$$

where,

x = Weight sample and thimble and oil

Y = Weight of empty thimble

Z = Weight of sample

Crude protein determination: For the purpose of determining crude protein, a modified version of the AOAC (1990) micro-Kjeldahl method was employed. The digestive process: Each of the defatted samples was weighed separately into a micro-Kjeldahl digestion flask containing three grams, along with a small amount of anti-bumping granules, and preweighed. In each flask, two grams of the catalyst combination (CuSO4: Na2SO4: SeO2, 5:1:02 w/w) were added. Next, 10 milliliters of nitrogen-free concentrated H2SO4 were added. The flasks were set in a fume cupboard, tilted on a heating mantle. Following 30 minutes of frothing-free heating at 30°C, the temperature was raised to 50°C for another 30 minutes, and eventually, complete heating (100°C) was reached to produce a clear solution. Another round of simmering below the boiling point was conducted for 30minutes to ensure complete digestion and conversion of nitrogen to ammonium sulphate. After digestion was completed, samples were allowed to cool and then transferred quantitatively to 100 mL volumetric flasks with washing and cooling to room temperature. Volumes were made up to mark with distilled water.

1. Using a 10 ml pipette, 5 ml of the digest's filtrate was placed into a 25 ml standard flask. After adding 2.5 milliliters of Alkaline Phenate, the mixture was well mixed by shaking it. After thoroughly shaking, 1 milliliter of sodium potassium tartrate was added, and then 2.5 milliliters of sodium hypochlorite. After that, distilled water was added to make the solution up to the 25 ml mark, and the absorbance of the finished product was measured at 630 nm

using a UV/visible spectrophotometer. The sample was handled in the same manner as the nitrogen standards**.**

CALCULATION

$$\%N = \frac{\text{Instrument Reading} \times \text{Slope Reciprocal} \times \text{Color Volume} \times \text{Digest Volume}}{\text{Weight of Sample} \times \text{Aliquot Taken}}$$

% Crude Protein = %Nitrogen×6.25 (AOAC, 1975)

Estimation of total carbohydrate: The total carbohydrate content of the diet samples was obtained by subtracting the sum of percentage crude protein, crude fat, Moisture, Fibre and ash from 100.

3.3 CHEMICALS

All chemicals used in the present study was purchased from the Chemistry Laboratory University of Benin, Edo State, Nigeria.

3.4 EXPERIMENTAL ANIMALS

Twenty adult Wistarrats, Eight weeks old, with average weight of 180g were purchased and housed in the Animal Laboratory, Department of Anatomy, University of Benin, Edo State, Nigeria under standard conditions(12 hours light and 12 hours dark cycle) throughout the duration of the experiment. They were allowed free access to food and water and were allowed to acclimatize to these housing conditions for 2 weeks prior to the experiment in which they were divided into five experimental groups each having five Wistar rats labelled A-E.

3.5 GROUPING OF ANIMALS

Group A - Control

Group B - $HgCl_2$ Only

Group C - $HgCl_2$ + 250mg/kg body weight of extract + $HgCl_2$500mg of extract only

Group D - $HgCl_2$ +500mg/kg body weight of extract

Group E - 500mg /kg body weight of extract only.

4g of the extract was concentrated with 100ml of water and administered to the animals in different doses according to their weights and the toxicant was administered to the animals one hour after the administration of the extract for 21days after which they were sacrificed. Administration was done orally using orogastric tube.

3.6 SACRIFICING THE ANIMALS

The animals were kept one after the other in a closed container containing chloroform anesthesia which made them unconcious. Then they were brought out one after the other and placed on a dissecting table where they were cut open using a dissecting scissors and their blood samples were collected using a 1.5ml syringe from the heart and poured in a well labeled plain bottle while their organs i.e cerebrum and cerebellum where harvested using a surgical blade and kept in a well labeled universal bottle containing formal saline.

3.7 BIOCHEMICAL ANALYSIS

Blood samples collected were taken to the laboratory to test for oxidative stress parameters such as SOD, MDA, Gluthatione, catalase and Gluthatione peroxide.

PROCEDURES

Determination of MDA Concentration

The approach of Guttridge and Wilkins (1982), which was a modification of Placer et al. (1966), was followed in order to determine the concentration of MDA. This assay is based on the idea that MDA, a byproduct of lipid peroxidation, generates a pink or crimson complex at 532 nm when heated with thiobarbituric acid (TBA) in the presence of an acid.

Assay Procedure

After adding an aliquot of the liver homogenate to 3.0 mL of TCA-TBA-HCl reagent, the mixture was well combined by spinning. The mixture was brought to a boil and left for fifteen minutes. Centrifugation at 1000 g for 10 min was used to extract the flocculent precipitate after cooling. At 535 nm, the clear supernatant's absorbance was calculated in relation to a reference blank..

Calculation

The MDA concentration of each sample was calculated as shown

$$\frac{O.D \times V_t \times 1000}{a \times V \times L \times Y}$$

where,

O.D = Absorbance of sample test at 535 nm

V_t = Total volume of the reaction mixture

a = Molar extinction coefficient of the product

L = Light path = 1.0 cm

V = Volume of sample homogenate used

Y = mg of tissue in the sample used

The unit of MDA is moles/mg wet tissue

Determination of Superoxide Dismutase (SOD) Activity
Principle

The Misra and Fridovich (1972) approach was used to evaluate the activity of SOD. The concentration of adrenochrome, which is produced when adrenaline auto-oxidizes quickly in an

aqueous solution, can be measured spectrophotometrically at 420 nm. Superoxide anions (O2-) must be present for auto-oxidation to occur. By catalyzing the breakdown of superoxide anions, superoxide dismutase (SOD) suppresses this auto-oxidation. Thus, SOD activity is measured by the degree of inhibition. An enzyme is said to be active when it produces 50% inhibition, or one unit.

Assay Procedure

2.5 mL of 0.05 M carbonate buffer (pH 10.2) was mixed with 0.2 mL of liver homogenate, and the mixture was left to equilibrate. 0.3 mL of newly made adrenaline (0.03 mM) was added as substrate to start the reaction. By inversion, the solution was combined. There were 2.7 mL of carbonate buffer and 0.3 mL of adrenaline in the reference tube and 2.5 mL of carbonate buffer, 0.2 mL of distilled water, and 0.3 mL of 0.03 mM adrenaline in the blank tube. For a duration of 120 seconds, the rise in absorbance at 420 nm resulting from the production of adrenochrome was observed every 30 seconds. It was determined that one unit of SOD activity was required to produce 50% inhibition of the oxidation of adrenaline to adrenochrome within 120 seconds.

Calculation

$$\%\ \text{inhibition} = \frac{\text{O. D}_{\text{test}} - \text{O. D}_{\text{reference}}}{\text{O. D}_{\text{test}}} \times \frac{100}{1}$$

$$\text{Enzyme Activity (units/mg protein)} = \frac{\%\text{inhibition}}{50 \times \text{Y}}$$

Where Y = mg of protein in the volume of the sample.

A unit of SOD activity was taken as the amount of SOD required to cause 50 % inhibition of the auto-oxidation of adrenaline to adrenochrome per minute.

Determination of Catalase Activity
Principle

This is predicated on Cohen.et al.'s (1970) approach. Based on the measurement of hydrogen peroxide's (H_2O_2) rate of breakdown following the addition of the enzyme-containing material, this estimate was made.

It is catalyzed by catalase: $O_2 + H_2O < 2H_2O_2$

The amount of hydrogen peroxide that decomposes is directly correlated with the enzyme concentration present in the sample. Measurement of the hydrogen peroxide generated in tissues involves reacting it with excess potassium permanganate ($KMNO_4$) and then using spectrophotometry to quantify the remaining $KMNO_4$ at 480 nm.

Assay Procedure

Test tubes with ice cold water were filled with 0.5 mL of liver homogenate, whereas the blank held 0.5 mL of distilled water. The sample and blank tubes were filled with 30 mM of cold, phosphate-buffered H_2O_2 (5 mL) at predetermined intervals, and the mixture was mixed by inversion. One milliliter of 6 M H_2SO_4 was quickly added to halt the reaction after three minutes. Following a thorough inversion of the tubes, 7 mL of 0.01 M $KMNO_4$ was added. It took three minutes to read the absorbance at 480 nm.

Calculation

The activity of catalase in each sample is calculated thus:

$$\frac{O.D/min \times Vt \times 1000}{M \times V \times L \times Y}$$

where,

O.D = Absorbance of sample test at 480 nm

Vt = Total volume of the reaction mixture = 13.5 mL

M = Molar extinction coefficient of H_2O_2 = $43.6M^{-1}$ cm^{-1}

L = Light path = 1.0 cm

V = Volume of sample homogenate used = 0.5 mL

Y = mg of protein in the tissue used

Determination of Glutathione Peroxidase Activity

Glutathione peroxidase (GPx) activity was measured according to the method described by Nyman (1959).

Principle

This is based on the oxidation of pyrogallol to purpuragallin by peroxidase, resulting in a deep brown colouration, which is read at 430 nm.

Procedure

To an aliquot of plasma (0.2 mL), 5 mL of phosphate-buffered H_2O_2, and 1.5 mL of pyrogallol were added. The reaction mixture was allowed to stand for 30 min at room temperature. A deep colour was formed, which was read at 430 nm.

Calculation

$$\text{Enzyme Activity} = \frac{\text{OD/min} \times \text{Vt} \times \text{Df}}{\text{E} \times \text{Vs} \times \text{Y}}$$

where OD = Absorbance of test

Vt = Total volume of the reaction mixture

Df = Dilution factor

E = Molar extinction coefficient (12/M/cm)

Vs = Volume of sample

Y = mg of protein used

Determination of Reduced Glutathione Concentration

The plasma concentration of reduced glutathione (GSH) was determined using the method described by Ellman (1959).

Reagents

5, 5^1-dithiobis-2-nitrobenzoic acid (DTNB), sodium citrate, and trichloroacetic acid (TCA)

Procedure

To 1.0 mL of plasma, 2.5 mL of 10 % TCA was added and centrifuged at 3000 g for 10 min. Then, 1.0 mL of the supernatant was treated with 0.5 mL of Ellman's reagent (0.0189 % DTNB and 1 % sodium citrate) and 3.0 mL of 0.3 M phosphate buffer (pH 8.0). The yellow colour developed was read immediately at 412 nm and expressed as µM GSH/g plasma.

Calculation

$$\text{The concentration of GSH} = \frac{A_{test} \times \text{Conc. of Standard}}{A_{standard}}$$

$$\% \text{ Glutathione Reduced} = \frac{(A_0 - A_1) \times 100}{A_0}$$

where A_0 = Absorbance of reference sample

A_1 = Absorbance of sample

3.8 HISTOLOGICAL ANALYSIS

3.8.1 PARAFFIN TISSUE PROCESSING

Tissue Processing according to Drury and Wellington (1980)

Following the fixation of the harvested tissues (cerebrum and cerebellum) in 10% formalin. The tissues were allowed to stand for about 72hours to achieve good tissue penetration, the tissues were processed as follows;

. Dehydration of tissues in an increasing grade of alcohol (from 70% to 90% and absolute alcohol) using ethanol as the choice of alcohol.

. Xylene as a clearing agent was used in clearing alcohol. Tissues were allowed to pass through two changes for the removal of alcohol from tissue samples.

.The tissues were infiltrated in three changes in a solution of molten paraffin wax in an oven at a temperature of 65-70oC. The first two changes were carried out for 15 mins each while the last change was for about 30 minutes.

. Embedding was carried out using an embedding mould, into which the molten paraffin wax was poured and the infiltrated tissues were placed in it in a longitudinal orientation to produce longitudinal section.

.The molten paraffin wax was allowed to cool, which then result in solidification to form tissue blocks. **.**Sectioning of the tissue blocks was done with the aid of a rotary microtome to cut tissue into thin-like sections of 5 microns' thickness.

3.8.2 HEMATOXYLIN AND EOSIN STAINING METHOD

A water bath at 30 degrees Celsius was used to cut and float the paraffin sections after satisfactory and good tissue portions that emerged as ribbons were chosen and soaked in 20% alcohol.

.The sectioned tissues were taken up on glass slides and allowed to air-dry

.• To remove superfluous paraffin wax from the tissue sections, they were immersed in xylene for 15 minutes. After that, they were hydrated by being passed through descending alcohol grades

(100%, 90%, and 70%) and finally into water, each of which lasted for roughly 5 minutes. The tissue was stained with HandE dyes. Hematoxylin was used to stain the tissues for ten minutes. Blueing is the term for washing tissues under running water.

- Sections were counter-stained with 1% Eosin for 5-10 minutes.
- Tissues were then rinsed in water.
- After that, the tissues underwent a quick dehydration process using increasing alcohol grades (from 70% to 90% and absolute alcohol) for five minutes. After five minutes of xylene clearing the tissue, the slides were mounted with a glass cover slip using an appropriate mountant, Xylene and Distrene Plasticizer (DPX).

3.9 DATA ANALYSIS

With the IBM statistical software for social science (SPSS), the data was analyzed. After cleaning the data, the Kolmogorov-Smirnov test was used to determine if it was normal. Whereas the organ weights were examined using post-hoc LSD and one-way analysis of variance (ANOVA), the animal body weights were examined using the dependent t test. Values were deemed significant at $p<0.05$, and tables and charts were utilized to show the results. Each animal research group was regarded as a single experimental unit, and all of the data produced for each animal were incorporated in the analysis.

CHAPTER FOUR

RESULTS

4.1 PHYTOCHEMICAL RESULTS

4.1.1 QUALITATIVE ANALYSIS

PARAMETER	PRESENCE
CARBOHYDRATE	+
REDUCING SUGAR	+
PHENOLIC	+
FLAVONIOD	+
STERIOD	++
ALKANOID	+
TANNIN	+
SAPONIN	++

4.1.2 QUANTITATIVE ANALYSIS

PROXIMATE ANALYSIS

PARAMETER	UNIT (%)
Moisture content	31.41
Ash content	3.32
Crude fat	1.125
Crude fibre	0.49
Crude Protein	10.76
Carbohydrate	53.67
Alkaloid	10.18

4.2 CHANGE IN BODY WEIGHT

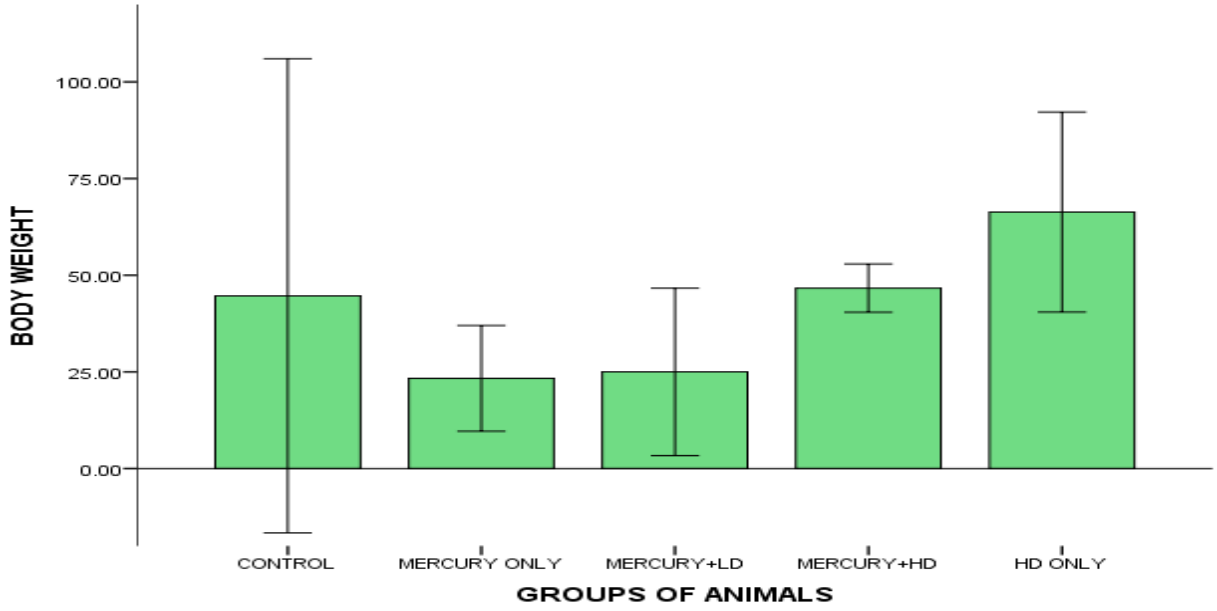


Fig 4.1 Bar chart showing the difference between the initial and final body weights of animals. There was no statistical significance among all the groups.

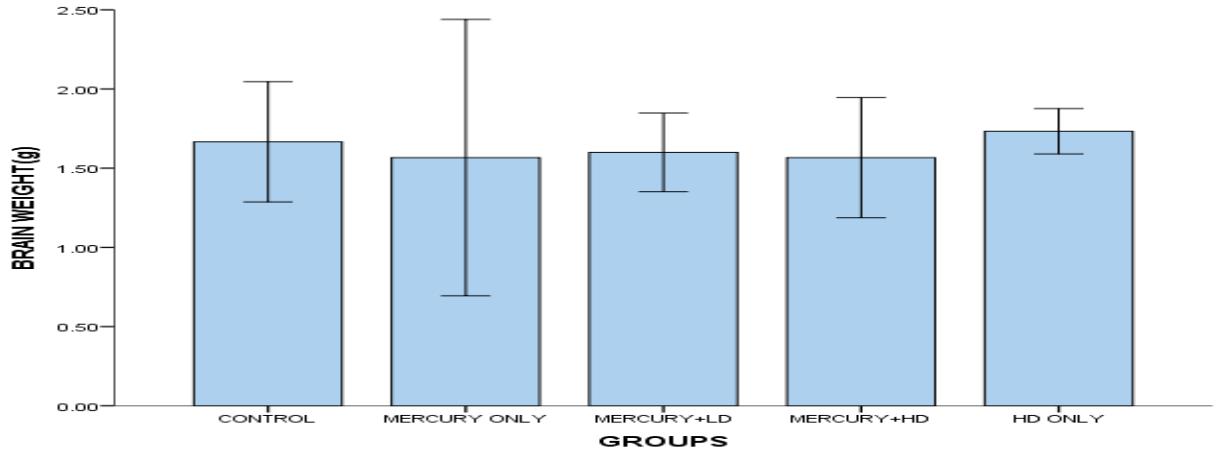


Fig 4.2 Bar chart showing the the mean brain weight of animals. There was no statistical significance among all the groups

4.3 RELATIVE BRAIN WEIGHT

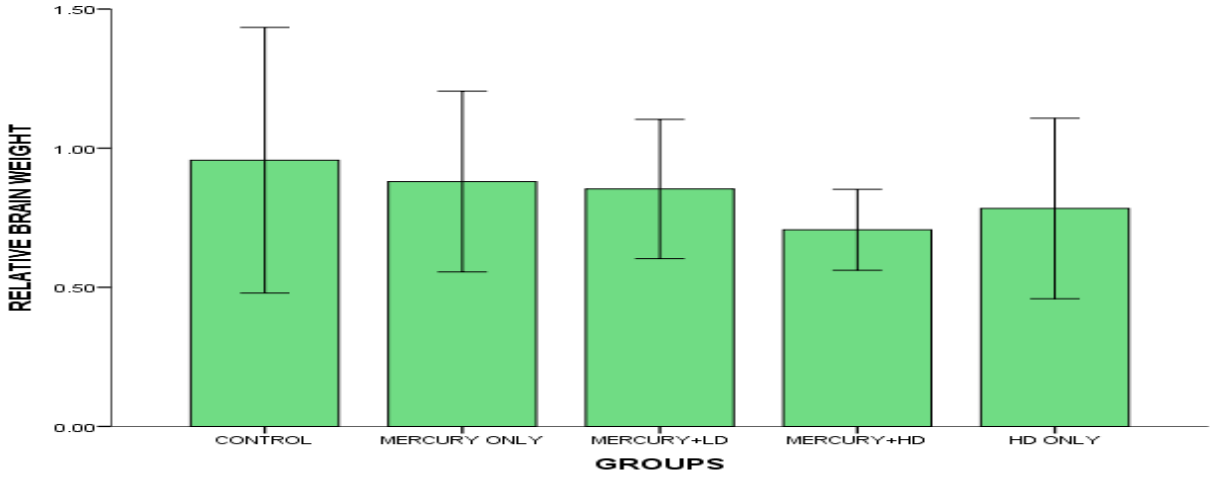


Fig 4.3 Bar chart showing the relative organ weights of animals. There was no statistical significance among all the groups

4.4 OXIDATIVE STRESS

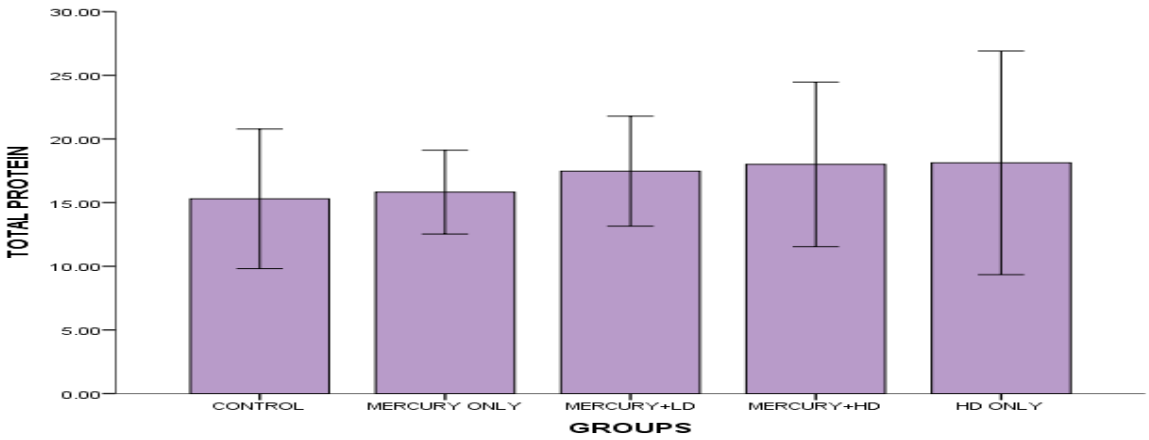


Fig 4.4 Bar chart showing the total protein in serum of experimental animals. Result shows no statistical significance among all groups

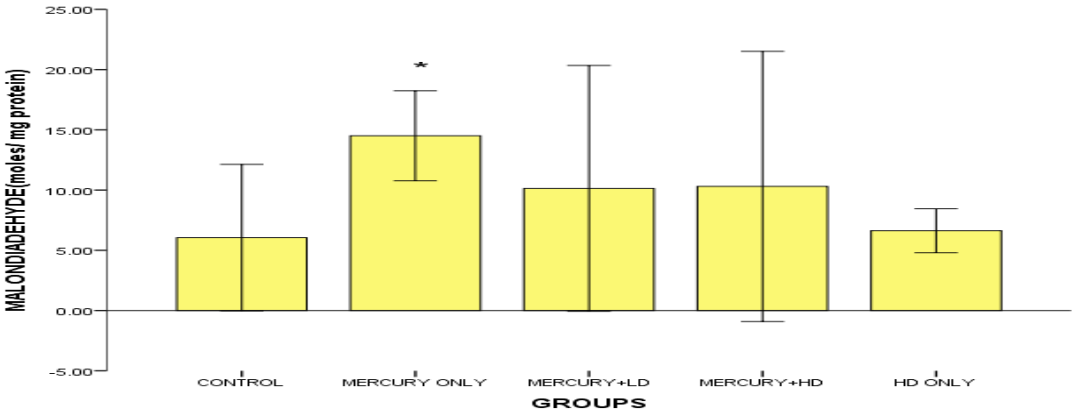


Fig 4.5 Bar chart showing test for malondiadehyde in serum of experimental animals. Result shows a statistically significant increase in the group administered with mercuric chloridealone.

*- indicates $p<0.05$ when compared to control

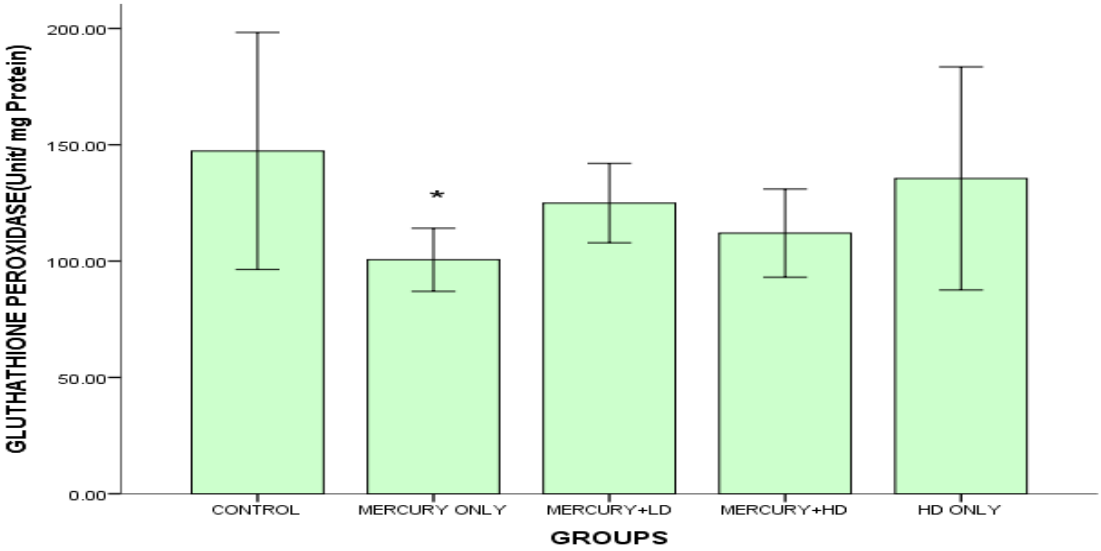


Fig 4.6 Bar chart showing test for glutathione peroxidase in serum of experimental animals. Result shows a statistically significant decrease in the group administered with mercuric chloridealone.

*- indicates $p<0.05$ when compared to control

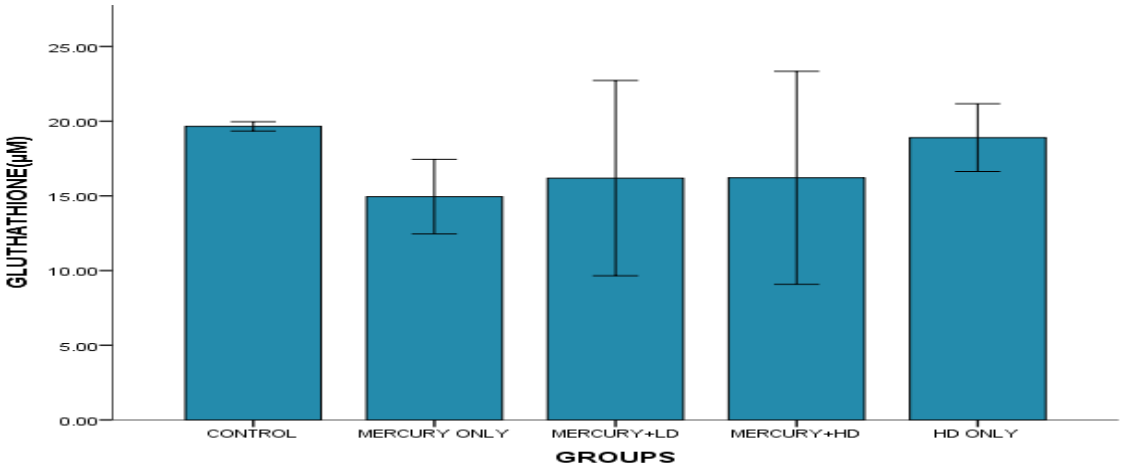


Fig 4.7 Bar chart showing test for glutathione in serum of experimental animals. Result shows no statistically significant difference among the groups

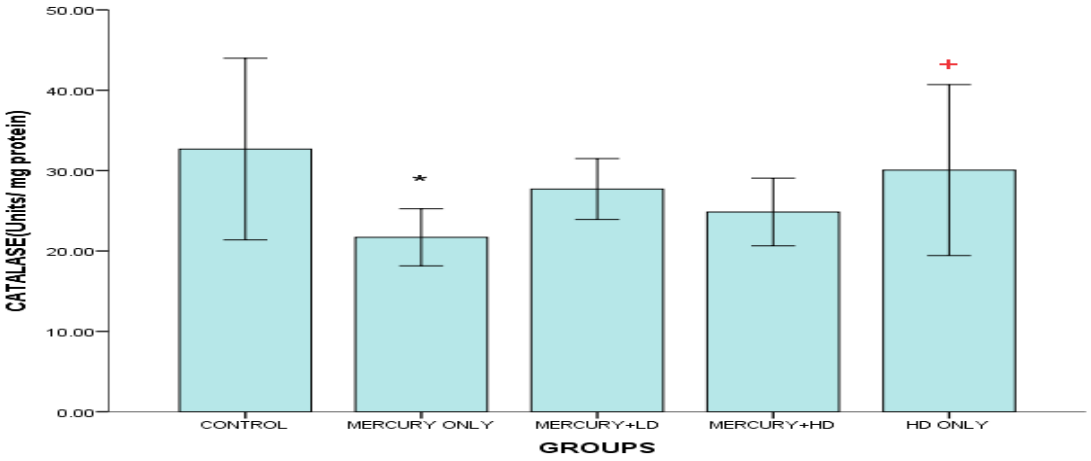


Fig 4.7 Bar chart showing test for catalase in serum of experimental animals. Result shows a statistically significant decrease in the group administered with mercuric chloridealone. Conversely, the group administered with the high dose of the extract had a statistically significant increase when compared to the mercury group

*-- indicates $p<0.05$ when compared to control

+ - indicates $p<0.05$ when compared to mercury only

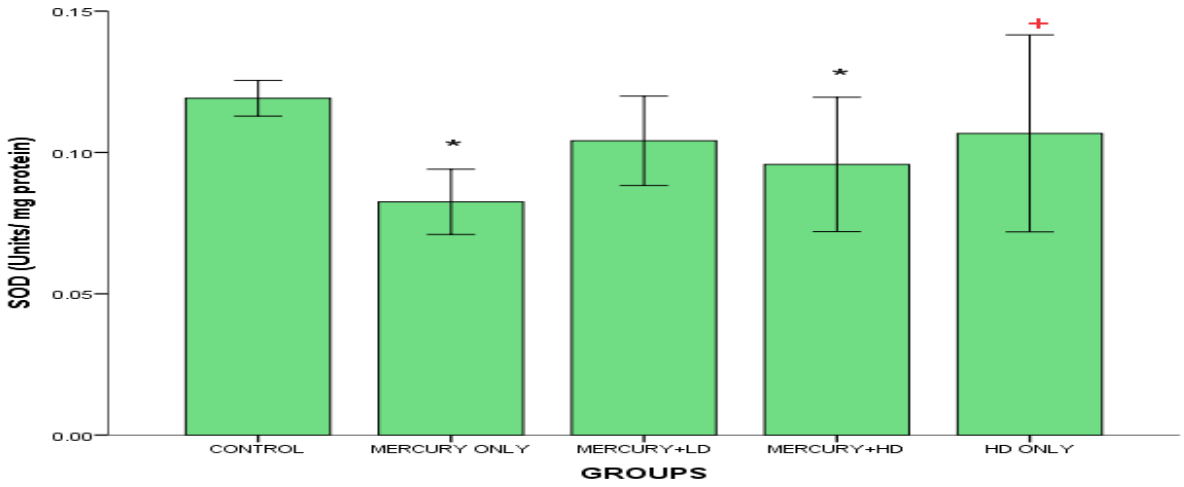


Fig 4.8 Bar chart showing test for SOD in serum of experimental animals. Result shows a statistically significant decrease in the group administered with mercuric chloridealone as well as the mercury+HD group. Additionally, the group administered with the high dose of the extract had a statistically significant increase when compared to the mercury group

*-- indicates $p<0.05$ when compared to control

+ - indicates $p<0.05$ when compared to mercury only

4.5 HISTOLOGY RESULTS

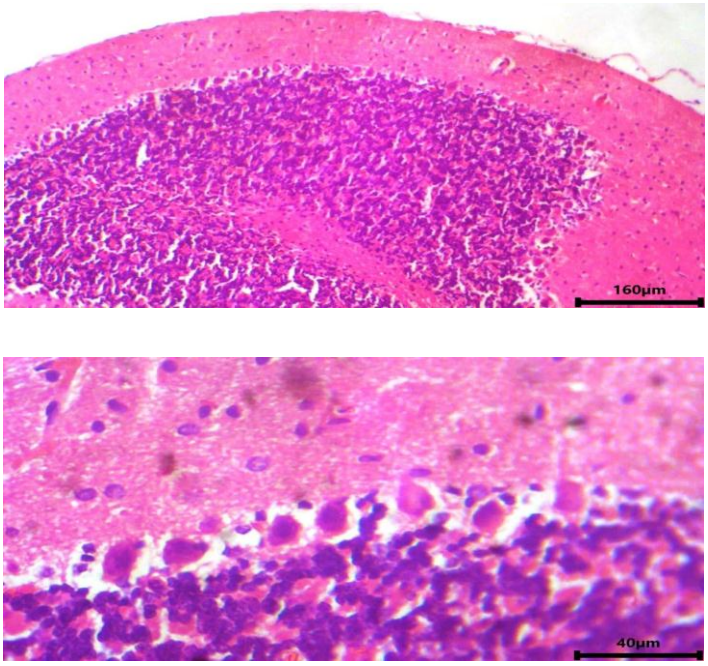


PLATE 1: Rat cerebellum, control showing normal histological layers of the cerebellar cortex: Inner Granular Layer, Middle Purkinje Layer and Outer Molecular Layer. (H&E, X400, scale bar = 40 µm and 160 µm)

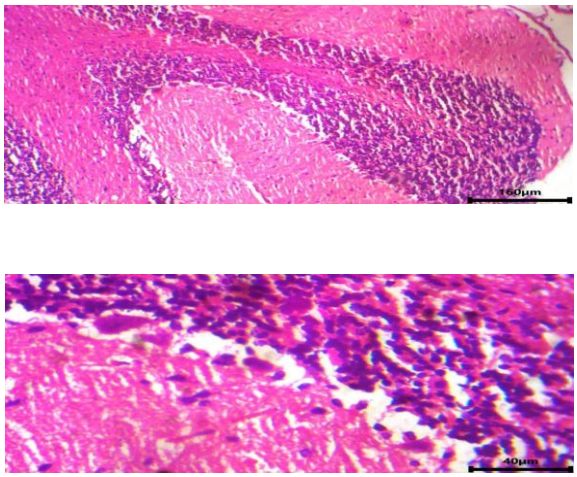


PLATE 2: Rat cerebellum group B treated with $HgCl_2$ only showing severe depletion and degeneration of purkinje cells (arrows) (H&E, x400, scale bar = 40 µm and 160 µm)

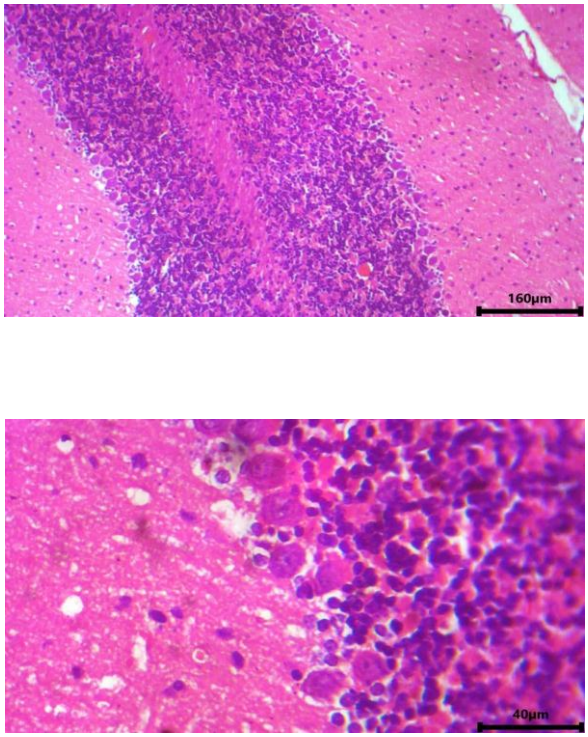


PLATE 3: Rat cerebellum of Group C treated with $HgCl_2$ and 250 mg/kg of extract showing normal architecture of cerebellar cortex and presence purkinje cells (H and E, x400, scale bar = 40 µm and 160 µm)

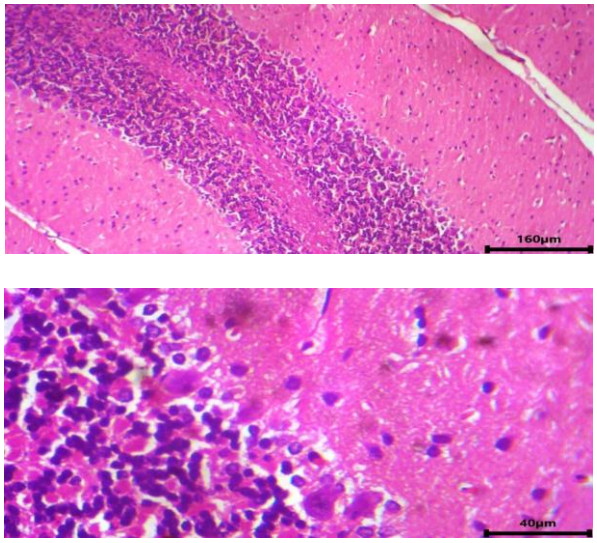
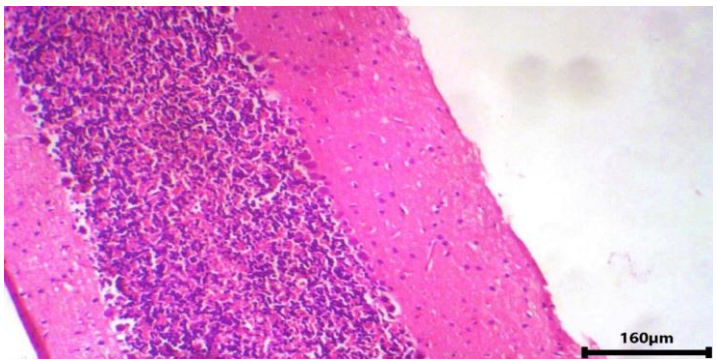


PLATE 4: Rat cerebellum of Group D treated with $HgCl_2$ and 500 mg/kg of extract showing normal architecture of cerebellar cortex and presence purkinje cells (H&E x400, scale bar = 40 µm and 160 µm)



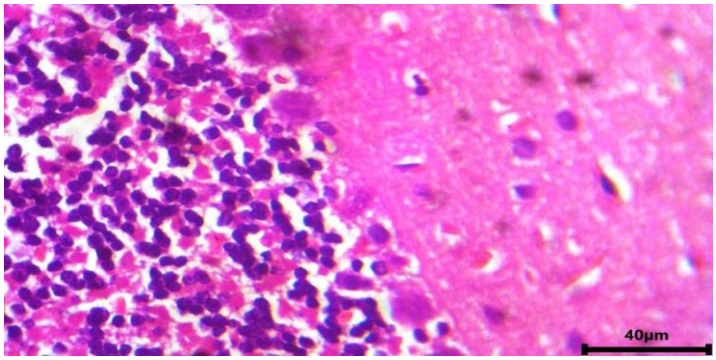


PLATE 5: Rat cerebellum of group E treated with extract only showing normal architecture of cerebellar cortex (H&E, x400, scale bar = 40 µm and 160 µm)

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

The primary integrative center for motor planning, facilitation of movement, and synchronization of muscular action is the cerebellum. Ataxia, dysmetria, and oculomotor dysfunction are only a few of the problems that can result from a serious lesion to the cerebellar cortex. Toxins enter the cerebellar cortex and attack a number of cells, including Purkinje cells, which are essential for regulating motor function. A number of neurological disorders have been linked primarily to abnormal Purkinje cell function or dysfunction (Cook et al., 2020).

This study sought to ascertain whether the ethanolic leaf extract of Centella Asiatica protected the cerebellar damage caused by mercuric chloride in Wistar rats. The findings of the investigation revealed no significant difference ($P > 0.05$) in body weight, organ weight, or relative organ weight across all groups. Mercury did cause a reduction in body weight, but it was not statistically significant when compared to the control group. These findings are consistent with a study by Said et al., 2021, which found no significant effect of mercuric chloride on the body weights of the animals.

According to the biochemical study, there was no statistically significant difference ($P>0.05$) between serum glutathione and total protein. On the other hand, group B ($HgCl_2$ only) showed a significant increase ($P<0.05$) in serum MDA (malondiadehyde) levels when compared to control. This disrupts the homeostasis between excitatory and inhibitory neurons, weakening cerebellar function during carbonyl stress and potentially leading to cancer, psychiatry, asthma, or cardiovascular disease. A number of neurological conditions, such as stroke, amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral

sclerosis (ALS), are linked to a substantial drop in SOD ($P<0.05$) in group B relative to control. Furthermore, group D (high dose of extract with HgCl2) showed significance, although when compared to mercuric chloridealone, only group E (high dose of extract) had a significance. When group B (HgCl2) was compared to the control, glutathione peroxidase likewise shown a significant drop, which may have led to cell senescence. In comparison to group A (control), group B (HgCl2) showed a substantial drop in catalase. A significant factor in the genesis of sensory discomfort and Parkison's illness is a decrease in catalase. Between group B (HgCl2 only) and group E (high dose extract only), there was also a significant difference. Group E (high dosage extract alone) showed an increase in catalase as compared to group B (HgCl2). These findings align with studies conducted by Gu et al. (2023) and Said et al. (2021) that reported exposure to mercuric chloride (HgCl2) results in decreased activity of enzymes such as catalase, glutathione peroxidase, and superoxide dismutase (SOD) and increased production of malondialdehyde (MDA) in the brain of mice.

Sections of the cerebellar cortex in Group A (control) displayed normal histological layers, namely the inner granular layer, middle Purkinje layer, and outer molecular layer, as determined by the photomicrography results. The purkinje layer in Group B (HgCl2 only) had significant purkinje cell depletion and degeneration, whereas Group C's cerebellar cortex showed normal architecture and purkinje cell presence, Group D's cerebellar cortex showed normal architecture and purkinje cell presence, and Group E also displayed normal architecture. It also conforms to a piece of writing that Ochai et al.

5.2 Conclusion

According to the study's findings, mercuric chloride significantly damaged the cerebellum, resulting in the depletion of the purkinje cell layer. Additionally, the ethanolic leaf extract of *Centella*

asiatica did not significantly improve the oxidative stress parameters. According to the results, there was a protective effect against the cerebellar cortical damage caused by mercuric chloride from a histological perspective. This is consistent with a book published in 2019 by Chiroma et al., which suggests that *Centella asciatica* may provide protection against morphological changes and cognitive decline.

5.3 Recommendation

To observe more beneficial effects of the extract on the organ, we advise giving the Centella asiatica ethanolic leaf extract at a higher dose and over a longer period of time. To clarify the plant extract's long-term effects on the cerebellum, we advise doing a chronic test.

REFERENCES

Ahmad, S., and Mahmood, R. (2019). Mercuric chloride toxicity in human erythrocytes: enhanced generation of ROS and RNS, hemoglobin oxidation, impaired antioxidant power, and inhibition of plasma membrane redox system. *Environmental Science and Pollution Research*, 26, 5645-5657.

Akakin, A., Peris-Celda, M., Kilic, T., Seker, A., Gutierrez-Martin, A., and Rhoton Jr, A. (2014). The dentate nucleus and its projection system in the human cerebellum: the dentate nucleus microsurgical anatomical study. *Neurosurgery*, 74(4), 401-425.

Ali, S., Awan, Z., Mumtaz, S., Shakir, H. A., Ahmad, F., Ulhaq, M., and Khan, M. A. (2020). Cardiac toxicity of heavy metals (cadmium and mercury) and pharmacological intervention by vitamin C in rabbits. *Environmental Science and Pollution Research*, 27, 29266-29279.

Almeer, R. S., Albasher, G., Kassab, R. B., Ibrahim, S. R., Alotibi F., Alarifi, S and Abdel Moneim, A. E. (2020). Ziziphus spina-christi leaf extract attenuates mercury chloride-induced testicular dysfunction in rats. *Environmental Science and Pollution Research*, 27, 3401-3412.

Andrzejewska, A., Dabrowska, S., Lukomska, B., and Janowski, M. (2021). Mesenchymal stem cells for neurological disorders. *Advanced Science*, 8(7), 2002944.

Antognoni F, Perellino N, Crippa S, Toso R, Danieli B, et al. (2011) Irbic acid, a dicaffeoylquinic acid derivative from Centella asiatica cell cultures. *African Journal of Traditional, Complemantary and Alternative Medicines* 23:147.

Anyanwu, B. O., Orish, C. N., Ezejiofor, A. N., Nwaogazie, I. L., and Orisakwe, O. E. (2020). Neuroprotective effect of Costus afer on low dose heavy metal mixture (lead, cadmium and mercury) induced neurotoxicity via antioxidant, anti-inflammatory activities. *Toxicology Reports*, 7, 1032-1038.

Arora, D., Kumar, M., and Dubey, S. D. (2002). Centella asiatica-A review of its medicinal uses and pharmacological effects. *Journal of Natural Remedies*, 2(2), 143–149.

Arpaia, N., Campbell, C., Fan, X., Dikiy, S., Van Der Veeken, J., Deroos, P., ... and Rudensky, A. Y. (2013). Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature*, 504(7480), 451-455.

Asgary, S., Movahedian, A., Keshvari, M., Taleghani, M., Sahebkar, A., and Sarrafzadegan, N. (2017). Serum levels of lead, mercury and cadmium in relation to coronary artery disease in the elderly: a cross-sectional study. *Chemosphere*, 180, 540-544.

Babykutty, S., Padikkala, J., Sathiadevan, P. P., Vijaykurup, V., Abdul Azis, T. K., Srinivas, P., and Gopala, S. (2009). Apoptosis induction of Centella asiatica on human breast cancer cells. *African Journal of Traditional, Complementary and Alternative Medicines*, 6(1), 9–16.

Barta, Č. (1970). Preparation of mercurous chloride monocrystals. *Kristall und Technik*, 5(4), 541-549.

Berg K., Puntervoll P., Valdersnes S., and Goksoyr A., (2010). Responses in the brain Proteome pf Atlantic cod (Gadus morhua) exposed to methylmercury. *Aquatic toxicology* vol. 100, issue 1,pg 51-65.

Berntssen, M. H., Aatland, A., and Handy, R. D. (2003). Chronic dietary mercury exposure causes oxidative stress, brain lesions, and altered behaviour in Atlantic salmon (Salmo salar) parr. *Aquatic toxicology*, 65(1), 55-72.

Bist, P., and Choudhary, S. (2022). Impact of heavy metal toxicity on the gut microbiota and its relationship with metabolites and future probiotics strategy: a review. *Biological trace element research*, 200(12), 5328-5350.

Bolton, E. E., Wang, Y., Thiessen, P. A., and Bryant, S. H. (2008). PubChem: integrated platform of small molecules and biological activities. *In Annual reports in computational chemistry* (Vol. 4, pp. 217-241). Elsevier.

Boylan H.M., Cain R.D., Kingston H.M. (2003): A new method to assess mercury emissions: a study of three coalfired electric-generating power station configurations. *Journal of the Air and Waste Management Association*, 53(11), 1318– 1325, 2003

Bridges, C. C., and Zalups, R. K. (2010). Transport of inorganic mercury and methylmercury in target tissues and organs. *Journal of Toxicology and Environmental Health, Part B*, 13(5), 385-410.

Brinkhaus, B., Lindner, M., Schuppan, D., and Hahn, E. G. (2000). Chemical, Pharmacological and Clinical Profile of the East Asian Medical Plant *Centella Aslatica. Phytomedicine* 7 (5), 427–448. doi:10.1016/s0944-7113(00)80065-3

Burger J., Jeitner C., Gochfeld M. (2011): Locational differences in mercury and selenium levels in 19 species of saltwater fish from New Jersey, *Journal of Toxicology and Environmental Health*, 74(13), 863–874..

Caglayan,C., Taslimi, P., Turk, C.,Kandemir, F.M., Demir, Y., and Gulcin, I.(2019). Purification and characterization of the carbonic anhydrase enzyme from horse mackerel (Trachurus trachurus) muscle and the impact of some metal ions and pesticides on enzyme activity. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, 226, 108605.

Camero C.M., Germanò M.P., Rapisarda A., D'Angelo V., Amira S., Benchikh F., Braca A., De Leo M. Anti-angiogenic activity of iridoids from Galium tunetanum. *Rev. Bras. de Farmacogn.* 2018;28:374–377. doi: 10.1016/j.bjp.2018.03.010. [CrossRef] [Google Scholar]

Canty, A. J., Raston, C. L., Skelton, B. W., and White, A. H. (1982). Structural studies of mercury (II) halide pyridine complexes [HgX 2 (py) 2], X= Cl, Br, or I. *Journal of the Chemical Society, Dalton Transactions*, (1), 15-18.

Cappelletti, S., Piacentino, D., Fineschi, V., Frati, P., D'Errico, S., and Aromatario, M. (2019). Mercuric chloride poisoning: symptoms, analysis, therapies, and autoptic findings. A review of the literature. *Critical reviews in toxicology*, 49(4), 329-341.

Cappello, T., Pereira, P., Maisano, M., Mauceri, A., Pacheco, M., and Fasulo, S. (2016). Advances in understanding the mechanisms of mercury toxicity in wild golden grey mullet (Liza aurata) by 1H NMR-based metabolomics. *Environmental pollution*, 219, 139-148.

Cavalcante G.M., da Silva Cabral A.E., Silva C.C. Leishmanicidal Activity of Flavonoids Natural and Synthetic: A Minireview. *Mintage J. Pharm. Med. Sci.* 2018;7:25–34. [Google Scholar]

Chippada, S. C., Volluri, S. S., Bammidi, S. R., and Vangalapati, M. (2011). In vitro anti-inflammatory activity of methanolic extract of Centella asiatica by HRBC membrane stabilisation. Rasayan J Chem, 4(2), 457-60. Retrieved September 2, 2017, from *rasayanjournal.co.in/vol-4/issue-2/35.pdf*

Chiroma, S. M., Baharuldin, M. T. H., Taib, C. N. M., Amom, Z., Jagadeesan, S., Adenan, M. I., and Moklas, M. A. M. (2019). Protective effect of Centella asiatica against D-galactose and aluminium chloride induced rats: Behavioral and ultrastructural approaches. *Biomedicine and Pharmacotherapy*, 109, 853-864

Chong J, Aziz Z (2013) A systematic review of the efficacy of Centella asiatica for improvement of the signs and symptoms of chronic venous insufficiency. *Evid Based Compliment Alternat Med* 11: 1-10

Chuarienthong P., Lourith N., Leelapornpisid P. Clinical efficacy comparison of anti-wrinkle cosmetics containing herbal flavonoids. *International Journal of Cosmetic Sci*ence 2010;32:99–106. doi: 10.1111/j.1468-2494.2010.00522.x. [PubMed] [CrossRef] [Google Scholar]

Cook AA, Fields E, Watt AJ. Losing the beat contribution of Purkinje cell firing dysfunction to disease, and its reversal. *Neuroscience.* 2020.

Crump, K. L., and Trudeau, V. L. (2009). Mercury-induced reproductive impairment in fish. Environmental Toxicology and Chemistry: *An International Journal*, 28(5), 895-907.

Danihelová M., Viskupičová J., Šturdík E. Lipophilization of flavonoids for their food, therapeutic and cosmetic applications. *Acta Chimica. Slovaca.* 2012;5:59–69. doi: 10.2478/v10188-012-0010-6. [CrossRef] [Google Scholar]

Delion, D. S., and Ghinescu, S. A. (2017). Geiger-Nuttall law for nuclei in strong electromagnetic fields. *Physical Review Letters*, 119(20), 202501.

Emran, T. B., Dutta, M., Uddin, M. M. N., Kumarnath, A., and Uddin, M. Z. (2015). Antidiabetic potential of the leaf extract of Centella asiatica in alloxan-induced diabetic rats. *Journal of Biological Sciences*, 4(1), 51–59.

Feliciano R.P., Pritzel S., Heiss C., Rodriguez-Mateos A. Flavonoid intake and cardiovascular disease risk. Curr. Opin. *Food Science.* 2015;2:92–99. doi: 10.1016/j.cofs.2015.02.006. [CrossRef] [Google Scholar]

Fisher, J. F., and World Health Organization. (2003). Elemental mercury and inorganic mercury compounds: human health aspects. World Health Organization.

Gao, P. C., Chu, J. H., Chen, X. W., Li, L. X., and Fan, R. F. (2021). Selenium alleviates mercury chloride-induced liver injury by regulating mitochondrial dynamics to inhibit the crosstalk between energy metabolism disorder and NF-κB/NLRP3 inflammasome-mediated inflammation. *Ecotoxicology and environmental safety*, 228, 113018.

George, M., Joseph, L., and Ramaswamy. (2009). Anti-allergic, anti-pruritic, and anti-inflammatory activities of Centella asiatica extracts. *African Journal of Traditional, Complementary and Alternative Medicines,* 6(4), 554–559.

Gohil K.J., Patel J.A., and Gajjar A. K. (2010). "Pharmacological review on Centella asiatica: A potential herbal cure-all". *Indian Journal of Pharmaceutical Sciences* 72.5 (2010): 546-556. 10.

Golla, P., and Tirupathi, H. (2016). To evaluate and compare antidepressant activity of Centella asiatica in mice by using forced swimming test. *International Journal of Basic and Clinical Pharmacology*, 5(5), 2017–2020.

Gonzalez P, Dominique Y, Massabuau JC, Boudou A, Bourdineaud JP. 2005. Comparative effects of dietary methylmercury on gene expression in liver, skeletal muscle, and brain of the zebrafish (Danio rerio). Environmental Science Technology 39: 3972–3980.

Gotu k (2015) University of Maryland Medical Center (MD): University of Maryland Medical Center; c2016. http://umm.edu/health/medical/altmed/herb/gotu-kola

Grace-Lynn Chong, Ibrahim Darah , Yeng Chen , Lachimanan Yoga Latha , Subramanion L. Jothy and Sreenivasan Sasidharan (2012). In Vitro Antioxidant Activity Potential of Lantadene A, *A Pentacyclic Triterpenoid of Lantana Plants. Molecules* 2012, 17.

Guo, S., Bezard, E., and Zhao, B. (2005). Protective effect of green tea polyphenols on the SH-SY5Y cells against 6-OHDA induced apoptosis through ROS-NO pathway. *Free Radical Biology and Medicine*, 39(5), 682–695

Hamidpour, R., Hamidpour, S., Hamidpour, M., Zarabi, M., Sohraby, M., Hamidpour, R. (2015). Medicinal property of gotu kola (Centella asiatica) from the selection of traditional applications to the novel phytotherapy. *Archives in Cancer Research*, 3(4), 1–7.

Han, Y., Kingston, H. M., Boylan, H. M., Rahman, G. M., Shah, S., Richter, R. C., ... and Bhandari, S. (2003). Speciation of mercury in soil and sediment by selective solvent and acid extraction. *Analytical and Bioanalytical Chemistry*, 375, 428-436.

Hashim P, Sidek H, Helan MHM, Sabery A, Palanisamy UD, Ilham M. Triterpene composition and bioactivities of centella asiatica. *Molecules*. 2011;16(2):1310–22.

Histology: A Text and Atlas by Michael H. Ross, Wojciech Pawlina (2010).6th ed. Lippincott Williams and Wilkins, Philadelphia. ISBN: 978-0-7817-7200-62.

Houston, M. C. (2007). The role of mercury and cadmium heavy metals in vascular disease, hypertension, coronary heart disease, and myocardial infarction. *Alternative Ther Health Medicine* 13(2), S128-S133.

https://www.hopkinsmedicine.org/health/conditions-and-diseases/anatomy-of-the-brain#:~:text=What%20is%20the%20brain%3F,central%20nervous%20system%2C%20or%20CNS.

Hussain, S., Rodgers, D. A., Duhart, H. M., and Ali, S. F. (1997). Mercuric chloride-induced reactive oxygen species and its effect on antioxidant enzymes in different regions of rat brain. *Journal of Environmental Science and Health Part B*, 32(3), 395-409.

Idris, F. N., and Nadzir, M. M. (2017). Antimicrobial Activity of Centella asiatica on Aspergillus niger and Bacillus Subtilis. *Chemical ngineering Transactions* 56, 1381–1386. doi:10.3303/CET1756231

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B., and Beeregowda, K. N. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary toxicology,* 7(2),60.

Javalkar, V., Khan, M., and Davis, D. E. (2014). Clinical manifestations of cerebellar disease. *Neurologic Clinics,* 32(4), 871-879.

Jayashree, G., Muraleedhara, K., Sudarslal, S., and Jacob, V. B. (2003). Anti-oxidant activity of Centella asiatica on lymphoma-bearing mice. *Fitoterapia,* 74(5), 431–434.

Johnson,C.D., et al. (2021). Mercury Chloride-Induced Cerebellar Damage: Mechanisms and Implications. *Environmental Toxicology*, 30(4), 489-502.

Johnson-Arbor, K., and Schultz, B. (2021). Effective decontamination and remediation after elemental mercury exposure: A case report in the united states. *Journal of Preventive Medicine and Public Health*, 54(5), 376.

Khatun J., Ashad I. and Debasis D., (2022). Effect of uncontrolled fertilization and heavy metal toxicity associated with arsenic (As), lead (Pb) and cadmium (Cd), and possible remediation. *Toxicology* 153274

Kumar, G. P., and Khanum, F. (2012). Neuroprotective potential of phytochemicals. *Pharmacognosy Review,* 6(12), 81–90.

Kumar, G. P., Anilkumar, K. R., and Naveen, S. (2015). Phytochemicals having neuroprotective properties from dietary sources and medicinal herbs. *Pharmacognosy Journal,* 7(1), 1–17.

Langford, N and Ferner, R. (1999). Toxicity of mercury. *Journal of Human Hypertension*.. 13(10): 651-6

Lanzendörfer G., Stäb F., Untiedt S. Cosmetic and Dermatological Preparations with Flavonoids. WO/1996/018379. *Molecules* 1996 Jun 20;

Liu, M., Zhang, Q., Maavara, T., Liu, S., Wang, X., and Raymond, P. A. (2021). Rivers as the largest source of mercury to coastal oceans worldwide. *Nature Geoscience*, 14(9), 672-677.

Manto, M. (2012). Toxic agents causing cerebellar ataxias. *Handbook of clinical neurology*, 103, 201-213.

Marambaud, P., Zhao, H., and Davies, P. (2005). Resveratrol promotes clearance of Alzheimer's disease amyloid-beta peptides. *Journal of Biological Chemistry*, 280(45), 37377–37382.

Martina Danihelová, Ernest Šturdík (2012). Nutritional And Health Benefits Of Buckwheat. *Potravinarstvo*, vol. 6, 2012, no. 3, p. 1-9.

Masoomi, I., Kamata, H., Yukimura, A., Ohtsubo, K., Schmid, M. O., and Scheffknecht, G. (2020). Investigation on the behavior of mercury across the flue gas treatment of coal combustion power plants using a lab-scale firing system. *Fuel Processing Technology,* 201, 106340.

Mato L, Wattanathorn J, Muchimapura S, Tongun T, Piyawatkul N, et al. (2009) Centella asiatica Improves Physical Performance and Health-related Quality of Life in Healthy Elderly Volunteer. *Evidence Based Complementary Alternative Medicine*.

Matsushima, K., Yagmurlu, K., Kohno, M., and Rhoton, A. L. (2016). Anatomy and approaches along the cerebellar-brainstem fissures. *Journal of neurosurgery*, 124(1), 248-263.

Meena, H., Pandey, H. R., and Ahmed, Z. (2012). Evaluation of antioxidant activity of two important memory enhancing medicinal plants Baccopa monnieri and Centella asiatica. *Indian Journal of Pharmacology*, 44(1), 114–117.

Mitoma, H., Manto, M., and Hampe, C. S. (2018). Time is cerebellum. *The Cerebellum*, 17, 387-391.

More, S. V., Kumar, H., Kang, S. M., Song, S. Y., Lee, K., and Choi, D. K. (2013). Advances in neuroprotective ingredients of medicinal herbs by using cellular and animal models of Parkinson's disease. *Evidence-Based Complementary and Alternative Medicine*, 2013(5), 1–15.

Mottet NK, Vahter ME, Charleston JS, and Friberg LT. 1997. "Metabolism of Methylmercury in the Brain and Its Toxicological Significance." *In Metal Ions in Biological Systems,* Volume 34: Mercury and Its Effects on Environment and Biology, edited by Sigel A and Sigel H, 371–403

Mukherjee, P. K., Kumar, V., and Houghton, P. T. (2007). Screening of indian medicinal plants for acetylcholinesterase inhibitory activity. *Phytotherapy Research*, 21(12), 1142–1145.

Mumtaz, S., Ali, S., Khan, R., Andleeb, S., Ulhaq, M., Khan, M. A., and Shakir, H. A. (2019). The protective role of ascorbic acid in the hepatotoxicity of cadmium and mercury in rabbits. *Environmental Science and Pollution Research*, 26, 14087-14096.

Nataraj, J., Manivasagam, T., Thenmozhi, J., and Essa, M. M. (2017). Neuroprotective effect of asiatic acid on rotenone-induced mitochondrial dysfunction and oxidative stress-mediated apoptosis in differentiated SH-SY5Y cells. *Nutrition Neuroscience*, 20(6), 351–359.

Netter Basic Science Series Saunders W.B.illustrated.Elsevier *Health Sciences,* 2013

Netter's Essential Histology by William K. Ovalle, Patrick C. Nahirney(2007). *Elsevier Saunders, Philadelphia*. ISBN: 978-1-929007-86-8

Ochai, J., Adebisi, S. S., Ibegbu, A. O., and Olatomide, O. D. Effect of Ethanolic Extract of Psidium Guajava Leaves on the Cerebellar cortex of Adult MaleWistarRats Treated with Mercuric Chloride. public health, 6, 7.

Orhan IE, Tareq M, Khan H. Centella asiatica (L.) Urban: From Traditional Medicine to Modern Medicine with Neuroprotective Potential. 2012;2012.

Paramita V., Kusumayanti H., Amalia R., Leviana W., Nisa Q.A. Application of Flavonoid and Anthocyanin Contents from Rambutan (Nephelium lappaceum) Peel as Natural Dyes on Cotton Fabric. Adv. Sci. Lett. 2018;24:9853–9855.
doi: 10.1166/asl.2018.13160. [CrossRef] [Google Scholar]

Patel K., Kumar V., Rahman M., Verma A., Patel D.K. New insights into the medicinal importance, physiological functions and bioanalytical aspects of an important bioactive compound of foods 'Hyperin': Health benefits of the past, the present, the future. *Beni-Suef University of Basic Applied Sciences*. 2018;7:31–42.
doi: 10.1016/j.bjbas.2017.05.009. [CrossRef] [Google Scholar]

Philippe Grandjean, Pal Weihe, Roberta F. White, Frodi Debes, Shunichi Araki, Kazuhito Yokoyama, Katsuyuki Murata, Nicolina Sørensen, Rasmus Dahl, Poul J. Jørgensen, (1997).Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicology and teratology* volume 19, issue 6, November–December 1997, pages 417-428.

R.F. Fan, J.X. Liu, Y.X. Yan, L. Wang, Z.Y. Wang Selenium relieves oxidative stress, inflammation, and apoptosis within spleen of chicken exposed to mercuric chloride. *Poultry Science*., 99 (2020), pp. 5430-5439

Rhee, H. M., and Choi, B. H. (1989). Hemodynamic and electrophysiological effects of mercury in intact anesthetized rabbits and in isolated perfused hearts. *Experimental and Molecular Pathology*, 50(3), 281-290.

Rizzetti, D. A., Altermann, C. D. C., Martinez, C. S., Peçanha, F. M., Vassallo, D. V., Uranga-Ocio, J. A., and Mello-Carpes, P. B. (2016). Ameliorative effects of egg white hydrolysate on recognition memory impairments associated with chronic exposure to low mercury concentration. *Neurochemistry International*, 101, 30-37.

Rocha, J. E., Guedes, T. A., Bezerra, C. F., Costa, M. D. S., Campina, F. F., de Freitas, T. S. and Coutinho, H. D. (2019). Mercuric chloridephytotoxicity reduction using antioxidative mechanisms evidenced by caffeic acid FTIR. *Applied Geochemistry*, 104, 109-115.

Roostaei, T., Nazeri, A., Sahraian, M. A., and Minagar, A. (2014). The human cerebellum: a review of physiologic neuroanatomy. *Neurologic clinics*, 32(4), 859-869.

Ross M. H., Pawlina W.: Histology: A text and atlas: With Correlated Cell and Molecular Biology, 6th edition, Lippincott Williams and Wilkins

Roy, D. C., Barman, S. K., and Shaik, M. M. (2013). Current Updates on Centella asiatica: Phytochemistry, Pharmacology and Traditional Uses. *Journal of Medicinal Plant Research.* 3. doi:10.5376/mpr.2013.03.0004

Said, E. S., Ahmed, R. M., Mohammed, R. A., Morsi, E. M., Elmahdi, M. H., Elsayed, H. S and Nadwa, E. H. (2021). Ameliorating effect of melatonin on mercuric chloride-induced neurotoxicity in rats. *Heliyon*, 7(7).

Sari, D. C. R., and Rochmah, M. A. (2015). The effects of ethanol extracts of Centella asiatica leaf on serial serum brain derived neurotrophin factor (BDNF) concentration of rats (Sprague Dawley) following chronic stress. *KnE Life Sciences*, 2, 159–167.

Sari, D. C. R., Aswin, S., Susilowati, R., Ar-Rochmah, M., Prakosa, D., Romi, M., et al. (2014). Ethanol Extracts of Centella asiatica Leaf Improves Memory Performance in Rats after

Chronic Stress via Reducing Nitric Oxide and Increasing Brain-Derived Neurotrophic Factor (BDNF) Concentration. *GSTF J. Psychology* 1, 9. doi:10.7603/s40790-014-0009-01

Sastry K.V. and Rao D.R., (1981). Enzymological and Biochemical cjanges produced by mercuric chloride in teleost fish, Channa Punctatus. *Toxicology Letters.*, 1981;9:321-326.

Sastry, K. V., and Sharma, K. (1980). Mercury induced haematological and biochemical anomalies in Ophiocephalus (Channa) punctatus. *Toxicology letters*, 5(3-4), 245-249.

Shan X., Cheng J., Chen K.l., Liu Y.M., Juan L. Comparison of Lipoxygenase, Cyclooxygenase, Xanthine Oxidase Inhibitory Effects and Cytotoxic Activities of Selected Flavonoids. *DEStech Transactions on Environment, Energy and Earth Sciences.* 2017 doi: 10.12783/dteees/gmee2017/16624. [CrossRef] [Google Scholar]

Shukla, A., Rasik, A. M., Jain, G. K., Shankar, R., Kulshrestha, D. K., and Dhawan, B. N. (1999). In vitro and in vivo wound healing activity of asiaticoside isolated from Centella asiatica. *Journal of Ethnopharmacology*, 65(1), 1–11.

Suhendrayatna, S., Arahman, N., Sipahutar, L. W., Rinidar, R., and Elvitriana, E. (2019). Toxicity and organ distribution of mercury in freshwater fish (Oreochromis niloticus) after exposure to water contaminated mercury (HgII). *Toxics*, 7(4), 58.

Sunilkumar, Parameshwaraiah, S., and Shivakumar, H. G. (1998). Evaluation of topical formulations of aqueous extract of Centella asiatica on open wounds in rats. *Indian Journal of Experimental Biology*, 36(6), 569–572.

Teixeira, F.B., de Oliveira, A.C.A., Le~ao, L.K.R., Fagundes, N.C.F., Fernandes, R.M., Fernandes, L.M.P., da Silva, M.C.F., Amado, L.L., Sagica, F.E.S., de Oliveira, E.H.C., Crespo-Lopez, M.E., Maia, C.S.F., Lima, R.R., 2018. Exposure to inorganic mercury causes oxidative stress, cell death, and functional deficits in the motor cortex. Front. Mol. *Neuroscience.* 11, 1–11

Thimmappa R., Geisler K., Louveau T., O'Maille P. and Osbourn A. (2014). Triterpene Biosynthesis in Plant. Annu. Rev. *Plant Biology*. 2014. 65:225-57

Valko M, Morris H, Cronin MT. (2005). Metals, toxicity and oxidative stress. *Current Medicinal Chemistry*. 12(10):1161-208.

Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals , metals and antioxidants in oxidative stressinduced cancer. *Chemico-Biological Interaction*. 2006;160:1–40.

Van Essen, D. C., Donahue, C. J., and Glasser, M. F. (2018). Development and evolution of cerebral and cerebellar cortex. *Brain Behavior and Evolution*, 91(3), 158-169.

Vasanthi N., muthukumaravel K., Sathick O., Sugumaran J., (2019). Toxic Effect of mercury on the freshwater fish Oroeochromis mossanbicus. Research *Journal of Life sciences, Bioinformatics, Pharmaceutical and chemical sciences*. DOI:10.26479/2019.0503.30

Villela A., van Vuuren M.S., Willemen H.M., Derksen G.C., van Beek T.A. Photo-stability of a flavonoid dye in presence of aluminium ions. *Dyes Pigment.* 2019;162:222–231. doi: 10.1016/j.dyepig.2018.10.021. [CrossRef] [Google Scholar]

Virtanen, J. K., Voutilainen, S., Rissanen, T. H., Mursu, J., Tuomainen, T. P., Korhonen, M. J., ... and Salonen, J. T. (2005). Mercury, fish oils, and risk of acute coronary events and cardiovascular disease, coronary heart disease, and all-cause mortality in men in eastern Finland. *Arteriosclerosis, thrombosis, and vascular biology*, 25(1), 228-233.

Visweswari, G., Prasad, K. S., Valluru, L., and Rajendra, W. (2010). The antiepileptic effect of Centella asiatica on the activities of Na+ /K+ , Mg2+ and Ca2+-ATPases in rat brain during pentyleneteteazol-induced epilepsy. *Indian Journal of Pharmacology*, 42(2), 82–86.

Waheed, R., El Asely, A. M., Bakery, H., El-Shawarby, R., Abuo-Salem, M., Abdel-Aleem, N and Abdeen, A. (2020). Thermal stress accelerates mercuric chloridetoxicity in Oreochromis niloticus via up-regulation of mercury bioaccumulation and HSP70 mRNA expression. *Science of the Total Environment*, 718, 137326.

Wijeweera P, Arnason J, Koszycki D, Merali Z (2006) Evaluation of anxiolytic properties of Gotukola–(Centella asiatica) extracts and asiaticoside in rat behavioral models. *Phytomedicine* 13: 668-676.

William K. O, Patrick C. N. Netter's Essential Histology, ClinicalKey 2012 Netter Basic Science Series Saunders W.B .*illustrated.Elsevier Health Sciences,* 2013

Yadav, K. K., and Trivedi, S. P. (2009). Sublethal exposure of heavy metals induces micronuclei in fish, Channa punctata. *Chemosphere*, 77(11), 1495-1500.

Yoshizawa, K., Rimm, E. B., Morris, J. S., Spate, V. L., Hsieh, C. C., Spiegelman, D and Willett, W. C. (2002). Mercury and the risk of coronary heart disease in men. *New England Journal of Medicine*, 347(22), 1755-1760.

Young B., Woodford P., O'Dowd G.: Wheater's Functional Histology: A Text and Colour Atlas, 6th edition, Churchill Livingstone Elsevier

Z. Zhou, D. Ma, P. Li, P. Wang, P. Liu, D. Wei, J. Wang, Z. Qin, Q. Fang, J. Wang (2020).Sirt1 gene confers Adriamycin resistance in DLBCL via activating the PCG-1alpha mitochondrial metabolic pathway. *Aging* Volume 12, Issue 12 pp 11364—11385

Zhao K., Yuan Y., Lin B., Miao Z., Li Z., Guo Q., Lu N. LW-215, a newly synthesized flavonoid, exhibits potent anti-angiogenic activity in vitro and in vivo. *Gene*. 2018;642:533–541. doi: 10.1016/j.gene.2017.11.065. [PubMed] [CrossRef] [Google Scholar]

Zhao L., Yuan X., Wang J., Feng Y., Ji F., Li Z., Bian J. (2019). A review on flavones targeting serine/threonine protein kinases for potential anticancer drugs. *Bioorganic and Medicinal Chemistry*. 2019;27:677–685. doi: 10.1016/j.bmc.2019.01.027. [PubMed] [CrossRef] [Google Scholar]

Zhu, Z., Yu, D., Yang, Y., Su, C., Huang, Y., Dong, Y. and Li, J. (2019). Gradient Li-rich oxide cathode particles immunized against oxygen release by a molten salt treatment. *Nature Energy*, 4(12), 1049-1058.

Zimbardo, P. G., Johnson, R. L., McCann, V., and Carter, C. (2003). *Psychology: core concepts* (pp. 647). Boston: Allyn and Bacon.