EVALUATING THE NEUROPROTECTIVE POTENTIAL OF AQUEOUS *ASPALATHUS LINEARIS* (ROOIBOS) LEAF EXTRACT ON MERCURY CHLORIDE-INDUCED TOXICITY IN THE CEREBELLUM OF ADULT WISTAR RATS

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BMS1705298

DEPARTMENT OF ANATOMY

SCHOOL OF BASIC MEDICAL SCIENCES

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF ANATOMY, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE (B.SC) DEGREE IN ANATOMY.

JANUARY, 2023

DECLARATION

I declare that:

- This project report is based on the experimental work undertaken by me in the Department of Anatomy, University of Benin, under the supervision of DR. ADAZE B. ENOGIERU.
- This work has not been previously submitted for the award of a degree elsewhere.
- All ideas and views are essentially based on this research and where the views of others have been expressed, such words duly acknowledged.

AKINKUADE, OLUWATUNMISE TRUTH

CERTIFICATION

This is to certify that this project titled 'Evaluating the Neuroprotective potential of Aqueous *Aspalathus linearis* (Rooibos) leaf extract on Mercury-chloride induced toxicity in the Cerebellum of Adult Wistar rats' will be carried out by AKINKUADE OLUWATUNMISE (BMS1705298) in the Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin.

Name of Project Supervisor ----------

Signature ----------

Date ----------

Name of Head of Department ----------

Signature ----------

Date ----------

Name of External Examiner ----------

Signature ----------

Date ----------

DEDICATION

I dedicate this project work to God Almighty for his unconditional love, care, strength, protection as well as a successful completion of this project work.

ACKNOWLEDGEMENT

I thank God for His grace upon my life and for seeing me through the completion of this project work

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ABSTRACT

Mercury is a common element in the environment that causes oxidative stress in those who are exposed, which in turn causes tissue damage. The role of natural antioxidants, especially those derived from plants is gaining a lot of attention with scientific evidence showing that vegetables, fruits and teas have protective effects and promote good health. *Aspalathus linearis*, commonly known as Rooibos, is well known for its rich content of different compounds with antioxidant properties. Studies have shown that it has anticarcinogenic, antimutagenic, anti-diabetic, antioxidant, cardio-protective effects. In this study, the neuroprotective activities of Aspalathus linearis (rooibos) was investigated in mercury chloride-induced neurotoxicity in experimental animals. Forty two adult Wistar rats, which weighed an average of 161g, were used for this study. The rats were grouped into seven groups (A, B, C, D, E, F and G), of six rats each. Group A served as the control and were given 1ml of sterile water, Group B ($HgCl_2$) were given 4mg/kg bodyweight of Mercury chloride only, Group C (RBT + $HgCl_2$) were administered 250mg/kg bodyweight aqueous leaf extract of *Aspalathus linearis* and 4mg/kg bodyweight of Mercury chloride, Group D (RBT + $HgCl_2$) were administered 500mg/kg bodyweight aqueous leaf extract of *Aspalathus linearis* and 4mg/kg bodyweight of Mercury chloride, Group E (RBT) were administered 500mg/kg bodyweight aqueous leaf extract of *Aspalathus linearis* only, Group F (Vit E + $HgCl_2$) were administered 500mg/kg bodyweight of vitamin E and 4mg/kg bodyweight of Mercury chloride and Group G(Vit E) were administered 500mg/kg bodyweight of vitamin E only. All administrations were given orally, through an orogastric tube and the experiment lasted for 28 days. At the end of the study period, the rats were weighed and open field test was performed. The rats were euthanized through cervical dislocation and their brains dissected out. Brain weight was recorded and antioxidant parameters such as MDA, GPx and SOD was investigated. Histology of the cerebellum was also examined in all groups. Result shows, in the neurobehavioural activity, for rearing and ambulation a decrease ($P < 0.05$) was observed in group B when compared with control, however in rearing and ambulation an increase ($P > 0.05$) was observed in group C, D and F when compared with group B. For grooming and immobility an increase ($P > 0.05$) was observed in group B when compared with control, however in grooming and immobility a decrease ($P < 0.05$) was observed in group C, D and F when compared with group B. Result for antioxidant activity showed oxidative stress (low antioxidant activity) in group B whereas the Control, RBT and Vit. E groups increase in antioxidant activity. Result from histology showed degeneration of the purkinje cells in the purkinje cell layer of the cerebellum in group B when compared with control, however treatment with RBT and Vit. E reversed the effect. In conclusion the findings showed *Aspalathus linearis* (rooibos) help to attenuate the neurotoxic effect of mercury-chloride on the cerebellum of adult Wistar rat

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Mercury chloride is an inorganic mercury salt and has proven to be the most toxic of the salts, likely due to its corrosiveness and high solubility (Magos et al., 2006). Human can be exposed to mercury by inhalation, ingestion and consumption via food chain. Following exposure, mercury ions are taken up by numerous organs, including the brain, intestine, kidney, liver, and placenta and the ions are accumulated in these organs (Bridges and Zalups, 2017). Brain tissues are more subjected to oxidative damage because of high concentration of polyunsaturated fatty acids that are predominantly susceptible to lipid peroxidation that plays a key role in necrosis and cell death (Teixeira et al., 2018).

Based on the extensive research done, it has been shown that mercury toxicity involves oxidative stress, inflammation, and apoptosis (Zhang et al., 2017). The inorganic ionic Mercury has great affinity for SH groups of biomolecules, such as glutathione (GSH) and sulfhydryl proteins, which may add to its toxicity (Hansen et al., 2006). Mercury can leave the cell to circulate in serum or lymph and be deposited in other organs or tissues, once the inorganic ionic Mercury bounds to GSH (Lorschieder et al., 1995). Mercury chloride is a pro-oxidant that induce oxidative stress (Khan et al., 2004). Oxidative stress occur when there is damage to macromolecules such as DNA, proteins and lipids due to the production of reactive oxygen species (ROS) such as, superoxide anion (O^{-2}), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH) exceeding the body defense mechanism (Valko et al., 2005). Excessive ROS generation causes interruption to lipid bilayer, nucleic acid damage and protein denaturation which result in oxidative damage, physiological disorder and eventually premature senescence and cell death (Vachhrajani et al., 1988).

Antioxidants in the human body can remove these radicals, resulting to balance oxidation and antioxidation (Dong-ping *et al*., 2017).

A fat-soluble antioxidant called vitamin E prevents the generation of ROS, which are created with fat when fat is subjected to oxidation. Scientists are examining whether vitamin E may

help prevent or postpone the chronic diseases linked to free radicals by decreasing the generation of free radicals and maybe through other mechanisms. As an antioxidant, the primary function of vitamin E is to scavenge loose electrons, or free radicals which can be harm cells. The human body needs to maintain a balance between free radicals and antioxidants such that when this equilibrium is interrupted, it can lead to oxidative stress (Arnarson, 2019). Free radicals are regularly formed in the body and in the absence of antioxidants these radicals would cause severe harm very quickly, eventually resulting in death. Scientists realized that free radical damage was involved in the early stages of artery-clogging atherosclerosis and might also be a factor in cancer, vision loss, and a number of other chronic disorder, antioxidant vitamins, particularly vitamin E, came to the public's notice. Antioxidants remove free radicals from the body cells and prevent or reduce the damage caused by oxidation.

Natural sources of vitamin E include certain vegetables, seeds and some fortified foods, it is also available as a dietary supplement. Antioxidants from natural sources play an important role in human health by neutralizing free radicals as well as reducing the risk of many diseases. Medicinal plants, fruits, vegetables, spices and teas are drawing a lot of attention because of their demonstrated health benefits, with scientific evidence demonstrating that phytochemicals in fruits, vegetables, spices and teas possess a high number of protective biological properties, including antioxidant, anti-inflammatory and other beneficial effects (Krzyzanowska et al., 2010).

Aspalathus linearis, commonly known as Rooibos, is a fynbos leguminous shrub indigenous to the Western and Northern Cape Provinces of South Africa, known for its health benefits. Traditionally, it is used to make herbal beverage that is naturally caffeine-free, low in tannin and rich in unique polyphenolic antioxidants (Joubert et al., 2008). Due to its rich content of different compounds, some unique, with antioxidant and other health properties, Rooibos is gaining more attention worldwide due to its potential for clinical purposes.

Scientific evidence confers numerous health benefits to Rooibos, for example antimutagenic, anticarcinogenic, antidiabetic, cardioprotective effect and modulating oxidative stress (Joubert *et al.,* 2008). These medical advantages are attributed to the presence of different polyphenolic compounds, including Aspalathin (Ajuwon et al., 2013; McKay and Blumberg, 2009). Earlier studies of *Aspalathus linearis* have shown its defensive effect. Aqueous extracts of Rooibos have been shown to possess antioxidant activities *in vitro* (Yoo et al., 2008). *In vivo* proof has

shown that aqueous Rooibos extracts are able to regulate oxidative stress by inhibiting lipid peroxidation and enlarging the glutathione redox status in rat sperm (Awoniyi et al., 2012), rat liver (Ajuwon et al., 2013) and in humans with an occupational risk (Nikolova et al., 2007) and at the risk of developing cardiovascular diseases (Marnewick et al., 2011). Immunomodulatory effects of rooibos have been previously reported both *in vitro* and *in vivo* (Kunishiro et al., 2001; Hendricks et al., 2010), while other studies also showed that Rooibos and two of its flavonoids (luteolin and quercetin) were able to reduce the secretion of pro-inflammatory cytokine, IL-6 and TNF-α using a LPS-stimulated macrophage model (Mueller et al., 2010).

1.2 JUSTIFICATION OF THE STUDY

Mercury is a widely distributed environmental and industrial xenobiotic. Consequently, the risk of mercury exposure for human populations is substantial and occurs through diverse routes such as occupational, dietary contamination, overuse of therapeutic or cosmetic agents, and fossil fuel emissions, among others (Magos et al., 2006; Park et al., 2012). Exposure to all forms of mercury including elemental, organic and inorganic can lead to a variety of pathologies involving numerous organ systems (Rice et al., 2014).

Mercury chloride has been shown to induce oxidative stress and mitochondrial dysfunction (Lund et al., 1993) which can result in alterations in calcium homeostasis and increased lipid peroxidation (Peraza et al., 1998). In addition, Mercury chloride may also increase radical oxygen species levels because of its ability to act as a catalyst for Fenton-type reactions (Peraza et al., 1998). Mercury poisoning may also cause chest pain or angina, especially in individuals under age 45 (Frustaci et al., 1999). There is also good evidence linking Mercury chloride with anemia including hemolytic anemia and aplastic anemia as mercury is thought to compete with iron for binding to hemoglobin which can result in impaired hemoglobin formation (Pyszel et al., 2005). Mercury chloride can cause immune, sensory, neurological, motor, and behavioral dysfunctions similar to traits defining or associated with autism (Bhardwaj et al., 2009) leading some to suggest that many cases of autism may be a form of mercury poisoning (Solt et al., 2010). It is therefore evident that the necessity to pervert the toxic effects of Mercury chloride is of major importance.

Aspalathus linearis (Rooibos) is a shrubby legume that is frequently used to make a mild tasting tisane rich in polyphenol antioxidants but with no caffeine and very little tannins. It is claimed to cure insomnia, allergies, and nervous breakdown as well as improve the appetite (Morton, 1983; Joubert et al., 2008). Currently, many of these claims are at various stages of formal substantiation; however, recent scientific endeavors suggest that Rooibos may confer various antioxidant-associated health benefits including antimutagenic, anticarcinogenic, anti-inflammatory, and antiviral properties and antiatherosclerotic effects (Joubert et al., 2008; Marnewick et al., 2005). Some of these health benefits were confirmed in humans where the consumption of fermented Rooibos improved the lipid profile as well as redox status in adults at risk of developing cardiovascular diseases (Marnewick et al., 2011). Studies have demonstrated *Aspalathus linearis* as a good source of unique phenolic compounds exhibiting its protective effect in various tissues (Marnewick et al., 2004; Pantsi et al., 2011; Awoniyi et al., 2011). Thus, it seems to be of interest to elucidate whether *Aspalathus linearis* is able to initiate its antioxidant properties in Mercury chloride-induced toxicity, an aspect which has not been systematically studied to date. An identification of its efficacy against Mercury chloride-induced toxicity in the Cerebellum would be of value in its promotion as a supplementary therapy.

1.3 AIM

The aim of this study is to investigate the neuroprotective potential of aqueous *Aspalathus linearis* (rooibos) leaf extract on Mercury-chloride induced toxicity in the Cerebellum of Adult Wistar rats.

1.4 SPECIFIC OBJECTIVES

The specific objective of this study is to investigate the neuroprotective potential of aqueous *Aspalathus linearis* leaf extract on;

- The neurobehavioral activities of rats treated with or without Mercury chloride.
- The antioxidant activities (Glutathione peroxidase, Superoxide dismutase and Malondialdehyde) in the cerebellum of rats treated with or without Mercury chloride.
- The histology of the cerebellum of rats treated with or without Mercury chloride.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 PLANT OF STUDY: ROOIBOS

Rooibos (*Aspalathus linearis*) which can likewise mean *red bush* is a broom-like member of the plant family Fabaceae that grows in South Africa (Dahlgren, 1980).The leaves are used in making herbal tea called Rooibos in South Africa and Bush tea, red tea or redbush tea in Great Britain. For ages the tea has been well known in South Africa and gained global popularity in the 2000s. The tea has an earthy flavour that is yerba mate or tobacco (Wipo, 2007; Curnow, 2012; Dahlgren, 1980). Formally classified as *Psoralea,* Rooibos is now thought to be part of the *Aspalathus* family (Dahlgren, 1980). In 1759, Burman gave the plant it specific name *linearis* for it's linear growing structure and needle-like leaves.

Figure 2.1: Rooibos Plant (wipo magazine, 2007)

Source: https://en.wikipedia.org/wiki/File:Rooibos_(Aspalathus_linearis)PICT2813.JPG

Dated: Jan 15, 2022

2.1.1 Production and processing

Rooibos is usually grown in the cederberg, a small mountainous area in the region of the Western Cape Province of South Africa (Dakora and Muofhe, 1999).

Generally, the leaves go through oxidation (Abudureheman *et al.,* 2022). It produces the distinctive reddish-brown colour of rooibos and enhances the flavour when it goes through oxidation. Unoxidised "green" rooibos is also produced, but the more demanding production

process for green rooibos (similar to the method by which green tea is produced) makes it more costly than traditional rooibos. It carries a malty and slightly grassy flavour not quite the same as its red counterpart (Standley *et al.,* 2001).

2.1.2 Uses

Rooibos tea is commonly prepared in a similar way as black tea, at times with the addition of milk, lemon, sugar, or honey. It is also served as lattes, cappuccinos or iced tea (Cape Point Press, 2014).

2.1.3 Chemical composition

Morton said in 1980 that rooibos has a high ascorbic acid (vitamin c) concentration when it is in its raw leaf form, which is lost when it is brewed into tea. Rooibos tea does not contain caffeine (Iswaldi *et al.,* 2011; Stander *et al.,* 2019) and it has less tannin compared to black tea or green tea (Morton, 1983) Rooibos contains polyphenols, like flavanols, flavones, flavanones, dihydrochalcones (Krafczyk *et al.,* 2009; Bramati, 2002), aspalathin (Ku *et al.,* 2015) and nothofagin (Joubert, 1996). Benzoic and cinnamic acids are present in the processed leaves and stems (Rabe *et al.,* 1994).

2.2 Biological activities

Rooibos present various antioxidant associated health benefit including antimutagenic, anticarcinogenic, antidiabetic, cardioprotective effect and modulating oxidative stress (Joubert *et al.,* 2008). Some of these health benefits were confirmed in humans (Marnewick *et al.,* 2011)

2.2.1 Antioxidant

Free radicals (unstable molecules that have lost an electron) can cause damage to the DNA in cells, resulting to cancer, and they can oxidize cholesterol resulting to clogged blood vessels, heart attack and stroke. Before the free radicals cause harm, antioxidant binds to free radicals

Some antioxidant are called polyphenols due to the fact that they contain a phenolic ring in their chemical structure. Polyphenols are common in plants and Rooibos tea contains polyphenol antioxidant that are potent free radical scavengers. The antioxidants present in rooibos help protect against free radical damage that can result to cancer, heart attack and stroke.

2.2.2 Antimutagenic

The process that result to a change or mutation in genetic is known as mutagenesis (Bronzetti, 1994). The mutation could involve a single gene or a large portion of DNA. Mutation is restricted to the tissues of the affected organise, when mutagenesis occurs in the DNA of somatic cell.

Mutation may be passed on to the later generations of organism if the mutation occur in the DNA of germ cells. Expression of proteins and enzymes in the cells may be affected as a result of damage to the DNA and this may cause abnormal development in the offspring.

Mutation normally occur at a low rate, but can be increased by the action of physical agents known as mutagens such as ionising radiation and ultra violet (UV) light. In the initiation stage of carcinogenesis, mutagenesis had been shown to have a role.

Antimutagens are different types of compounds that are able to decrease or inhibit the effect of mutagens (Bronzetti, 1994). When tested in an *in vitro* assay, rooibos showed protective effect against DNA damage (Marnewick 2002).

2.2.3 Anticarcinogenic

Cancer is caused by mutations in the genetic material of cells leading to uncontrollable proliferation of cells. Cell division and damage to DNA (which converts DNA lesions to mutations) are two critical factors responsible for the formation of mutations.

Anticarcinogens inhibit one or more stages of the carcinogenic process and prevent or delay the formation of cancer (Ho *et al*., 1994). Due to the presence of phenolic compound in Rooibus, it is able to suppress mutation and thereby preventing cancer (Van der merwe *et al.,* 2006).

2.2.4 Cardioprotective

Rooibos help to promote heart health in humans (Pantsi *et al.,* 2011), it inhibit the activity of a specific enzyme called angiotensin-converting enzyme (ACE). This enzyme involve in development of cardiovascular disease. ACE inhibitor is use to treat hypertension and heart disease.

2.2.5 Antidiabetic effect

Due to the rapid increase in cases of diabetes mellitus worldwide (Suksomboon *et al.,* 2011), there has been interest in the use of plant-derived polyphenols as nutraceuticals to prevent the onset and progression of diabetes mellitus.

Rooibos is a rich source of uncommon glycosylated plate polyphenols with various critical health-promoting properties, including the treatment and prevention of diabetes mellitus.

2.2.6 Modulating oxidative stress

The human body constantly generate free radicals (hydroxyl and superoxide radicals) and other reactive oxygen species (ROS) (nitric oxide, hydrogen peroxide, peroxynitrile and hypochlorous acid) as a result of aerobic metabolism.

The brain is one of the most sensitive target tissues to oxidative stress (Boveris *et al.,*), due to it high demand for oxygen.

Rooibos is a good source of antioxidant due to it large proportion of polyphenolic compounds.

2.3 CHEMICAL OF STUDY

2.3.1 Mercury (II) Chloride

Mercury (II) Chloride is the inorganic compound of mercury and chlorine ($Hgcl_2$) that has been referred to throughout history as sulema or corrosive sublimate (Chisholm, 1911). It is a toxic white crystalline solid that is also a molecular chemical and a scientific reagent. Mercury chloride was used in the treatment of syphilis before more effective therapies became available and it was discovered that it is harmful (Chisholm, 1911).

2.3.1.1 Synthesis

Chlorine reacts with mercury or mercury (l) chloride to form mercuric chloride. It can also be produced by the addition of hydrochloric acid to a hot, concentrated solution of mercury (I) compounds such as the nitrate:

$$Hg_2(NO_3)_2 + 4HCl \rightarrow 2HgCl_2 + 2H_2O + 2NO_2$$

Volatile HgCl2, which can be separated by sublimation, is also produced by heating a mixture of solid mercury (ll) sulphate and sodium chloride (Chisholm, 1911).

It implies that mercury chloride is not a free element in nature.

2.3.1.2 Properties

Mercury chloride has a tendency to be sublime because it is a linear triatomic molecule rather than salt. Each mercury atom is bonded to two chloride ligands with the Hg-Cl distance of 2.38Å in the crystal; six more chlorides are more distant at 3.38Å (Wells, 1984).

2.3.2 Applications

The primary use of mercuric chloride is as a catalyst in the conversion of acetylene to vinyl chloride, the precursor to polyvinyl chloride: $C2H2 + HCl \rightarrow CH2=CHCl$ For this application, concentrations of about 5 weight percent of mercuric chloride are supported on carbon. The thermal cracking of 1,2- dichloroethane has surpassed this technology. Mercuric chloride also has major uses as a reagent in the synthesis of organic compounds and analytical chemistry, as well as a depolarizer in batteries (Matthias *et al*., 2006) It is being used in plant tissue culture to sterilise the surface of explants like leaf or stem nodes.

2.3.2.1 As a chemical reagent

Mercuric chloride is occasionally used to form an amalgam with metals, like aluminium (Deng, Wang and Danheiser, 2015). Aluminium strips are quickly covered by a thin layer of the amalgam after being treated with a mercuric chloride aqueous solution. Normally, a thin layer of oxide covers aluminium to protect it, thus making it inert. Aluminium can go through variety reactions after amalgamation. For instance, after the oxide layer is removed, the exposed aluminium will immediately react with water to produce $Al(OH)^3$ and hydrogen gas. In the Barbier reaction, halocarbons and amalgamated aluminium react. These alkylaluminium compounds are nucleophilic and can be used in a similar fashion to the Grignard reagent. In the synthesis of organic materials, amalgamated aluminium is also used as a reducing agent. Zinc is also commonly amalgamated using mercuric chloride.

Mercuric chloride may be used as a stabilising agent for chemicals and analytical samples. It is important to take precautions to ensure that any mercuric chloride discovered does not overwhelm the signals of other sample components, as is possible in gas chromatography (Foreman *et al*., 1992).

2.3.2.2 Historical use in preservation

During the late 19th and early 20th centuries, objects were painted with or dipped in a "mercuric solution" for the preservation of anthropological and biological specimens. This was done to stop molds, mites, and moths from destroying the specimen. Crystalline mercuric chloride were scattered over objects in drawers to protect them (Goldberg, 1996). It has a little role in tanning, and wood was preserved by kyanizing (soaking in mercuric chloride) (Freeman *et al.,* 2003). In 1830 and 1856, mercuric chloride was one of the three chemicals used in both Europe and United State for railroad tie wood treatment. Limited railroad ties were treated in the United States until there were concerns over lumber shortages in the 1890s (Jeffrey, 1999). The process was generally abandoned because mercuric chloride was water-soluble and not effective for the long term, as well as being highly poisonous. Furthermore, less harmful treatment processes like copper sulphate, zinc chloride, and ultimately creosote were discovered. In the 1890s and early 1900s, some railroad ties were subjected to limited kyanizing (Jeffrey, 2011).

2.3.2.3 Historic use in medicine

In early 20th century, mercuric chloride was a popular over-the-counter disinfectant recommended for everything from fighting measles germs (Gettysburg, 1908) and eradicating red ants (Child, 1832). It is one of the most popular and effective household antiseptics, but Carlin Philips, a doctor from New York, stated in 1913 that it should only be available by prescription because it is so corrosive and deadly (Philips,1913). Later that same month, a group of physicians in Chicago made the same demand. The product frequently caused accidental poisonings and was used as a suicide method (The Day Book, 1913).

In the Middle Age, Arab physicians employed it to treat wounds (Maillard *et al*., 2007). Until modern medicine declared it hazardous for use, Arab physicians continued to utilize it till the 20th century.

Before the development of antibiotics mercuric chloride was frequently used to cure syphilis. It was injected, ingested, inhaled, and topically administer. Treatment for syphilis with mercuric chloride and poisoning while receiving the treatment were both so frequent that the latter's symptoms were frequently mistaken for those of syphilis. This use of "salts of white

mercury" is referred to in the English-language folk song "The Unfortunate Rake" (Pimple, Pedroni and Berdon, 2002).

Before the development of antibiotics, Yaws was treated with mercuric chloride (labelled as Corrosive Sublimate). To treat ulcerative symptoms, it was given topically. Evidence of this is found in Jack London's book The Cruise of the Snark in the chapter entitled "The Amateur M.D."

2.3.3 Toxicity

Mercury dichloride is highly toxic compound (Wikipedia, 2022), both acutely and as a cumulative poison. Its toxicity is not just because of the mercury it contains, but also because of its corrosive properties, which can result in major internal injuries such as stomach, mouth, and throat ulcers as well as corrosive damage to the intestines. Mercuric chloride also tends to accumulate in the kidneys, causing severe corrosive damage which can lead to acute kidney failure. Mercuric chloride is a cumulative toxin, but unlike other inorganic mercury salts, it does not easily pass across the blood-brain barrier.

Common side effects of acute mercuric chloride poisoning include burning sensations in the mouth and throat, stomach pain, abdominal discomfort, lethargy, vomiting of blood, corrosive bronchitis, severe irritation to the gastrointestinal tract, and kidney failure. Chronic exposure can cause symptoms more common with mercury poisoning, such as insomnia, delayed reflexes, excessive salivation, bleeding gums, exhaustion, tremors, and dental problems.

Acute exposure to large amounts of mercuric chloride can lead to death in as little as 24 hours, usually due to acute kidney failure or damage to the gastrointestinal tract. In other cases, victims of acute exposure have taken up to two weeks to die (Toxnet).

2.4 ORGAN OF STUDY

2.4.1 Cerebellum

The cerebellum, also known as little brain is a major feature of the hindbrain of all vertebrates (Hodos, 2009).

The cerebellum in humans plays an important role in motor control. However, its well-established functions are movement-related. It is also thought to be involved in some cognitive processes like attention and language, as well as emotional regulation processes like fear regulation and pleasure response (Wolf, Rapoport and Schweizer, 2009; Schmahmann and Caplan, 2006). The cerebellum in humans does not initiate movement but contributes to coordination, precision and accurate timing. It gets information from spinal cord sensory systems and from other parts of the brain, and integrates these informations to fine-tune motor activity (Fine, Lonita and Lohr, 2002). In humans, damage to the cerebellum typically result in abnormalities in fine movement, equilibrium, posture and motor learning. (Fine, Lonita and Lohr, 2002).

In addition to its direct role in motor control, the cerebellum is important for numerous types of motor learning, including the ability to adapt to changes in sensorimotor relationships. In other to explain sensorimotor calibration in terms of synaptic plasticity within the cerebellum, several theoretical models has been made. These models were developed based on the observation that each cerebellar purkinje cell receives two dramatically different types of input: one is made up of thousands of weak inputs from the parallel fibers of the granule cells, and the other is an extremely strong input from a single climbing fiber (Albus, 1971). The fundamental idea behind the Marr–Albus theory is that the climbing fibres act as a "teaching signal", which induces a long-lasting change in the strength of parallel fibres inputs. Theories of this nature have received some support from observations of long-term depression in parallel fiber, but their validity remains controversial (Purves *et al*., 2007).

2.4.2 Structure

At the level of gross anatomy, the cerebellum is made up of a layer of cortex that is tightly folded, with white matter underneath and a fluid-filled ventricle at the base. The white matter contains four deep nuclei of the cerebellum that are embedded in it. Each region of the cortex is made up of the same small set of neuronal elements, arranged in a highly stereotyped geometry. At an intermediate level, the cerebellum and its supporting structures can be

divided into several hundred or thousand independently functioning modules called "microzones" or "microcompartments".

2.4.3 Gross anatomy

The cerebellum is located in the posterior cranial fossa. The fourth ventricle, pons and medulla are located in front of the cerebellum (Standring *et al*., 2008). All of its connections with other parts of the brain travel through the pons; it is separated from the overlying cerebrum by a layer of leathery dura mater called the tentorium cerebelli. Anatomists classify the cerebellum as part of the metencephalon, which also includes the pons; the metencephalon is the upper part of the rhombencephalon , also known as "hindbrain". The cerebellum is split into two hemispheres, just like the cerebral cortex; it also contains a narrow midline zone (the vermis). Conventionally, the overall structure is divided into 10 smaller "lobules" by a series of massive fold. The cerebellum has more neurons than the entirety of the rest of the brain because of its large number of tiny granule cells, but takes up only 10% of the total brain volume (Llinas, Walton and Lang, 2004). The amount of neurons in the cerebellum is related to the amount of neurons in the neocortex. Numerous mammalian species share a constant ratio whereby the cerebellum has roughly 3.6 times as many neurons as the neocortex (Herculano-Houzel, 2010). The unusual surface appearance of the cerebellum hides the fact that most of its volume is made up of a very tightly folded layer of gray matter which is the cerebellar cortex. Each ridge or gyrus in this layer is known as folium. It is estimated that, if the human cerebellar cortex were completely unfolded, it would give rise to a layer of neural tissue that is approximately 1 meter long and 5 centimeters wide on average—a total surface area of about 500 square cm, packed within a volume of dimensions 6 cm × 5 cm × 10 cm (Llinas, Walton and Lang, 2004). Underneath the gray matter of the cortex lies white matter, made up largely of myelinated nerve fibres running to and from the cortex. Embedded within the white matter—which is sometimes called the arbor vitae (tree of life) because of its branched, tree-like appearance in cross-section—are four deep cerebellar nuclei, composed of gray matter (Ghez and Fahn, 1985). Three pairs of cerebellar peduncles connect the cerebellum to various area of the nervous system. These are the superior cerebellar peduncle, the middle cerebellar peduncle and the inferior cerebellar peduncle, all of which are named by their position relative to the vermis. The superior

cerebellar peduncle is mainly an output to the cerebral cortex, carrying efferent fibres via thalamic nuclei to upper motor neurons in the cerebral cortex. The fibres arise from the deep cerebellar nuclei. The middle cerebellar peduncle is connected to the pons, which is where it receives all of its input from the pons majorly from the pontine nuclei. The input to the pons is from the cerebral cortex and is relayed from the pontine nuclei via transverse pontine fibres to the cerebellum. The middle peduncle is the largest of the three and its afferent fibres are grouped into three separate fascicles taking their inputs to different parts of the cerebellum. The inferior cerebellar peduncle receives input from afferent fibres from the vestibular nuclei, spinal cord and the tegmentum. Output from the inferior peduncle is via efferent fibres to the vestibular nuclei and the reticular formation. The inferior cerebellar peduncle transmits modulatory information from the inferior olivary nucleus to the entire cerebellum (Purves et al., 2011).

2.4.4 Subdivisions

Three lobes can be identified within the cerebellum based on the surface appearance: the anterior lobe (above the primary fissure), the posterior lobe (below the primary fissure), and the flocculonodular lobe (below the posterior fissure). These lobes separate the cerebellum from rostral to caudal (in humans, top to bottom). However, in terms of function, there is a more signigicant distinction along the medial-to-lateral dimension. Leaving out the flocculonodular lobe, which has separate connections and functions, the cerebellum can be divided functionally into a medial sector called the spinocerebellum and a larger lateral sector called the cerebrocerebellum (Ghez and Fahn, 1985). The cerebellar vermis is a narrow strip of protruding tissue along the midline. (Vermis is Latin for "worm") (Ghez and Fahn, 1985). The smallest region, which is the flocculonodular lobe, is often referred to as the vestibulocerebellum. It is the oldest part in evolutionary terms (archicerebellum) and participates majorly in balance and spatial orientation; the vestibular nuclei are its primary connections, although it also receives visual and other sensory input. Damage to this region causes disturbances of balance and gait (Ghez and Fahn, 1985).The spinocerebellum, also known as paleocerebellum is the medial zone of the anterior and posterior lobes. The primary function of this sector of the cerebellum is to fine-tune body and limb movements. It receives proprioceptive input from the dorsal columns of the spinal cord (including the spinocerebellar tract) and from the cranial trigeminal nerve, as well as from visual and auditory systems (Snider and Stowell, 1944). It sends fibres to deep cerebellar nuclei that, in turn, project to both the cerebral cortex and the brain stem, thus providing modulation of descending motor

systems (Ghez and Fahn, 1985). The cerebrocerebellum, also known as neocerebellum, is made up of the lateral zone, which in humans is by far the largest part. It only receives input from the cerebral cortex (especially the parietal lobe) via the pontine nuclei (forming cortico-ponto-cerebellar pathways), and sends output mainly to the ventrolateral thalamus (in turn connected to motor areas of the premotor cortex and primary motor area of the cerebral cortex) and to the red nucleus (Ghez and Fahn, 1985). There is disagreement about the best way to describe the functions of the lateral cerebellum: It is thought to be involved in planning movement that is about to occur(Kingsley, 2000), in evaluating sensory information for action (Ghez and Fahn, 1985), and in a number of purely cognitive functions, such as determining the verb which best fits with a certain noun (as in "sit" for "chair") (Petersen *et al* 1989; Timmann and Daun, 2007; Strick, Dum and Fiez, 2009; Buckner, 2013).

2.4.5 Microanatomy

Two types of neurons play dominant roles in the cerebellar circuit and they are Purkinje cells and granule cells. Three types of axons also play dominant roles: mossy fibres and climbing fibres (which enter the cerebellum from outside), and parallel fibres (which are the axons of granule cells). There are two main pathways through the cerebellar circuit, originating from mossy fibres and climbing fibres, both eventually terminating in the deep cerebellar nuclei (Llinas, Walton and Lang, 2004). Mossy fibres project directly to the deep nuclei, but also give rise to the following pathway: mossy fibres → granule cells → parallel fibres → Purkinje cells → deep nuclei. Climbing fibres project to Purkinje cells and also send collaterals directly to the deep nuclei (Llinas, Walton and Lang, 2004). The mossy fibres and climbing fibres inputs each carry fibres-specific information; the cerebellum also receives dopaminergic, serotonergic, noradrenergic, and cholinergic inputs that presumably perform global modulation (Schweighofer, Doya and Kuroda, 2004). Three layers makes up the cerebellar cortex. At the bottom lies the thick granular layer, densely packed with granule cells, along with interneurons, majorly Golgi cells but also including Lugaro cells and unipolar brush cells. In the middle lies the Purkinje layer, a confined zone that contains the cell bodies of Purkinje cells and Bergmann glial cells. At the top lies the molecular layer, which contains the flattened dendritic trees of Purkinje cells, along with the huge array of parallel fibres penetrating the Purkinje cell dendritic trees at right angles. This outermost layer of the cerebellar cortex also contains two types of inhibitory interneuron, which are stellate cells and basket cells. Both stellate and basket cells form GABAergic synapses onto Purkinje cell dendrites (Llinas, Walton and Lang, 2004).

2.4.6 Molecular layer

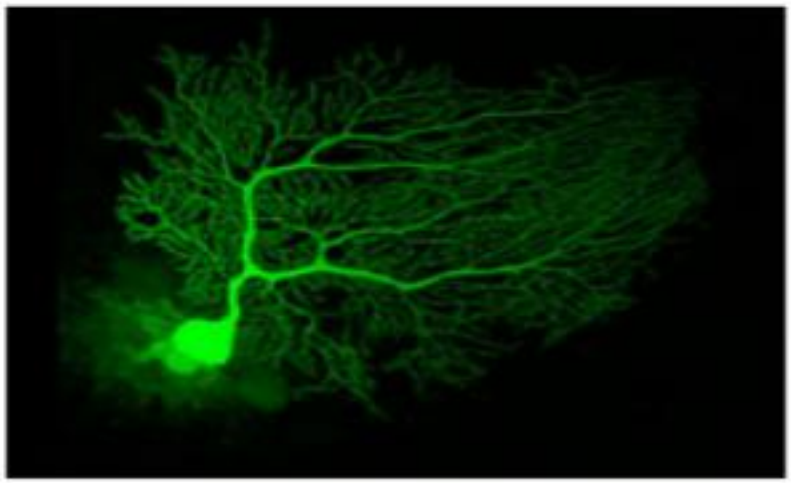
The top, outermost layer of the cerebellar cortex is the molecular layer. This layer contains the flattened dendritic trees of Purkinje cells, and the huge array of parallel fibres, from the granular layer, that penetrate the Purkinje cell dendritic trees at right angles. The molecular layer also contains two types of inhibitory interneuron: stellate cells and basket cells. Both stellate and basket cells form GABAergic synapses onto Purkinje cell dendrites (Llinas, Walton and Lang, 2004).

2.4.7 Purkinje layer

Purkinje cells are one of the most distinctive neurons in the brain, and one of the earliest types to be recognized—they were first described by the Czech anatomist Jan Evangelista Purkyně in 1837. They are distinguished by the shape of their dendritic tree: The dendrites branch very profusely, but are severely flattened in a plane perpendicular to the folds of the cerebellum. Thus, the dendrites of a Purkinje cell form a dense planar net, through which parallel fibres pass at right angles (Llinas, Walton and Lang, 2004). The dendrites are covered with dendritic spines, each of which receives synaptic input from a parallel fibres. Purkinje cells receive more synaptic inputs than any other type of cell in the brain—estimates of the number of spines on a single human Purkinje cell run as high as 200,000 (Llinas, Walton and Lang, 2004). The large, spherical cell bodies of Purkinje cells are packed into a narrow layer (one cell thick) of the cerebellar cortex, known as the Purkinje layer. Their axons travel into the deep cerebellar nuclei after emitting collaterals that affect nearby parts of the cortex, where they make on the order of 1,000 contacts each with several types of nuclear cells, all within a small domain. Purkinje cells use GABA as their neurotransmitter, and therefore exert inhibitory effects on their targets (Llinas, Walton and Lang, 2004).

Purkinje cells form the heart of the cerebellar circuit, and their large size and distinctive activity patterns have made it very simple to study their response patterns in behaving animals using extracellular recording techniques. Purkinje cells usually emit action potentials at a high rate even in the absence of the synaptic input. Mean rates in awake, behaving animals usually average around 40 Hz. The spike trains display a mixture of what are called simple and complex spikes. A simple spike is a single action potential followed by a refractory period of about 10 ms; a complex spike is a patterned sequence of action potentials with very short inter-spike intervals and declining amplitudes (Eccles, Llinás and Sasaki, 1966). Physiological studies have shown that complex spikes (which occur at baseline rates

around 1 Hz and never at rates much higher than 10 Hz) are reliably associated with climbing fibres activation, while simple spikes are produced by a combination of baseline activity and parallel fibres input. Complex spikes are often followed by a pause of several hundred milliseconds during which simple spike activity is suppressed (Simpson, Wylie and De Zeeuw, 1996). A specific, recognizable feature of Purkinje neurons is the expression of calbindin (Whitney *et al*., 2008). Calbindin staining of rat brain after unilateral chronic sciatic nerve injury suggests that Purkinje neurons may be newly generated in the adult brain, initiating the organization of new cerebellar lobules (Rusanescu and Mao, 2017).



A mouse Purkinje cell injected with fluorescent dye

Figure 2.2 A mouse Purkinje cell injected with fluorescent dye

Source: https://www.britannica.com/media/1/484088/162705

Dated: Jan 15, 2022

Cerebellar granule cells, in contrast to Purkinje cells, are one of the smallest neurons in the brain. They are also known as the most numerous neurons in the brain: In humans, estimates of their total number average around 50 billion, which means that about 3/4 of the brain's neurons are cerebellar granule cells (Llinas, Walton and Lang, 2004). Their cell bodies are tightly arranged into a thick layer at the bottom of the cerebellar cortex. A granule cell emits only four to five dendrites, each of which ends in an enlargement known as a dendritic claw (Llinas, Walton and Lang, 2004). These enlargements are sites of excitatory input from mossy fibres and inhibitory input from Golgi cells (Llinas, Walton and Lang, 2004). The thin,

unmyelinated axons of granule cells rise vertically to the upper (molecular) layer of the cortex, where they split in two, with each branch traveling horizontally to form parallel fibres; the splitting of the vertical branch into two horizontal branches gives rise to a distinctive "T" shape. The human parallel fibres runs for an average of 3 mm in each direction from the split, for a total length of about 6 mm (about 1/10 of the total width of the cortical layer) (Llinas, Walton and Lang, 2004). As they run along, the parallel fibres pass through the dendritic trees of Purkinje cells, contacting one of every 3–5 that they pass, making a total of 80–100 synaptic connections with Purkinje cell dendritic spines (Llinas, Walton and Lang, 2004). Granule cells use glutamate as their neurotransmitter, and therefore exert excitatory effects on their targets (Llinas, Walton and Lang, 2004).

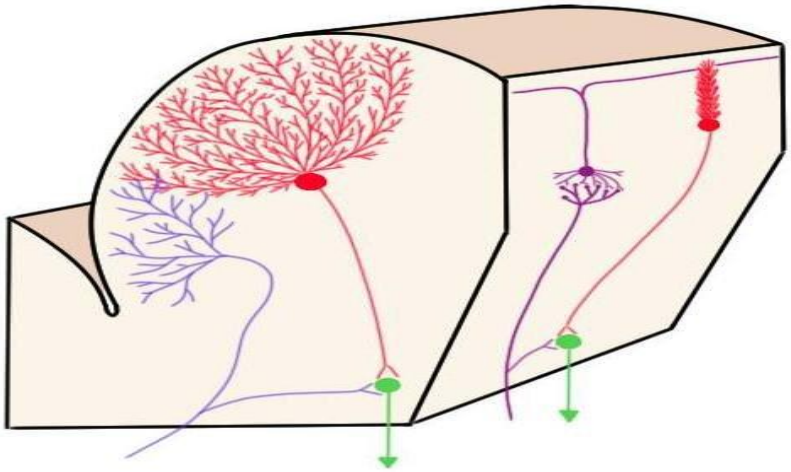


Figure 2.3 showing the cerebellar cortex

Source: https://www.google.com/url

Dated: Jan 15, 2022

Granule cells receive all of their input from mossy fibres, but outnumber them by 200 to 1 (in humans). Thus, the information in the granule cell population activity state is the same as the information in the mossy fibres, but recoded in a much more expansive way. Due to the granule cells' are so small and so densely packed, it is difficult to record their spike activity in behaving animals, so there is little data to use as a basis for theorizing. The most popular concept of their function was proposed in 1969 by David Marr, who suggested that they could encode combinations of mossy fibres inputs. The idea is that with each granule cell receiving input from only 4–5 mossy fibres, a granule cell would not respond if only a single

one of its inputs were active, but would respond if more than one was active. This combinatorial coding scheme would potentially allow the cerebellum to make much finer distinctions between input patterns than the mossy fibres alone would permit (Marr, 1969).

2.4.8 Mossy fibres

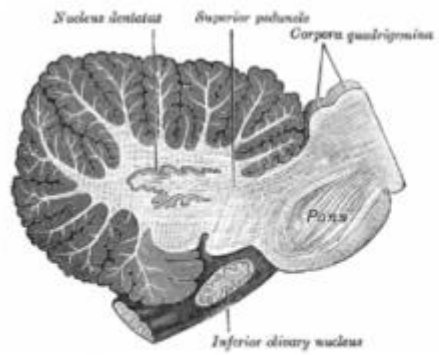
Mossy fibres enter the granular layer from their points of origin, many arising from the pontine nuclei, others from the spinal cord, vestibular nuclei etc. In the human cerebellum, the total number of mossy fibres has been estimated at 200 million (Llinas, Walton and Lang, 2004). These fibres form excitatory synapses with the granule cells and the cells of the deep cerebellar nuclei. Within the granular layer, a mossy fibres generates a series of enlargements called rosettes. The contacts between mossy fibres and granule cell dendrites take place within structures called glomeruli. Each glomerulus has a mossy fibres rosette at its center, and up to 20 granule cell dendritic claws contacting it. Golgi cells terminals infiltrate the structure and make inhibitory synapses onto the granule cell dendrites. The entire assemblage is surrounded by a sheath of glial cells (Llinas, Walton and Lang, 2004). Each mossy fibres sends collateral branches to several cerebellar folia, generating a total of 20– 30 rosettes; thus a single mossy fibres makes contact with an estimated 400–600 granule cells (Llinas, Walton and Lang, 2004).

2.4.9 Climbing fibres

Purkinje cells also receive input from the inferior olivary nucleus on the contralateral side of the brainstem via climbing fibres. Although the inferior olive lies in the medulla oblongata and receives input from the spinal cord, brainstem and cerebral cortex, its output goes entirely to the cerebellum. A climbing fibre gives off collaterals to the deep cerebellar nuclei before entering the cerebellar cortex, where it splits into about 10 terminal branches, each of which gives input to a single Purkinje cell (Llinas, Walton and Lang, 2004). In striking contrast to the 100,000-plus inputs from parallel fibres, each Purkinje cell receives input from exactly one climbing fibres; but this single fibres "climbs" the dendrites of the Purkinje cell, winding around them and making a total of up to 300 synapses as it goes (Llinas, Walton and Lang, 2004). The net input is so strong that a single action potential from climbing fibres is capable of producing an extended complex spike in the Purkinje cell: a burst of several spikes in a row, with diminishing amplitude, followed by a pause during which activity is suppressed. The climbing fibres synapses cover the cell body and proximal dendrites; this zone is devoid of parallel fibres inputs (Llinas, Walton and Lang, 2004). Climbing fibres fire at low rates,

but a single climbing fibres action potential induces a burst of several action potentials in a target Purkinje cell (a complex spike). The contrast between parallel fibres and climbing fibres inputs to Purkinje cells (over 100,000 of one type versus exactly one of the other type) is perhaps the most provocative feature of cerebellar anatomy, and has motivated much of the theorizing. In fact, the function of climbing fibres is the most controversial topic concerning the cerebellum. There are two schools of thought, one following Marr and Albus in holding that climbing fibres input serves primarily as a teaching signal, the other holding that its function is to shape cerebellar output directly. Both views have been defended in great length in numerous publications. In the words of one review, "In trying to synthesize the various hypotheses on the function of the climbing fibres, one has the sense of looking at a drawing by Escher. Each point of view seems to account for a certain collection of findings, but when one attempts to put the different views together, a coherent picture of what the climbing fibres are doing does not appear. For the majority of researchers, the climbing fibres signal errors in motor performance, either in the usual manner of discharge frequency modulation or as a single announcement of an 'unexpected event'. For other investigators, the message lies in the degree of ensemble synchrony and rhythmicity among a population of climbing fibres (Simpson, Wylie and De Zeeuw, 1996)

Deep Nuclei



Sagittal cross-section of human cerebellum, showing the dentate nucleus, as well as the pons and inferior olivary nucleus

Figure 2.4: showing Deep Nuclei

Source: https://en.wikipedia.org/wiki/File:Gray707.png

Dated: Jan 15, 2022

The deep nuclei of the cerebellum are clusters of gray matter lying within the white matter at the core of the cerebellum. They are, with the minor exception of the nearby vestibular nuclei, the only sources of output from the cerebellum. These nuclei receive collateral projections from mossy fibres and climbing fibres as well as inhibitory input from the Purkinje cells of the cerebellar cortex. The four nuclei (dentate, globose, fastigial and emboliform) each communicate with different parts of the brain and cerebellar cortex. (The emboliform and the globose nuclei are also referred to as combined in the interposed nucleus). The interposed and fastigial nuclei belong to the spinocerebellum. The dentate nucleus, which in mammals is much larger than the others, is formed as a thin, convoluted layer of gray matter, and only communicates with the lateral parts of the cerebellar cortex. The flocculus of the flocculonodular lobe is the only part of the cerebellar cortex that does not project to the deep nuclei—its output goes to the vestibular nuclei instead (Llinas, Walton and Lang, 2004). Most of the neurons in the deep nuclei have large cell bodies and spherical dendritic trees with a radius of about 400 μm, and use glutamate as their neurotransmitter. These cells project to various targets outside the cerebellum. Intermixed with them are a lesser number of small cells, which use GABA as a neurotransmitter and project exclusively to the inferior olivary nucleus, the source of climbing fibres. Thus, the nucleo-olivary projection provides inhibitory feedback to match the excitatory projection of climbing fibres to the nuclei. There is evidence that each small cluster of nuclear cells projects to the same cluster of olivary cells that send climbing fibres to it; there is strong and matching topography in both directions (Llinas, Walton and Lang, 2004). When a Purkinje cell axon enters one of the deep nuclei, it branches to touch both large and small nuclear cells, but the total number of cells contacted is only about 35 (in cats). Conversely, a single deep nuclear cell receives input from approximately 860 Purkinje cells (again in cats) (Llinas, Walton and Lang, 2004).

2.4.10 Blood Supply

The cerebellum is provided with blood from three paired main arteries: the superior cerebellar artery (SCA), the anterior inferior cerebellar artery (AICA), and the posterior inferior cerebellar artery (PICA). The SCA supply the upper region of the cerebellum. It divide at the upper surface and branches into the pia mater where the branches anastomose with those of the anterior and posterior inferior cerebellar arteries. The AICA supply the front part of the undersurface of the cerebellum. The PICA arrives at the undersurface, where it divide into a medial branch and a lateral branch. The medial branch continue backward to the cerebellar notch between the two hemispheres of the cerebellum; while the lateral branch

supply the under surface of the cerebellum, as far as its lateral border, where it anastomoses with the AICA and the SCA.

2.4.11 Function

The strongest clues to the function of the cerebellum have come from examining the effects of damage to it. Animals and humans with cerebellar dysfunction show, in particular, problems with motor control, on the same side of the body as the damaged part of the cerebellum. They continue to be able to generate motor activity but lose precision, uncoordinated, producing erratic, or incorrectly timed movements. A standard test of cerebellar function is to reach with the tip of the finger for a target at arm's length: A healthy person will move the fingertip quickly and straight, whereas a person with cerebellar damage will reach slowly and erratically, with many mid-course corrections. Deficits in non-motor functions are more challenging to detect. Thus, the general conclusion reached decades ago is that the primary function of the cerebellum is to calibrate the detailed form of a movement, not to initiate movements or to decide which movements to execute. Prior to the 1990s the function of the cerebellum was almost universally believed to be purely motor-related, but newer research have brought that view into question. Functional imaging studies have shown cerebellar activation in relation to language, mental imagery and attention; correlation studies have shown interactions between the cerebellum and non-motor areas of the cerebral cortex; and a variety of non-motor symptoms have been recognized in people with damage that appears to be confined to the cerebellum (Rapp, 2001; Doya, 2000). In particular, the cerebellar cognitive affective syndrome or Schmahmann's syndrome (Manto and Mariën, 2015) has been described in adults (Schmahmann and Sherman, 1998) and children (Levisohn *et al*., 2000). Estimates based on functional mapping of the cerebellum using functional MRI suggest that more than half of the cerebellar cortex is interconnected with association zones of the cerebral cortex (Buckner *et al*., 2011). Kenji Doya has argued that the cerebellum's function is best understood not in terms of the behaviours it affects, but the neural computations it performs; the cerebellum consists of a large number of more or less independent modules, all with the same geometrically regular internal structure, and therefore all, it is presumed, performing the same computation. If the input and output connections of a module are with motor areas (as many are), then the module will be involved in motor behaviour; but, if the connections are with areas involved in non-motor cognition, the module will show other types of behavioral correlates. Thus the cerebellum has been implicated in the regulation of many differing functional traits such as affection, emotion and behaviour

(Hernáez-Goñi *et al*., 2010; Turner *et al.,* 2007). The cerebellum, Doya proposes, is best understood as predictive action selection based on "internal models" of the environment or a device for supervised learning, in contrast to the basal ganglia, which perform reinforcement learning, and the cerebral cortex, which performs unsupervised learning (Doya, 2020; Doya, 1999). Three decades of brain research have led to the proposal that the cerebellum generates optimized mental models and interacts closely with the cerebral cortex, where updated internal models are experienced as creative intuition ("a ha") in working memory (Manto, Marvel and Vandervert , 2022).

2.4.12 Clinical significance

Damage to the cerebellum often cause motor-related symptoms, the details of which depend on the part of the cerebellum involved and how it is damaged. Damage to the flocculonodular lobe may show up as a lack of equilibrium and in particular an altered, irregular walking gait, with a wide stance caused by difficulty in balancing. Damage to the lateral zone typically causes problems in skilled voluntary and planned movements which can cause errors in the direction, force, speed and amplitude of movements. Other manifestations include hypotonia (decreased muscle tone), dysmetria (problems judging distances or ranges of movement), dysarthria (problems with speech articulation), dysdiadochokinesia (inability to perform rapid alternating movements such as walking), impaired check reflex or rebound phenomenon, and intention tremor (involuntary movement caused by alternating contractions of opposing muscle groups) (Schmitz, 2003; Mariën and Manto, 2016). Damage to the midline portion may affect whole-body movements, whereas damage localized more laterally is more likely to disrupt fine movements of the hands or limbs. Damage to the upper part of the cerebellum tends to result in gait impairments and other problems with leg coordination; damage to the lower part is more likely to result in uncoordinated or poorly aimed movements of the arms and hands, as well as difficulties in speed (Ghez and Fahn, 1985).This complex of motor symptoms is known as ataxia. To identify cerebellar problems, neurological examination includes assessment of gait (a broadbased gait being indicative of ataxia), finger-pointing tests and assessment of posture (Fine, Lonita and Lohr, 2002). If cerebellar dysfunction is indicated, a magnetic resonance imaging scan can be used to obtain a detailed picture of any structural alterations that may exist (Gilman, 1998). The list of medical problems that can produce cerebellar damage is long, including stroke, hemorrhage, swelling of the brain (cerebral edema), tumors, alcoholism, physical trauma such as gunshot wounds or explosives,

and chronic degenerative conditions such as olivopontocerebellar atrophy (National Institute of Health, 2014; Yuhas, 2016) Some forms of migraine headache may also produce temporary dysfunction of the cerebellum, of variable severity (Vincent and Hadjikhani, 2007). Infection can result in cerebellar damage in such conditions as the prion diseases (National Institute of Health, 2014) and Miller Fisher syndrome, a variant of Guillain–Barré syndrome.

Others include

- Aging (Horvath *et al.,* 2015).
- Developmental and degenerative disorders (Albert and Porter, 2006).
- Pain (Moulton *et al*., 2010; Baumann *et al*., 2015).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 REAGENTS /CHEMICALS

All reagents and chemicals would be of analytical grade. They include potassium permanganate, Distilled water, Na_2HPO_4, NaH_2PO_4,H_2SO_4, Hydrogen Peroxide, Na_2CO_3, $NaHCO_3$, EDTA-Disodium, Hydrochloric acid, Adrenaline, Pyrogallol, Trichloroacetic acid, cadmium chloride, ethanol , olive oil, alcohol (50%, 70%, 90%,100%), xylene, paraffin, formal saline, Mercury chloride, vitamin E.

3.2 EQUIPMENTS

Surgical latex gloves, orogastric tubes, sample bottles, plastic cages, open field maze, weighing balance, mortar and pestle, refrigerator, rotary microtome, binocular microscope, oven, water bath, paraffin dispenser, dissecting set, measuring cylinder, conical flask, volumetric flask, glass rods.

3.3 COMPUTER SOFTWARE

Adobe Photoshop CS6, version 13.0, x64 (manufactured by Adobe Systems Software Ireland Limited) and Graph pad prism software, version 7 (developed by Graphpad software, Inc; Released in 2016).

3.4 PLANT EXTRACT

600g of Rooibos tea was placed in two 2000ml beakers. 3250ml of distilled water at boiling point was poured into the two beakers such that it covered the sample to an approximate level of 3cm above. While allowing it to cool, it was intermittently stirred to encourage exhaustive extraction. Filtering was done using cheese cloth; 2700ml of the water extract was obtained while the residue was discarded. The liquid from the extract was centrifuge and the supernatant was stored while the sediment was discarded.

3.5 ANIMALS

The animals for this study were bred at the Animal House, Department of Anatomy, School of Basic Medical Sciences, College of Medical Science, University of Benin, Benin City, Edo State, Nigeria. They were kept in polypropylene cages under normal room temperature and were fed with Topfeeds Growers Mash (manufactured by Premier feed mills Co. Ltd, 1 Eagle Flour Road, Lagos/Ibadan expressway Toll point, Ibadan, Oyo State, Nigeria). Animals were

weighed weekly before commencement and throughout the duration of the experiment using a digital weighing scale calibrated in gram and recorded to the nearest whole number. Protocols for this experiment were in accordance with the guide for the care and use of laboratory animals (National Research Council of the National Academies, 2011).

The study protocol was submitted for review and approval by the Ethical Committee of the College of Medical Sciences, University of Benin.

3.6 DOSAGE

The chosen dosage of aqueous *Aspalathus linearis* leaf extract was 250mg/kg bodyweight and 500mg/kg bodyweight as previously reported (Kebe, 2016).

While the dosage of vitamin E was 500mg/kg bodyweight (Alina et al., 2014).

In addition the dosage of Mercury chloride was 4mg/kg in accordance with that used by (Nabil et al., 2020) and this is the dose that is known to induce toxicity in Wistar rats.

The administration of the plant extract and chemical requires a solvent medium for absorption and water was used as the medium for this study.

3.7 RESEARCH DESIGN

A total of forty two adult Wistar rats were used for this study. They were divided into seven groups (A, B, C, D, E, F and G) with six rats each after acclimatization to animal house conditions for two weeks with free access to feed and water.

Groups	**Treatment**
A	**1ml of sterile water**

B	**4mg/kg of HgCl**
C	**250mg/kg of RBT + 4mg/kg of HgCl**
D	**500mg/kg of RBT + 4mg/kg of HgCl**
E	**500mg/kg of RBT**
F	**500mg/kg of vit. E + 4mg/kg of HgCl**
G	**500mg/kg of vit. E**

Table 3.1: showing experimental design

All administrations were given orally with an orogastric tube, throughout 28 days of study.

3.8 NEUROBEHAVIORAL ACTIVITY (OPEN FIELD TEST)

To assess the effects of treatments on neurobehavioral activities, the open field test was performed. This test was performed according to the method of Olopade et al., (2017). The open-field test was used to evaluate anxiety as well as the locomotory and exploratory activities of rats. This test was based on subjecting an animal to an unknown environment whose escape was prevented by surrounding walls. Briefly, each rat will be placed in an open field, a 72 by 72 cm square box with lines on the floor dividing it into 18 by 18 cm square that allowed the definition of central and peripheral parts. Each animal will then be placed in the center of the field and the following parameters will be measured:

- Rearing: This is a measure of anxiety. It is the total number of erect position exhibited by the rats.
- Grooming: This is when the rats scratch their faces. It also indicates anxiety.
- Ambulation: Refers to the locomotive activity in rats. It is the number of floor units entered with all four paws rearing frequency, it is carried out using open field apparatus with video cameras to view movement of the rat in the open field
- Immobility: This is the inability of rats to move.

These parameters were assessed by the same set of observers who ensured the arena was cleaned with 70% ethanol to eliminate olfactory bias and allowed to dry before introducing a fresh animal.

3.9 ESTIMATION OF OXIDATIVE STRESS

After harvesting the brain, it was blotted free of blood and weighed immediately using an electronic weighing balance calibrated in milligram and recorded to the nearest two decimal places. The harvested and weighed brains was washed twice in cold phosphate-buffered saline (PBS), homogenized using acid-washed sand and PBS in porcelain mortar and pestle. The homogenate was centrifuged at 10,000g for 15 minutes at 4°C. The supernatant was collected for the estimation of the various biochemical assays.

3.10 Estimation of Malondialdehyde (MDA) Activity

Malondialdehyde would be determined using the thiobarbituric acid assay (Buege and Aust, 1978).

3.10.1 Principle

Malondialdehyde, which is a product of lipid peroxidation, reacts with thiobarbituric acid to give a red species.

3.10.2 Preparation of reagent

Stock TCA-TCB-HCL would be prepared by mixing 15g of trichloroacetic acid, 0.375g of thiobarbituric acid and 0.25N hydrochloric acid. This solution would be mildly heated to assist in the dissolution of the thiobarbituric acid.

3.10.3 Procedure

A volume of plasma (1.0ml) would be added to 2.0ml of TCA-TBA-HCL and mixed thoroughly. The solution would be heated for 15 minutes in a boiling water bath. After cooling, the flocculent precipitate would be removed by centrifuging at 1000g for 10 minutes. The absorbance would be determined at 535mn against a blank.

The concentration MDA would be determined using the formula

MDA (unit/mg protein) = $\frac{A \times Vt \times 1000}{\mathrm{M} \times \mathrm{V} \times \mathrm{l} \times \mathrm{Y}}$

A = absorbance of sample test at 535nm

V_t= total volume of the reaction = 3ml

M = molar extinction co-efficient of product= $1.56 \times 10^5 m^{-1}cm^{-1}$

l = light path= 1cm

V= volume of tissue extract used = 1ml

Y = mg tissue in the volume of sample used

3.11 Estimation of Glutathione Peroxidase (GPx) Activity

This was determined by the method of Nyman (1959).

3.11.1 Principle

This was based on the oxidation of pyrogallol to purpurogallin by peroxidase activity, resulting in a deep brown colour desposition, read at 430nm.

3.11.2 Preparation of reagent

Pyrogallol (20mM): 0.2552g of pyrogallol was dissolved in 100ml of distilled water.

3.11.3 Procedure

To an aliquot of plasma (0.2ml), 2.5ml of phosphate buffer, 2.5ml of H_2O_2, 1.5ml of distilled water and 2.5ml of pyrogallol was added. The reaction was allowed to stand for 30 minutes at room temperature. A deep brown colour was formed which was read at 420nm.

3.11.4 Calculation

$$\text{Activity} = \frac{\text{OD/Min} \times \text{VtDf}}{\text{E} \times V_s \times \text{Y}}$$

OD = Absorbance of test

V_t = Total volume of reaction of reaction mixture

Df = Dilution factor = 1

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

V_s= volume of sample

Y = mg of protein used

3.12 Estimation of Superoxide Dismutase (SOD)

This was determined according to the method of Misra and Fridovich (1972).

3.12.1 Principle

Adrenaline undergoes autoxidation rapidly to adrenochrome whose concentration can be determined at 420 nm with the aid of a spectrophotometer. The auto-oxidation of adrenaline depends on the presence of superoxide anions.

Superoxide dismutase inhibits auto-oxidation of adrenaline by catalysing the breakdown of superoxide anion. The degree of inhibition reflects the activity of SOD which is determined at 420 nm.

3.12.2 Preparation of reagents

Carbonate buffer (0.05 M) pH 10.2: this was prepared by dissolving 0.2014 g of Na_2CO_3. 0.2604 g of $NaHCO_3$ and 0.0372 g of EDTA in 100 ml of distilled water.

Hydrochloric acid (0.005 M): this was prepared by adding 0.044 concentrated HCl to 99.96 ml of distilled water.

Adrenaline solution (0.3 mM): this was prepared by dissolving 0.01098 g of Adrenaline in 100 ml of 0.005 M HCl solution.

3.12.3 Procedure

Plasma volume of 0.2 ml was mixed with 2.5 ml of carbonate buffer and 0.3 ml of adrenaline solution, 0.2 ml of distilled water was mixed with 2.5 m of carbonate buffer and 0.3 ml adrenaline as the reference sample. These were mixed and absorbance read at 420 nm.

$$\% \text{ inhibition} = \frac{(O.D_{test} - O.D_{ref}) \times 100}{O.D_{test}}$$

Enzyme activity can thus be calculated

$$\text{SOD activity (Unit/ mg protein)} = \frac{\% \text{ inhibition}}{}$$

50 x Y

3.13 HISTOLOGY OF THE CEREBELLUM

On the completion of the neurobehavioral tests, the rats were euthanized through cervical dislocation. The skulls were opened and the brain of the rats were harvested, blotted free of blood and weighed immediately using an electronic weighing balance calibrated in milligram and recorded to the nearest two decimal places. The relative brain weights were calculated as follows:

The relative brain weight will then be calculated as

$$\text{Relative brain weight} = \frac{\text{Absolute brain weight (g)}}{\text{Body weight of rat (g)}} \times 100$$

The brain was divided into two sagittal sections and the right hemisphere was post fixed in 10% neutral buffered formalin for about 12-24 hours The brain was dehydrated by placing in ascending grades of alcohol (50%, 70%, 90%) spending 2-4 hours in each solution and then it's placed in two changes of absolute alcohol, spending about 24 hours in each. After dehydration, the brain was cleared in two changes of xylene where it'll spend about 2 hours each time. It was then embedded in molten paraffin wax at a temperature of about 60°c for about 2-4 hours twice. A tissue block was then formed by placing the tissue in a metal block filled with molten paraffin wax and then the block was left to cool. The tissue was then placed on the rotary microtome (bright B5143, Huntington, England) for sectioning. These sections were then be transferred into water bath (40°c) to allow the spreading of folded ribbons of sections after which the sections were mounted on new glass slides which were dried at 40°c on a slide drier to enhance the adherence of sections to slides. The tissues were then stained using haematoxylin and eosin stain.

3.13.1 HAEMATOXYLIN AND EOSIN STAINING PROCEDURES

Tissue sections were deparaffinized in two changes of xylene for two minutes in each change and passed through two changes of absolute alcohol for four minutes each. They were hydrated using a series of descending grades of alcohol until water was used. Procedures of Haematoxylin and Eosin adopted on the sections were described by Drury and Wallington (1980). The sections were:

- Dewaxed in two changes of xylene for two minutes in each change
- Rehydrated in descending grades of alcohol (absolute II, absolute I, 95%, 90%, 70% and 50% ethanol) for two minutes each
- Rinsed in distilled water for three minutes
- Stained in haematoxylin for 15-20 minutes
- Excess haematoxylin stain were removed by rinsing well in running tap water for two to three minutes (sections were examined microscopically at this stage to confirm sufficient degree of staining)
- Differentiated in acid alcohol (0.5% HCL in 70% ethanol for two to three minutes
- Rinsed well in running water for 10-15 minutes
- Counterstained in 1% aqueous eosin for two to four minutes
- Excess stain were washed off in running water and examined under microscope
- Dehydrated rapidly in ascending grades of ethanol (50% through absolute ethanol), cleared in xylene and mounted in a synthetic resin medium (DPX).

3.14 PHOTOMICROGRAPHY

The processed slides were captured with a LABO® trinocular microscope (Labo Microsystems GmbH, Germany) on which were mounted an Omax 9.0MP USB Digital Microscope Camera (made in Korea). The camera features 9 megapixels (3488 × 2616 pixel) high-resolution colour digital camera and 0.5X reduction lens. It was connected to a laptop on which was installed ToUpView software (version ×64, 3.7.71.49; built-in 2016). A panoramic

view of the slides were captured using ×4 and ×10 objective lenses. The images were merged and processed automatically with Adobe Photoshop CS6 (version 13.0, ×64) for final output.

3.15 STATISTICAL ANALYSIS

Data generated were analysed using Graph Pad Prism statistical package, version 7 (developed by Graphpad software Inc; released in 2016). Statistical significance was determined by means of Analysis of Variance (ANOVA), followed by turkey's multiple comparison post-hoc test.

CHAPTER 4

4.0 RESULTS

4.1 EFFECT OF TREATMENT ON NEUROBEHAVIOURAL ACTIVITY

Figure 4.1(Rearing) shows the measure of anxiety. It is the total number of erect position exhibited by the rats. Here, a significant decrease ($P < 0.05$) was observed in group B when compared with control. However, an increase ($P > 0.05$) was observed in group C, D, E and F when compared with group B but it was not significant. Figure 4.2 (Grooming) shows when the rats scratch their faces. It also indicates anxiety. Here, a significant increase ($P < 0.05$) was observed in group B when compared with control. However, a significant decrease ($P < 0.05$) was observed in group C, D and F when compared with group B. Though, an increase ($P > 0.05$) was observed in group E and G when compared with control but it was not significant. Figure 4.3(Ambulation) shows the locomotive activity in rats. Here, a significant decrease ($P < 0.05$) was observe in group B when compared with control. However, a significant increase ($P < 0.05$) was observed in group C, D and F when compared with group B. Though, a decrease ($P > 0.05$) was observed in group E and G when compared with control. Figure 4.4(Immobility) shows the inability of rats to move. Here, an increase ($P < 0.05$) was observed in group B when compared with control. However, a significant decrease ($P < 0.05$) was observed in group C, D and F when compared with group B. Though, an increase ($P > 0.05$) was observed in group E and G when compared with control, it was not significant.

Note

- Control was compared with HgCl and indicated with ϕ
- HgCl was compared with 250 + HgCl, 500 + HgCl and Vit. E + HgCl and indicated with *
- Control was compared with RB alone and Vit. E alone

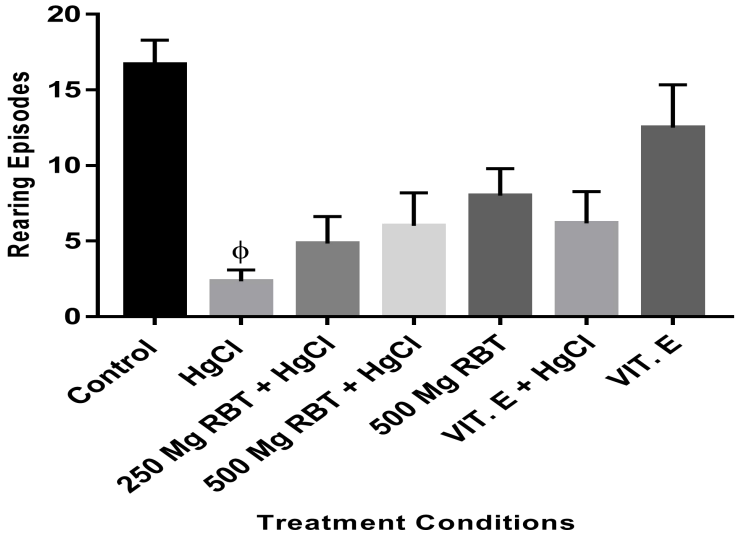


Figure 4.1: Bar chart showing the rearing activity across experimental groups.

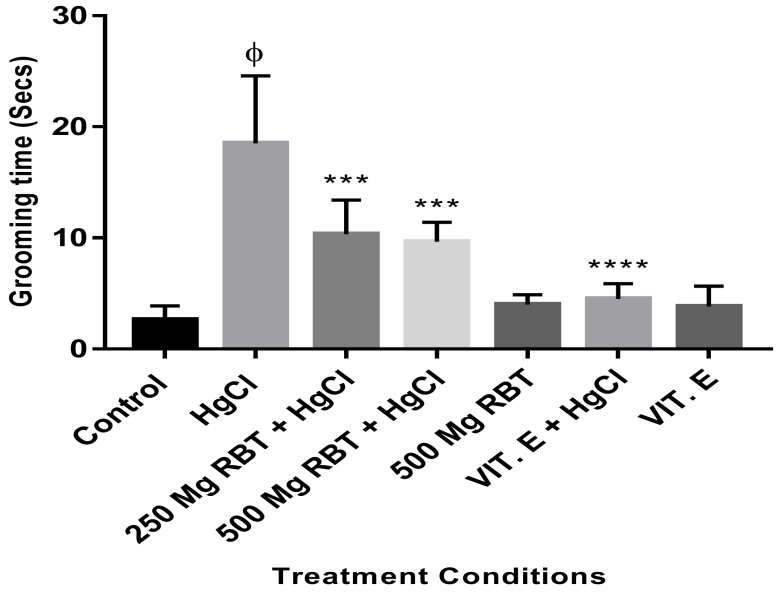


Figure 4.2: Bar chart showing the grooming activity across experimental groups.

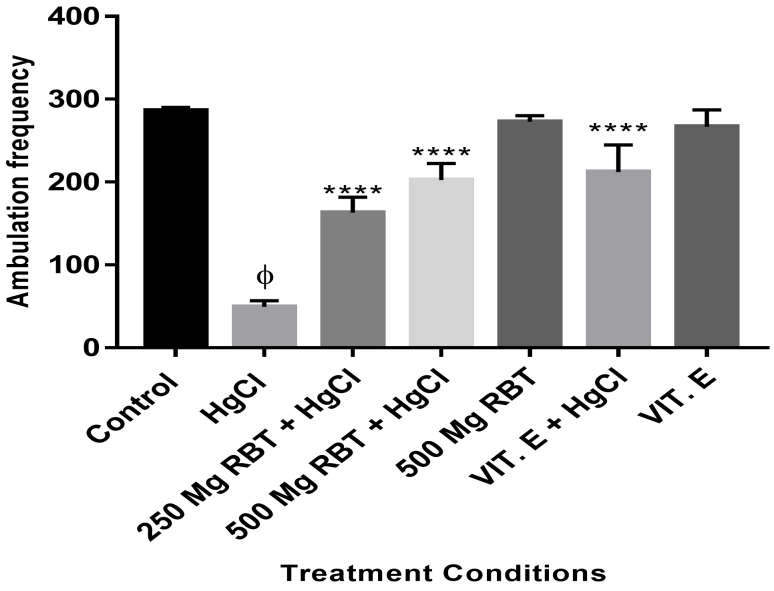


Figure 4.3: Bar chart showing the ambulation activity across experimental groups.

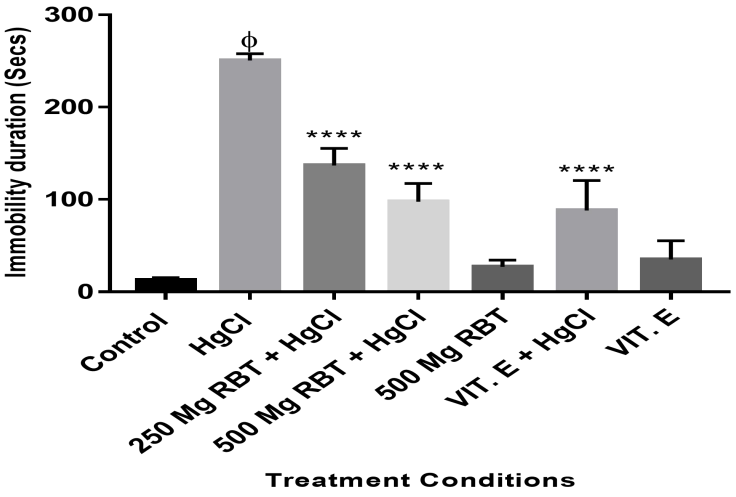


Figure 4 4: Bar chart showing the immobility activity across experimental groups.

4.2 EFFECT OF TREATMENT ON ANTIOXIDANT ACTIVITY

Figure 4.5-4.7 illustrates the activity of antioxidant enzymes in experimental groups A-G

Here, a significant increase in MDA ($P < 0.05$) was observed in group B when compared with control. However, a significant decrease in MDA ($P < 0.05$) was observed in group C, D and F when compared with group B. Though, no significant change in MDA was observed in group E and G when compared with control. Furthermore, a significant decrease in GPx and SOD ($P < 0.05$) was observed in group B when compared with control. Conversely, an increase in GPx and SOD ($P>0.05$) was observed in group C, D and F when compared with group B but it was not significant. Though, no significant change in GPx and SOD was observed in group E and G when compared with control

Note

- Control was compared with HgCl and indicated with ϕ
- HgCl was compared with 250 + HgCl, 500 + HgCl and Vit. E + HgCl and indicated with *
- Control was compared with RB alone and Vit. E alone

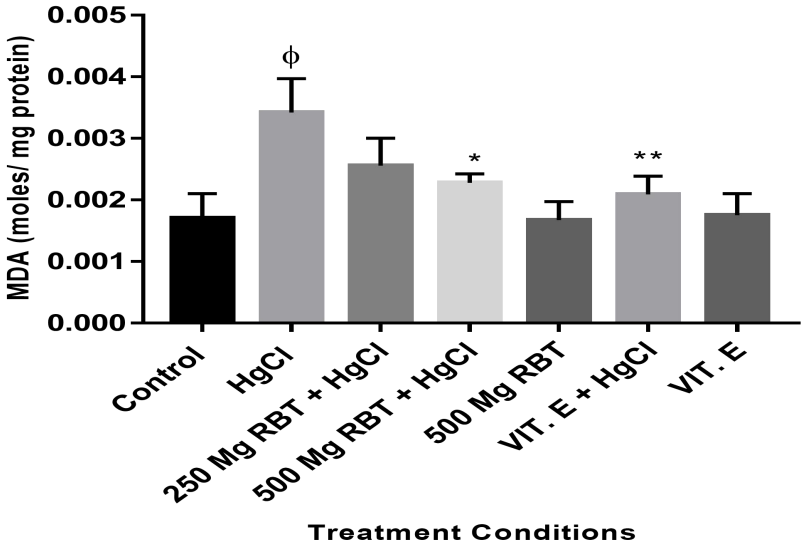


Figure 4.5: Bar chart showing MDA level across experimental groups.

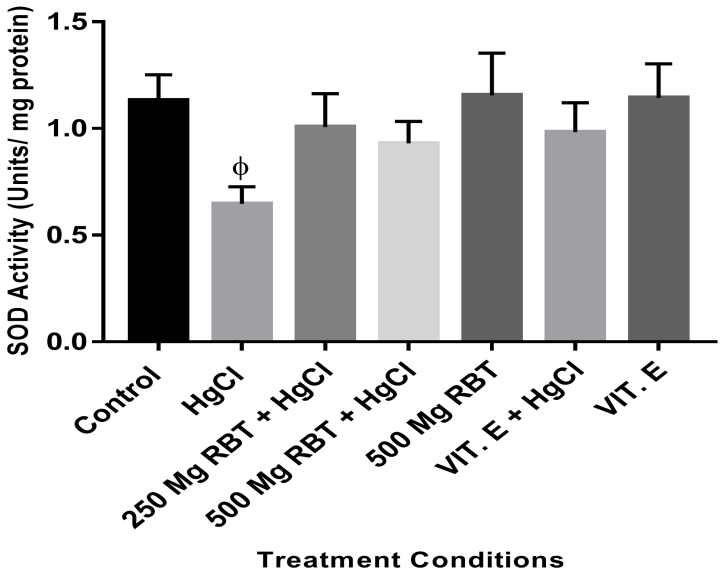


Figure 4.6: Bar chart showing SOD level across experimental groups.

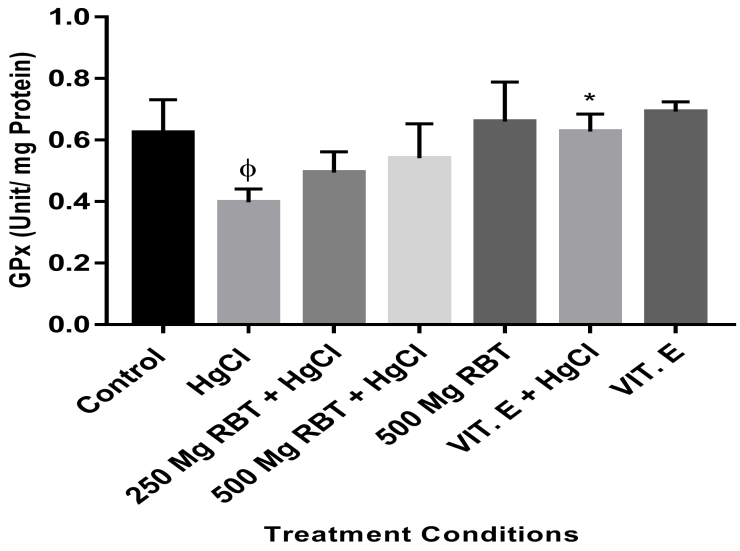


Figure 4.7: Bar chart showing GPx level across experimental groups.

4.3 HISTOLOGICAL FINDINGS

Photomicrograph from the histological section of the cerebellum shows that the purkinje cells in the purkinje cell layer in group A, C, D, E and F are normal. However the purkinje cell in the purkinje cell layer in group B degenerate

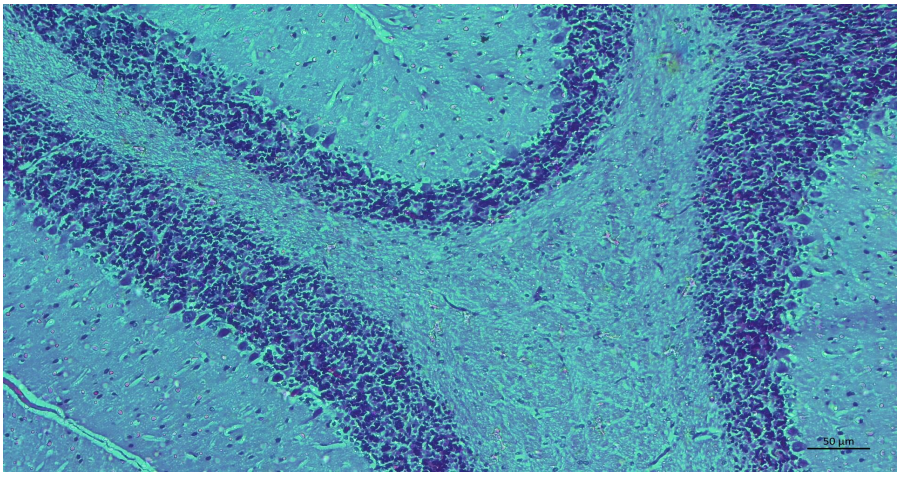


Figure 4.8: Representative histology of the cerebellum in plate A (control) rats showing normal histological structure of cerebellum layers.

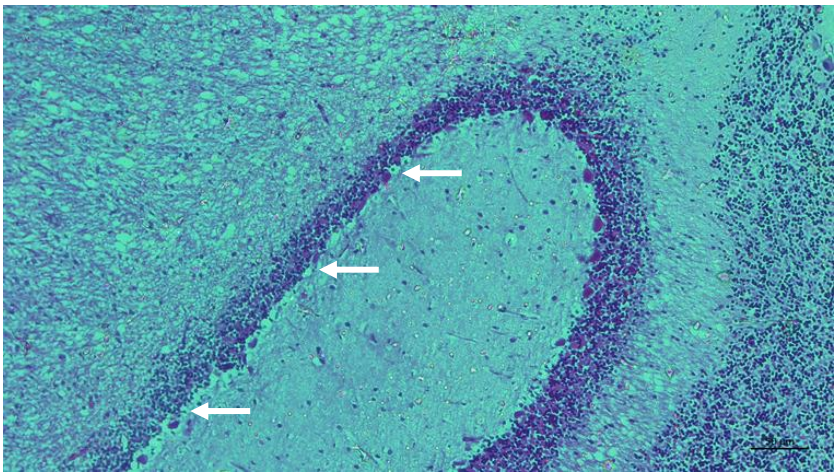


Figure 4.9: Representative histology of the cerebellum in plate B ($HgCl_2$) rats showing depletion and degeneration of the Purkinje cells in the Purkinje cell layer of the cerebellum.

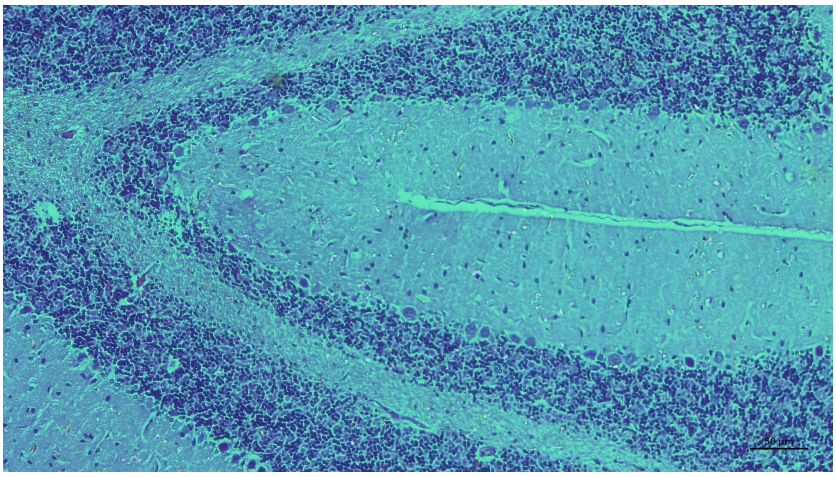


Figure 4.10: Representative histology of the cerebellum in plate C (RBT +$HgCl_2$) showing normal architecture and presence of Purkinje cells.

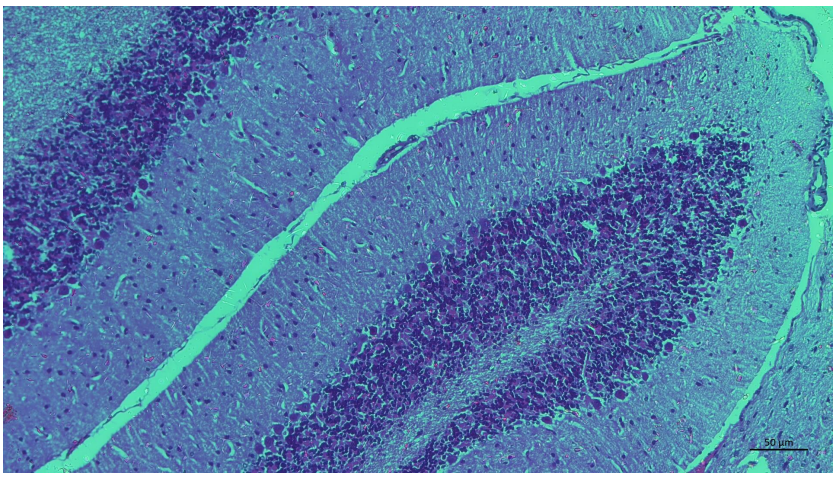


Figure 4.11: Representative histology of the cerebellum in plate D (RBT + $HgCl_2$) showing relatively normal architecture and presence of Purkinje cells.

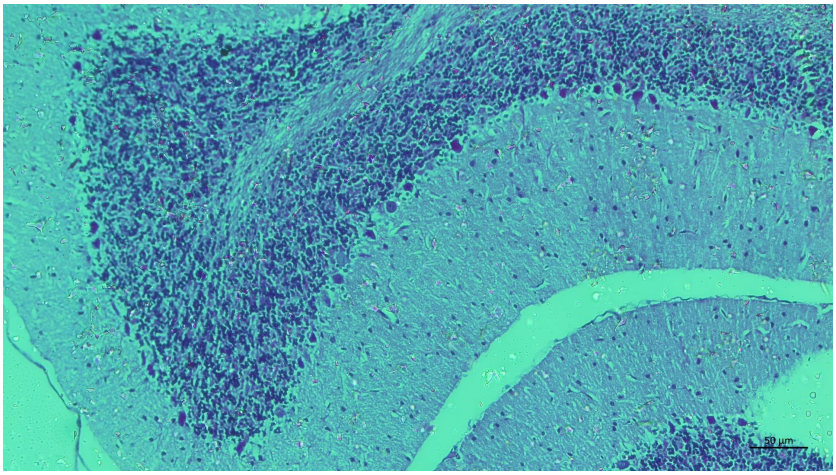


Figure 4.12: Representative histology of the cerebellum in plate E (RBT) rats showing normal histological structure of cerebellum layers.

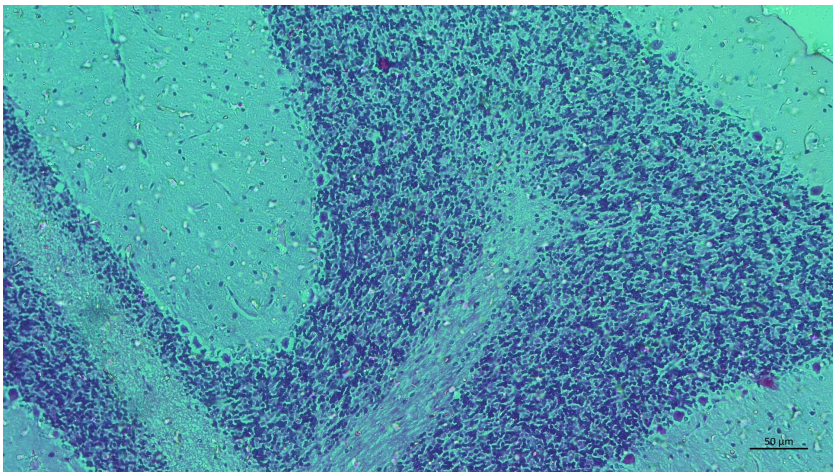


Figure 4.13: Representative histology of the cerebellum in plate F (Vit. E +$HgCl_2$) showing a normal histological structure of cerebellum layers.

CHAPTER 5

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

Mercury chloride is an inorganic mercury salt and has proven to be the most toxic of the salts, likely due to its corrosiveness and high solubility (Magos et al., 2006). Human can be exposed to mercury by inhalation, ingestion and consumption via food chain. Following exposure, mercury ions are taken up by numerous organs, including the brain, intestine, kidney, liver, and placenta and the ions are accumulated in these organs (Bridges and Zalups, 2017). Hence, this study aims to evaluate the protective activity of *Aspalathus linearis* on mercury chloride-induced cerebral toxicity.

Open field test provides information about the locomotory and exploratory activities of rats (Olapade et al., 2017). It also provides simultaneous measures of locomotion and anxiety. (Milan 2003). The findings show that the parameter of grooming and immobility shows a significant increase in the group ($Hgcl_2$) treated with only mercury chloride this increase in the frequency of their anxiety and locomotion rats when compared with groups treated with rooibos which showed a significant decrease this implies that rooibos was able to mitigate the impaired locomotion that was induced by mercury chloride.

Antioxidants scavenge free radicals from the body cells and prevent or reduce the damage caused by oxidation. Results from this study show that there was a significant increase ($P < 0.05$) in $HgCl_2$ in MDA when compared with control while there was a significant decrease ($P < 0.05$) in SOD and GPx in the cerebellum of the group treated with mercury chloride only when compared to control. While in Group C, D and F showed a significant decrease ($P < 0.05$) in MDA, it showed an increase ($P>0.05$) in both GPx and SOD activity when compared to $HgCl_2$ but it was not significant. This shows that mercury chloride is able to alter antioxidant activities by inhibiting antioxidant enzymes and generation of reactive oxygen species. Impaired antioxidant defences can be as a result of the inhibitory effects of mercury chloride on various enzymes, which in turn causes the cells to be more vulnerable to oxidative stress. However, an increase was observed in the antioxidant rooibos pretreated groups when compared to Mercury Chloride in SOD and GPx with the exception of MDA which showed significant decrease. In addition to ROS, oxidative stress may also induce uncontrolled lipid peroxidation, which in turn, can result in cell injuries via DNA damage and

directly inhibit proteins (Spiteller et al., 2007). Malondialdehyde (MDA) is a stable end product of lipid peroxidation and therefore can be used as an indirect measure of the cumulative lipid peroxidation. Our results showed an increase in cerebellum MDA level when compared to the control indicating high level of lipid peroxidation. Conversely, a decrease was observed in cerebellum MDA level rooibos pretreated groups when compared to Mercury Chloride only treated group. This implies that the rooibos was able to protect against Mercury Chloride-induced oxidative stress.

Histological findings from the control showed the normal histology of the cerebellum. However, mercury chloride treated group showed degenerated of Purkinje cells in the Purkinje cell layer. This agrees with a similar histological data from a previous study that mercury chloride exposure causes acute neurodegeneration of Purkinje cells, eosinophilic swelling seen adjacent to Purkinje cell bodies. Purkinje cells were observed without a prominent nucleolus and well-defined nuclear membrane and Pyknosis of Purkinje cells. (Ghosh B et al., 2021). In consequence with administration of the extract; rooibos was able reverse the degeneration of purkunje cells in the cerebellum of adult Wistar rats. These show a considerable neuroprotective impact of it.

Our findings also shows that the rats treated with rooibos seed extract had similar histological appearance when compared to that of control. This therefore signifies that the 250mg/kg and 500mg/kg doses of extract had no toxic effect on the cerebellum adult Wistar rats.

5.2 CONCLUSION

The findings of this study suggest a therapeutic role of *Aspalathus linearis* (rooibos) against oxidative damage and in prevention of Mercury Chloride-induced alterations in the oxidative stress biomarkers. Treatment with *Aspalathus linearis* (rooibos) reduced the peroxidation rate and restored the antioxidant capacity. Therefore, it is concluded that Mercury Chloride exposure led to varying degree of changes in antioxidant defence mechanism and tissue architecture and treatment with *Aspalathus linearis* (rooibos) provided protection from damage due to Mercury Chloride in adult Wistar rats.

REFERENCES

Abudureheman, Buhailiqiemu; Yu, Xiaochun; Fang, Dandan; Zhang, Henghui (2022). "Enzymatic Oxidation of Tea Catechins and Its Mechanism" (https://www.mdpi.com/1420-3049/27/3/942). Molecules. 27 (3): 942.

Albert RK, Porter RS, eds. (2006). The Merck Manual of Diagnosis and Therapy (18th ed.). Whitehouse Station, *New Jersey*: Merck Research Libraries. pp. 1886–1887.

Albus JS (1971). "A theory of cerebellar function". *Mathematics Biosciences*. 10 (1–2): 25–61. CiteSeerX10.1.1.14.7524.

Alina Antache, V Cristea, lulia Grecu, Mirela Cretu, Seria Zootehnie (2014). The synergistic influence of thymus vulgaris and vitamin E on growth performance and oxidative stress at Oreochromis niloticus species. 62, 85-90

Arnarson., A. (2019). https://www.healthline.com/nutrition/antioxidants-explained. Accessed 27/04/2022.

Awoniyi, D. O., Aboua, Y. G., Marnewick, J. L., Du Plesis, S. S., & Brooks, N. L. (2011). Protective effects of rooibos (*Aspalathus linearis*), green tea (*Camellia sinensis*) and commercial supplements on testicular tissue of oxidative stress-induced rats. *African Journal of Biotechnology.* 10(75):17317-17322.

Awoniyi, D. O., Aboua, Y. G., Marnewick, J., & Brooks, N. (2012). The effects of rooibos (*Aspalathus linearis*), green tea (*Camellia sinensis*) and commercial rooibos and green tea supplements on epididymal sperm in oxidative stress-induced rats. *Phytotherapy Research.* 26(8):1231-1239.

Baumann O, Borra RJ, Bower JM, Cullen KE, Habas C, Ivry RB, Leggio M, Mattingley JB, Molinari M, Moulton EA, Paulin MG, Pavlova MA, Schmahmann JD, Sokolov AA (2015). "Consensus paper: *therole of the cerebellum in perceptual processes*". Cerebellum. 14 (2): 197-220.

Bhardwaj, A., Kar, J. P., Thakur, O. P., Srivastava, P., & Sehgal, H. K. (2009). Electrical characteristics of PbSe nanoparticle/Si heterojunctions. *Journal of Nanoscience and Nanotechnology.* 9(10):5953-5957.

Boveris A, Chance B (1973) The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. *Biochemistry Journal* 134:707-716

Bramati, Lorenzo (2002). "Quantitative Characterization of Flavonoid Compounds in Rooibos Tea (Aspalathus linearis) by LC−UV/DAD". *Journal of Agricultural and Food Chemistry*. 50 (20): 5513–5519.

Bridges, C. C., & Zalups, R. K. (2017). Mechanisms involved in the transport of mercuric ions in target tissues. *Archives of toxicology*: 91(1):63-81.

Bronzetti, G. (1994). Antimutaens in food. In: *Food science and technology* (edited by B. White). Pp. 390-395, Cambridge:Elsevier science Publishers Limited.

Buckner RL (October 2013). "The cerebellum and cognitive function: 25 years of insight from anatomy and neuroimaging" (https://doi.org/10.1016%2Fj.neuron.2013.10.044). *Neuron*. 80 (3): 807–15

Buckner RL, Krienen FM, Castellanos A, Diaz JC, Yeo BT (2011). "The organization of the human cerebellum estimated by intrinsic functional connectivity" (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3214121). *Journal of Neurophysiology*. 106 (5): 2322–45.

Buege, J.A., Aust, S.D. (1978). Microsomal lipid peroxidation. Method in Enzymology. 52:302-310

Bursill Christina, Mavis Abbey, Paul Roach. *Atherosclerosis* 193(1), 89-93, 2007

Canda, Oluwafemi Omoniyi Oguntibeju, Jeanine L Marnewick. *Oxidative medicine and cellular longevity* 2014

Cape Point Press (2014) "Rooibos tea cappuccino or latte - " (http://capepointpress.com/rooibos-cappuccino-l atte/). Cape Point Press. 6 March 2014. Retrieved 20 July 2017.

Child, Lydia Maria (1832). The American Frugal Housewife (https://www.gutenberg.org/files/13493/13493-h/13493-h.htm#page21) (12th ed.). p. 21.

Chisholm, Hugh, ed. (1911). "Corrosive Sublimate" (https://en.wikisource.org/wiki/1911_Encyclop%C3%A 6dia_Britannica/Corrosive_Sublimate). Encyclopædia Britannica. Vol. 7 (11th ed.). Cambridge University Press. p. 197.

Curnow, Robyn (2012). "South Africa's rooibos a hit with tea lovers across the world | CNN

Deng, James; Wang, Yu-Pu; Danheiser, Rick L. (2015). "Synthesis of 4,4-Dimethoxybut-1-yne" (https://doi.org/10.15227%2Forgsyn.092.0013). *Organic Syntheses*. 92: 13–25.

Dong-ping, X., Ya, L., Xiao, M., Tong, Z., Yue, Z., Jie, Z., Jiao-Jiao, Z., Hua-Bin, L. (2017). Natural Antioxidants in foods and Medicinal plants; Extraction, Assessment and Resources. *International Journal of Molecular Sciences*. 18(1):96.

Doya K (1999). "What are the computations of the cerebellum, the basal ganglia and the cerebral cortex?". *Neural Networks*. 12 (7–8): 961–974.

Doya K (December 2000). "Complementary roles of basal ganglia and cerebellum in learning and motor control". *Current Opinion in Neurobiology*. 10 (6): 732–9.

Drury, R.A., Wallington, E.A. (1980). Carleton's Histological Techniques. *Oxford University Press, New York.* 5th Edition. 195.

Eccles JC, Llinás R, Sasaki K (1966). "The excitatory synaptic action of climbing fibres on the Purkinje cells of the cerebellum" (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1357472). *Journal of Physiology*. 182 (2): 268–96.

Fine EJ, Ionita CC, Lohr L (2002). "The history of the development of the cerebellar examination". *Seminars in Neurology*. 22 (4): 375–84.

Foreman, W. T.; Zaugg, S. D.; Faires, L. M.; Werner, M. G.; Leiker, T. J.; Rogerson, P. F. (1992). "Analytical interferences of mercuric chloride preservative in environmental water samples: Determination of organic compounds isolated by continuous liquid-liquid extraction or closed-loop stripping". *Environmental Science & Technology*. 26 (7): 1307.

Freeman, M.H. Shupe, T.F. Vlosky, R.P. Barnes, H.M. (2003). Past, present and future of the wood preservation industry. *Forest Products Journal*. 53(10) 8–15

Frustaci, A., Magnavita, N., Chimenti, C., Caldarulo, M., Sabbioni, E., Pietra, R., ... & Maseri, A. (1999). Marked elevation of myocardial trace elements in idiopathic dilated cardiomyopathy compared with secondary cardiac dysfunction. *Journal of the American College of Cardiology.* 33(6):1578-1583.

Ghez C, Fahn S (1985). "The cerebellum". In Kandel ER, Schwartz JH (eds.). *Principles of Neural Science,* 2nd edition. New York: Elsevier. pp. 502–522.

Ghosh, B. and Biswas, S., 2021. Polymeric micelles in cancer therapy: State of the art. *Journal of Controlled Release*, *332*, pp.127-147.

Gilman S (1998). "Imaging the brain. Second of two parts". *New England Journal of Medicine*. 338(13): 889–96.

Goldberg, Lisa (1996). "A History of Pest Control Measures in the Anthropology Collections, National Museum of Natural History, Smithsonian Institution". Retrieved April 17, 2005. JAIC. 35 (1): 23–43.

Halliwell B (1994) free radicals, antioxidant and human disease:Curiosity, cause, or consequence? Lancet 344; 721-724.

Hansen JM, Zhang H, Hones DP (2006) Differential oxidation of thio-redoxin-1, thioredoxin-2, and glutathione by metal ions. *Free Radic Biol Med* 40:138–145.

Hendricks, R., & Pool, E. J. (2010). The in vitro effects of rooibos and black tea on immune pathways. *Journal of Immunoassay and Immunochemistry*. 31(2):169-180.

Herculano-Houzel S (2010). "Coordinated scaling of cortical and cerebellar numbers of neurons" (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2839851). *Frontiers in Neuroanatomy*. 4: 12.

Hernáez-Goñi P, Tirapu-Ustárroz J, Iglesias-Fernández L, Luna-Lario P (2010). "Participación del cerebelo en la regulación del afecto, la emoción y la conducta" [The role of the cerebellum in the regulation of affection, emotion and behavior]. *Revista de Neurología* (in Spanish). 51 (10): 597–609.

Ho, C., Ferraro, T., Chen, Q., Rosen, R.T. & Huang, M. (1994). Phytochemicals in teas and rosemary & their cancer-preventive properties. In: *Food Phytochemicals for Cancer Prevention.* Washington DC: American chemical society. 11. Pp. 3-19.

Hodos W (2009). "Evolution of Cerebellum". *Encyclopedia of Neuroscience*. Berlin, Heidelberg: Springer. pp. 1240–1243.

Horvath S, Mah V, Lu AT, Woo JS, Choi OW, Jasinska AJ, Riancho JA, Tung S, Coles NS, Braun J, Vinters HV, Coles LS (2015). "The cerebellum ages slowly according to the epigenetic clock" (https://www.aging-us.com/article/100742/text). *Aging*. 7 (5): 294–306.

Iswaldi, I; Arráez-Román, D; Rodríguez-Medina, I; Beltrán-Debón, R; Joven, J; Segura-Carretero, A; Fernández-Gutiérrez, A (2011). "Identification of phenolic compounds in aqueous and ethanolic rooibos extracts (Aspalathus linearis) by HPLC-ESI-MS (TOF/IT)". *Analytical and Bioanalytical Chemistry.* 400 (10): 3643–54.

Jeffrey A. Oaks (1999): "Date Nails and Railroad Tie Preservation" (3 vol.; 560 p.), published in 1999 by the Archeology and Forensics Laboratory, University of Indianapolis; Pg. 19-75

Jeffrey D. *Cambridge University press,* 2011

Joubert, E.; Gelderblom, W.C.A.; Louw, A.; de Beer, D. (2008). "South African herbal teas: Aspalathus linearis, Cyclopia spp., and Athrixia phylicoides – a review" (https://linkinghub.elsevier.com/retrieve/pii/S0378874108003334). *Journal of Ethnopharmacology.* 119 (3): 376–412.

Joubert, E. (1996). "HPLC quantification of the dihydrochalcones, aspalathin and nothofagin in rooibos tea (Aspalathus linearis) as affected by processing". *Food Chemistry*. 55 (4): 403–411.

Khan A, Atkinson A, Graham T, Thompson S, Ali S, et al. (2004) Effects of inorganic mercury on reproductive performance of mice. *Food Chem Toxicol*. 42:571–577.

Kingsley RE (2000). Concise Text of Neuroscience (2nd ed.). Lippincott Williams & Wilkins. ISBN 978-0-683-30460-2.

Krafczyk, Nicole; Woyand, Franziska; Glomb, Marcus A. (2009). "Structure-antioxidant relationship of flavonoids from fermented rooibos". *Molecular Nutrition & Food Research*. 53 (5): 635–42.

Krzyzanowska, J., Czubacka, A., & Oleszek, W. (2010). Bio-Farms for Nutraceuticals: Functional Food and Safety Control by Biosensors. *Landes Bioscience and Springer Science*. 74-99.

Ku, S. K.; Kwak, S; Kim, Y; Bae, J. S. (2015). "Aspalathin and Nothofagin from Rooibos (Aspalathus linearis) inhibits high glucose-induced inflammation in vitro and in vivo". *Inflammation*. 38 (1): 445–55.

Kunishiro, K., Tai, A., & Yamamoto, I. (2001). Effects of rooibos tea extract on antigen-specific antibody production and cytokine generation in vitro and in vivo. *Bioscience, biotechnology, and biochemistry*. 65(10):2137-2145.

Levisohn L, Cronin-Golomb A, Schmahmann JD (2000). "Neuropsychological consequences of cerebellar tumour resection in children: *cerebellar cognitive affective syndrome in a paediatric population"* (https://doi.org/10.1093%2Fbrain%2F123.5.1041). Brain. 123 (5): 1041-50.

Llinas RR, Walton KD, Lang EJ (2004). "Ch. 7 Cerebellum". In Shepherd GM (ed.). *The Synaptic Organization of the Brain*. New York: Oxford University Press. ISBN 978-0-19-515955-4.

Lorschieder FL, Vimy MJ, Summers AO (1995) Mercury exposure from “silver” tooth filling: *emerging evidence questions a traditional dental paradigm*. FASEB J. 9:504–508.

Lund, B. O., Miller, D. M., & Woods, J. S. (1993). Studies on Hg (II)-induced H2O2 formation and oxidative stress in vivo and in vitro in rat kidney mitochondria. *Biochemical pharmacology*. 45(10):2017-2024.

Magos, L., & Clarkson, T. W. (2006). Overview of the clinical toxicity of mercury. *Annals of clinical biochemistry.* 43(4):257-268.

Maillard, Adam P. Fraise, Peter A. Lambert, Jean-Yves (2007). Principles and Practice of Disinfection, Preservation and Sterilization. Oxford: *John Wiley & Sons*. p. 4. ISBN 978-0470755068.

Mariën P, Manto M (2016). The linguistic cerebellum. London, UK: *Academic Press*. pp. 337–351. ISBN 978-0-12-801608-4.

Marnewick JL. *Personal communication.* August 2002

Marnewick JL, Rautenbach F, Venter I, *et al.,* (2002) Effects of rooibos (Aspalathus linearis) on oxidative stress and biochemical parameters in adults at risk for cardiovascular disease. *Journal of Ethnopharmacology*. 2011;133(1):46-52

Marnewick, J. L., Batenburg, W., Swart, P., Joubert, E., Swanevelder, S., & Gelderblom, W. C. A. (2004). Ex vivo modulation of chemical-induced mutagenesis by subcellular liver fractions of rats treated with rooibos (*Aspalathus linearis*) tea, honeybush (*Cyclopia intermedia*) tea, as well as green and black (*Camellia sinensis*) teas. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*. 558(1-2):145-154.

Marnewick, J. L., Rautenbach, F., Venter, I., Neethling, H., Blackhurst, D. M., Wolmarans, P., & Macharia, M. (2011). Effects of rooibos (*Aspalathus linearis*) on oxidative stress and biochemical parameters in adults at risk for cardiovascular disease. *Journal of ethnopharmacology*. 133(1):46-52.

Marnewick, J., Joubert, E., Joseph, S., Swanevelder, S., Swart, P., & Gelderblom, W. (2005). Inhibition of tumour promotion in mouse skin by extracts of rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia intermedia*), unique South African herbal teas. *Cancer letters*. 224(2):193-202.

Marr D (1969). "A theory of cerebellar cortex" (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1351491). *Journal of Physiology*. 202 (2): 437–70.

Mckay D, Blumbery JB. *Afrofood industry Hi-Tech* 20(6), 40-42, 2009.

Misra, H.P., Fridovich, I. (1972). The role of superoxide anion in the autooxidation of epinephrine and simple assay for superoxide dismutase. *The journal of biological chemistry*. 247:3170-3175

Monte, A.S., Greicy, C.S., Roger, S.M., Joanna, K.S., Júnia, V.S., Rafaela, C.C. (2013). Prevention and reversal of ketamine-induced schizophrenia related behavior by minocycline in mice: possible involvement of antioxidant and nitrergic pathway. *Journal of Psychopharmacology*. 11:1032–1043.

Morton, Julia F. (1983). "Rooibos tea, Aspalathus linearis, a caffeine-less, low-tannin beverage". *Economic Botany*. 37 (2): 164–73

Moulton EA, Schmahmann JD, Becerra L, Borsook D (2010). "The cerebellum and pain: passive integrator or active participator?" (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2943015). *Brain Research Reviews*. 65 (1): 14–27.

Mueller, M., Hobiger, S., & Jungbauer, A. (2010). Anti-inflammatory activity of extracts from fruits, herbs and spices. *Food chemistry*. 122(4):987-996.

Muller, C. J. F., Joubert, E., De Beer, D., Sanderson, M., Malherbe, C. J., Fey, S. J., & Louw, J. (2012). Acute assessment of an aspalathin-enriched green rooibos (*Aspalathus linearis*) extract with hypoglycemic potential. *Phytomedicin*. 20(1):32-39.

Muofhe, M.L.; Dakora, F.D. (1999). "Nitrogen nutrition in nodulated field plants of the shrub tea legume Aspalathus linearis assessed using 15N natural abundance". *Plant and Soil*. 209 (2): 181–186.

Nabil, A., Elshemy, M. M., Asem, M., & Gomaa, H. F. (2020). Protective effect of DPPD on mercury chloride-induced Hepatorenal toxicity in rats. *Journal of Toxicology.* 56:27.

National Institutes of Health (2014): "*NINDS Ataxias and Cerebellar or Spinocerebellar Degeneration Information Page*" (http://www.ninds.nih.gov/disorders/ataxia/ataxia.htm).. 16 April 2014.

Nikolova, V., Petrova, S., Petkova, V., Pavlova, S., Michailova, A., & Georgieva, T. (2007). Antioxidative effects of rooibos tea on workers occupationally exposed to lead. *Toxicology Letters.* (172):S120-S121.

Nyman, M. (1959). Serum hemoglobin; methodological and clinical studies*. Scandinavian Journal of Clinical and Laboratory Investigation.* 11:1-169.

Olopade, F.E., Shokunbi M.T., Siren A. (2012). The relationship between the ventricular dilation, neuropathological and neurobehavioural changes in hydrocephalic rats. *Fluids and Barriers of the CNS*. 9(1):19.

Pantsi, W.G., Marnewick, J.L., Esterhuyse, A.J., Rautenbach, F. & Van Rooyen, J. 2011. Rooibos (*Aspalathus linearus*) offers cardiac protection against ischaemial reperfusion in the isolated perfused rat heart*. Phytomedicine*, 18(14):1220-12208

Park, J. D., & Zheng, W. (2012). Human exposure and health effects of inorganic and elemental mercury. *Journal of preventive medicine and public health.* 45(6):344.

Peraza, M. A., Ayala-Fierro, F., Barber, D. S., Casarez, E., & Rael, L. T. (1998). Effects of micronutrients on metal toxicity. *Environmental Health Perspectives*. 106(suppl 1):203-216.

Petersen SE, Fox PT, Posner MI, Mintun M, Raichle ME (1989). "Positron emission tomographic studies of the processing of single words". *Journal of Cognitive Neuroscience*. 1 (2): 153–70.

Purves D, Augustine GJ, Fitzpatrick D, Hall WC, LaMantia AS, White LE (2011). *Neuroscience* (5th ed.).Sunderland, Mass.: Sinauer. pp. 417–423. ISBN 978-0-87893-695-3.

Purves D, Augustine GJ, Fitzpatrick D, Hall WC, LaMantia AS, White LE (2007). *Neuroscience* (4th ed.). New York: W. H. Freeman. pp. 197–200. ISBN 978-0-87893-697-7.

Pyszel, A., Wróbel, T., Szuba, A., & Andrzejak, R. (2005). Effect of metals, benzene, pesticides and ethylene oxide on the haematopoietic system. *Medycyna pracy*. 56(3):249-255.

Rabe, C; Steenkamp, JA; Joubert, E; Burger, JF; Ferreira, D (1994). "Phenolic metabolites from rooibos tea (Aspalathus linearis)". *Phytochemistry.* 35 (6): 1559–1565.

Rapp B (2001). The Handbook of Cognitive Neuropsychology: What Deficits Reveal about the Human Mind. *Psychology Press*. p. 481. ISBN 978-1-84169-044-5.

Rebelo, A.G.; Mtshali, H.; von Staden, L. (2006). "Large-leaf Sugarbush" (http://redlist.sanbi.org/species.php?species=799-26). Red List of South African Plants. version 2020.1. *South AfricanNational Biodiversity Institute*. Retrieved 13 August 2020.

Rice, K. M., Walker Jr, E. M., Wu, M., Gillette, C., & Blough, E. R. (2014). Environmental mercury and its toxic effects. *Journal of preventive medicine and public health*. 47(2):74.

Rusanescu G, Mao J (2017). "Peripheral nerve injury induces adult brain neurogenesis and remodelling" (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5264155). *Journal of Cellular and Molecular Medicine.* 21 (2): 299–314.

Schmahmann JD, Caplan D (2006): "Cognition, emotion and the cerebellum" (https://doi.org/10.1093%2Fbrain%2Fawh729). *Brain*. 129 (Pt 2): 290–2.

Schmahmann JD, Sherman JC (1998). "The cerebellar cognitive affective syndrome" (https://doi.org/10.1093%2Fbrain%2F121.4.561). *Brain*. 121 (4): 561–79.

Schweighofer N, Doya K, Kuroda S (2004). "Cerebellar aminergic neuromodulation: towards a functional understanding". *Brain Research. Brain Research Reviews.* 44 (2–3): 103–16.

Simpson JI, Wylie DR, De Zeeuw CI (1996). "On climbing fiber signals and their consequence(s)". Behav. *Brain Science*. 19 (3): 384–398.

Snider RS, Stowell A (1 November 1944). "Receiving Areas of the Tactile, Auditory, and Visual Systems in the Cerebellum". *Journal of Neurophysiology*. 7 (6): 331–357.

Solt, I., & Bornstein, J. (2010). Childhood vaccines and autism--much ado about nothing?. Harefuah. 149(4):251-5.

Spiteller, G., 2007. The important role of lipid peroxidation processes in aging and age dependent diseases. *Molecular biotechnology*, *37*(1), pp.5-12.

Stander, Maria A.; Joubert, Elizabeth; De Beer, Dalene (2019). "Revisiting the caffeine-free status of rooibos and honeybush herbal teas using specific MRM and high resolution LC-MS methods". *Journal of Food Composition and Analysis*. Elsevier BV. 76: 39–43.

Standley, L; Winterton, P; Marnewick, JL; Gelderblom, WC; Joubert, E; Britz, TJ (2001). "Influence of processing stages on antimutagenic and antioxidant potentials of rooibos tea". *Journal of Agricultural and Food Chemistry.* 49 (1): 114–7.

Standring S, Borley NR, et al., eds. (2008). "Chapter 20". Gray's anatomy : *the anatomical basis of clinical practice* (40th ed.). London: Churchill Livingstone. p. 297. ISBN 978-0-8089-2371-8.

Strick PL, Dum RP, Fiez JA (2009). "Cerebellum and nonmotor function". *Annual Review of Neuroscience*. 32: 413–34.

Suksomboon N., Poolsup N., Boonkues S.,Suthisisang C.C. *Meta-analysis of the effect of herbal supplement on glycemic control in type 2 diabetes Journal Ethnopharmacol*. 2011;137:1328-1333.

Teixeira, F. B., de Oliveira, A. C., Leão, L. K., Fagundes, N. C., Fernandes, R. M., & Fernandes, L. M. (2018). Exposure to inorganic mercury causes oxidative stress, cell death, and functional deficits in the motor cortex. *Front Mol Neuroscience.* 11:125.

The Day Book (1913): "Want Sale of Bichloride of Mercury Restricted" (https://chroniclingamerica.loc.gov/lccn/sn83045487/1913-06-23/ed-1/seq-8/).. Chicago, IL. 1913-06-23. Retrieved 2021-09-25.

Timmann D, Daum I (2007). "Cerebellar contributions to cognitive functions: a progress report after two decades of research". *Cerebellum*. 6 (3): 159–62.

Vachhrajani K, Makhija S, Chinoy N, Chowdhury A (1988) Structural and functional alterations in testis of rats after mercuric chloride treatment. *Jounal Reprod Bio Compara Endocrinol*. 8:97–104.

Valko M, Morris H, Cronin MTD (2005) Metals, toxicity and oxidative stress. *Currurent Medical Chemistry*. 12:161–208.

Vincent M, Hadjikhani N (2007). "The cerebellum and migraine" (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3761082). Headache. 47 (6): 820–33.

Wells, A.F. (1984) Structural Inorganic Chemistry, Oxford: Clarendon Press. ISBN 0-19-855370-6.

Whitney ER, Kemper TL, Rosene DL, Bauman ML, Blatt GJ (2008). "Calbindin-D28k is a more reliable marker of human Purkinje cells than standard Nissl stains: a stereological experiment". *Journal of Neuroscience Methods*. 168 (1): 42–7.

Wikipedia (2022): Mercury (II) chloride, toxicity

Wipo Magazine (2007). "(Making the Origin Count: Two Coffees). And a Tea" (https://www.wipo.int/wipo_magazine/en/2007/05/article_0002.html). www.wipo.int.

Wolf U, Rapoport MJ, Schweizer TA (2009). "*Evaluating the affective component of the cerebellar*

Yoo, K. M., Lee, C. H., Lee, H., Moon, B., & Lee, C. Y. (2008). Relative antioxidant and cytoprotective activities of common herbs. *Food chemistry*. 106(3):929-936.

Zhang, H., Tan, X., Yang, D., Lu, J., Liu, B., Baiyun, R., & Zhang, Z. (2017). Dietary luteolin attenuates chronic liver injury induced by mercuric chloride via the Nrf2/NF-κB/P53 signaling pathway in rats. *Oncotarget.* 8(25):40982.