ACTIVITY OF VITAMIN E IN THE CEREBELLUM OF WISTAR RATS TREATED WITH ALUMINIUM CHLORIDE

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

IN PARTIAL FULFULMENT FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc) DEGREE IN ANATOMY OF THE UNIVERSITY OF BENIN, BENIN CITY

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DECLARATION

I declared 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 view are essentially based on this research. And where the views of others have been expressed, such words were duly acknowledged

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NEGBENEBOR ENAHOLO HUMPHERY

CERTIFICATION

This is to certify that this project work titled "**ACTIVITY OF VITAMIN E IN THE CEREBELLUM OF WISTAR RATS TREATED WITH ALUMINIUM CHLORIDE**" was carried out by **NEGBENEBOR ENAHOLO HUMPHERY** in the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City.

ADAZE B. ENOGIERU (Ph.D)	**DATE**
Supervisor	

ADAZE B. ENOGIERU (Ph.D)	**DATE**
Head of Department	

EXTERNAL EXAMINER	**DATE**

DEDICATION

This project is dedicated to God Almighty for his divine guidance, wisdom and strength that has seen me through my academic journey.

To my parents Mr. and Mrs. Negbenebor for their love and care and support. I will continue to make you proud.

ACKNOWLEDGEMENT

I will like to express my deepest gratitude to everyone who supported me throughout thye completion of this project. Their encouragement, guidiance and assistance have been invaluable.

First and foremost, I extend my appreciation to my parents (Mr. & Mrs NEGBENEBOR) for their unwavering love. Their belief in me has been a constant source of motivation despite attempts to give up. And I am truly grateful for their sacrifices.

And to my supervisor and Head of Department Dr Adaze B. Enogieru, for his constant push, patience, insightful feedback throughout my journey. And his lovely wife Mrs Radiance Enogieru. Thank you so much for the love.

A special thanks to my best lecturer Dr Vitalis. I never knew I would get to love him so much. But he has shown me love more than I ever deserved.

To my friends and family, Eric Negbenebor and wife, Odogbo Samuel, Triumph, Choice, Wisdom, Kelvin, Maxwell, Agbono Deborah, Prestige, Elohor, Courage, Enoghase Raymond especially and respectfully and lots more who I may not remember to mention at this time. I say thank you to you all.

To my big mummy Mrs Olotu Okhuosuri. Thank you mummy for the motivations as a grandmother. May God continue blessing you as you retire.

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ABSTRACT

Vitamin E, a fat-soluble antioxidant, plays a crucial role in protecting cellular membranes from oxidative damage. Its neuroprotective properties have garnered attention in recent studies, particularly concerning neurotoxicity induced by aluminum chloride (AlCl3). Exposure to AlCl3 has been linked to cognitive deficits and neurodegenerative changes in the brain, making it a significant concern in neurobiology. Research has demonstrated that Vitamin E administration can mitigate the adverse effects of AlCl3 by reducing oxidative stress and inflammation in the brain. This research aims to explore the activity of Vitamin E in the cerebellum of Wistar rats treated with aluminum chloride. A total of twenty-eight (28) adult Wistar rats with an average weight of 180g were used for this study. They were randomly assigned into four groups (A, B, C, and D) with each group consisting of Seven rats. Group A served as control, Group B was administered 5mg/kg of Aluminum chloride, Group C was administered 5mg/kg of Aluminum chloride + Vitamin E and Group E was administered Vitamin E only. Administration lasted for 28 days and was done via oral route. Neurobehavioural activity was assessed after administration on the 28th day. The rats were ;sacrificed after the neurobehavioural activity was assessed. The key findings of this study suggest that Vitamin E administration mitigated the adverse effects of aluminum chloride exposure on the cerebellum of Wistar rats by reducing oxidative stress, improving antioxidant enzyme activity, and preventing neurodegeneration in the Purkinje cell layer. The findings of this study indicate that Vitamin E can effectively protect the cerebellum of Wistar rats against the neurotoxic effects of aluminum chloride exposure by modulating oxidative stress and improving antioxidant defense mechanisms.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

The cerebellum plays a crucial role in motor coordination, balance, and cognitive functions (Stoodley, 2011). However, exposure to neurotoxic substances such as aluminum chloride ($AlCl_3$) has been linked to cerebellar dysfunction, oxidative stress, and neurodegeneration (Abbas *et al*., 2022).

Aluminum chloride is a widely used industrial compound known for its role as a Lewis acid and catalyst in organic reactions (Murty *et al*., 2006). However, its neurotoxic properties have raised significant concerns. Chronic exposure to aluminum compounds has been associated with neurological disorders, including Alzheimer's disease and other forms of neurodegeneration (Dey and Singh, 2022). Studies have shown that aluminum can cross the blood-brain barrier (Song *et al*., 2008), accumulate in neural tissues, and induce oxidative stress (Yuan *et al*., 2012), mitochondrial dysfunction (Xu *et al*., 2017), and apoptosis (Rahimzadeh *et al*., 2022). Specifically, in the cerebellum, aluminum exposure has been linked to impaired motor coordination, neuronal loss, and altered synaptic transmission (Kaur *et al*., 2021).

The cerebellum, often referred to as the "little brain," is responsible for coordinating voluntary movements, maintaining balance, and fine-tuning motor activities (Abg-Abd-Wahab *et al*., 2019). It consists of an intricate network of neurons, including Purkinje cells, granule cells, and deep cerebellar nuclei, which are essential for its function (Miall, 2022). Damage to the cerebellum due to toxic insults like aluminum exposure can lead to ataxia, tremors, and cognitive deficits (Manto, 2021). The histological structure of the cerebellum, with its three distinct cortical layers—the

molecular layer, Purkinje cell layer, and granular layer—makes it particularly susceptible to oxidative stress and excitotoxicity induced by aluminum compounds (Dell'Acqua *et al*., 2013).

Vitamin E is a fat-soluble antioxidant that plays a vital role in protecting neural tissues from oxidative damage (Grimm *et al*., 2016). It consists of tocopherols and tocotrienols, which function by scavenging reactive oxygen species (ROS) and stabilizing cell membranes (Papas, 2019). Studies have demonstrated that vitamin E can mitigate neuronal degeneration by reducing lipid peroxidation (Petrovic *et al*., 2020, modulating inflammatory responses (Lewis *et al*., 2018), and enhancing mitochondrial function (Abadi *et al*., 2013). Given its potent antioxidant properties, vitamin E has been proposed as a therapeutic agent against neurotoxicants, including aluminum-induced toxicity.

1.2 Statement of Research Problem

Neurotoxicity induced by aluminum chloride ($AlCl_3$) has been implicated in various neurodegenerative disorders, including Alzheimer's disease and cerebellar dysfunction (Abbas *et al*., 2022). Chronic exposure to aluminum compounds has been associated with oxidative stress, neuronal apoptosis, and impaired neurotransmission, leading to progressive motor and cognitive deficits (Yuan *et al*., 2012; Xu *et al*., 2017; Rahimzadeh *et al*., 2022). The cerebellum, which plays a crucial role in motor coordination and balance, is particularly vulnerable to neurotoxic insults due to its high metabolic activity and extensive neural circuitry. However, despite the well-documented neurotoxic effects of aluminum, there remains a limited understanding of its specific impact on cerebellar structure and function.

1.3 Significance of Study

This study is significant as it explores the impact of aluminum chloride-induced neurotoxicity on the cerebellum and evaluates the potential neuroprotective role of vitamin E. The findings will contribute to the understanding of aluminum-related neurodegeneration and provide insights into antioxidant-based therapeutic strategies.

Understanding how aluminum chloride affects the cerebellum is crucial, as this brain region is responsible for motor coordination, balance, and cognitive functions. By identifying specific histopathological changes and oxidative stress markers, the study will offer valuable insights into the mechanisms of aluminum-induced neuronal damage.

The study will also assess whether vitamin E, a well-known antioxidant, can counteract the harmful effects of aluminum toxicity. If proven effective, vitamin E could be considered as a potential therapeutic agent for preventing or reducing cerebellar dysfunction caused by environmental neurotoxins. This would further support the use of antioxidants in mitigating neurodegenerative processes.

Given the widespread presence of aluminum in food, water, medications, and industrial products, the study has important public health implications. Understanding the neurotoxic effects of aluminum chloride could help inform regulatory policies and raise awareness about potential risks associated with prolonged exposure. This knowledge may lead to better guidelines for safe levels of aluminum consumption and exposure in humans.

The research also has the potential to serve as a foundation for further investigations into the broader impact of aluminum toxicity on the nervous system. By establishing a link between aluminum-induced cerebellar damage and the protective effects of vitamin E, this study may pave the way for future clinical applications. It could contribute to the development of antioxidant-based neuroprotective strategies, particularly for individuals at risk of neurodegenerative disorders.

By investigating the role of vitamin E in preserving cerebellar integrity, this study may have broader implications for conditions such as Alzheimer's disease, Parkinson's disease, and other neurodegenerative disorders where oxidative stress is a major contributing factor. If vitamin E demonstrates neuroprotective benefits, it could become an essential component in dietary and pharmacological interventions aimed at reducing the burden of such diseases.

Overall, this study will contribute to the fields of neuroscience, toxicology, and public health by providing critical insights into the dangers of aluminum exposure and the potential benefits of vitamin E in maintaining brain health.

1.4 Aim of the Study:

The aim of this study is to investigate the activity of Vitamin E in the cerebellum of rats treated with aluminum chloride.

1.5 Specific Objectives

The specific objectives of the study are to investigate the activity of vitamin E on:

- The brain and body weight changes in rats treated with or without aluminum chloride.
- The neurobehavioral activity (Open Field Test and Movement Initiation Test) of rats treated with or without aluminum chloride.
- The antioxidant activities (Superoxide dismutase, Catalase, Glutathione peroxidase, and Glutathione) in rats treated with or without aluminum chloride.
- The lipid peroxidation (Malondialdehyde) concentration in Wistar rats treated with or without aluminum chloride.
- The histology of the Cerebellum in rats treated with or without aluminum chloride.

CHAPTER TWO

LITERATURE REVIEW

2.1 ALUMINUM CHLORIDE

Aluminum chloride, also known as aluminum trichloride, is an inorganic compound with the formula $AlCl_3$. It forms a hexahydrate with the formula $[Al(H_2O)^6]Cl_3$, containing six water molecules of hydration. Both the anhydrous form and the hexahydrate are colourless crystals, but samples are often contaminated with iron(III) chloride, giving them a yellow colour. The anhydrous form is commercially important. It has a low melting and boiling point. It is mainly produced and consumed in the production of aluminum, but large amounts are also used in other areas of the chemical industry (Helmboldt *et al*., 2007). The compound is often cited as a Lewis acid. It is an inorganic compound that reversibly changes from a polymer to a monomer at mild temperature.

2.1.1 HISTORY

The salt was known in the 18th century as muriate of alumina, marine alum, argillaceous marine salt (Fourcroy, 1790). muriated clay (Berthollet, 1791), It was first chemically studied in the 1830s (Gambold, 1835).

Anhydrous

AlCl3 adopts three structures, depending on the temperature and the state (solid, liquid, gas). Solid AlCl3 has a sheet-like layered structure with cubic close-packed chloride ions. In this framework, the Al centres exhibit octahedral coordination geometry (Wells, 1984). Yttrium(III) chloride adopts the same structure, as do a range of other compounds. When aluminum trichloride is in its melted state, it exists as the dimer Al_2Cl_6, with tetracoordinate aluminum. This change in structure is related to the lower density of the liquid phase (1.78 g/cm3) versus solid aluminum trichloride (2.48 g/cm3). Al_2Cl_6 dimers are also found in the vapour phase. At higher

temperatures, the Al_2Cl_6 dimers dissociate into trigonal planar $AlCl_3$ monomer, which is structurally analogous to BF_3 . The melt conducts electricity poorly (Greenwood and Earnshaw, 1984), unlike more ionic halides such as sodium chloride. Aluminum chloride monomer belongs to the point group D3h in its monomeric form and D_2h in its dimeric form.

Hexahydrate

The hexahydrate consists of octahedral $[Al(H_2O)_6]^{3+}$ cation centers and chloride anions (Cl^-) as counterions. Hydrogen bonds link the cation and anions (Andress and Carpenter, 1934). The hydrated form of aluminum chloride has an octahedral molecular geometry, with the central aluminum ion surrounded by six water ligand molecules. Being coordinatively saturated, the hydrate is of little value as a catalyst in Friedel-Crafts alkylation and related reactions.

2.1.2 USES

Alkylation and acylation of arenes

$AlCl_3$ is a common Lewis-acid catalyst for Friedel-Crafts reactions, both acylations and alkylations (Olah, 1963). Important products are detergents and ethylbenzene. These types of reactions are the major use for aluminum chloride, for example, in the preparation of anthraquinone (used in the dyestuffs industry) from benzene and phosgene (Greenwood and Earnshaw, 1984) In the general Friedel-Crafts reaction, an acyl chloride or alkyl halide reacts with an aromatic system as shown (Olah, 1963): The alkylation reaction is more widely used than the acylation reaction, although its practice is more technically demanding. For both reactions, the aluminum chloride, as well as other materials and the equipment, should be dry, although a trace of moisture is necessary for the reaction to proceed (Nenitzescu and Cantuniari, 1933). A general problem with the Friedel-Crafts reaction is that the aluminum chloride catalyst sometimes is required in full stoichiometric quantities, because it complexes strongly with the products. This complication sometimes generates a large amount of corrosive waste. For these and similar reasons, the use of aluminum chloride has often been displaced by zeolites (Helmboldt *et al*.,

2007). Aluminum chloride can also be used to introduce aldehyde groups onto aromatic rings, for example via the Gattermann-Koch reaction which uses carbon monoxide, hydrogen chloride and a copper(I) chloride co-catalyst (Wade, 2003).

Other applications in organic and organometallic synthesis

Aluminum chloride finds a wide variety of other applications in organic chemistry (Galatsis, 1999). For example, it can catalyse the ene reaction, such as the addition of 3-buten-2-one (methyl vinyl ketone) to carvone (Snider, 1980).

It is used to induce a variety of hydrocarbon couplings and rearrangements (Rieke *et al*., 1979; Shama and Wamser, 1983). Aluminum chloride combined with aluminum in the presence of an arene can be used to synthesize bis(arene) metal complexes, e.g. bis(benzene)chromium, from certain metal halides via the Fischer– Hafner synthesis. Dichlorophenylphosphine is prepared by reaction of benzene and phosphorus trichloride catalyzed by aluminum chloride (Buchner and Lockhart, 1951).

Medical

Topical aluminum chloride hexahydrate is used for the treatment of hyperhidrosis (excessive sweating) (McConaghy and Fosselman, 2018; Nawrocki and Cha, 2019)

Reactions

Anhydrous aluminum chloride is a powerful Lewis acid, capable of forming Lewis acid-base adducts with even weak Lewis bases such as benzophenone and mesitylene (Olah, 1963). It forms tetrachloroaluminate ($[AlCl_4]^-$) in the presence of chloride ions. Aluminum chloride reacts with calcium and magnesium hydrides in tetrahydrofuran forming tetrahydroaluminates.

Reactions with Water

Anhydrous aluminum chloride is hygroscopic, having a very pronounced affinity for water. It fumes in moist air and hisses when mixed with liquid water as the Cl− ligands are displaced with H2O molecules to form the hexahydrate $[Al(H_2O)_6]Cl^3$. The anhydrous phase cannot be regained on heating the hexahydrate. Instead HCl is lost leaving aluminum hydroxide or alumina (aluminum oxide)

$[Al(H_2O)_6]Cl_3 \rightarrow Al(OH)_3 + 3HCl + 3H_2O$

Like metal aquo complexes, aqueous AlCl3 is acidic owing to the ionization of the aquo ligands:

$[Al(H_2O)_6]_3+ \rightleftharpoons [Al(OH)(H_2O)_5]^{2+}+H^+$

Aqueous solutions behave similarly to other aluminum salts containing hydrated Al^{3+} ions, giving a gelatinous precipitate of aluminum hydroxide upon reaction with dilute sodium hydroxide:

$AlCl_3 + 3NaOH \rightarrow Al(OH)_3 + 3NaCl$

Synthesis

Aluminum chloride is manufactured on a large scale by the exothermic reaction of aluminum metal with chlorine or hydrogen chloride at temperatures between 650 and 750 °C (1,202 and 1,382 °F) (Greenwood and Earnshaw, 1984).

$2Al + 3Cl_2 \rightarrow 2AlCl_3$

$2Al + 6HCl \rightarrow 2AlCl_3 + 3H_2$

Aluminum chloride may be formed via a single displacement reaction between copper(II) chloride and aluminum.

$2Al + 3CuCl_2 \rightarrow 2AlCl_3 + 3Cu$

In the US in 1993, approximately 21,000 tons were produced, not counting the amounts consumed in the production of aluminum (Helmboldt *et al*., 2007). Hydrated aluminum trichloride is prepared by dissolving aluminum oxides in hydrochloric acid. Metallic aluminum also readily dissolves in hydrochloric acid ─ releasing hydrogen gas and generating considerable heat. Heating this solid does not produce anhydrous aluminum trichloride, the hexahydrate decomposes to aluminum hydroxide when heated:

$[Al(H_2O)_6]Cl_3 \rightarrow Al(OH)^3 + 3HCl + 3H_2O$

Aluminum also forms a lower chloride, aluminum(I) chloride (AlCl), but this is very unstable and only known in the vapour phase (Greenwood and Earnshaw, 1984).

2.1.3 Natural Occurrence

Anhydrous aluminum chloride is not found as a mineral. The hexahydrate, however, is known as the rare mineral chloraluminite (Aneto, 2012). A more complex, basic and hydrated aluminum chloride mineral is cadwaladerite (Graedel, 1989; Aneto, 2012).

2.1.4 Safety

Anhydrous $AlCl_3$ reacts vigorously with bases, so suitable precautions are required. It can cause irritation to the eyes, skin, and the respiratory system if inhaled or on contact (Thá *et al*., 2022).

2.2 CEREBELLUM

Cerebellum is a Latin word meaning little brain. It is the largest part of the hind brain and weighs about 150 g. It is enshrined in posterior cranial fossa beneath the tentorium cerebelli behind the pons and medulla oblongata. Cerebellum is separated from the pons and medulla by the cavity of fourth ventricle (Figure 1). Cerebellum is connected to brainstem by three large bundles of fibres called cerebellar peduncles. Superior peduncle connects cerebellum with mid brain, middle with pons and inferior with medulla oblongata (Singh, 2020).

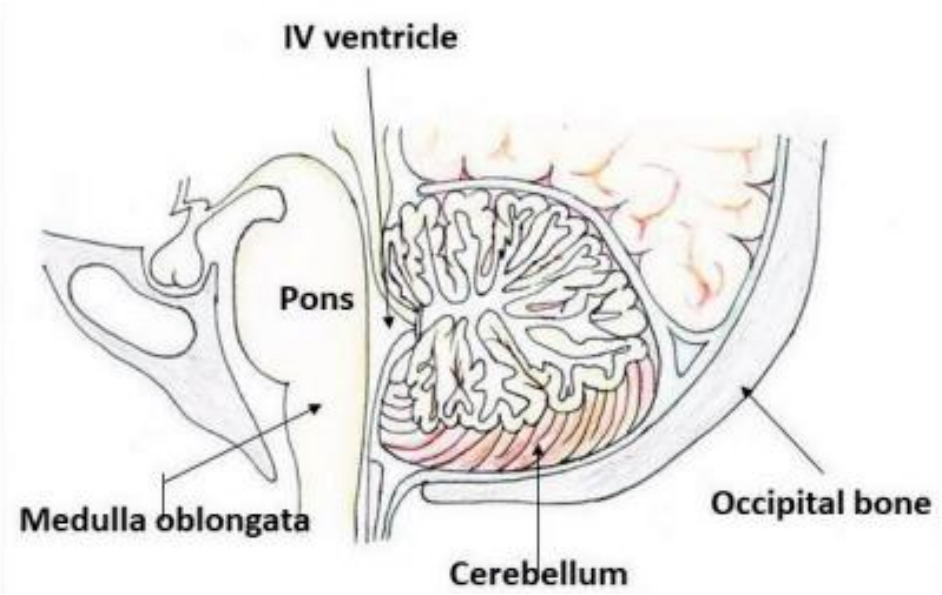

Figure 1. The location and connections of cerebellum

2.2.1 Gross anatomy

Grossly cerebellum comprises of three parts: two surfaces, two notches and three well demarcated fissures (Figure 2A and B).

Parts

Cerebellum consists of two large bilateral lobes called cerebellar hemispheres. These two lobes are united to each other by a median worm like portion, vermis. Superior and inferior aspect of vermis are known as superior and inferior vermis, respectively. Superior vermis is continuous with the hemispheres but the inferior vermis is separated from hemispheres by deep furrow, the vallecular (Singh, 2020).

Surfaces

Superior surface of the cerebellum is convex, and two cerebellar hemispheres are continuous with each other on this surface. Inferior surface presents deep furrow known as vallecular which separates two cerebellar hemispheres. The floor of the vallecular is occupied by inferior vermis.

Notches

There is a wide shallow gap known as anterior cerebellar notch on the anterior aspect of cerebellum. The anterior cerebellar notch lodges pons and medulla. Similarly, posteriorly there is posterior cerebellar notch which lodges falx cerebelli.

Fissures

Three fissures are related to cerebellum viz. horizontal, postero-lateral and primary fissures.

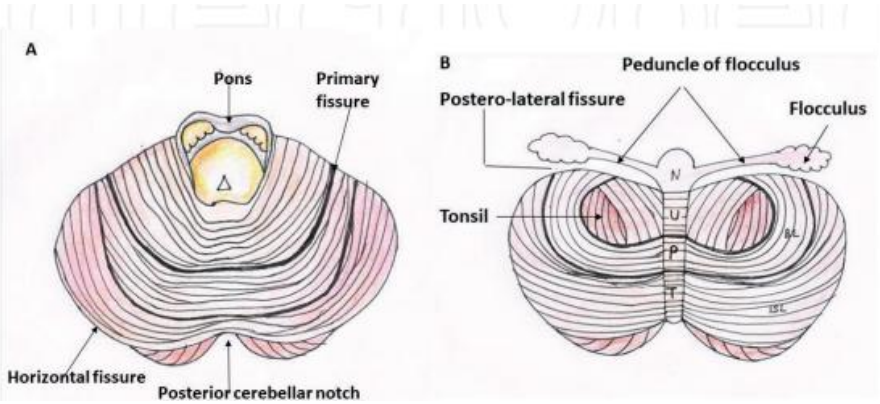

Figure 2. (A) Schematic diagram showing superior surface of the cerebellum; (B) schematic diagram showing subdivisions of the cerebellum on the inferior surface. N = nodule, U = uvula, P = pyramid, T = tuber, BI = biventral lobule, ISL = inferior semilunar lobule.

Horizontal fissure is most prominent and courses along the lateral and posterior margins of the cerebellum. It separates the superior and inferior surfaces of the cerebellum. Postero-lateral fissure is located on the inferior surface of the cerebellum and separates the flocculonodular lobe from the rest of the cerebellum also known as corpus cerebelli. Primary fissure is situated on the superior surface and divides the corpus cerebelli into anterior and posterior (middle) lobes.

2.3.2 Subdivisions of cerebellum

Anatomical subdivisions

Cerebellum is divided by postero-lateral fissure into flocculonodular lobe and corpus cerebelli which is further divided by primary fissure into anterior and posterior lobes. Flocculonodular lobe is located on the inferior surface in front of postero-lateral fissure and comprises of nodule of inferior vermis and a pair of floccule which are connected to the nodule by peduncles. Anterior lobe is situated on the superior surface anterior to primary fissure. Vermal portion of anterior lobe consists of lingual, central lobule, and culmen. Posterior lobe is located between the primary fissure on superior surface and postero-lateral fissure on inferior surface. This lobe includes both surfaces of cerebellum. Superior surface of posterior lobe consists of declive and folium while inferior surface consists of tuber, pyramid and uvula (Chaurasia *et al*., 2009, Kulkarni *et al*., 2012). Subdivisions of vermis and cerebellar hemispheres are described in

Table 1. **Subdivisions of vermis and cerebellar hemispheres.**

Lobes	Subdivisions of Vermis	Subdivisions of Cerebellar hemispheres
Anterior lobe	Lingula	No lateral extension
	Central lobe	Ala
	Culmen	Quadrangular lobule
Posterior lobe	Declive	Lobulus simplex
	Folium	Superior Semilunar lobule
	Tuber	Inferior Semilunar Lobule
	Pyramid	Biventral lobule
	Uvula	Tonsil
Flocculonodular lobe	Nodule	Flocculus

Morphological subdivisions

Phylogenetically cerebellum is divided into three subdivisions: Archicerebellum, Paleocerebellum and Neocerebellum. Archicerebellum (vestibular cerebellum): it is the oldest part of cerebellum and first to appear in aquatic vertebrates. Fishes and lower amphibians possess only this component of the cerebellum. Archicerebellum comprises of flocculonodular lobe and lingula and has mainly vestibular connections. It maintains equilibrium, tone and posture of trunk muscles. Lobes Subdivisions of vermis Subdivisions of cerebellar hemispheres Anterior lobe Lingula No lateral extension Central lobule Ala Culmen Quadrangular lobule Posterior lobe Declive Lobulus simplex Folium Superior semilunar lobule Tuber Inferior semilunar lobule Pyramid Biventral lobule Uvula Tonsil Flocculonodular lobe Nodule Flocculus Table 1. Subdivisions of vermis and cerebellar hemispheres. Neurodegenerative Diseases - Molecular Mechanisms and Current Therapeutic Approaches 4 Figure 3. Structure of cerebellar cortex along with intrinsic neurons and their processes. Paleocerebellum (spinal cerebellum) appears next in terrestrial vertebrates with the appearance of limbs. It includes anterior lobe except lingula and pyramid and uvula. It is concerned with spinocerebellar connections and responsible for tone, posture and crude movements of the limbs. Neocerebellum (cerebral cerebellum) is the most recent part of cerebellum to develop. It develops in primates and associated with the enlargement of telencephalone and cerebral cortex. It is very prominent in higher mammals. Neocerebellum includes posterior lobe except pyramid and uvula. It is mainly cortico-ponto-cerebellar connections and is concerned with smooth performance of skilled voluntary movements (Chaurasia *et al*., 2009, Kulkarni *et al*., 2012).

2.2.3 Histology of cerebellum

Cerebellum consists of outer layer of grey matter, the cerebellar cortex and inner layer of white matter. Masses of grey matter, intracerebellar nuclei lie embedded in the white matter. Cerebellar cortex is folded to form narrow leaf like bands called folia. Each folium consists of central core of

white matter surrounded by thin layer of grey matter. Central core of white matter is arranged in the form of the branching tree so called arbor vitae cerebelli.

Grey matter Main features of grey matter are

(a) cerebellar cortex and

(b) intracebellar nuclei.

2.2.4 Structure of cerebellar

Cerebellar cortex composed of three distinct layers:

(a) outer molecular layer,

(b) intermediate Purkinje cell layer, and

(c) inner granular layer cortex (Figure 3).

Molecular (plexiform) layer

This layer consists of unmyelinated nerve fibres derived from axons of granule, stellate and basket cells, dendrites of Purkinje and Golgi cells. It also contains

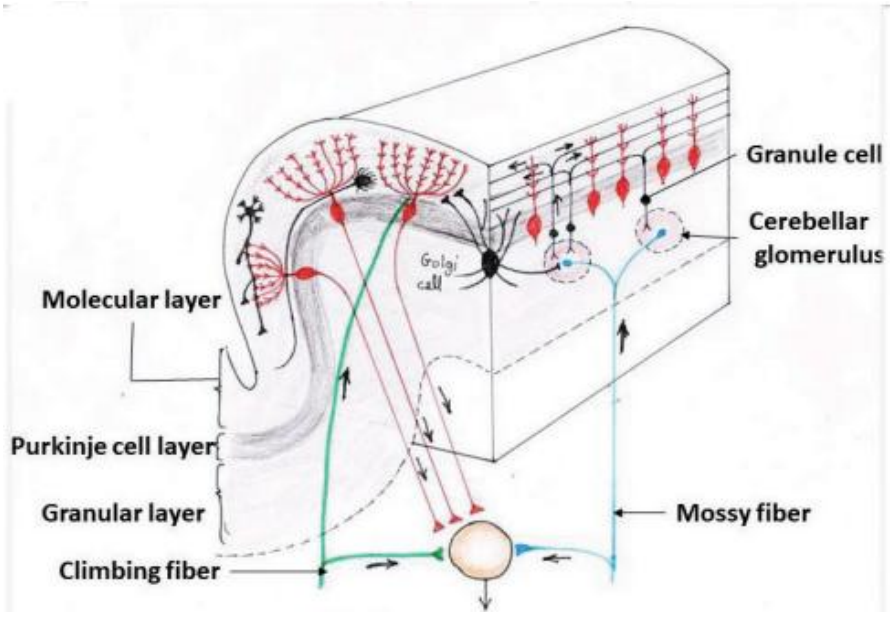

Figure 3. Structure of cerebellar cortex along with intrinsic neurons and their processes

stellate and basket cells. Stellate cells possess short process and are found scattered near the surface. The axons of these cell synapse with the dendrites of Purkinje cells. Basket cells contains little cytoplasm but have extensive processes. The axons of these cells follow transverse course parallel to the cortical surface and synapse with dendrites of Purkinje cells.

Purkinje cell layer

Purkinje cell layer consists of single layer of flask shaped Purkinje cells. Dendrites of these cells travel upwards into the molecular layer in which these cells undergo profuse branching. The dendrites of Purkinje cells synapse with collaterals of basket cells, axons of granule cells and climbing fibres. Axons of Purkinje cells travel through granular layer into white matter where they form synaptic connections with intracerebellar nuclei and exert inhibitory influence on these nuclei.

Granular layer

The inner granular layer composed of numerous granule cells and few Golgi cells. Each granule cell possesses 4–5 dendrites which synapse with mossy fibres. Axons of these cells courses into molecular layer where these bifurcates and branches pass parallel to the long axis of cerebellar folium. These fibres are known as parallel fibres and synapse with the dendrites of Purkinje cells. Golgi cells are prominent but scanty, and their dendrites ramify in molecular layer. Human cerebellum contains about 30–50 billion granule cells, 30 million Purkinje cells and 100 million stellate and basket cells. Purkinje cells, granule cells, stellate cells, basket cells and Golgi cells constitute intrinsic neurons of cerebellar cortex. All intrinsic neurons except granule cells are inhibitory and such collection of inhibitory neurons is not found anywhere in the central nervous system except cerebellum (Singh, 2020).

Intracerebellar nuclei

Intracerebellar nuclei (Figure 4) also known as central nuclei are collection of grey matter embedded in white matter. As these are situated close to roof of IV ventricle on each side of midline hence also referred as roof nuclei. From lateral to medial side, these are

(1) Dentate nucleus

(2) Emboliform nucleus

(3) Globose nucleus

(4) Fastigial nucleus

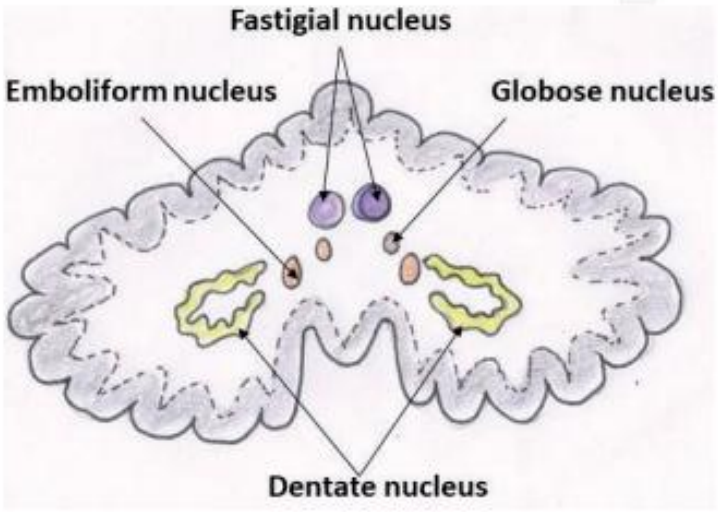

Figure 4. Intracerebellar nuclei.

Dentate nucleus

It is the most prominent cerebellar nucleus and largest in primates including human beings. It belongs to neocerebellum and receives afferent from it. Its shape is like crumpled purse with

hilum directed ventro-medially and its interior is filled with white matter consisting of efferent fibres forming most of the superior cerebellar peduncle.

Emboliform nucleus

It is oval shaped and located medial to dentate nucleus. It belongs to paleocerebellum, and this nucleus receives fibres from paleocerebellum and gives fibres to red nucleus via superior cerebellar peduncle.

Globose nucleus

It is rounded in shape and situated between emboliform and fastigial nuclei. It has similar connections as emboliform nucleus. Emboliform and globose nuclei together are known as nucleus interpositus.

Fastigial nucleus

This nucleus is situated in the midline in the vermis and smaller than dentate nucleus but larger than nucleus interpositus. It belongs to archicerebellum receiving afferents from it conveying efferents to vestibular and reticular nuclei.

2.2.5 White matter

White matter of cerebellum composed of three types of fibres viz. intrinsic, afferent and efferent. Intrinsic fibres are limited to cerebellum and connect different regions of cerebellum either of same hemispheres or of the two cerebellar hemispheres. Afferents and efferents connect cerebellum to other parts of central nervous system.

2.2.6 Connections of cerebellum

Afferent fibres

Cerebellum acquires information from cerebral cortex, spinal cord, vestibular apparatus, red nucleus and tectum of midbrain through afferent fibres. Cerebellum accrues input from cerebral

cortex through cortico-ponto-cerebellar, cerebro-olivocerebellar and cerebro-reticulo-cerebellar pathways. Cerebellum receives information from spinal cord through anterior spinocerebellar, posterior spinocerebellar and cuneocerebellar tracts and that from vestibular apparatus either directly or after relaying in the vestibular nuclei (Chaurasia *et al*., 2009, Kulkarni *et al*., 2012). Afferent fibres reach the cerebellum through middle and inferior cerebellar peduncle and are of two types: (a) climbing fibres and (b) mossy fibres. Climbing fibres arises in the inferior olivary nucleus and each fibre after giving collateral to the intracerebellar nuclei synapses with the Purkinje cell. Mossy fibres are the main afferent fibres of cerebellum, and each mossy divides into 30–40 terminal swellings known as rosette which synapses with dendrites of granule cells and axons of Golgi cells. The structure formed by the rosette along with its synapses with granule and Golgi cells is known as cerebellar glomerulus which is spherical in shape surrounded by a capsule of neuroglial cells. One climbing fibre synapses with single Purkinje cell; however, one mossy fibre synapses with many granule cells, and each granule cell synapses with thousands of Purkinje cells. Thus, one climbing fibre influences one Purkinje cells while one mossy fibres multitude of Purkinje cells. Both climbing and mossy fibres have excitatory effect on Purkinje cells.

Efferent fibres

Cerebellum provides output to red nucleus, thalamus, vestibular nuclei and reticular formation through efferent fibres via Purkinje cells. Majority of axons of Purkinje cells synapse with neurons of intracerebellar nuclei which in turn project to other parts of nervous system but few Purkinje cells from flocculonodular lobe and vermis directly end in lateral vestibular nuclei. Efferent fibres from dentate, emboliform and globose nuclei travel through superior cerebellar peduncle and those from fastigial nucleus through inferior cerebellar peduncle.

Intrinsic cerebellar circuitry

All the afferent fibres to the cerebellum viz. climbing and mossy fibres are excitatory to the cells of cerebral cortex, and their collaterals are also excitatory to the intracerebellar nuclei. The

climbing fibres excite the Purkinje cells directly but mossy fibres excite the Purkinje cells through granule cells which in turn excite basket and stellate cells but basket and stellate cells inhibit the Purkinje cells. Mossy fibres in addition also excite Golgi cells which in turn inhibit granule cells. Purkinje cells inhibit intracerebellar nuclei which in turn control muscular activity through motor areas of brainstem and cerebral cortex.

Cerebellar peduncles

The afferent and efferent fibres of the cerebellum together form three bundles, cerebellar peduncles on each side. These peduncles are superior, middle and inferior. Superior cerebellum connects cerebellum to the midbrain, middle to the pons and inferior to the medulla oblongata. Superior cerebellar peduncle (brachium conjunctivum) ascends upward from the anterior cerebellar notch to the tectum of the midbrain. These peduncles form the supero-lateral boundary of the fourth ventricle. It conveys mainly efferent fibres from the cerebellum arising in dentate nucleus. Middle cerebellar peduncle (brachium pontis) is the largest of three peduncles and consists principally of afferent fibres to the cerebellum. It bridges the basilar part of the pons and cerebellar hemispheres. Inferior cerebellar peduncle (restiform body) connects dorso-lateral part of medulla oblongata and cerebellar hemispheres and composed of afferent fibres to the cerebellum from spinal cord, olivary nucleus, reticular formation of the medulla and vestibular nuclei. Neurodegenerative Diseases - Molecular Mechanisms and Current Therapeutic Approaches (Chaurasia *et al*., 2009).

2.2.7 Fibres transmitted by cerebellar peduncles

Superior cerebellar peduncle

This peduncle conveys both afferent and efferent fibres.

Afferent fibres

1. Anterior spino-cerebellar tract arise from the cells of laminae V-VII of the spinal cord. This tract convey proprioceptive and exteroceptive impulses from lower limb and lower part of the body and maintenance of posture and movement of lower limb.

2. Tecto-cerebellar tract originate from the superior and inferior colliculi of the midbrain tectum and are projected to the vermal and paravermal regions of declive, folium, tuber and pyramid and carry information from visual and auditory system.

3. Trigemino-cerebellar fibres arise from superior sensory and spinal nucleus of the trigeminal nerve and are projected to the culmen and declive.

4. Ceruleo-cerebellar is nor-adrenergic fibres arising from locus ceruleus and inhibiting Purkinje cells.

5. Hypothalamo-cerebellar fibres are cholinergic fibres originating from hypothalamus.

Efferent fibres

1. Dentato-thalamic fibres arise from dentate nucleus and projected to area 4 and 6 of the motor cortex regulating motor functions.

2. Cerebello-rubral fibres erupt from nucleus interpositus and end in contralateral red nucleus.

3. Cerebello-olivary fibres bud from dentate nucleus and terminate in inferior olivary nucleus.

4. Cerebello-reticular fibres originate from fastigial nucleus and end in reticular nuclei.

Middle cerebellar peduncle

Afferent fibres

1. Ponto-cerebellar fibres originate from pontine nuclei of basilar part of the pons and projected partly to contralateral neocerebellum and partly to contralateral paleocerebellum. Pontine nuclei receive fibres from cerebral cortex forming cerebro-ponto-cerebellar tract.

2. Reticulo-cerebellar tract arise from reticular formation of brainstem and are projected to the vermal region of the cerebellum.

3. Some serotonergic fibres from raphe nuclei of the pons reach the cerebellum through this peduncle.

Efferent fibres

No efferent fibres pass through this peduncle

Inferior cerebellar peduncle

Afferent fibres

1. Posterior spino-cerebellar tract arises from thoracic nucleus (Clarke's column) of the spinal cord conveying proprioceptive and exteroceptive impulses from lower limb to paleocerebellum.

2. Cuneo-cerebellar tract (posterior external arcuate fibres) arise from ipsilateral accessory cuneate nucleus of medulla transmitting proprioceptive and exteroceptive impulses from upper limb and upper trunk and project on culmen and pyramid of vermis.

3. Anterior external arcuate fibres originate from arcuate nucleus of both sides and project into the neocerebellum.

4. Vestibulo-cerebellar tract primarily arise from vestibular nerve and secondary from medial and inferior vestibular nuclei forming juxta-restiform body and projecting to ipsilateral flocculonodular lobe, uvula and lingual. Few fibres end in fastigial nuclei.

5. Olivo-cerebellar tract sprout from contralateral inferior olivary nucleus and project into the neocerebellum but few fibres terminate into deep nuclei.

6. Parolivo-cerebellar tract arise from medial and dorsal accessory olivary nuclei and project on to the contralateral neocerebellum.

7. Reticulo-cerebellar tract buds from lateral and paramedian reticular nuclei of the medulla oblongata and project on to the neocerebellum.

Efferent fibres

1. Cerebello-vestibular fibres sprout from ipsilateral flocculonodular and fastigial nuclei of both sides. These fibres travel through juxta-restiform body and projected to vestibular nuclei

2. Cerebello-reticular fibres arise from fastigial nuclei of both sides and reaches the pontine and reticular formation. Fibres arising from contralateral fastigial nuclei form hook bundle of russel.

3. Cerebello-olivary fibre's origin is unknown and connect cerebellum with the inferior olivary nucleus.

2.2.8 Functions of cerebellum

1. Cerebellum maintains equilibrium, muscle tone, posture and coordinates skilled voluntary movements by regulating the grade of muscle tension between agonist and antagonist muscles

2. Sherrington named cerebellum as the head ganglion of the proprioceptive system as various sensory inputs from the vestibular, visual and auditory systems, stretch receptors of muscle spindle and Golgi tendon organ, tactile and pressure receptors of head and body are relayed in the

cerebellum. The sensory impulses are processed in the intrinsic cerebellar circuitry and integrated into the motor system by cerebral motor cortex, red nucleus, vestibular nuclei and reticular formation.

3. If the movement is to be carried out, cerebral cortex sends information to anterior horn cells of spinal cord to initiate movement, and it also sends impulses to cerebellum about the movement to be executed. Cerebellum also receives proprioceptive information from the muscles and joints about the actual movement occurring. The cerebellum compares both these information about movement and if any difference is noted in information concerning intended and actual movement, the cerebellum sends the information to cerebral cortex and anterior horn cells of the spinal cord to correct the discrepancy so that movement carried out is accurate in time, rate, range, force and direction.

2.2.9 Arterial supply of the cerebellum

The cerebellum is irrigated by three pairs of cerebellar arteries.

a. Superior cerebellar artery, branch of basilar artery irrigates superior surface of the cerebellum.

b. Anterior inferior cerebellar artery, branch of basilar artery supplies anterior part of inferior surface of cerebellum.

c. Posterior inferior cerebellar artery, branch of vertebral artery irrigates posterior part of inferior surface of cerebellum.

2.2.10 Applied anatomy

Cerebellar lesions may occur due to trauma, vascular occlusion, tumour or other pathologies producing cerebellar syndrome. Cerebellar syndrome is grouped in three types viz. Archicerebellar, paleocerebellar and neocerebellar syndromes.

Archicerebellar syndrome

In this syndrome, predominantly flocculonodular is affected by tumour, medulloblastoma. The patient is unable to maintain equilibrium while standing and falls on closing the eyes. This is called positive Romberg's sign. In addition to this, the patient walks on a wide base with legs well apart and sways from side to side.

Paleocerebellum syndrome

Lesion of this part of cerebellum produces hypotonia (decreased muscle tone) of limb muscles manifesting as:

a. Instability of joints resulting in flail joints.

b. Abnormal tendon reflex, for example, oscillating movements of leg are produced when patellar tendon is tapped (Pendular knee jerk).

c. Unable to maintain equilibrium while walking exhibiting ataxic gait.

14.3 Neocerebellum syndrome

Lesion in this part of cerebellum leads to incoordination known as asynergia and tremor which manifests in form of:

a. Ataxia due to incoordination of muscles of trunk, pectoral and pelvic girdles. The patient tends to fall on the side of lesion and to prevent fall patient stands or walk on a broad base.

b. Dysmetria culminates into past pointing where the patient is not able to measure the distance for performing intended task. This is tested by finger-nose test in which the patient is supposed to touch the tip of nose by finger but in this disorder the patient either over or under shoots the tip of nose.

c. Intention tremors appear during purposeful movements and disappear during rest. These tremors are coarse, arrhythmic and occur at the end of the movement.

d. Dysdiadochokinesis/adiadochokinesis is the inability to perform alternate movements with rapidity such as supination and pronation.

e. Rebound phenomenon occurs when the action of agonist muscle is not checked by corresponding antagonist muscle. If the patient is asked to push the palm of physician and when physician removes his hand, the hand of the patient moves back (rebounds) and hits the physician as the patient is unable to stop the pushing act immediately.

f. Dysarthria/scanning speech occurs due to incoordination of muscles responsible for speech. The speech is slurred, prolonged, explosive and with pauses at wrong places.

g. Nystagmus results in oscillation of eye ball due to incoordination of extraocular muscles.

In addition to above mentioned diseases, cerebellum is affected by certain neurodegenerative diseases which are elaborated below: Cerebellar degenerative diseases:

a.Spinocerebellar ataxia

This condition involves mutation in genes causing degenerative changes in neurons of cerebellum including brainstem and spinal cord. If a parent is affected by this disease, there is 50% chance of inheriting the disease (Lublin *et al*.,2013). It is hereditary progressive degenerative disease often fatal. The disease is associated with progressive incoordination of gait, hand, speech and eye. No treatment is available only symptomatic relief can be provided to affected individuals.

b. Multiple sclerosis

In this condition, both genetic and environmental factors influence the outcome of diseases (Lublin *et al*., 2013). The myelin sheath enveloping the neurons is damaged resulting in delayed and interrupted impulses from and to the cerebellum (Dayalu *et al*., 2015). It is incurable condition.

c. Paraneoplastic disorders

In this disorder, the person's autoimmune system especially T-cells become active in response to malignant tumours resulting in degeneration of neurons of cerebellum causing impaired ability to talk, walk, sleep, maintain balance and coordinate muscle activity (Dayalu *et al*., 2015).

This disorder is more common in middle aged individuals with lung, ovarian and breast cancer (Dayalu *et al*., 2015).

d. Chronic alcohol abuse

This condition is more prevalent in men than women. Chronic alcohol intake reduces vitamin B1 absorption and utilisation leading to degeneration of cerebellar neurons (Dayalu *et al*., 2015). It is most common cause of nutritional spinocerebellar ataxia.

e. Parkinson's disease

Lesions of basal ganglion and cerebellum produce abnormal movements or changes in tone. Tremors occur both with the lesions of basal ganglion and cerebellum. However, tremors in cerebellar lesions occur only during movement, hence also known as intention tremors, while in basal ganglion diseases, like Parkinson's disease, tremors are observed during resting state. In addition to this, other signs of Parkinson's disease like mask face, clasp knife rigidity, lead pipe rigidity and hypokinesia/akinesia are not observed in cerebellar neurodegenerative diseases (Dayalu *et al*., 2015).

f. Alzheimer's disease

This chronic neurodegenerative disease is characterised by gradual onset of dementia. Alzheimer's disease is the cause of dementia in 60–70% cases. Most common early symptom is difficulty in remembering recent events (Burns *et al*., 2009). Later on other symptoms like problems with language, disorientation and mood swings appear slowly. Though the causes of this disease can be various, the risk attributed to genetics is estimated to be around 70% (Ballard *et al*.,

2011). In this disease, there is degeneration and loss of neurons and synapses in various parts of brain resulting in atrophy (reduction in size) of related regions. Amyloid plaques and neurofibrillary tangles are found deposited in the neurons of the brain (Wenk *et al*., 2003).

g.Huntington's disease

Huntington's disease is also known as Huntington's chorea. It is an inherited disorder caused by mutation in the huntingtin gene which codes for huntingtin protein. Mutant huntingtin gene produces mutant and defective protein which is toxic to neurons of brain causing degenerative changes in brain. This causes problem with mood and mental abilities associated with lack of coordination and unsteady gait. Gradually coordinated movements become difficult and person is unable to talk (Dayalu *et al*., 2015). There is as no treatment for the disease except for the supportive treatment.

2.3 VITAMIN E

2.3.1 INTRODUCTION

Evans and Bishop's research from 1922 demonstrated the presence of an unrecognized dietary element essential for the reproductive health of rats. At that time, the most significant function of vitamin E was understood to be the maintenance of normal gestation in pregnant rats, preventing embryo resorption, which occurred in its absence (Niki *et al*., 2012). Green lettuce, dried alfalfa leaves, wheat, and oats were found to be sources of this unidentified dietary element, referred to as factor X. In 1936, Evans isolated factor X from wheat germ oil, identified it with the molecular formula C29H50O2, and introduced the term α-tocopherol (Evans *et al*., 1962). In 1938, Fernholz published the structural formula of α-tocopherol. Tocotrienols were identified much later in the early 1960s compared to tocopherol (Janiszowska *et al*., 1976). Olcott found that the lipid components in vegetable oils contain antioxidants that prevent lard from undergoing oxidative deterioration. It has since become clear that vitamin E protects biological molecules from

oxidative damage and serves as an important antioxidant both in living organisms and in laboratory conditions (Traber *et al*., 2007). In recent years, there has been a marked increase in interest regarding the non-antioxidant functions of vitamin E in cellular signaling, gene regulation, membrane dynamics, and neural functions (Aziz *et al*., 2007). However, many issues remain to be clarified and debated. To understand the role of vitamin E in both living organisms and laboratory settings, it is essential to have reliable information based on solid chemical evidence.

2.3.2 SOURCE

A variety of foods are rich in vitamin E, with fruits and seeds being among the top sources. Leafy green vegetables also play an important role in providing vitamin E. This vitamin can only be produced by plants and organisms that perform photosynthesis (Mene-saffrane., 2017). The synthesis of stereospecific tocopherols, or RRR-tocopherols, is facilitated by enzymes (Della., 2005). Tocopherols and tocotrienols can be extracted, purified, or concentrated from vegetable oils and other materials derived from higher plants. Another source of vitamin E comes from supplements found in food, which often contain significantly higher levels than what is present in natural foods. A common technique employed to enhance the shelf life of vitamin E in dietary supplements and fortified foods is esterification, which helps retain its antioxidant properties.

Natural oils differ in their amounts and combinations of tocopherols and tocotrienols based on the species of plant, even among plants of the same species. While higher plants are abundant in tocopherols, only a few non-photosynthetic tissues contain tocotrienols. For example, γ-tocopherol is more commonly found in certain edible oils like corn, rapeseed, and soybean oils, whereas α-tocopherol predominates in palm, olive, and sunflower oils. The primary sources of tocotrienols are palm, rice, and annatto, featuring tocopherol–tocotrienol ratios of 25:75, 50:50, and 0.1:99.9 respectively. Palm oil contains substantial amounts of α-, γ-, and δ-tocotrienols. In rice bran oil, the most abundant vitamin E isoform is γ-tocotrienol, while β-tocopherol is one of

the main isoforms found in wheat germ oil (Schwartz *et al*., 2008). Notably, annatto seeds had no tocopherols present in their lipid fraction, but contained mostly δ-tocotrienol (Frega *et al*.,1998).

2.3.3 FUNCTION AND APPLICATION

Applied in fields such as medicine, pharmaceuticals, cosmetics, and food products, vitamin E is an essential micronutrient for health. It is believed that vitamin E plays a significant role in promoting overall health and in the prevention and/or treatment of various diseases and disorders. The recommended daily intake of vitamin E for individuals is 15 mg (22.4 IU, International Unit).

The functions of vitamin E have been demonstrated or theorized, including: (1) providing antioxidant protection against free radicals, particularly peroxyl radicals and singlet oxygen; (2) stabilizing membranes by forming complexes with destabilizing molecules to maintain the structure's amphipathic balance; (3) acting as a physiological regulator of enzymatic activity and cellular signaling, cell proliferation, and gene expression—roles that are not directly linked to its antioxidant capabilities; (4) inhibiting platelet coagulation; (5) preventing diseases, including age-related damage to the eyes and skin, neurological issues, and cardiovascular diseases; and (6) serving as a biocompatible modifier of biomaterials and medical devices, such as high molecular weight polyethylene used in knee and hip implants. Tocotrienols are also thought to have additional beneficial health effects beyond those of tocopherols. Some of these benefits include reducing blood cholesterol levels and enhancing immune system function (Sen *et al*., 2007).

As a dietary supplement, vitamin E is often utilized either on its own or alongside other micronutrients like vitamin C to promote health and reduce the likelihood of diseases believed to be caused by harmful oxidative damage to biological components. Certain foods and drinks may be enriched with vitamin E. While malnutrition and genetic disorders can lead to a deficiency in vitamin E, it is uncommon since regular diets typically provide sufficient amounts of the nutrient. Vitamin E deficiency can occur in premature infants with very low birth weights. Additionally, individuals with fat-malabsorption conditions and genetic disorders that result in decreased levels

of selenoproteins (Saito *et al*., 2015) or those who have a defective or absent liver α-tocopherol transfer protein (α-TTP) are at a higher risk of vitamin E deficiency and may require higher doses of supplemental vitamin E.

CHAPTER THREE

METHODOLOGY

3.1 REAGENT / CHEMICALS

All reagents and chemicals were of analytical grade. They included Nickel chloride, distilled water, Normal saline, EDTA Disodium, Citicoline, alcohol (50%, 70%, 90%, 100%), xylene, paraffin, mountant, methylated spirit, formal saline.

3.2 EQUIPMENTS

Surgical latex glove, weighing balance, orogastric tube, measuring cylinder, conical flask volumetric flask, polypropylene cages, mortar and pestle, refrigerator, oven, sample bottles, water bath, paraffin dispenser, dissecting set, glass rods, rotary microtome, binocular microscope.

3.3 EXPERIMENTAL ANIMALS

The animals used in this study were bred at the Animal House, Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria. Wistar rats were housed in polypropylene cages at normal room temperature. Throughout the twenty-eight-day study period, the animals were fed with topline Grower Mash (Premier feed co.Ltd., Ibadan, Oyo State, Nigeria) and provided with unrestricted access to water. Prior to the start of the experiment and throughout its duration, the animals' weights were measured weekly using a digital weighing scale calibrated in grams, and the values were recorded to the nearest whole number

3.4 RESEARCH DESIGN

A total of twenty-eight (28) adult Wistar rats with an average weight of 180g were used for this study. Following a two-week period of acclimatization to the animal house environment with unrestricted access to food and water. They were randomly assigned into four groups (A, B, C, and D) with each group consisting of seven rats. The study was approved by the Research Ethical

Committee of the College of Medical Sciences, University of Benin. All administrations were delivered both intraperitoneally using a 5ml syringe and needle and orally using an orogastric tube throughout the entire 28-day study period.

3.5 EFFECTS OF TREATMENT ON NEUROBEHAVIOURAL ACTIVITIES

In order to evaluate the impact of the treatments on neurobehavioral activities, a range of neurobehavioral assessment tests were conducted. These tests encompass Open field test, and movement initiation test.

GROUPS	DOSAGE
GROUP A (CONTROL)	1 ml of distilled water.
GROUP B	5mg/kg body weight of Aluminum chloride ($AlCl_2$) only.
GROUP C	5mg body weight of Aluminum chloride ($AlCl_2$) + Vitamin E
GROUP E	Vitamin E only.

3.6. OPEN FIELD TEST.

This test was performed according to the method of Olopade *et al*., (2012). The open field test is used to elevate anxiety as well as the as the locomotory and exploratory activities of rats. This test is based on subjecting an animal to an unknown environment whose escape is 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 was then 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 positions 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 unit entered with all four paws rearing frequency, it is carried out using open field apparatus with video camera to view movement of the rat in the open field.

Immobility: This is the inability of rats to move.

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

3.7 MOVEMENT INITIATION TEST

This test was conducted according to established methods of Brown *et al* (1999) and Santiago *et al* (2012). This was used to evaluate the ability of the animal to initiate purposeful movements. The rat was held by its trunk with its hind limbs and one forelimb lifted above the surface of a table so that the weight of the rat's body is supported by one forelimb alone. The time to initiate one step was recorded for each forelimb. Initiation times for both forelimbs was averaged together to make one score.

3.8 BRAIN OXIDATIVE STRESS PARAMETERS

The brain was removed from the body after being harvested, blotted of blood, and weighed right away with an electronic balance calibrated in milligrams and recorded to the nearest two decimal places. The harvested and weighed brains was homogenized with acid-washed sand and PBS in a porcelain mortar and pestle after being washed twice in cold phosphate-buffered saline (PBS). Centrifuging the homogenate at 10,000 g for 15 minutes at 4 °C. The supernatant was gathered to estimate the results of several biochemical experiments.

3.8.1 Estimation of Catalase (CAT) activity

This was determined by the method of Cohen *et al.* (1970).

Principle: Catalase is present in nearly all animal, plant, and bacteria cells. It acts to prevent the accumulation of noxious H_2O_2 which is converted to O_2 and H_2O.

Preparation of reagent: 0.01M $KMnO_4$ was prepared by dissolving 0.158g of $KMnO_4$ in 100 ml of distilled water. Phosphate buffer (pH 7.4); 0.426 of $NaHPO_4$ NaH_2PO_4 was weighed and dissolved in 100ml of distilled water. 6M H_2SO_4: 32.3ml of conc. H_2SO_4 was added to 66.7 ml of distilled water. 30Mm H_2O_2 solution: this was prepared by measuring 0.34ml of 30% of H_2O_2 in 1001ml of phosphate buffer.

Procedure: To a known volume of plasma, (0.5ml), 5.0ml of H_2O_2 would be added. This was mixed by inversion and allowed to stand for 30 minutes. The reaction was stopped by adding 6M H_2SO_2. The absorbance was taken at 480nm within 30-60 seconds against distilled water.

Calculation: Activity = $\frac{OD / \times \min \times Vt}{M \times V \times L \times Y}$

OD= absorbance

L= light path =1cm

V_t =total volume of the reaction sample

M= molar extinction co-efficient of H_2O_2 (40/M/cm)

3.8.2 Estimation of Malondialdehyde (MDA) activity

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

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

Preparation of reagent: Stock TCA-TCB-HCL was 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.

Procedure: A volume of plasma (1.0ml) was 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;

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

A = absorbance of sample test at 535nm

Vt= total volume of the reaction = 3ml

M = molar extinction co-efficient of product= 1.56 × 105m-1cm-1 l = light path= 1cm

V= volume of tissue extract used = 1ml

Y = mg tissue in the volume of sample used

3.8.3 Estimation of Glutathione Peroxidase (GPx) activity

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

Principle: This is based on the oxidation of pyrogallol to purpurogallin by peroxidase activity, resulting in a deep brown color disposition, read at 430nm.

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

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 color was formed which was read at 420nm.

Calculation: Activity = $\frac{OD}{Min \times VtDf}$

$E \times Vs \times Y$

OD = Absorbance of test

Vt = Total volume of reaction of reaction mixture

Df = Dilution factor = l

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

Vs= volume of sample

Y = mg of protein used

3.8.4 Estimation of Superoxide Dismutase (SOD)

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

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

Preparation of reagents: Carbonate buffer (0.05 M) pH 10.2: this would be 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 would be prepared by adding 0.044 concentrated HCl to 99.96 ml of distilled water. Adrenaline solution (0.3 mM): this would be prepared by dissolving 0.01098 g of Adrenaline in 100 ml of 0.005 M HCl solution.

Procedure: A plasma volume of 0.2 ml would be mixed with 2.5 ml of carbonate buffer and 0.3 ml of adrenaline solution, and 0.2 ml of distilled water was mixed with 2.5 m of carbonate buffer and 0.3 ml adrenaline as reference sample. These would be mixed and absorbance read at 420 nm. % inhibition = $\left(\frac{\text{O.D test} - \text{O.D ref}) \times 100}{O.D\ test}\right)$

Enzyme activity can thus be calculated:

SOD activity (Unit/ mg protein) $= \frac{inhibition}{50 \times Y}$

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

3.9 HISTOLOGY OF THE CEREBELLUM.

The rats were euthanized by cervical dislocation, once the neurobehavioral tests were finished. Rats' brains were removed from their skulls, blotted clean of blood, and instantly weighed using an electronic balance calibrated in milligrams and recorded to the nearest two decimal places. The relative brain weights were calculated as follows:

Relative brain weight= $\frac{absolute\ brain\ weight\ (g) \times 100}{body\ weight\ of\ rat\ (g)}$

Two sagittal slices were created from the collected tissue. For histopathology, the right hemisphere of each brain was maintained in 10% phosphate-buffered formalin. They were each dehydrated for an hour at room temperature using ethanol concentrations of 70%, 90%, absolute ethanol I, and absolute ethanol II. Two xylene changes at room temperature, lasting an hour each, removed dehydrated tissue. The tissues were soaked in two separate batches of molten paraffin

wax for one hour each at 60 degrees Celsius before being embedded in multi-block paraffin wax moulds. The paraffin wax embedded method used was Drury and Wallington (1980) to prepare the tissues. The paraffin-blocked tissues were cut into smaller pieces and put on a wooden chuck for rotary microtome sectioning. A rotary microtome was used to slice the tissue blocks into sections that were 5m thick. To spread the parts' folded ribbons, the sections were placed in a water bath at 40 degrees Celsius. These pieces were fixed to a fresh, spotless glass slide. To increase the adherence of the sections to the slides, these were dried at 40°C using a slide drier.

3.10 HEMATOXYLIN 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 would be used. Procedures of Hematoxylin 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 hematoxylin for 15-20 minutes
- Excess hematoxylin stain would be removed by rinsing well in running tap water for two to three minutes (sections would be 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 would be washed off in running water and examined under a microscope;
- Dehydrated rapidly in ascending grades of ethanol (50% through absolute ethanol), cleared in xylene, and mounted in a synthetic resin medium (DPX).

3.11PHOTOMICROGRAPHY

A binocular microscope equipped with a Leica CC50 USB Digital Microscope Camera was used to take pictures of the treated slides. The camera has a 0.5X reduction lens and a 9 megapixel (3488 x 2616 pixel) high quality color digital camera. A laptop was then linked to it. The use of 4 and 10 objective lenses produced a panoramic image of the slides.

3.12 STATISTICAL ANALYSIS

Data was analyzed using Graphpad Prism statistical package (version 9). Statistical significance ($P<0.05$) was determined by means of analysis of variance (ANOVA), followed by turkey's multiple comparison post-hoc test. Results were presented as mean ± standard error of mean (mean ± SEM).

CHAPTER FOUR

RESULT

4.1 Effect of treatment on Weight

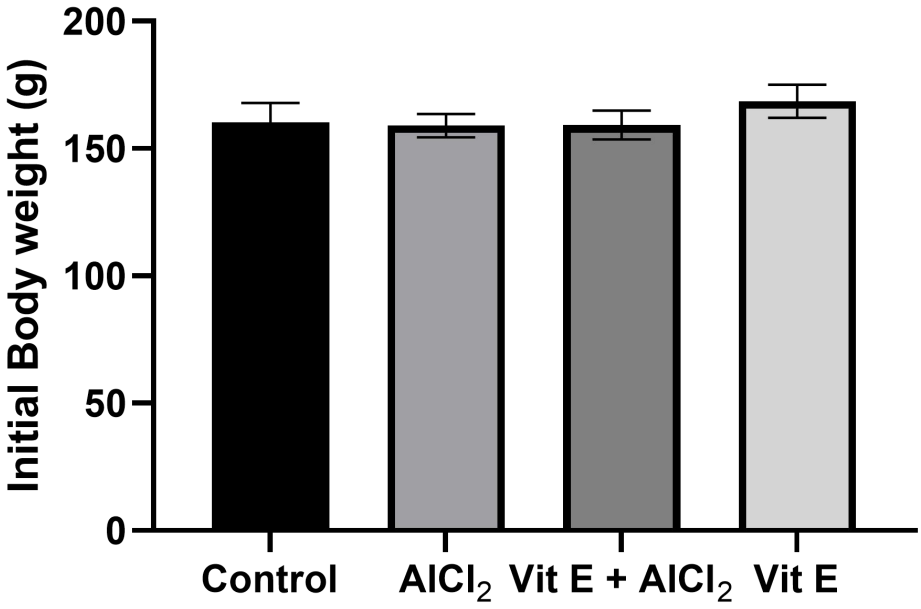

Figure 1: Initial weights of control and treatment groups after 28 days.

Values are given as mean ± SEM of each group.

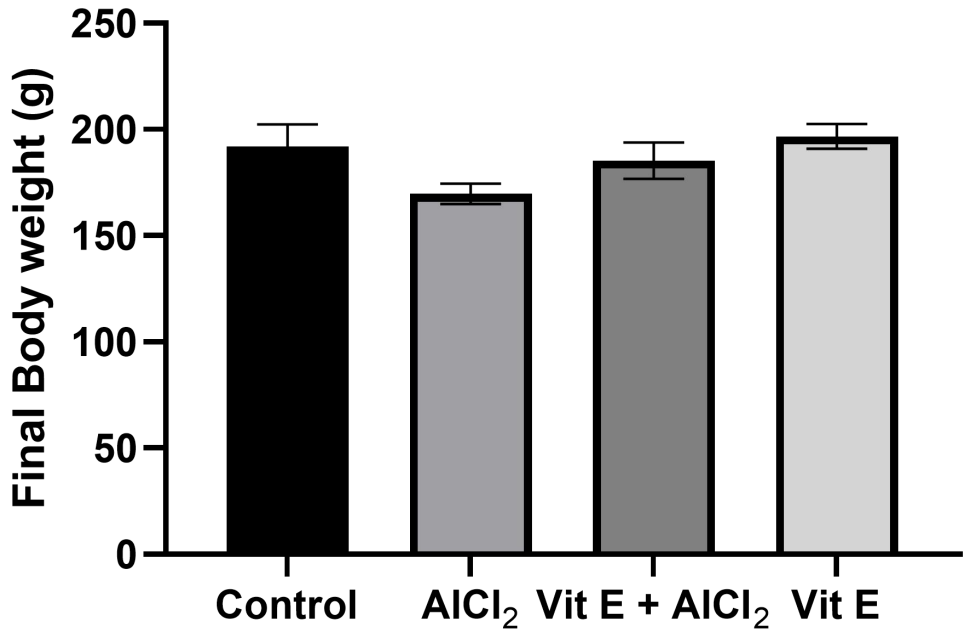

Figure 2: Final weights of control and treatment groups after 28 days.

Values are given as mean ± SEM of each group.

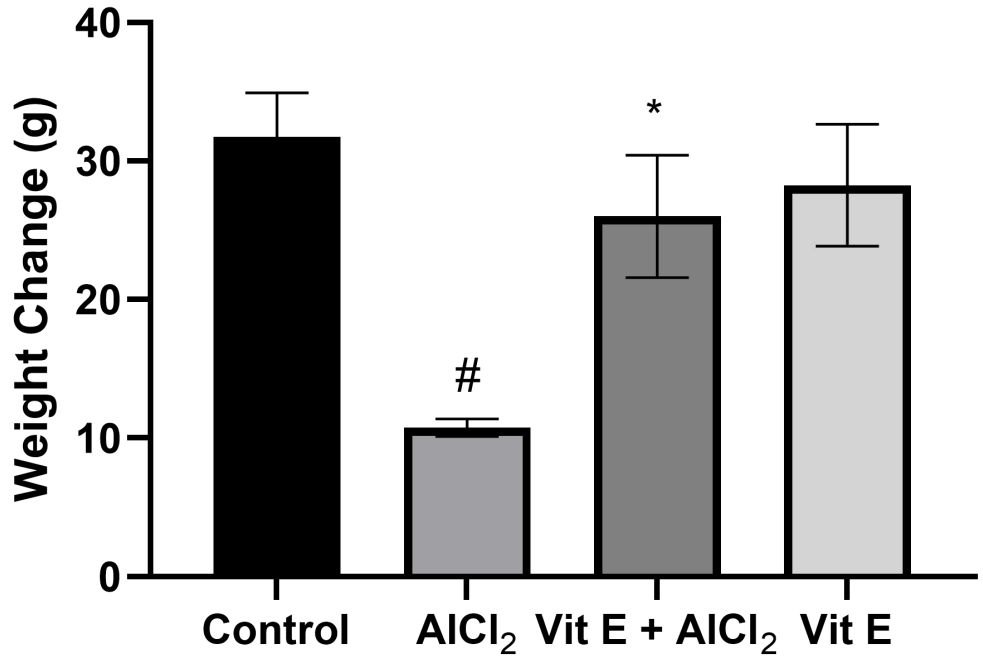

Figure 3: Weights change of control and treatment groups after 28 days.

Values are given as mean ± SEM of each group. # $p<0.05$ compared with the control group; * $p<0.05$ compared with $AlCl_2$ group

4.2 Effect of treatment on Neurobehavioural activity (OFT)

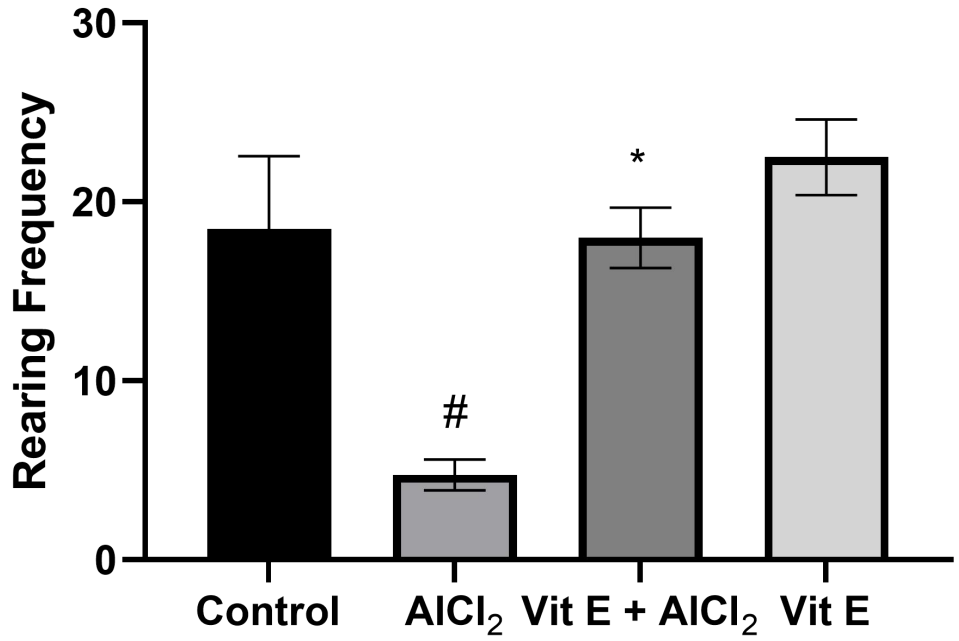

Figure 4: Rearing frequency of control and treatment groups after 28 days. Values are given as mean ± SEM of each group. # $p<0.05$ compared with the control group; * $p<0.05$ compared with $AlCl_2$ group

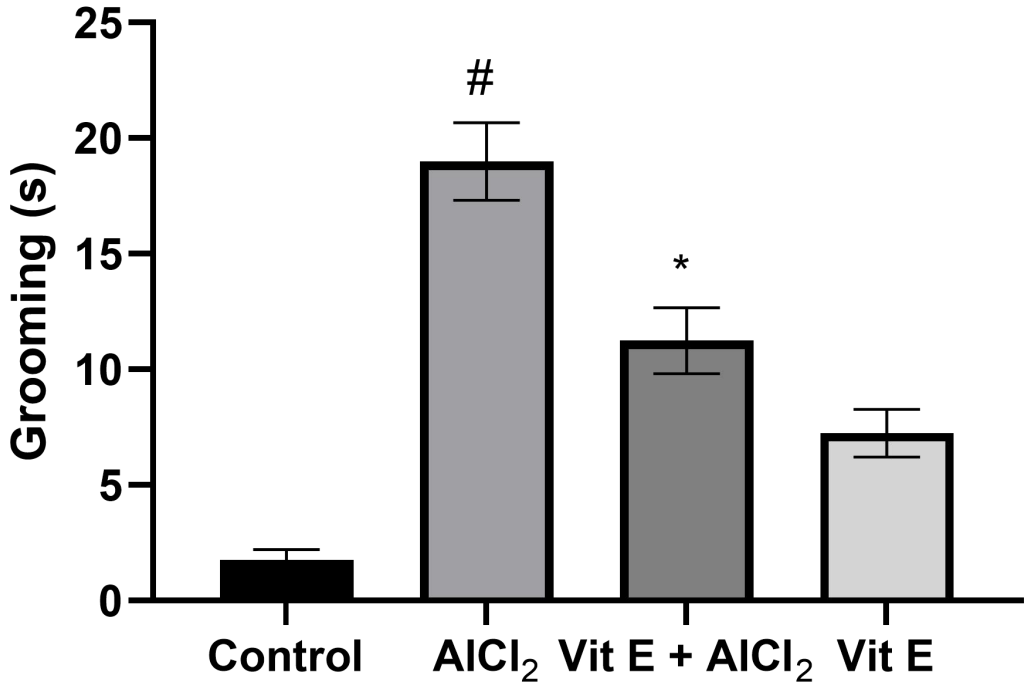

Figure 5: Grooming of control and treatment groups after 28 days. Values are given as mean ± SEM of each group. # $p<0.05$ compared with the control group; * $p<0.05$ compared with $AlCl_2$ group

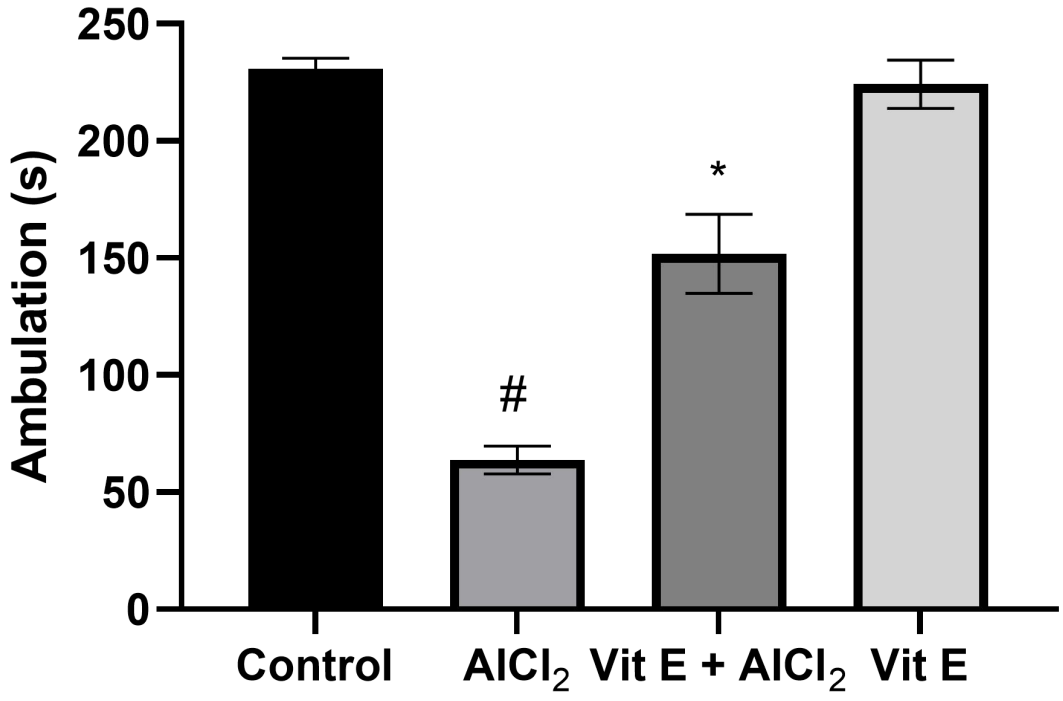

Figure 6: Ambulation of control and treatment groups after 28 days. Values are given as mean ± SEM of each group. # $p<0.05$ compared with the control group; * $p<0.05$ compared with $AlCl_2$ group

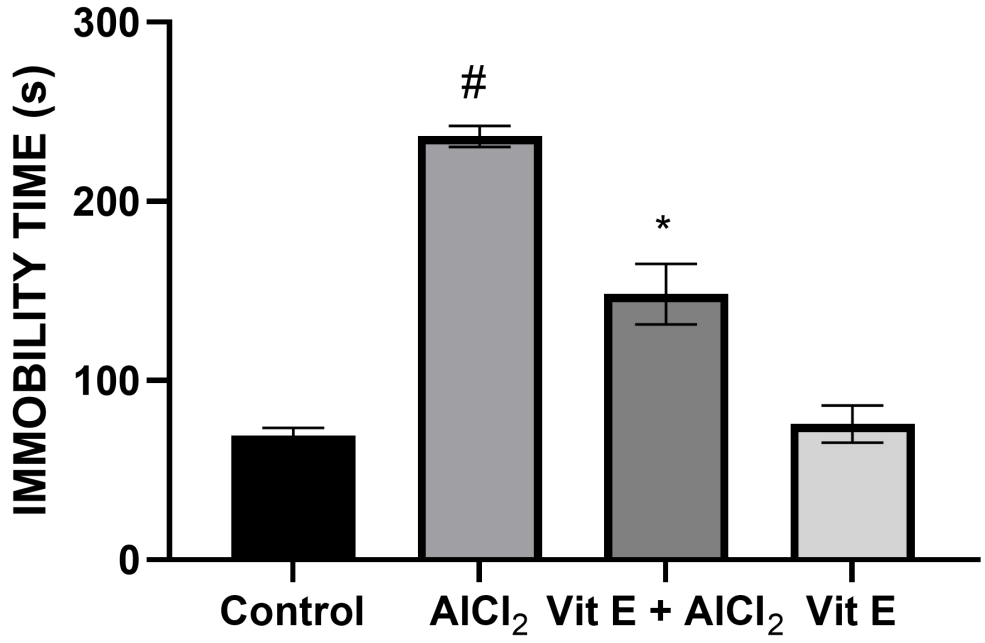

Figure 7: Immobility of control and treatment groups after 28 days. Values are given as mean ± SEM of each group. # $p<0.05$ compared with the control group; * $p<0.05$ compared with $AlCl_2$ group

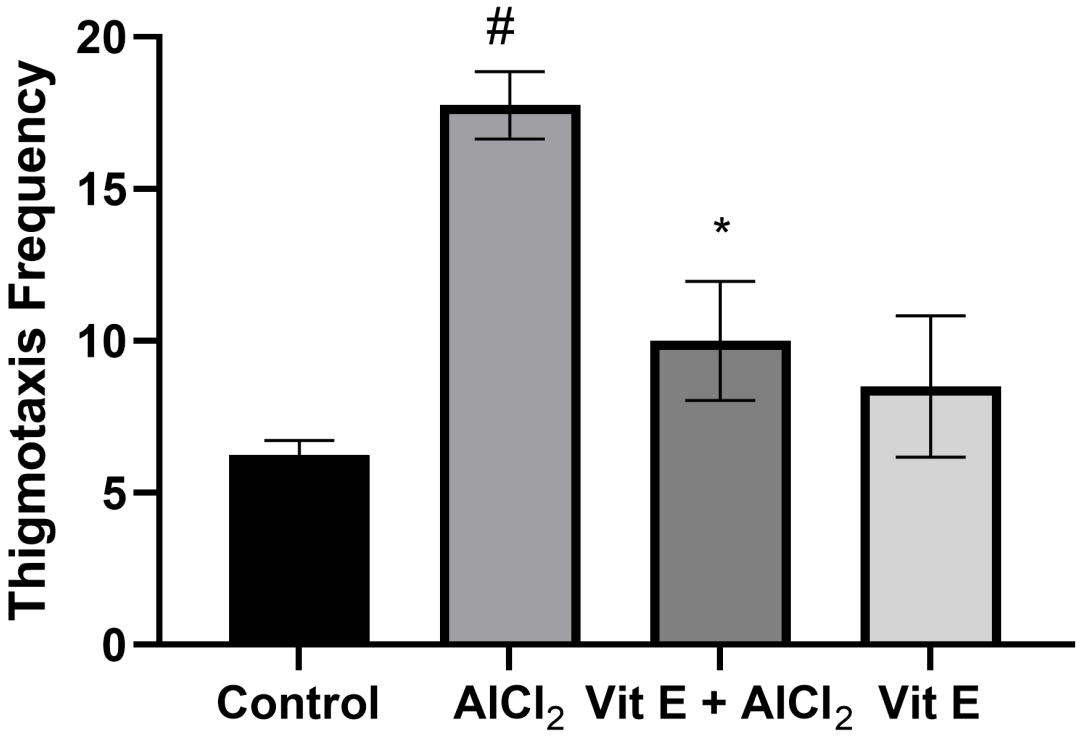

Figure 8: Trigmotaxis of control and treatment groups after 28 days. Values are given as mean ± SEM of each group. # $p<0.05$ compared with the control group; * $p<0.05$ compared with $AlCl_2$ group

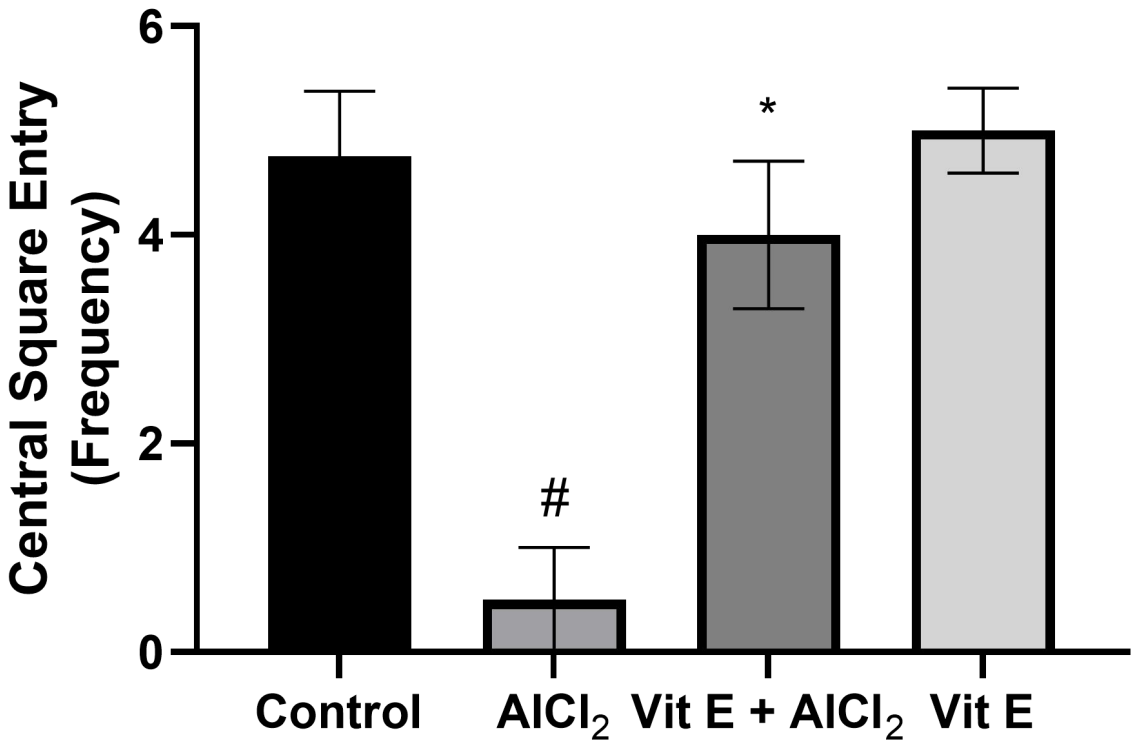

Figure 9: Central square entry of control and treatment groups after 28 days. Values are given as mean ± SEM of each group. # $p<0.05$ compared with the control group; * $p<0.05$ compared with $AlCl_2$ group

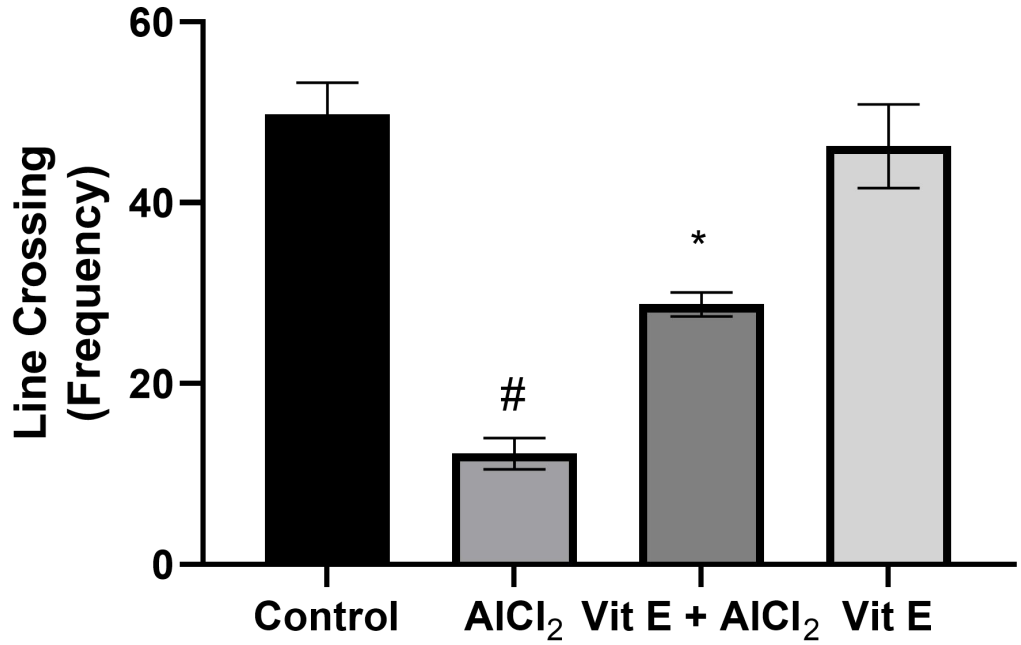

Figure 10: Line crossing of control and treatment groups after 28 days. Values are given as mean ± SEM of each group. # $p<0.05$ compared with the control group; * $p<0.05$ compared with $AlCl_2$ group

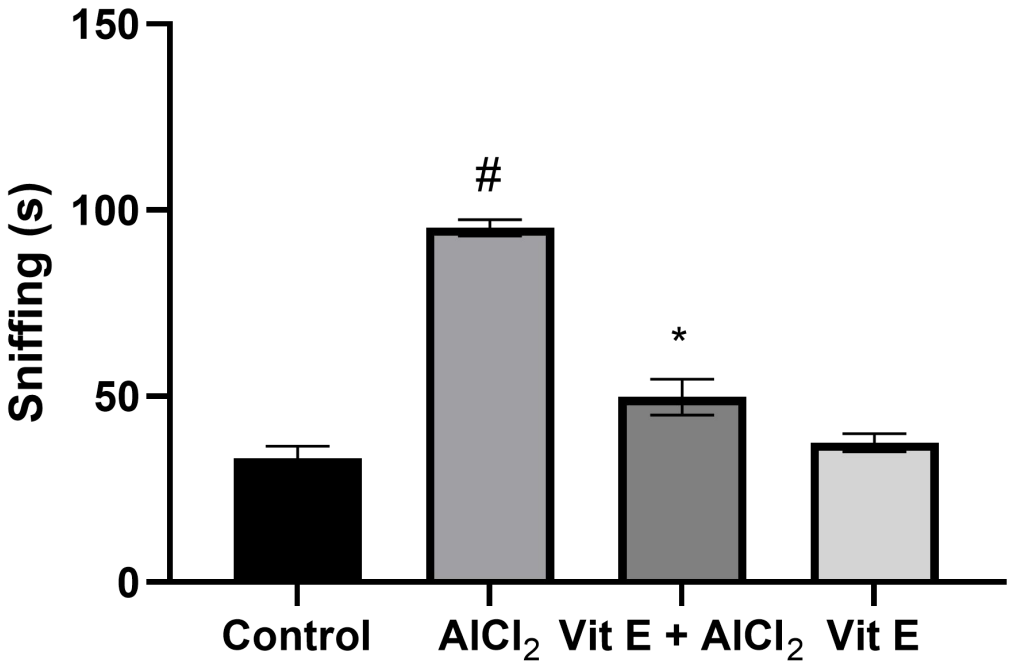

Figure 11: Sniffing of control and treatment groups after 28 days. Values are given as mean ± SEM of each group. # $p<0.05$ compared with the control group; * $p<0.05$ compared with $AlCl_2$ group

4.3 Effect of treatment on Neurobehavioural activity (MOVEMENT INITIATION TEST)

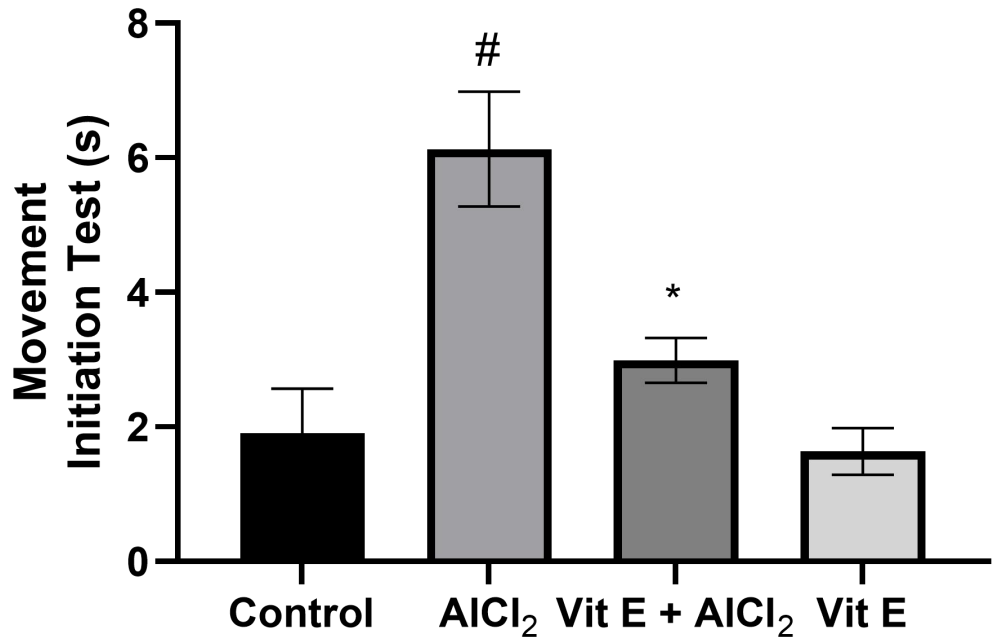

Figure 12: Movement initiation of control and treatment groups after 28 days. Values are given as mean ± SEM of each group. # $p<0.05$ compared with the control group; * $p<0.05$ compared with $AlCl_2$ group

4.4 Effect of treatment on Antioxidant enzymes activity

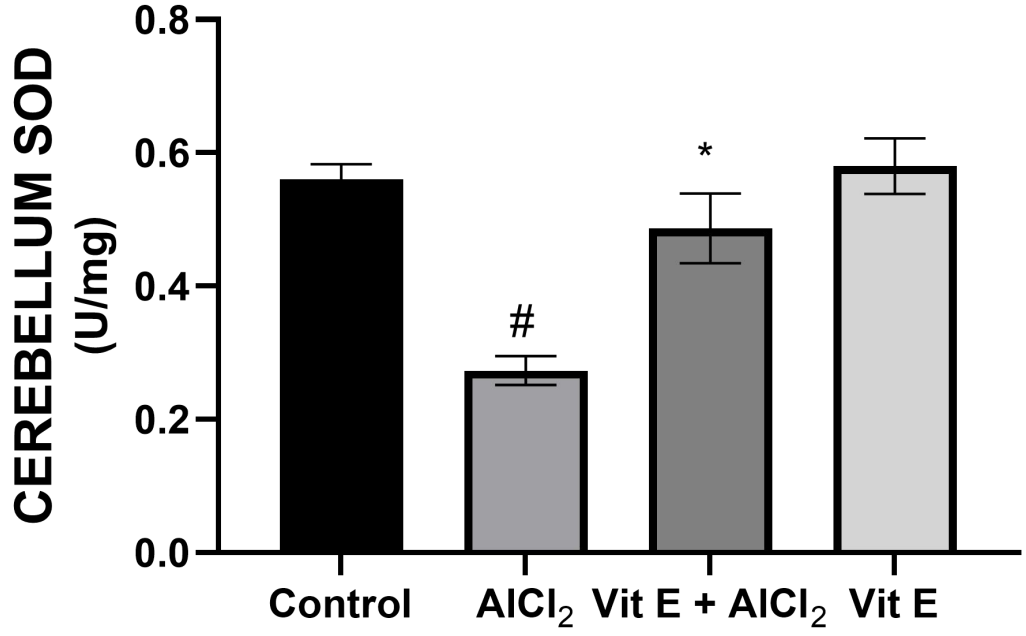

Figure 13: Activity of Superoxide Dismutase in the cerebellum of control and treatment groups after 28 days. Values are given as mean ± SEM of each group. $^{\#}$ $p<0.05$ compared with the control group; * $p<0.05$ compared with $AlCl_2$ group

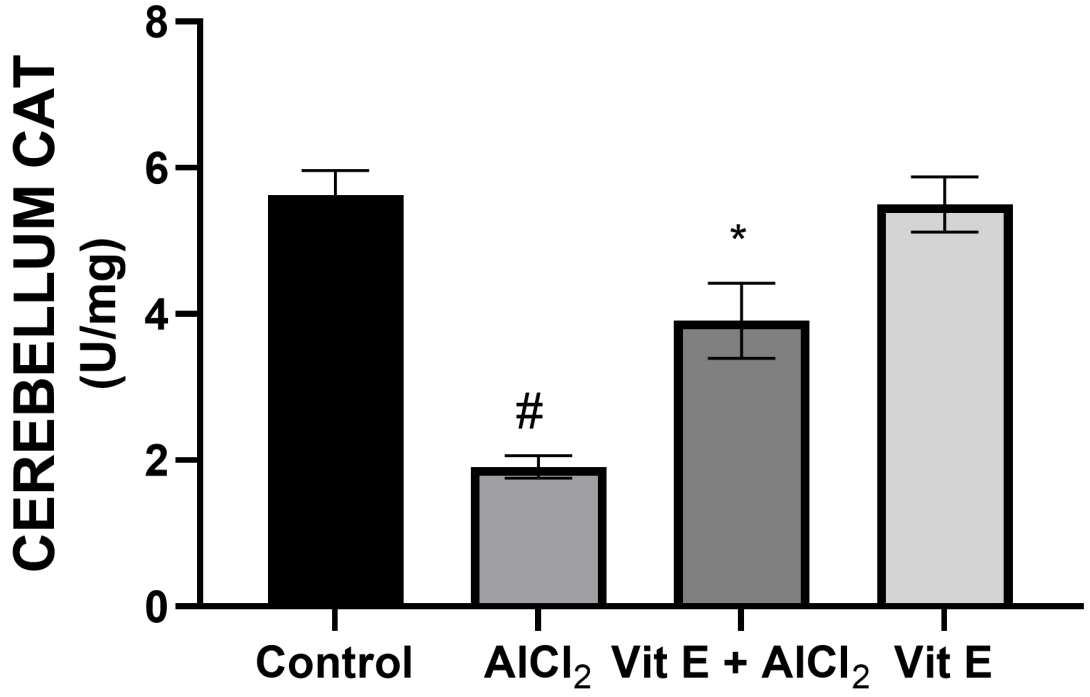

Figure 14: Activity of Catalase in the cerebellum of control and treatment groups after 28 days. Values are given as mean ± SEM of each group. # $p<0.05$ compared with the control group; * $p<0.05$ compared with $AlCl_2$ group

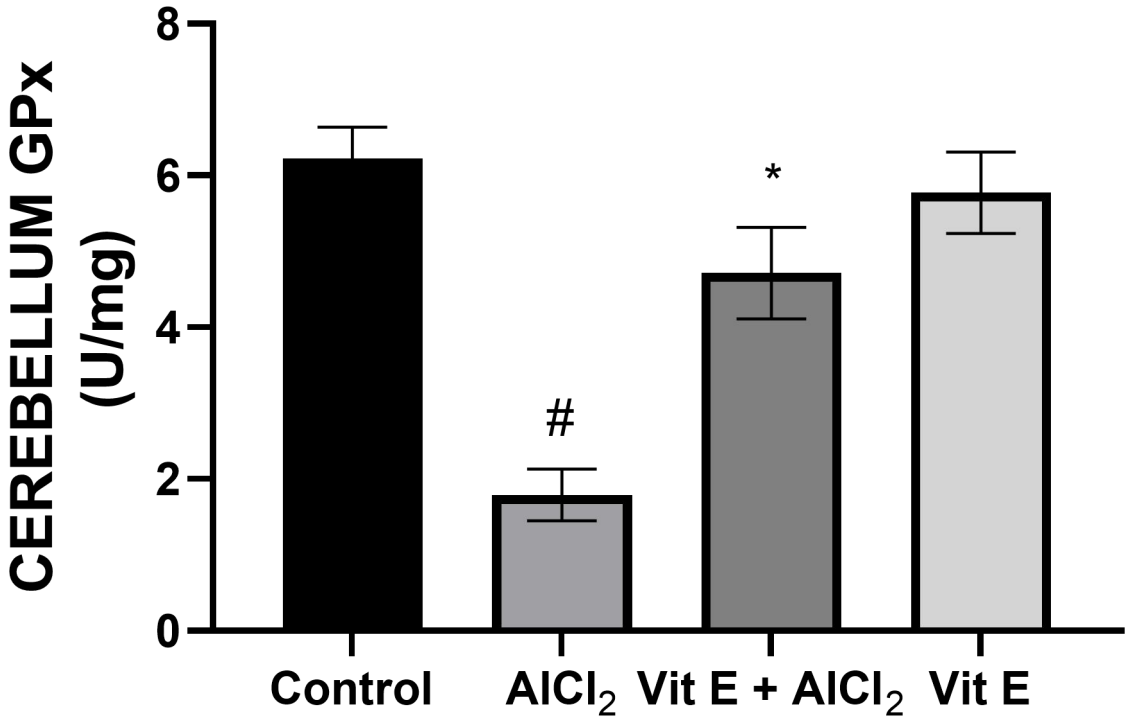

Figure 15: Activity of Glutathione peroxidase in the cerebellum of control and treatment groups after 28 days. Values are given as mean ± SEM of each group. $^{\#}$ $p<0.05$ compared with the control group; * $p<0.05$ compared with $AlCl_2$ group

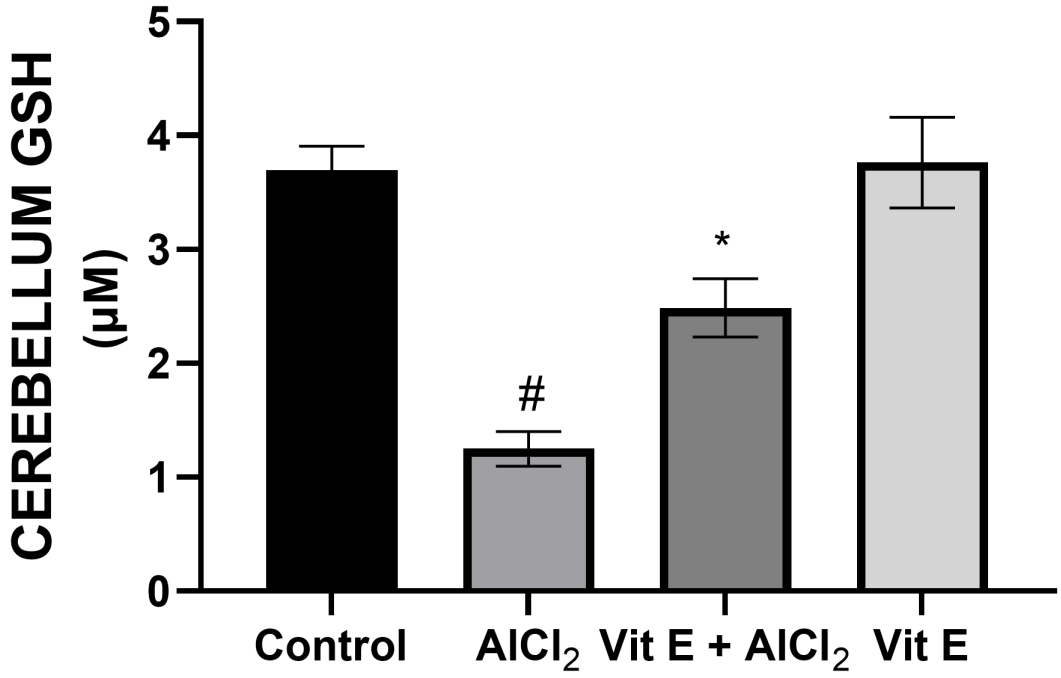

Figure 15: Activity of Reducing glutathione in the cerebellum of control and treatment groups after 28 days. Values are given as mean ± SEM of each group. # $p<0.05$ compared with the control group; * $p<0.05$ compared with $AlCl_2$ group

4.5 Effect of treatment on Lipid peroxidation

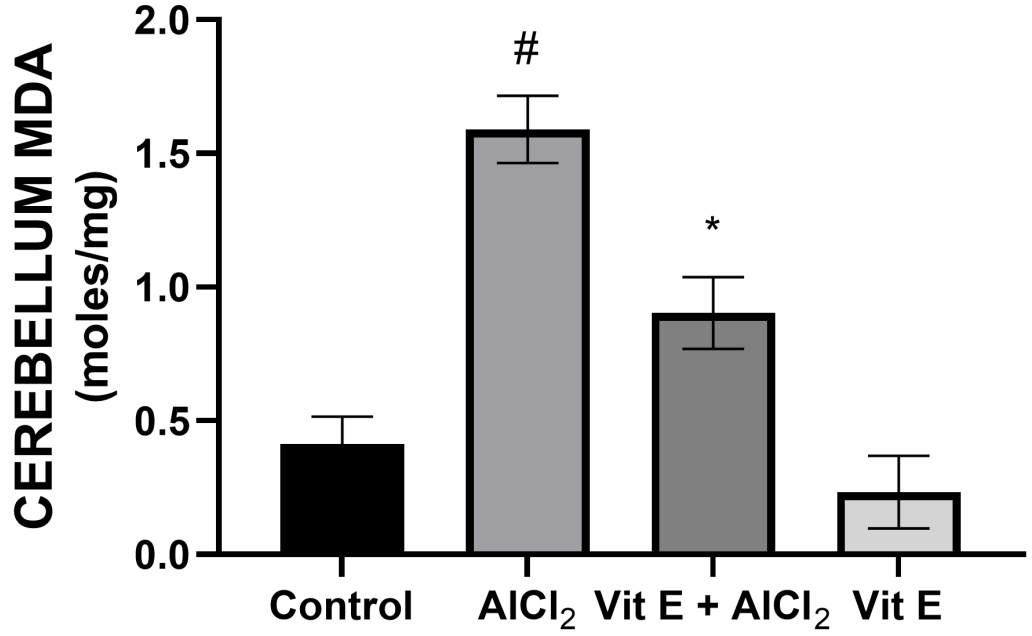

Figure 17: Lipid peroxidation concentration (Malondialdehyde) in the cerebellum of control and treatment groups after 28 days. Values are given as mean ± SEM of each group. $^{\#}$ p<0.05 compared with the control group; * p <0.05 compared with $AlCl_2$ group.

4.6 Effect of treatment on Histology

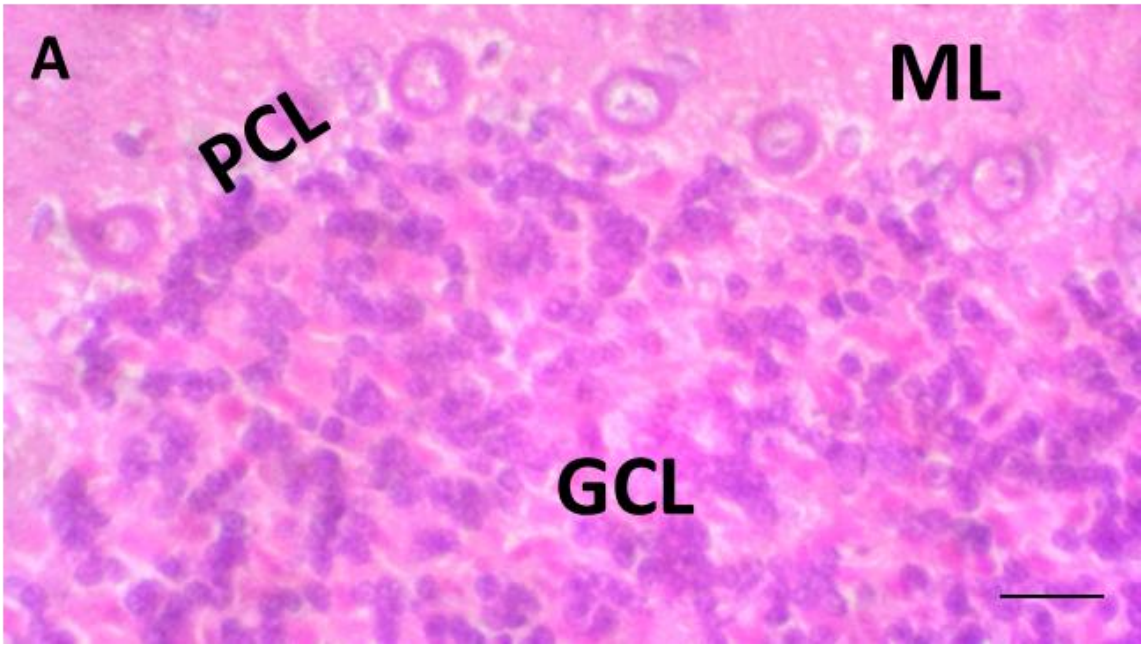

Plate 1: Representative histology of the cerebellum in control and treatment rats. (A) Normal histological structure of cerebellum layers – Molecular layer (ML); Purkinje Cell layer (PCL); Granular Cell layer (GCL);

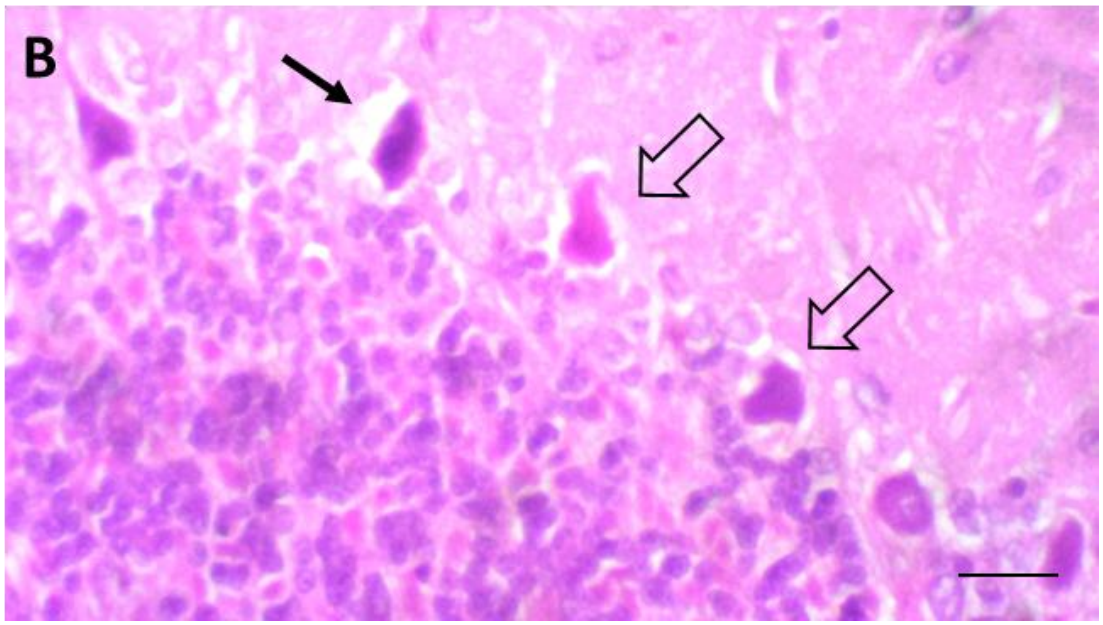

Plate 2: Represent cerebellum of rats treated with 5mg/kg of Aluminum chloride showing degenerating Purkinje cells (big arrows), with nuclei appearing irregular, darkly stained and pyknotic. Also observed are vacuolations in the Purkinje layer [arrow]

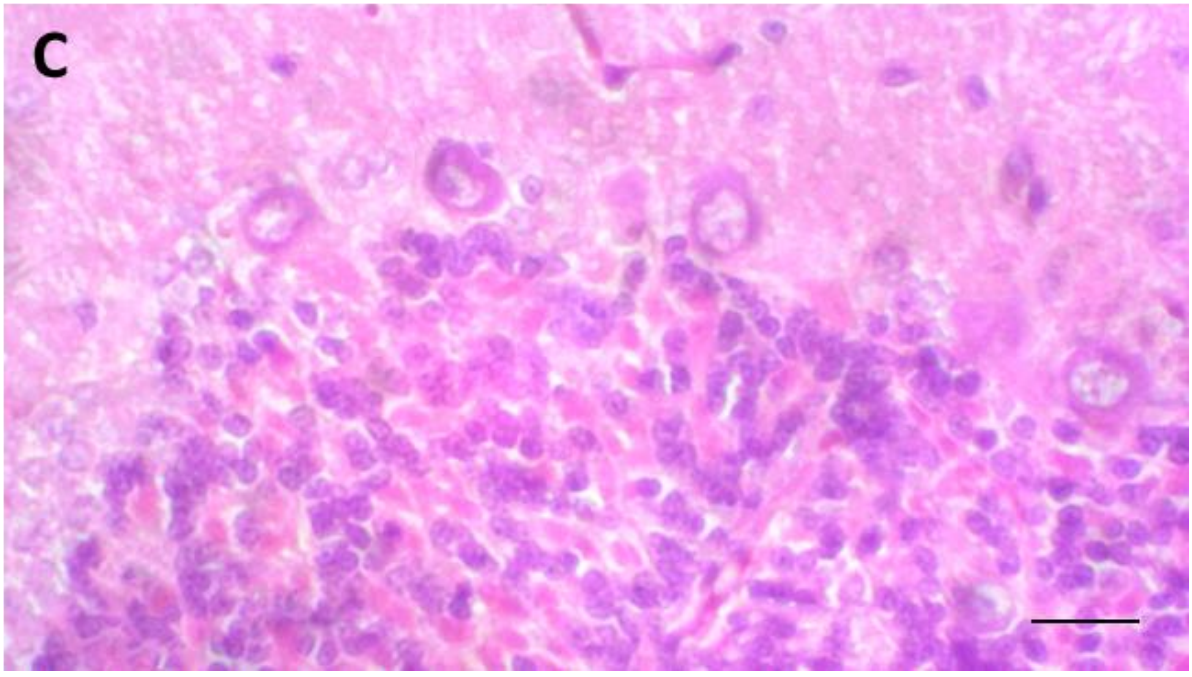

Plate 3: Represents rats treated with 5mg/kg of Aluminum chloride and Vitamin E showing Normal histological feature of the cerebellum observed (HandE 400x; Scale bar: 25µm)

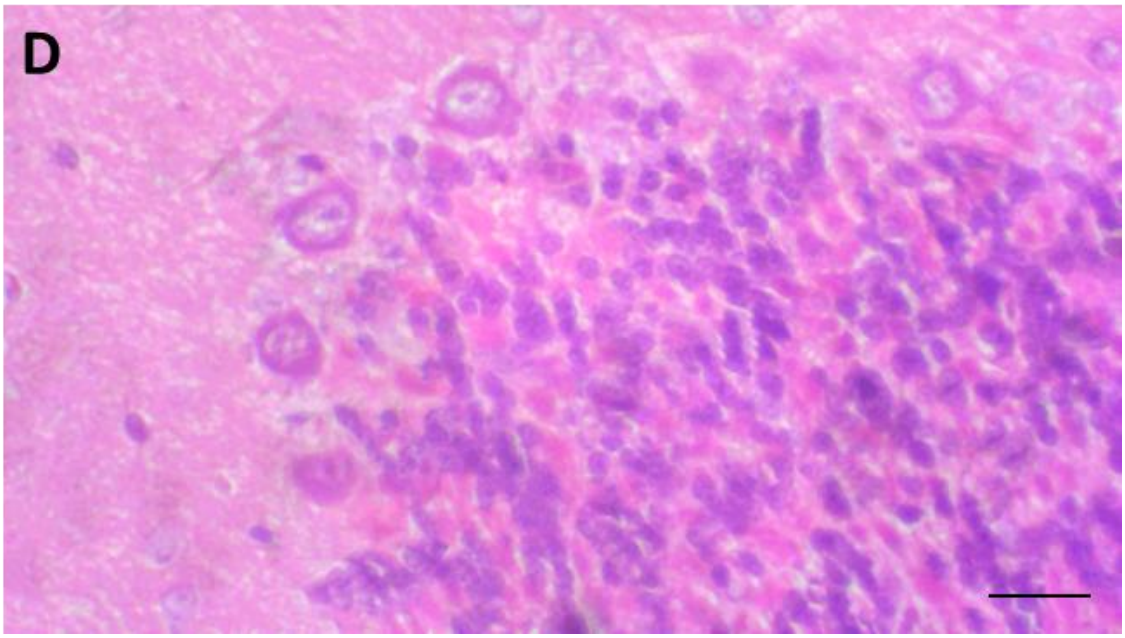

Plate 4: Represent rats treated with Vitamin E only showing Normal histological feature of the cerebellum observed (HandE 400x; Scale bar: 25µm)

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

The experimental results from this study highlight the pathological changes induced by aluminum chloride ($AlCl_3$) exposure in the cerebellum of Wistar rats and the ameliorative potential of Vitamin E. The weight changes observed indicate a significant reduction in body weight in the $AlCl_3$-treated group compared to the control, suggesting systemic toxicity and metabolic impairment, which aligns with previous findings that chronic aluminum exposure can lead to weight loss due to oxidative stress and metabolic disruption (Dey and Singh, 2022). However, Vitamin E co-administration mitigated this weight loss, likely due to its antioxidant properties, which counteract oxidative damage.

Neurobehavioral assessments, including the open field test (OFT) and movement initiation test, revealed that $AlCl_3$ exposure significantly impaired motor activity, as evidenced by reduced ambulation, grooming, rearing frequency, and central square entry, alongside increased immobility and thigmotaxis. These behavioral deficits are indicative of neurotoxicity and anxiety-like behavior, which have been associated with aluminum-induced neurodegeneration (Vlasak *et al*., 2023). The mechanism underlying these behavioral impairments is primarily linked to oxidative stress-mediated neuronal damage, leading to alterations in neurotransmitter systems and synaptic dysfunction (Tchekalarova and Tzoneva, 2023). The significant improvement in movement parameters observed in the $AlCl_3$ + Vitamin E group supports the hypothesis that

Vitamin E exerts neuroprotective effects by scavenging free radicals and stabilizing neuronal membranes, which is consistent with prior studies demonstrating its efficacy in ameliorating heavy metal-induced neurotoxicity (Nehru and Anand, 2005).

Antioxidant enzyme activity measurements in the cerebellum further substantiate the oxidative damage induced by $AlCl_3$. The significant reduction in superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and reducing glutathione (GSH) levels in the $AlCl_3$ group is indicative of a compromised antioxidant defense system, which facilitates neuronal damage and apoptosis (Flora *et al*., 2009). This aligns with the well-established role of aluminum in generating reactive oxygen species (ROS), leading to lipid peroxidation, protein oxidation, and DNA damage (Exley, 2013). The significant increase in malondialdehyde (MDA) levels further confirms enhanced lipid peroxidation, a hallmark of oxidative stress-induced cellular damage. The restoration of antioxidant enzyme activity and reduction in MDA levels in the $AlCl_3$ + Vitamin E group demonstrate the ability of Vitamin E to counteract oxidative damage, as also reported in previous studies where Vitamin E supplementation significantly improved antioxidant status in metal-induced neurotoxicity models (Sadek *et al*., 2019).

Histopathological analysis of the cerebellum provides concrete evidence of $AlCl_3$-induced neurodegeneration. The presence of degenerating Purkinje cells with pyknotic nuclei and vacuolations in the Purkinje cell layer indicates neuronal apoptosis and structural disintegration, which are hallmark features of aluminum-induced cerebellar toxicity (Abdelhameed *et al*., 2023). These histopathological changes correlate with the observed behavioral and biochemical alterations, reinforcing the role of oxidative stress in aluminum-induced neuronal damage. Notably, Vitamin E co-administration preserved

the cerebellar architecture, preventing Purkinje cell degeneration and maintaining normal histological features. This neuroprotective effect is attributed to Vitamin E's ability to inhibit lipid peroxidation, stabilize cell membranes, and reduce neuroinflammation, corroborating earlier findings that antioxidant therapy can effectively mitigate heavy metal-induced neurotoxicity (Tchekalarova and Tzoneva, 2023).

In comparison with previous studies, the findings of this research align with existing literature on aluminum neurotoxicity. Studies by Nehru and Anand (2005) and Flora *et al*. (2009) similarly reported significant oxidative stress, neurobehavioral deficits, and neuronal degeneration following aluminum exposure, with Vitamin E proving effective in ameliorating these toxic effects. However, some studies have suggested that the degree of neuroprotection conferred by Vitamin E may vary depending on dosage and duration of exposure, as prolonged exposure to high doses of aluminum may overwhelm antioxidant defenses (Sadek *et al*., 2019). This study strengthens the evidence supporting the use of Vitamin E as a neuroprotective agent, highlighting its potential therapeutic role in mitigating environmental neurotoxicants.

5.2 CONCLUSION

In conclusion, this study demonstrates that aluminum chloride induces significant oxidative stress, neurobehavioral deficits, and cerebellar neurodegeneration, while Vitamin E effectively mitigates these effects through its antioxidant and neuroprotective properties.

5.3 RECOMMENDATIONS:

Further studies should explore the long-term effects of aluminum chloride exposure and the sustained efficacy of Vitamin E treatment. Additional research should investigate the optimal dosage and duration of Vitamin E supplementation for neuroprotection. Future studies should consider other antioxidant compounds or combination therapies to enhance neuroprotection against aluminum-induced toxicity.

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