INVESTIGATING THE PROTECTIVE PROPERTIES OF CITICOLINE ON NICKEL CHLORIDE-INDUCED CEREBRAL TOXICITY IN ADULT WISTAR RATS

BY

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FEBRUARY, 2025

DECLARATION

I declare that

1. This project is based on the experimental work undertaken by me in the Department of
   Anatomy, University of Benin, under the supervision of IDEMUDIA O. USMANll
2. This work has never been submitted for the award of a degree elsewhere.
3. All odeas and views are solely based on this research, and whete the views of others
   have been expressed, such words were duly acknowledged.

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DAVID JACOB PRECIOUS ALEKOSE

CERTIFICATION OF PROJECT ON PLAGIARISM

We the undersigned attest and declare that the project undertaken by **DAVID JACOB PRECIOUS ALEKOSE** titled: **INVESTIGATING THE PROTECTIVE PROPERTIES OF CITICOLINE ON THE NICKEL CHLORIDE-INDUCED CEREBRAL TOXICITY IN WISTAR RATS** has successfully passed the anti-plagiarism test and does not violate any copyrighys regulations

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IDEMUDIA O. USMAN **DATE**

(SUPERVISOR)

_____ _____

Dr. ADAZE B. ENOGIERU (PhD) **DATE**

(Ag. HEAD OF DEPARTMENT)

CERTIFICATION

This is to certify that this research work titled **"INVESTIGATING THE PROTECTIVE PROPERTIES OF CITICOLINE ON NICKEL CHLORIDE-INDUCED CEREBRAL NEUROTOXICITY IN ADULT WISTAR RATS"** for the award of a degree of Bachelor of Science (B.Sc.) in Anatomy was carried out by **DAVID JACOB PRECIOUS ALEKOSE (MISS)** under the supervision of **DR IDEMUDIA O. USMAN**. All literatures used in this study have been acknowledged and properly referenced.

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IDEMUDIA O. USMAN **DATE**

(SUPERVISOR)

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Dr. ADAZE B. ENOGIERU (PhD) **DATE**

(HEAD OF DEPARTMENT)

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PROFESSOR O. EGWU **DATE**

EXTERNAL EXAMINER

DEDICATION

This project is dedicated to God Almighty , the divine source of all wisdom and guidance, and my loving family for all their support.

ACKNOWLEDGEMENT

My sincere and profound appreciation goes to God Almighty for providing me with the strength, wisom and guidance throughout this project. I would also like to extend my heartfelt thanks to my entire family, my grandparents who never stopped encouraging me, my parents, my siblings who always provide for me. My uncle's and Aunt's and everyone who has supported me in one way or the other. Most importantly, my supervisor, Dr Idemudia and my HOD, Dr Adaze. My friends that have always been there for me, Joshua, Godschoice, Ifeanyi, Abundance, and others too many to mention that has always been there in one way or the other. I am most grateful for all your contributions.

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ABSTRACT

Nickel chloride is known for its neurotoxicity and its exposure poses significant threat to the cerebrum, an essential brain organ for a lot of human day to day activities. This study investigates the neuroprotective effects of vitamin B6 on the cerebral integrity in nickel chloride exposed Wistar rats. After acclimatization for 14 days, fourty-one rats were weighed and divided to six groups of (n = 7). Group A (control) was administered 1ml of distilled water. Group B ($NiCl^2$) was administered 5mg/kg body weight (BW) of Nickel chloride only. Group C (Citicoline-1) was administered 25mg/kg BW of vitamin B6 only. Group D (Citicoline-2) was administered 50mg/kg BW of vitamin B6 only. Group E ($NiCl^2$+ Citicoline-1) was administered 25mg/kg BW of vitamin B6 and 5mg/kg BW of Nickel chloride. Group F ($NiCl^2$ + Citicoline-2) was administered 50mg/kg BW of vitamin B6 and 5mg/kg BW of Nickel chloride. The rats were intraperitoneally administered nickel chloride and orally administered vitamin B6 for 28 days, with vitamin B6 pretreatment an hour before nickel chloride administration. Neurobehavioral, (Y-maze), antioxidant enzymes, lipid peroxidation, molecular dockingand histological alterations were evaluated at the end of the experiment. Results showed that the treatment with nickel chloride effectuated ($p<0.05$) cognitive and memory deficits in the rats, decreased ($p<0.05$) the activities of antioxidant enzymes, increased ($p<0.05$) lipid peroxidation. Pretreatment of Nickel chloride rats with Citicoline attenuated ($p<0.05$) the neurobehavioral alterations, enhanced ($p<0.05$) the activity of antioxidant enzymes and mitigated ($p<0.05$) oxidative stress. Histological assessment provided evidence that Citicoline protected against nickel chloride induced neurodegeneration in the cerebrum. These findings indicate that Citicoline may have a neuroprotective effect against nickel chloride induced toxcity.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

According to Branco *et al*. (2021), the term "neurotoxicity" describes the detrimental effects of chemicals on the peripheral nerves, brain, and spinal cord as well as the structures and functions of the nervous system. According to Santos-Sacramento *et al*. (2021), exposure to specific chemicals, such as industrial pollutants, pesticides, and heavy metals, can cause neurotoxicity and have a negative impact on cognitive function, neurological development, and general brain health. According to Das *et al*. (2008) & Denkhaus and Salnikow (2002), the nervous system is prone to nickel toxicity, with the brain being regarded as a primary target organ. Numerous neurological disorders, including Parkinson's and Alzheimer's illnesses, have been linked to nickel exposure (Anyachor *et al.,* 2022). Nickel chloride is a toxic substance that has been shown to induce cerebral neurotoxicity in animals, leading to oxidative stress, inflammation, and neuronal damage (Liu *et al.*, 2013). The mechanism of nickel chloride-induced cerebral neurotoxicity involves the generation of reactive oxygen species (ROS), which can damage cellular components, including DNA, proteins, and lipids (Halliwell, 2007). Nickel chloride has also been shown to induce inflammation in the brain, leading to the activation of microglia and the release of pro-inflammatory cytokines (Zhang *et al.,* 2015). Food, air, water, tobacco smoke, skin contact with nickel-plated objects, nickel coins, stainless steet, jewellery, and prosthetic body parts are ways humans are exposed to nickel (Das and Buchner, 2007). Nickel modifies the intracellular chemical microenvironment by increasing the concentration of ionized calcium, lipid peroxidation, cyclooxygenase, nitric oxide synthase, leukotriene B4, prostaglandin E2, interleukins, Capase, complement activation, tumor necrosis factor-a, heat shock protein 70 kDa,

and hypoxia-inducible factor-1a (Das *et al*., 2020). Reports indicate that nickel exposure produces reactive oxygen species and compromises the antioxidant system (Gopal *et al*., 2009; Lamtai et al., 2018; Topal *et al*., 2015). Oxidative damage and lipid peroxidation can however be ameliorated by elimination of free radicals by the action of antioxidants (Gupta *et al*., 2009).

Citicoline, also known as CDP-choline, is a naturally occurring compound that has been widely used as a dietary supplement and has been shown to have various pharmacological effects, including neuroprotective, anti-inflammatory, and antioxidative activities (Secades & Frontera, 1995). The protective activity of citicoline on nickel chloride-induced cerebral neurotoxicity has not been fully investigated, and therefore, this study aims to explore the potential neuroprotective effects of citicoline on nickel chloride-induced cerebral neurotoxicity in adult Wistar rats. Citicoline has been shown to have neuroprotective effects in various animal models of neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and stroke (D'Orlando & Sandage, 1995). The neuroprotective effects of citicoline are thought to be mediated by its ability to increase the levels of neurotransmitters, such as acetylcholine and dopamine, and to reduce oxidative stress and inflammation (Savci *et al.,* 2003). Citicoline has also been shown to have antioxidative effects, reducing the levels of ROS and increasing the levels of antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase (Wurtman et al., 2000).

1.2 AIM AND OBJECTIVES OF THE STUDY

1.2.1 AIM

This study is aimed at investigating the neuroprotective properties of Citicoline on the nickel chloride induced cerebral toxicity in adult Wistar rats.

1.2.2 SPECIFIC OBJECTIVES

The specific objectives of the study are to investigate the neuroprotective activity of Citicoline on :

1. the brain and body weight changes in rats treated with or without nickel chloride.
2. the neurobehavioral activity (Novel object recognition test, Y-maze test) of rats treated with or without nickel chloride.
3. the antioxidant enzyeme activities (superoxide dismutase, catalase) in the cerebrum of rats treated with or without nickel chloride.
4. the lipid peroxidation concentration in the cerebrum of rats treated with or without nickel chloride.
5. the histology of the cerebrum in rats treated with or without nickel chloride.
6. the in-silico molecular docking against NF-Kb.

1.3 STATEMENT OF RESEARCH PROBLEM

Neurotoxicity describes the detrimental effects of chemicals on the peripheral nerves, brain and spinal cord as well as the structure and function of the nervous system (Branco *et al*., 2021). The advancement of Science and technology and widespread industrialization, has exposed humans to heavy metals which have been proven useful but its pollution harmful (Das *et al.*, 2019). Nickel is a metal that is widely found in the environment (Das *et al*., 2008) and is widely employed in a variety of consumer and industrial application (Huang *et al* ., 2003), its exposure however, increases the nervous system's vulnerability to a range of neurobehavioral and

neurodegenerative disorders. Exposure to nickel alters the intracellular chemical microenvironment by elevating the levels of ionized calcium, capases, complement activation, tumor necrosis factor-a, lipid peroxidation, and reducing the activity of antioxidant enzyme, ultimately resulting in cognitive dysfunction and impairment of learning and memory (Gopal *et al*., 2009; Lamtai *et al*., 2018; Topal *et al*., 2015; Das S *et al*., 2020). Nickel exposure which causes oxidative stress can be mitigated by enhancing the body's antioxidant defenses using prevention or treatment with antioxidants. The research problem of this study is to investigate the protective activity of citicoline on nickel chloride-induced cerebral neurotoxicity in adult Wistar rats. Specifically, the study aims to answer the following questions:

-Can citicoline protect against nickel chloride-induced cerebral toxicity in adult Wistar rats?

-What are the mechanisms of action of citicoline in protecting against nickel chloride-induced cerebral toxicity?

1.4 JUSTIFICATION OF STUDY

Citicoline is an essential drug that is vital to human health. Its shortage has been linked to major health problems in humans, including memory loss, confusion, difficulty in concentrating,anxiety, depression, irritability, physically and mental exhaustion, headaches, dizziness and tremors. According to Adibhatla *et al.* (2001), Citicoline seems to increase a brain chemical called phosphatidylcholine. Citicoline might also increase the amounts of other chemicals that send messages in the brain. It was originally used as a drug to help improve memory and brain function after a stroke. The purpose of this study is to acess the

neuroprotective effect of citicoline on the cerebrum of Wistar rats. This study is justified for several reasons:

- Nickel chloride is a toxic substance that is widely used in various industrial and commercial applications, and exposure to it can have serious health consequences, including cerebral neurotoxicity (Liu *et al.,* 2013)

- Citicoline is a naturally occurring compound that has been shown to have neuroprotective effects in various animal models of neurodegenerative diseases, and it may have potential therapeutic applications in the treatment of nickel chloride-induced cerebral neurotoxicity (D'Orlando & Sandage, 1995).

- The mechanisms of action of citicoline in protecting against nickel chloride-induced cerebral neurotoxicity are not fully understood, and this study aims to investigate the potential mechanisms of action of citicoline in protecting against nickel chloride-induced cerebral neurotoxicity.

- The results of this study may have important implications for the development of new therapeutic strategies for the treatment of nickel chloride-induced cerebral neurotoxicity and other neurodegenerative diseases.

CHAPTER TWO

LITERATURE REVIEW

2.1 CITICOLINE

Citicoline is a naturally occurring chemical compound that supports brain function and health. It's also known as cytidine 5'-diphosphocholine or CDP-choline. In the human body, citicoline plays a crucial role in forming neuron cell membranes. Citicoline was first identified by Kennedy and his colleagues in 1955, it was then synthesized in 1956 (Kennedy, 1955). It's known to have neuroprotective properties. Trusted Source such as promoting brain metabolism and increasing neurotransmitter levels in the central nervous system (Secades, 1995). Citicoline is available as a dietary supplement that is chemically identical to the naturally occurring compound. It belongs to a class of substances called nootropic agents, which are used to improve thinking ability and memory (Baldi, 2018). The dietary supplement is available over the counter in the United States and as a prescription drug in Europe and Japan. In a clinical setting, healthcare professionals may give citicoline therapy as a medication you take by mouth, as an injection into a muscle, or as an intravenous medication that flows directly into your vein (Marie Lorraine Johnson, 2023). Citicoline (cytidine-5′-diphosphocholine; CDP-choline) is an endogenous mononucleotide, composed of ribose, cytosine, pyrophosphate, and choline, and an essential precursor for the synthesis of neuronal plasma membrane phospholipids, important as a rate-limiting step in phosphatidylcholine synthesis (Cavalu, 2024). Citicoline can also be an exogenous source for the synthesis of acetylcholine, a key neurotransmitter, and is a member of the group of molecules that play important roles in cellular metabolism, such as nucleotides that form the basic structural units found in nucleic acids (Grieb, 2015). Kennedy and colleagues first identified citicoline in

1955 and synthesized it in 1956, Since then it has been studied extensively in Europe, Japan, and the United States (Alexander G. Schauss *et al.,* 2020).

Citicoline and choline are both dietary supplements that support brain health, but they are not the same. Citicoline supplements release two main substances into your body: cytidine and choline. Once absorbed, they cross the blood-brain barrier and reach your central nervous system, where they produce a range of neuroprotective effects. In contrast, choline supplements provide only choline, an essential nutrient that supports fat metabolism, liver health, and brain function.As a dietary supplement, citicoline is used to boost brain performance and improve memory. It has also been investigated as a drug to treat a wide range of conditions, including stroke, dementia, bipolar disorder, and depression (Cavalu *et al.,* 2024)

While some research has been promising, results have been mixed. Many studies have found evidence to suggest that citicoline improves memory and brain function (Marie Lorraine Johnson, 2023). A small 2021 study with 100 participants found that citicoline dietary supplements improved overall memory in older adults with age-related memory impairment (Gavillet, 2021). A 2023 review also noted that citicoline is beneficial for memory function in older adults (Parikh, 2023). A 2022 review of more than 800 studies on citicoline concluded that it improves memory and attention in generally healthy people and in people with vascular dementia. In addition, a 2008 study suggests that citicoline supplements increase energy use and energy reserves in the brain (Hurtado, 2008). However, some of these studies were performed in animals, and most studies in humans used small sample sizes. More research is needed to explore the potential benefits of citicoline for brain function and memory.Citicoline has been investigated for the treatment of various neurological conditions, including: stroke, traumatic brain injury, Parkinson’s disease, Alzheimer’s disease. Because citicoline occurs naturally in your body, it has

low toxicity and a low risk of side effects. Research, including a small 2021 trial, suggests that citicoline is safe and well tolerated. Side effects are rare and mild. The side effects of citicoline may include: headache, nausea, stomach pain, diarrhea, constipation, restlessness. Research suggests that citicoline interacts with the drug levodopa, a dopaminergic agent used to treat Parkinson's disease (Marie Lorraine Johnson *et al.,* 2023)

The chemical name of citicoline is 5′-O-[hydroxy({hydroxy[2-(trimethylammonio) ethoxy] phosphoryl} Moxy) phosphoryl] cytidine. It contains two major structural components, choline and cytidine (Secades, 1995). Choline is bound to the ribose ring through a pyrophosphate bond (Kennedy, 1955). This chemical bridge between ribose and choline gives citicoline the chemical property to be easily broken down and readily resynthesized given the favorable conditions or the presence of relevant enzymes (Baldi, 2018). This property is important to the delivery of citicoline to the CNS, as citicoline cannot cross the blood-brain barrier while choline and cytidine can, hence citicoline has to be hydrolyzed to cytidine and choline in the liver and resynthesized in the brain via the pyrophosphate bridge (Grieb, 2015). Ribose and cytosine make citicoline a component important for RNA biology, though the exact role of which has not yet been deciphered (Muneeb A. Faiq et al., 2019). It is speculated that nucleic acid synthesis may be one of the roles of citicoline in the light of its chemical composition (Cavalu, 2024). Further research is needed to fully understand the role of citicoline in RNA biology and its potential applications in medicine.

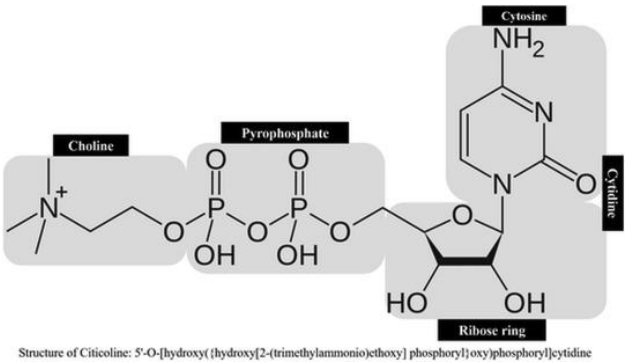


Fig 2.1 Molecular structure of citicoline

2.1.1 DISTRIBUTION OF CITICOLINE

There are two forms of citicoline available as either a dietary supplement ingredient or as a pharmacotherapeutic: citicoline sodium and citicoline free-base. Whereas citicoline free-base has been available as a dietary supplement in the United States for three decades, citicoline sodium is primarily used in other countries as a drug or para-drug for the treatment of neurological disorders. Citicoline is also available for intravenous use and as a topical in solution, for use as eye drops. When taken orally, citicoline is hydrolyzed in the intestinal tract and in the circulation to form choline and cytidine, which is the nucleoside of cytosine. Citicoline provides the brain with a source of choline and cytidine, which are efficiently used in the Kennedy cycle to generate phospholipids (Roman *et al.* 2011). Although choline on its own is preferentially used for the synthesis of acetylcholine, cytidine is highly efficiently used in the brain for the synthesis of various nucleotides. Studies in neuronal cell lines showed that cytidine administration increased the incorporation of choline into membrane phosphatidylcholine. In terms of safety, choline is a substance with a low level of toxicological concern. Administering choline with cytidine, in the form of citicoline, lowers the toxicity index by 20-fold.5 Furthermore, citicoline

administration is significantly different from the administration of choline in cases of cerebral ischemia caused by stroke and other conditions. Citicoline's therapeutic effects in such conditions stem from its ability to: increase the synthesis of phosphatidylcholine, the primary component of neuronal membranes. Beyond phosphatidylcholine action in maintaining phospholipid metabolism, one of its mechanisms of action is to restore and preserve the structure/function integrity of neuronal membranes after an acute ischemic stroke or brain damage from traumatic head injuries, enhance acetylcholine synthesis, thereby ameliorating the symptoms resulting from ischemic loss of cholinergic neurons, promote the synthesis of several other membrane phospholipids, including phosphatidylethanolamine and phosphatidylserine, to contribute to the repair and regeneration of axons and synapses, serve as agonists at nicotinic acetylcholine receptors, which play a critical role in maintaining normal cognition, and prevent the accumulation of free fatty acids and generation of free radicals at the site of ischemia, thereby preventing the initiation of a proinflammatory cascade of events. However, as will be discussed in this chapter, citicoline has also been studied for its effects on eye health and visual function, substance abuse, infectious diseases, and metabolic diseases. Citicoline is distributed to various tissues and organs, including the brain, liver, and kidneys. In the brain, citicoline is taken up by neurons and glial cells, where it is converted into phosphocholine and then into phosphatidylcholine, a major component of cell membranes (Wurtman et al., 2000). The brain uptake of citicoline is rapid, with significant amounts of the compound being detected in the brain within 30 minutes after oral administration (Hurtado et al., 2005).

2.1.2 ABSORPTION OF CITICOLINE

You can get choline from both animal- and plant-based foods, including eggs and shiitake mushrooms. Choline is a nutrient needed for many bodily processes, including metabolism,

neurotransmitter synthesis, brain development, and more. While your body naturally makes small amounts of this nutrient, it's not enough to fulfill your needs, so you need to get some from your diet. Adult men and women need 550 mg and 425 mg of choline per day, respectively, but 90% of the U.S. population does not meet this recommended Intake.Because choline is important for fetal growth and development, the need for this nutrient increases during pregnancy and breastfeeding. As such, pregnant people need 450 mg of choline per day, while those who are breastfeeding need 550 mg. Despite this, many prenatal supplements contain little, if any, choline. That's why it's essential that pregnant or breastfeeding people opt for high quality prenatal supplements and add choline-rich foods to their diet. Fortunately, this nutrient is found in many animal- and plant-based foods.Citicoline is a brain chemical that can be obtained from dietary supplements or from foods that contain choline. Choline is an essential nutrient that can be found in many foods, including: Organ meats, such as liver and kidneys, Fish, such as salmon, tuna, and cod, Cruciferous vegetables, such as broccoli, cauliflower, and Brussels sprouts, Almonds, Kidney beans, Whole eggs, Caviar, Soybeans, Wheat germ, fruits, such as avocados, berries, tangerines, apples, and kiwi. Dietary supplements: Citicoline is available as a dietary supplement in the United States (Katherine Marengo, 2023).

Citicoline can modulate the cellular CDP pathway (Weiss, 1995). Following ingestion, a large portion of citicoline is hydrolyzed in the small intestine in choline and cytidine, which are then absorbed and transported to the liver. Absorption follows a biphasic process with a first peak at one hour after ingestion followed by a sharp decline and then by a second larger peak at 24 h. Citicoline is metabolized in liver and elimination occurs mainly via respiratory CO2 and urinary excretion in two phases with an elimination half-life is 56 h for CO2 and 71 h for urinary excretion (Georges Emile Grau, 2009)

Citicoline is absorbed from the gastrointestinal tract and is then distributed to various tissues and organs, including the brain, liver, and kidneys (D'Orlando & Sandage, 1995). The absorption of citicoline is rapid, with peak plasma concentrations reached within 1-2 hours after oral administration (Savci et al., 2003). The bioavailability of citicoline is relatively high, with approximately 90% of the administered dose being absorbed into the bloodstream (Parker et al., 2011)

2.1.3 ELIMINATION OF CITICOLINE

Citicoline is eliminated from the body primarily through the kidneys, with approximately 70% of the administered dose being excreted in the urine within 24 hours (Savci et al., 2003). The elimination half-life of citicoline is relatively short, ranging from 2-4 hours, indicating that the compound is rapidly cleared from the body (Parker et al., 2011).

2.1.4 BIOLOGICAL ACTIVITIES

Dietary choline can impact systemic immunity, but it remains unclear whether this is primarily via direct impacts on immune cells or secondary effects of altered metabolic function. To determine whether increased choline concentrations (3.2, 8.2, 13.2 μm) in cell culture alter the function of bovine innate and adaptive immune cells, we isolated cells from dairy cows in early and mid-lactation as models of immuno-compromised and competent cells, respectively. Phagocytic and killing capacity of isolated neutrophils were linearly diminished with increasing doses of choline. In contrast, lymphocyte proliferation was linearly enhanced with increasing doses of choline. Furthermore, increasing doses of choline increased the mRNA abundance of

genes involved in the synthesis of choline products (betaine, phosphatidylcholine, and acetylcholine) as well as muscarinic and nicotinic acetylcholine receptors in a quadratic and

linear fashion for neutrophils and monocytes, respectively. Phagocytic and killing capacity of neutrophils and proliferation of lymphocytes were not affected by stage of lactation or its interaction with choline or LPS. In neutrophils from early lactation cows, choline linearly increased the mRNA abundance of muscarinic and nicotinic cholinergic receptors, whereas choline-supplemented monocytes from mid-lactation cows linearly increased the mRNA abundance of several genes coding for choline metabolism enzymes. These data demonstrate that choline regulates the inflammatory response of immune cells and suggest that the mechanism may involve one or more of its metabolic products (Zeisha SH, 1981).Although most people in the United States don't get recommended amounts of choline, few people have symptoms of choline deficiency. One reason might be that our bodies can make some choline. However, if a person's choline levels drop too low, he or she can experience muscle and liver damage as well as deposits of fat in the liver (a condition called nonalcoholic fatty liver disease (NAFLD) that can damage the liver). Getting too much choline can cause a fishy body odor, vomiting, heavy sweating and salivation, low blood pressure, and liver damage. Some research also suggests that high amounts of choline may increase the risk of heart disease [U.S. Department of Agriculture (USDA), 2022].

2.1.4.1 ANTI-MICROBAL ACTIVITY

It's been demonstrated that citicoline can stop some types of bacteria from growing. According to research, choline in the cells shows strong antibacterial properties against Streptococcus pneumoniae which remains one of the most common causes of bacterial meningitis and despite effective antimicrobial therapy, continues to be associated with high mortality and morbidity (L. Letkowitz *et al.,* 1997). About one third of the survivors, especially children, experience

neurological sequelae including deafness, mental retardation, focal neurologic deficits, and cognitive impairment (N. Rasmussen *et al.,* 1984; J.S. Bradley *et al.,* 1998; Van de Beek *et al.,* 2002). One of the anti-infection mechanisms of citicoline is through the ubiquitin-proteasome system (UPS). It primarily regulates protein degradation and turnover within cells, playing essential roles in various cellular processes, including immune responses(Wang J and Maldonado MA, 2006). However, while alterations in this system can affect immune function, there is limited direct evidence linking the anti-infection properties of citicoline specifically to its modulation of the UPS. The UPS plays a crucial role in the early stages of viral replication, particularly in endocytosis and viral maturation (Nathaniel M *et al.,* 2021). Viral infections, such as COVID-19 can manipulate the ubiquitin system by producing de-ubiquitinated proteins and accumulating ubiquitin conjugates (Nathaniel *et al.,* 2021). As a regulator of UPS activity, citicoline can impede UPS function and thus hinder viral replication through various mechanisms, including inhibiting protein synthesis, inducing endoplasmic reticulum stress and promoting cell death (Nathaniel *et al.,* 2021). While citicoline shows promise as a potential treatment, its effectiveness as an anti-infection agent, and particularly as an antibacterial agent remains controversial, and requires further research.

2.1.4.1.1 ANTIMICROBIAL EFFECTS AGAINST BACTERIA

Citicoline has been shown to exhibit potent antimicrobial effects against a wide range of bacteria, including Gram-positive and Gram-negative bacteria (Hurtado et al., 2005). The compound has been shown to inhibit the growth of bacteria such as E. coli, S. aureus, and Pseudomonas aeruginosa, and has been shown to be effective against antibiotic-resistant strains of bacteria (Savci et al., 2003). Additionally, citicoline has been shown to enhance the effects of antibiotics

against bacteria, and has been proposed as a potential adjuvant therapy for the treatment of bacterial infections (Parker et al., 2011).

2.1.4.1.2 ANTIMICROBIAL EFFECTS AGAINST VIRUSES

Citicoline has also been shown to exhibit antimicrobial effects against viruses, including influenza virus, herpes simplex virus, and human immunodeficiency virus (HIV) (D'Orlando & Sandage, 1995). The compound has been shown to inhibit the replication of viruses by interfering with their metabolic pathways and disrupting their cell membranes (Savci et al., 2003). Additionally, citicoline has been shown to modulate the host immune response against viral infections, and has been proposed as a potential adjuvant therapy for the treatment of viral infections (Wurtman et al., 2000).

2.1.4.1.3 ANTIMICROBIAL EFFECTS AGAINST FUNGI

Citicoline has been shown to exhibit antimicrobial effects against fungi, including C. albicans and Aspergillus fumigatus (Parker et al., 2011). The compound has been shown to inhibit the growth of fungi by interfering with their metabolic pathways and disrupting their cell membranes (Savci et al., 2003). Additionally, citicoline has been shown to modulate the host immune response against fungal infections, and has been proposed as a potential adjuvant therapy for the treatment of fungal infections (Wurtman et al., 2000).

2.1.4.2 ANTI-INFLAMMATORY ACTIVITY

Citicoline possesses anti-inflammatory properties, inhibiting pro-inflammatory cytokines (TNF-α, IL-1β, IL-6 and monocyte chemoattractant protein-1/MCP-1) while enhancing the generation of anti-inflammatory cytokines, such as IL-10, IFN-γ and TGF-β. Based on available evidence, citicoline exhibits potential to improve neurological disorders in CNS infections. It has anti-

infective properties, beneficial for both TB and viral infections such as COVID-19 (Nathaniel *et al.*, 2021). CNS TB and COVID-19 in the patient described herein can both cause brain oxidative stress and inflammation, leading to neuronal damage. The antioxidant and anti-inflammatory properties of citicoline may reduce tissue damage and improve neurological outcomes (Gupta JK *et al.,* 2016; Qureshi I and Endres JR, 2010; Nathaniel *et al.,* 2021: Al-Kuraishy HM, *et al,* 2022). Additionally, its neuroprotective, neuromodulatory and neurorestorative properties may aid in brain tissue repair, restore normal neural signaling and neurotransmitter balance, and enhance cognitive and motor functions caused by these infections (Gupta JK *et al.,* 2016; Qureshi I and Endres JR, 2010; Nathaniel *et al.,* 2021: Al-Kuraishy HM, *et al,* 2022)). Considering the current evidence supporting the safety and multiple beneficial properties of citicoline, it may serve as a valuable adjuvant treatment for patients, such as in the case presented herein. However, the careful assessment and monitoring of the patient's response to citicoline are essential. Further research and clinical trials investigating the efficacy of citicoline in CNS infections would be beneficial to strengthen its role in treatment strategies.

2.1.4.2.1 ANTI-INFLAMMATORY EFFECTS ON NEUROINFLAMMATION

Citicoline has been shown to exhibit anti-inflammatory effects on neuroinflammation, which is a key component of various neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and multiple sclerosis (Hurtado et al., 2005). The compound has been shown to inhibit the production of pro-inflammatory cytokines and to reduce oxidative stress in the brain, which can contribute to neuroinflammation (Savci et al., 2003). Additionally, citicoline has been shown to modulate the immune response in the brain, which can help to reduce neuroinflammation and promote neuroprotection (Wurtman et al., 2000).

2.1.4.2.2 ANTI-INFLAMMATORY EFFECTS ON CARDIOVASCULAR INFLAMMATION

Citicoline has been shown to exhibit anti-inflammatory effects on cardiovascular inflammation, which is a key component of various cardiovascular diseases, including atherosclerosis and hypertension (Parker et al., 2011). The compound has been shown to inhibit the production of pro-inflammatory cytokines and to reduce oxidative stress in the cardiovascular system, which can contribute to cardiovascular inflammation (Savci et al., 2003). Additionally, citicoline has been shown to modulate the immune response in the cardiovascular system, which can help to reduce cardiovascular inflammation and promote cardiovascular health (Wurtman et al., 2000).

2.1.4.2.3 ANTI-INFLAMMATORY EFFECTS ON GASTROINTESTINAL INFLAMMATION

Citicoline has been shown to exhibit anti-inflammatory effects on gastrointestinal inflammation, which is a key component of various gastrointestinal diseases, including inflammatory bowel disease and gastritis (Hurtado et al., 2005). The compound has been shown to inhibit the production of pro-inflammatory cytokines and to reduce oxidative stress in the gastrointestinal system, which can contribute to gastrointestinal inflammation (Savci et al., 2003). Additionally, citicoline has been shown to modulate the immune response in the gastrointestinal system, which can help to reduce gastrointestinal inflammation and promote gastrointestinal health (Wurtman et al., 2000).

2.1.4.3 ANTIOXIDATIVE ACTIVITY

Citicoline functions as an antioxidant by inhibiting the accumulation of free fatty acids (FFA), free radicals, lipid peroxidation and sphingomyelin damage (Gupta JK *et al.,* 2016 and Endres JR,

2010). In ischemic conditions, nerve cell damage and death result in the deposition of FFA, glycerol and arachidonic acid in the lesion, with the subsequent accumulation of metabolites, such as prostaglandins and thromboxane causing further damage over time. Citicoline also stimulates glutathione synthesis and enhances glutathione reductase activity, essential antioxidants that help prevent cell damage (Gupta JK *et al.,* 2016; Qureshi I and Endres JR, 2010; Al-Kuraishy HM *et al.,* 2022). These actions inhibit lipid peroxidation and the activation of PLA2, suppressing inflammation and neuronal cell death caused by oxidative stress. This was confirmed in another study, where citicoline was shown to reduce the biomarker of oxidative stress, malondialdehyde (Al-kuraishy HM *et al.,* 2022). In COVID-19, there is a decrease in choline levels, which is a source of phospholipid synthesis. Citicoline, a source of choline, can prevent PLA2 activity on the mitochondrial membrane and reduce phospholipid hydrolysis (Nathaniel M *et al.,* 2021). However, the referred studies are reviews; hence, the level of evidence is not very high (Gupta JK *et al.,* 2016; Qureshi I and Endres JR, 2010; Nathaniel *et al.,* 2021: Al-Kuraishy HM, *et al,* 2022). Additionally, some studies did not solely focus on CNS infections and lacked the descriptions of their literature search.

2.1.4.3.1 ANTIOXIDATIVE EFFECTS ON NEURODEGENERATIVE DISEASES

Citicoline has been shown to exhibit antioxidative effects on neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and multiple sclerosis (Hurtado et al., 2005). The compound has been shown to reduce oxidative stress in the brain, which is a key component of neurodegenerative diseases, by scavenging ROS and inhibiting lipid peroxidation (Savci et al., 2003). Additionally, citicoline has been shown to modulate antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase, which can help to reduce oxidative stress and promote neuroprotection (Wurtman et al., 2000).

2.1.4.3.2 ANTIOXIDATIVE EFFECTS ON CARDIOVASCULAR DISEASES

Citicoline has been shown to exhibit antioxidative effects on cardiovascular diseases, including atherosclerosis and hypertension (Parker et al., 2011). The compound has been shown to reduce oxidative stress in the cardiovascular system, which is a key component of cardiovascular diseases, by scavenging ROS and inhibiting lipid peroxidation (Savci et al., 2003). Additionally, citicoline has been shown to modulate antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase, which can help to reduce oxidative stress and promote cardiovascular health (Wurtman et al., 2000).

2.1.4.3.3 ANTIOXIDATIVE EFFECTS ON CANCER

Citicoline has been shown to exhibit antioxidative effects on cancer, including breast cancer, lung cancer, and colon cancer (Hurtado et al., 2005). The compound has been shown to reduce oxidative stress in cancer cells, which is a key component of cancer progression, by scavenging ROS and inhibiting lipid peroxidation (Savci et al., 2003). Additionally, citicoline has been shown to modulate antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase, which can help to reduce oxidative stress and promote cancer prevention (Wurtman et al., 2000).

2.2 CHEMICAL OF STUDY: NICKEL

After iron, oxygen, magnesium and silicon, nickel (Ni) is the fifth most plentiful element in terms of weight and the twenty-fourth most abundant element in the Earth's crust. The majority of nickel on Earth is inaccessible due to its location in the molten outer core of the planet iron-nickel alloy, 10% of which is represented by nickel and is located below the mantle and above the solid inner core of Earth. Elemental nickel, a silver-white solid metal with excellent thermal

and electrical conductivity, is found in nature in conjunction with sulphur, arsenic, and antimony. Although they are less common, other valences of nickel (+1 and +4) may exist in addition to the +2 oxidation state. A naturally occurring element, nickel can be found in a variety of mineral forms (Schaumlöffel, 2012). White nickel salts of mild inorganic acids, nickel sulphides and nickel oxides are poorly water-soluble, nickel salts of strong acids (chloride, nitrate, and sulphate) and organic salts are readily soluble in water (Denkhaus and Salnikow, 2002). The compounds nickel chloride, carbonate, nitrate, sulphate, acetate, hydroxide, and oxide are very important to the commercial world. Nickel chloride, because of its special physical and chemical characteristics, nickel is a metal that is widely found in the environment (Das *et al.,* 2008) and is widely employed in a variety of consumer and industrial applications (Huang *et al.,* 2013). Also, for a number of animal species, nickel is also recognized to be a necessary component. Its absence may have a negative impact on various bodily processes (Samal and Mishra, 2011).

2.2.1 NICKEL EXPOSURE

Nickel is a common element in soil, water, air and the biosphere, and is found naturally in the crust of the Earth. The crust of the planet contains 0.008% nickel on average. There are several compounds made of nickel as well. Compounds of nickel that dissolve in water comprise nickel oxide, nickel sulphide, and nickel subsulphide; insoluble nickel compounds include nickel chloride and nickel sulphate (Zhao *et al.,* 2009). Nickel carbonyl is a volatile, extremely poisonous liquid with certain industrial applications. Natural processes such as windblown dust, volcanic eruptions, plants, forest fires, and meteoric dust release nickel into the atmosphere. The primary human-caused sources of atmospheric nickel emissions include the burning of coal and oil, incineration of waste in municipal areas, manufacture of steel and other nickel alloys, and electroplating. The ambient air in metropolitan areas contains 1-10 ng m-3 of nickel. Levels

between 110 and 180 ng m-3 have been found in industrialized areas and big cities. The primary nickel compounds found in contaminated air seems to be elemental nickel, nickel sulphate, oxides, and sulphides. The two primary ways that the general public is exposed to nickel are through food and cigarette smoke (Cempel and Nikel, 2006). With one cigarette, around 0.04-0.58 μg of nickel are emitted into the atmosphere. Thus inhaling 40 cigarettes a day could result in 2-23 μg of nickel (Klein and Costa, 2022). It has been calculated that am adults typical daily intake of nickel from food is around 152 μg (Cempel and Nikel, 2006). Inhaling ambient air or consuming water tainted with nickel can potentially expose the general public to low concentrations of nickel (Klein and Costa, 2022).

A healthy human intake of nickel would be less than 100 μg/day, and even as low as 25-35 μg/day has been proposed based on research on animals (Nielsen, 2020). The majority of people meet this amount since the average daily food intake of nickel is greater than 70 ug/day. For the purpose of food and dietary supplements labelling, daily values for nickel have not been defined. Several detrimental health effects have been linked to prolonged exposure to high nickel levels. Reaction similar to asthma and respiratory symptoms like coughing and wheeze can be brought on by inhaling nickel compounds. An allergic contact dermatitis characterized by redness, itching, and skin lesions can results from prolonged skin contact with nickel. Additionally, exposure to nickel has been associated with a higher risk of lung and nasal malignancies, especially for those employed in the nickel processing and refining industries.

2.2.2 PHARMACOKINETICS AND TOXIC KINETICS

2.2.2.1. Absorption

Inhalation, ingestion, and, to a very small amount, skin exposure are the ways in which nickel can be absorbed. The solubility of the molecule affects the absorption of nickel, with the following general sequence of absorption occuring: nickel carbonyl > soluble nickel compounds > insoluble nickel compounds. Nickel chloride can be absorbed through various routes, including inhalation, ingestion, and dermal contact (ATSDR, 2005). Inhalation of nickel chloride particles can occur through occupational exposure, such as in nickel refining and processing industries (IARC, 1990). Ingestion of nickel chloride can occur through contaminated food and water, as well as through accidental ingestion of nickel-containing products (Liu *et al.*, 2013). Dermal contact with nickel chloride can occur through skin contact with nickel-containing products, such as jewelry and coins. The solubility of the substance and the size of the particles affect how much of the inhaled marerial is absorbed. Because smaller particles are able to enter the respiratory tract more deeply than bigger ones, there is a greater relative absorption. More of the smaller chemicals are absorbed swiftly decreasing their mucociliary clearance potential before being ingested. In an absorption study, 27% of nickel sulphate given to humans in drinking water was systematically absorbed compared with 1% when it was given in food (Klein and Costa, 2022). The thyroid, adrenal glands, and lungs had the greatest quantities of nickel in human autopsy investigations involving non-occupationally exposed subjects. The kidneys, heart, liver, brain, spleen and pancreas have all been found to contain lower amount of nickel (Cempel and Nikel, 2006). Breast milk had been reported to contsi nickel, which crosses the placenta (Motas *et al.,* 2021).

2.2.2.2 DISTRIBUTION

Once absorbed, nickel chloride is distributed to various tissues and organs, including the liver, kidneys, lungs, and brain (Liu *et al.*, 2013). The distribution of nickel chloride is influenced by its chemical properties, such as its solubility and lipophilicity (Klaassen *et al*., 2013). Nickel chloride can bind to proteins and other biomolecules, which can affect its distribution and toxicity (Liu *et al.*, 2013). The average crustal abundance of nickel is estimated to be 0.008%, making it a reasonably plentiful element in the Earth's crust. It can be found in primary and secondary mineral deposits, frequently in combination with other metals like iron, copper, cobalt, and members of the platinum group. While secondary nickel deposits can come from the weathering and erosion of primary deposits, original nickel deposits are usually generated by magmatic processes, such as ultramafic and mafic intrusions.

Sulphide ore deposits, which include nickel in the form of nickrl sulphides like pentlandite and nickeliferous pyrrhotite, account for the majority of the world's nickel production (Wang *et al.,* 2020). The main methods used to mine these sulphide ores are open-pit or underground mining. Another significant source of nickel production is lateralitic nickel deposits, which are created in tropical temperatures by the weathering of ultramafic rocks. These deposits are found mostly in areas like Indonesia, the Philippines and New Caledonia (Elias, 2002).

2.2.2.3 EXCRETION

The elimination of nickel chloride from the body is a complex process that involves multiple pathways, including urinary excretion, fecal excretion, and biliary excretion (Klaassen *et al*., 2013). Urinary excretion is the primary route of elimination for nickel chloride, with the majority of the dose being excreted in the urine within 24 hours (Liu *et al*, 2013). Fecal excretion is also

an important route of elimination, with a significant portion of the dose being excreted in the feces (Klaassen *et al*., 2013). Biliary excretion is a minor route of elimination, with only a small portion of the dose being excreted in the bile (Liu *et al.,* 2013). The majority of ingested nickel is eliminated through the urine, while some may also be expelled through tears, saliva, sweat, and milk. Nickel that is not absorbed from the gastrointestinal tract is excreted in the feaces (Huang *et al.,* 2022). A urinary elimination half-life (for absorbed nickel) of 17-48 hours has been reported in a human oral exposure study (Zhao *et al.,* 2009).

2.2.2.4 TOXICOKINETICS

The toxicokinetics of nickel chloride are influenced by its chemical properties, such as its solubility and lipophilicity (Klaassen *et al.*, 2013). The half-life of nickel chloride in the body is relatively short, ranging from 1-5 days (Liu *et al*., 2013). The bioavailability of nickel chloride is influenced by its route of administration, with inhalation and ingestion being the most effective routes of exposure (ATSDR, 2005).

2.2.2.5 HEALTH EFFECTS

The health effects of nickel chloride are influenced by its toxicokinetics and toxicodynamics (Liu *et al.,* 2013). Exposure to nickel chloride has been associated with various health effects, including cancer, neurological damage, and reproductive toxicity (IARC, 1990) [4]. The mechanisms of nickel chloride toxicity are complex and involve multiple pathways, including oxidative stress, inflammation, and DNA damage (Liu *et al*., 2013).

2.2.3 MECHANISM OF NICKEL TOXICITY

By increasing the concentration of ionized calcium, lipid peroxidation, cyclooxygenase, constitutive nitric oxide synthase, leukotriene B4, prostaglandin E2, interleukins, tumor necrosis

factor-α, caspases, complement activation, heat shock protein 70 kDa, and hypoxia-inducible factor-1α, nickel modifies the intracellular chemical microenvironment. The spread of metastatic, is caused by the oxidative stress that nickel induces. Exposure to nickel has been found to cause the production of reactive oxygen species, which in turn causes the expression of p53, nuclear factor kappa-light-chain- enhancer of activated B cells (NF-kβ), activator protein-1 (AP-1), and Mitogen-activated protein kinase (MAPK) to increase (Das *et al.,* 2020).

The main molecular pathways of nickel-induced neurotoxicity are oxidative stress and mitochondrial dysfunctions. The loss of mitochondrial deoxyribonucleic acid (DNA) and disruption to the mitochondrial membrane potential are the usual causes of mitochondrial damage brought on by nickel-induced neurotoxicity, which lowers the production of adenosine triphosphate (ATP). Damage to mitochondrial functioning increases ROS and exacerbates oxidative stress by interfering with tye mitochondrial transport chain. According to Lamtai *et al.* (2018) and Topal *et al.* (2015), nickel exposed neurons create ROS and show signs of a compromised cell antioxidant system. When lipid peroxidation (LPO) is activated, it starts a chain reaction that produces a lot of free radicals, which can covalently alter proteins and DNA, making them both genotoxic and cytotoxic (Das *et al.,* 2008). Prior to structural alteration in biological membranes (Lamtai *et al.,* 2018) or other lipid-containing components, these leads to a reduction in membrane fluidity and the deactivation of many enzymes that are attached to the membrane. All of these cause degenerative problems in the central nervous system and cause cellular death (Lamtai *et al.,* 2018; Aslinjensipriya *et al.,* 2022). Furthermore, by weakening the antioxidant system, nickel can potentially cause oxidative stress. It is commonl6 recognized that when coupled, superoxide dismutase (SOD) and catalase (CAT) provide a strong defensive mechanism against free radicals (Lamtai *et al.,* 2018). Nickel exposed rats have decreased levels

of the antioxidant enzymes superoxide dismutase, catalase, glutathione S-transferases (GST), glutathione peroxidase (GPx) activity (Adedara *et al.,* 2020; Ijomone *et al.,* 2018). These point to a compromised antioxidant defense mechanism, which in turn causes a build up of more cytotoxic radicals.

An inflammatory pathway is triggered by an overexposure to nickel through the tumor necrosis factor α (TNF)-α signalling pathway. After nickel exposure, there was a significant increase in the levels of pro-inflammatory cytokines, such as TNF-α and interleukin-1β (1L-1β), and inflammatory biomarkers including myeloperoxidase (MPO) activity (Adedara *et al.,* 2020; Ijomone *et al.,* 2018). A well known "master regulator" of the inflammatory response, TNF-α is responsible for attracting immune cells to areas of tissue damage, making it a crucial oxido-inflammatory stress biomarker (Aratani, 2018; Ndrepepa, 2019).Nickel has been found to be the external stimulation that starts the apoptotic process. Capase-3 is a crucial executioner enzyme in the apoptotic cascade, and nickel promotes it's expression.

The stimulation of apoptotic cell death is shown by the elevated level of this protein in the cerebrum and cerebellum (Adedara *et al.,* 2020; Ijomone *et al.,* 2018) found that exposed rats' hippocampus and striatum had higher levels of caspase-3 expression. Both intrinsic and extrinsic routes can activate caspase-3, an effector or executioner caspase, in an apoptotic cell (Ghavami *et al.,* 2009; Mcllwain *et al.,* 2013). Prior in vitro research has shown that nickel compounds activate caspase-3 in cultivated cells, which in turn initiates the apoptotic process.

According to Hussian *et al.* (2011) and Siddiqi *et al.* (2012), the release of cytochrome c from the mitochondria causes the release of caspase-3. Additionally, it was demonstrated that the intrinsic apoptotic mechanism after nickel treatment were initiated by an increase in the pro-

apoptotic protein Bax and a concurrent decrease in the anti-apoptotic protein Bel-2 (Guo *et al.,* 2016; Huang *et al.,* 2013).

2.3. THE CEREBRUM

The cerebrum is the largest part of the brain, located superiorly and anteriorly in relation to the brainstem. It consists of two cerebral hemispheres (left and right), separated by the falx cerebri of the dura mater. Embryologically, the cerebrum is derived from the prosencephalon (Sadler, 2018).The cerebrum makes up most of your brain and includes the frontal, parietal, temporal, insular and occipital lobes. The cerebrum is the upper part of the brain, handling many different functions, including muscle movements, language, processing what your senses pick up and more (Kolb, 2019). The cerebrum is the largest part of the brain, and it handles a wide range of responsibilities. Located at the front and top of your skull, it gets its name from the Latin word "*cerebra*" meaning “brain.” The cerebrum is instrumental in everything a person do in day-to-day life, ranging from thoughts to actions. In essence, it’s responsible for the brain functions that allow us to interact with our environment and make us who we are (Lezak, 2012). Scientists have been studying the brain for years, trying to unlock just how it works and how to diagnose and treat conditions that affect it. While experts know a lot about how the cerebrum works, there’s much that’s not fully understood. Fortunately, advances in technology and medical science have helped drive growth in what experts understand about the brain (Gazzaniga, 2018).

The ancient Greek physician Galen (129-216 AD) is credited with being the first to describe the cerebrum.Andreas Vesalius (1514-1564) published detailed anatomical drawings of the brain,

including the cerebrum, in his book "De humani corporis fabrica". Thomas Willis (1621-1675) described the structure and function of the cerebrum, including its role in controlling movement and processing sensory information. Franz Joseph Gall (1758-1828) developed the theory of phrenology, which posited that the shape and size of the skull could reveal information about an individual's intelligence, personality, and character. Paul Broca (1824-1880) discovered the language center in the left hemisphere of the cerebrum, now known as Broca's area. Carl Wernicke (1848-1905) identified the area responsible for language comprehension in the left hemisphere of the cerebrum, now known as Wernicke's area. Santiago Ramón y Cajal (1852-1934) discovered the structure of neurons and their connections in the cerebrum, earning him the Nobel Prize in Physiology or Medicine in 1906. Roger Sperry (1913-1994) conducted pioneering research on the lateralization of brain function, demonstrating the specialized roles of the left and right hemispheres of the cerebrum.

The cerebrum is the largest part of your brain and includes parts above and forward of the cerebellum. The cerebrum is the part of the brain that starts and manages conscious thoughts; meaning, things that a person actively think about or do.The cerebellum is a small part of the brain located at the bottom of this organ near the back of the head. It processes and regulates signals between other parts of the brain and body, and is involved in coordinating functions of the body (for example, walking). The cerebrum handles much of the brain’s “conscious” actions (American Association of Neurological Surgeons, 2024).

That means it’s responsible for elements that require thinking, including the five senses: the cerebrum manages and processes everything the senses take in. That includes sight, sound, smell, taste and touch.

Language: Various parts of the cerebrum control the ability to read, write and speak.

Working memory: This is a type of short-term memory.

Behavior and personality: Part of the cerebrum is the frontal lobe, which manages personality and behavior. It's the part of the brain that acts as a filter to stop someone from doing or saying things they might later regret.

Movement: Certain areas of the cerebrum send signals that tell the muscles what to do when they are needed.

Learning, logic and reasoning: Different areas of the cerebrum work together when a person need to learn a new skill, make a plan of action or puzzle out a problem (Jawabri, 2021)

The cerebrum works together with other parts of the brain, especially the cerebellum, to help with daily activities. An example of this is picking up a pencil off a table. The cerebrum sends the signals to the muscles in the arms, and the cerebellum helps calculate and control movements, so the hand goes right to the pencil without missing. The cerebellum not only manages conscious thoughts, but also planning and actions. That includes when a person decide to be physically active, choose what to eat for a meal or set aside time to see a healthcare provider for any reason. Because of this, the cerebrum plays a critical role in the health and well-being of the entire body (Jones, 2007; Jawabri, 2021)

There are some interesting facts about the cerebrum some of which includes:

Crossed representation. When you do something with one side of the body, the other side of the brain is usually behind that process. An example of this is having a stroke on the left side of the brain and feeling its effects on the right side of the body. The brain is very adaptable. The brain

can “rewire” itself. This ability can happen as we learn new skills or help us recover from injuries to our brain. The brain has specialized areas. Different parts of the brain are responsible for different abilities and skills. However, that’s also fed into the disproven myth that some people are “left-brained” or “right-brained" (American Brain Foundation, 2023).

Any condition affecting the brain can affect the cerebrum, including mental health conditions. Some major examples include: Alzheimer’s disease, Anxiety disorders, Attention-deficit hyperactivity disorder (ADHD), Stroke, Concussion and traumatic brain injuries, Congenital disorders (conditions that occur at birth, such as Menkes disease), Dementia, Depression,Dizziness, Epilepsy, Immune and inflammatory conditions (an example of this is fibromyalgia), Genetic disorders (conditions you have at birth that you inherited from one or both parents, such as Wilson’s disease), Infections (these can happen because of bacteria, viruses, parasites and fungi), Parkinson’s disease, Post-traumatic stress disorder (PTSD), Schizophrenia, Vitamin deficiencies and nutrition problems (such as low vitamin B12 levels and hypothyroidism) (National Institute of Neurological Disorders and stroke (US), 2024).

Many symptoms are possible when a person has a condition that affects the cerebrum. Some of the most common symptoms include: Aphasia: Problems with the speech centers in the cerebrum can affect your ability to speak or understand others who are speaking.

Ataxia: This is a loss of coordination. It can make you clumsy, causing balance problems or trouble using your hands for common tasks, Behavior changes and confusion, Dizziness, Headaches and migraines, Memory problems, Paralysis.

This can affect various parts of the body, Shaking or tremors. Loss of muscle coordination can cause parts of the body, especially the hands, to shake, Trouble concentrating or thinking,

Vision problems. The cerebrum plays a role in controlling the eyes and how the brain processes what is seen. Vision problems can range from blurred or distorted vision to blindness.Many types of tests can help diagnose conditions that affect the brain, including the cerebrum. Common tests include: Blood tests (these can look for anything from immune system issues to toxins and poisons, especially certain metals like copper). Computerized tomography (CT) scan, Electroencephalogram (EEG), Electromyogram, Evoked potentials test, Genetic testing, Magnetic Resonance Imaging (MRI), Position Emission Tomography (PET) scan, sleep studies, spinal tap (lumber puncture), X-rays (American Brain Foundation, 2023).

2.3.1 Gross Anatomy of the Cerebrum

The cerebrum is inside of the skull, at the top and front of the head, and makes up the largest part of the brain. The outer surface of the cerebrum, the cerebral cortex, is mostly smooth but has many wrinkles, making it look something like a walnut without its shell. These are grooves known as the sulci and gyrus (Bui and Das, 2021; Maldonado *et al.,* 2023). The cerebrum is divided into lobes.

Frontal: This lobe handles things like attention, behavior control, the ability to speak and certain types of muscle movements.

Parietal: This area handles touch, temperature and pain signals. It also helps with how a person sees the world around them, especially judging distance from and the size of objects. It also plays a role in processing sound, languages spoken, ability to use numbers and count, and organization of information and decision making.

Temporal: This area helps to understand language when other people are speaking. It also helps to recognize people and objects. This part also helps to connect emotions with memories.

Insular (deep inside of the brain, underneath the frontal, parietal and temporal lobes): This part of the brain handles taste senses. It may also help process certain types of emotions like compassion and empathy.

Occipital: This lobe manages much of the eyes' sensory input, including the ability to see movement and colors (Bui and Das, 2021).

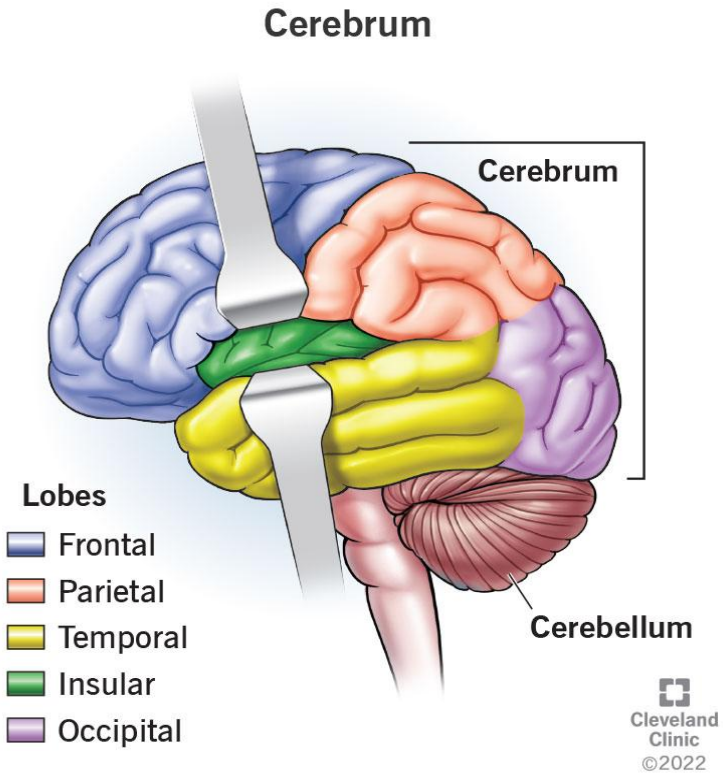


Fig 2.2 The Brain showing the lobes of the cerebrum

A few structures that are part of the cerebrum stand out because they have very specific purposes. These include the:

Cerebral cortex: This is a thin layer of brain tissue on the surface of the cerebrum (the word "cortex" comes from the Latin word for "bark," as in the outer layer of a tree trunk) (Jawabri, 2021). The cerebral cortex is responsible for a wide range of functions, including sensory perception, movement, and cognition (Kandel et al., 2000). The different layers and types of cells in the cerebral cortex work together to enable the brain to process and integrate sensory information, control movement, and facilitate thought, perception, and memory.

Thalamus: This part of the brain acts like a relay station, sorting input from senses and sending it to various parts of the cerebrum (except for smell, which bypasses the thalamus and goes directly to the cerebrum) (Jones, 2007).

Hypothalamus: This part of the brain (whose name means "under the thalamus") manages functions in the nervous and endocrine systems, both of which help with controlling other systems and processes throughout the body. An example of this is how your hypothalamus helps manage body's temperature, heart rate and blood pressure.

Hippocampus: This structure with the temporal lobe helps manage and store memories in areas of the cerebrum and fetches them when needed (Theibert AB, 2020).

There's a reason why different parts of the brain handle the same information as others. That's because the information is interconnected in many ways. The average adult brain is between 3.5 and 4 times the volume of a regulation baseball, and the cerebrum makes up about 80% of the overall brain volume. That means the cerebrum is about 3 to 3.2 times the volume of a baseball. The average adult brain weighs between 2.6 pounds (lbs.) and 3.1 lbs. The cerebrum makes up

about 2 lbs. to 2.5 lbs. of that total weight. The tissue of your brain is roughly: 77% water, 11% lipids (fats), 8% proteins, 4% other (Leaze C *et al.,* 2017).

2.3.2 HISTOLOGY OF THE CEREBRUM

Microscopically, the cerebral cortex is composed of cell bodies of billions of neurons, their dendrites, myelinated and unmyelinated axons which altogether form a unique, multilayered arrangement. In addition, it contains a dense population of supporting glial cells which include oligodendrocytes, astrocytes, microglia, and ependymal cells, and blood vessels. The neuronal cells of the cortex consist of six main cell types. These are the pyramidal cells (the main output neurons of the cerebral cortex), fusiform cells, stellate (granular) cells, basket cells, horizontal cells of Cajal-Retzius and cells of Martinotti (Society for Neuroscience, 2018). Histologically, the cerebral cortex is organized into six layers or horizontal laminae based on the size and shape of the neuronal bodies.

The cerebral cortex is the outermost layer of the brain, responsible for processing sensory information, controlling movement, and facilitating thought, perception, and memory (Kandel et al., 2000). The histology of the cerebral cortex is complex and consists of multiple layers of neurons, glial cells, and blood vessels, which work together to enable the brain to function properly (Peters & Jones, 1984). This essay will review the histology of the cerebral cortex, its structure, and function, and discuss the different types of cells and layers that make up this complex tissue.

2.3.2.1 LAYERS OF THE CEREBRAL CORTEX

The cerebral cortex is divided into six distinct layers, each with its own unique characteristics and functions (Brodmann, 1909). The layers are numbered from 1 to 6, with layer 1 being the most superficial and layer 6 being the deepest. The layers are as follows:

- Layer 1: Molecular layer, contains few neurons and is composed mainly of glial cells and nerve fibers.

- Layer 2: External granular layer, contains small granular neurons and is involved in the processing of sensory information.

- Layer 3: External pyramidal layer, contains large pyramidal neurons and is involved in the integration of sensory information.

- Layer 4: Internal granular layer, contains small granular neurons and is involved in the processing of sensory information.

- Layer 5: Internal pyramidal layer, contains large pyramidal neurons and is involved in the integration of sensory information.

- Layer 6: Multiform layer, contains a variety of neuronal types and is involved in the processing of sensory information.

2.3.2.2 TYPES OF CELLS IN THE CEREBRAL CORTEX

The cerebral cortex contains a variety of cell types, including neurons, glial cells, and blood vessels (Peters & Jones, 1984). Neurons are the primary functional units of the brain and are responsible for processing and transmitting information. There are several types of neurons in the cerebral cortex, including pyramidal neurons, granular neurons, and interneurons. Glial cells,

such as astrocytes and oligodendrocytes, provide support and maintenance functions for neurons, including the provision of nutrients and the removal of waste products.

CHAPTER THREE

METHODOLOGY

3.1 REAGENTS/CHEMICALS

All reagents and chemicals are of analytical grade. They include distilled water, Nickel Chloride, alcohol (50%, 70%, 90%, 100%), xylene, paraffin, normal saline, and 10% formalin.

3.2 EQUIPMENT

Weighing balance, orogastric tube, surgical latex glove, sample bottles, plastic cages, mortar and pestle, refrigerator, industrial grinding machine, oven, water bath, paraffin dispenser, dissecting set, measuring cylinder, conical flask volumetric flask, glass rods, rotary microtome, binocular microscope.

3.3 COMPUTER SOFTWARE

GraphPad Prism statistical package Version 10 (manufactured by GraphPad software, Inc; released in 2016).

3.4 DETERMINATION OF DOSAGE

The dosage of citicoline would be 250 mg/kg body weight for low dose and 500 mg/kg body weight for high dose, 5 mg/kg body weight of nickel would be used to induce neurotoxicity in adult Wistar rats. This is the established and reported dose for inducing neurotoxicity in rats Ijomone *et al.* (2018)

3.5 ANIMAL CARE AND MANAGEMENT

Forty-two (42) adult Wistar rats would be procured from the Animal House, Department of Anatomy, University of Benin, Benin City. They would be kept in clean cages and allowed to acclimatize for two weeks. They would be allowed access to standard rat chow and water ad libitum throughout the entire study period of twenty-eight (28) days. They would be weighed weekly before commencement and throughout the experiment using a digital weighing scale calibrated in grams and recorded to the nearest whole number.

3.6 EXPERIMENTAL DESIGN

A total of forty two adult wistar rats weighing between 140g and 170g were used for this study. They were randomly assigned into six groups (A,B ,C, D, E, and F) of six rats each and they were allowed to acclimatized for three weeks with access to food and water. The rats in Group A served as Control and received distilled water. The rats in Group B received 5mg/kg BW if Nickel chloride. Group C received 0.25mg/kg BW of Citicoline and 5mg/kg BW of Nickel chloride. Group D received 0.5mg/kg BW of Citicoline and 5mg/kg BW of Nickel chloride. Group E received 0.25mg/kg BW of Citicoline and Group F received 0.5mg/kg BW of Citicoline. Citicoline and Nickel-chloride were dissolved separately in distilled water daily and were administered orally and intraperitoneally, for a period of 28 days. This study was approved by the Research Ethical Committee of the College of Medical Sciences, University of Benin with approval number CMS/REC/2024/622..

Table 3.1 Experimental design of the research

Groups	**Dosage**
GRUOP A (control)	Distilled water only
GROUP B	5mg/kg body weight of Nickel chloride
GROUP C	0.25mg/kg body weight of Citicoline+ 5mg/kg body weight of Nickel chloride
GROUP D	0.5mg/kg body weight of Citicoline+ 5mg/kg body weight of Nickel chloride
GROUP E	0.25mg/kg body weight of Citicoline
GROUP F	0.5mg/kg body weight of Citicoline

3.7 NEUROBEHAVIORAL TESTS

To evaluate the effects of treatments on neurobehavioural functions, a range of neurobehavioural assessment tests were conducted. These assessments involved several evaluations including Y-maze tests.

3.7.1 Y-Maze Test

The Y-maze test (YMT) is used to measure the spontaneous alternations performance, which allowed the evaluation of cognitive searching behavior, as an index for the cognitive dysfunction (Monte *et al.,* 2013). The animals were gently placed individually in the Y-maze apparatus, which consists of three identical arms (33×11×12cm each) in which the arms are symmetrically

separated at 120° (Dall"igna *et al.,* 2007). Specifically, each mouse was placed at the end of arm A, and allowed to explore all the three arms (labelled A, B, and C) freely for 5 mins, and the following parameters were taken: number of arm visits and sequence (alternations) of arm visits visually. An alternation is defined as entries in all three arms on consecutive occasions (Monte *et al.,* 2013). The percentage of alternation was calculater as total of alternations (total arm entries-2), as previously described (Dall"igna *et al.,* 2007). After each rat session, the observation chamber was cleaned with 10% ethanol to remove residual odor.

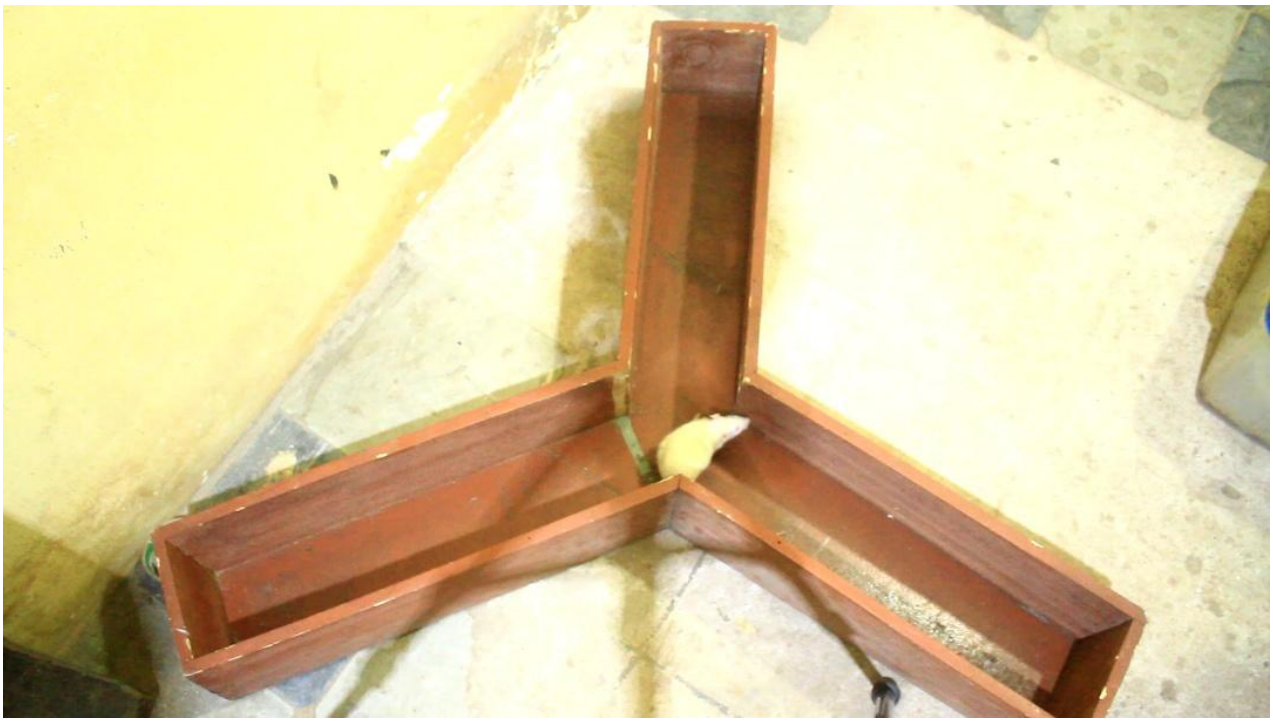


Figure 3.1 Y-maze test apparatus

3.8 BRAIN OXIDATIVE STRESS PARAMETERS

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 then washed twice in cold phosphate buffered

saline (PBS), homogenized using acid-washed sand and PBS in porcelain mortar and pestle. The homogenate would be centrifuged at 10,000 g for 15 minutes at 4°C. The supernatant will be collected for the estimation of the various biochemical assays.

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 accumulation of noxious H2O2 which is converted to O2 and H2O.

Preparation of reagent

0.01M KMnO4 was prepared by dissolving 0.158g of KMnO4 in 100ml of distilled water

Phosphate buffer (pH 7.4); 0.426 of NaHPO4 NaH2PO4 was weighed and dissolved in 100ml of distilled water

6M H2SO4: 32.3ml of conc. H2SO4 was added to 66.7ml of distilled water.

30Mm H2O2 solution: this was prepared by measuring 0.34ml of 30% of H2O2 in 1001 ml of phosphate buffer.

Procedure

To a known volume of plasma, (0.5ml), 5.0ml of H2O2 was added. This was mixed by inversion and allowed to stand for 30 minutes. Reaction was stopped by adding 6M H2SO2

The absorbance was taken at 480 nm within 30-60 seconds against distilled water.

Calculation

Activity =

OD= absorbance

L= light path =1cm

Vt =total volume of reaction sample

M= molar extinction co-efficient of H2O2 (40/M/cm)

3.8.2 ESTIMATION OF GLUTATHIONE PEROXIDASE (GPx) ACTIVITY

This was determined by the method of Rotruck *et al*. (1973)

Principle

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

Preparation of reagent

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

Procedure

To an aliquot of plasma (0.2 ml), 2.5 ml of phosphate buffer, 2.5ml of H_2O_2, 1.5ml of distilled water and 2.5 ml 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

$$\text{activity} = \frac{OD/Min \times VtDf}{E \times Vs \times Y}$$

OD = Absorbance of test

Vt = Total volume of reaction of reaction mixture

Df = Dilution factor = 1

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

Vs= volume of sample

Y = mg of protein used

3.8.3 ESTIMATION OF SUPEROXIDE DISMUTASE (SOD)

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

Principle

Adrenaline undergo 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 inhibit 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 was prepared by dissolving 0.2014 g of Na2CO3. 0.2604 g of NaHCO3 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.

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 reference sample. These were mixed and absorbance read at 420 nm.

% inhibition =

O.D test

Enzyme activity will thus be calculated

SOD activity (Unit/ mg protein) = % inhibition

50 x Y

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

3.8.4 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 15 g of trichloroacetic acid, 0.375 g of thiobarbituric acid and 0.25 N hydrochloric acid. This solution was mildly heated to assist in the dissolution of the thiobarbituric acid.

Procedure

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

The concentration MDA was determined using the formula

MDA (unit/mg protein) = (A×Vt× 1000)

$$(M \times V \times l \times Y)$$

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

3.9 HISTOLOGY OF THE CEREBRUM

The rats were be sacrificed through cervical dislocation. The skulls was opened and the brain of the rats was 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 cerebrum of the rats were removed from the skull,, 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 will be calculated as follows:

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

For Histopathology the extracted hippocampus was preserved in 10 % formalin. The tissues were processed via paraffin wax embedded method of Drury., *et al* (1980).

Hematoxylin and Eosin Staining Procedures

Fixed tissues were put through the following slides to obtain micro thin slides for photomicrography. They were hydrated using a series of descending grades of alhol until water was used. Procedure of hematoxylin and eosin adopted sections was 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 was removed by rinsing well in running tap water for two to three minutes (sections were observed at this stage microscopically 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.

•Counter stained in 1% aqueous eosin for two to four minutes;

•Excess stain was 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).

3.10 PHOTOMICROGRAPHY

The processed slides were captured with a binocular microscope on which was mounted an Omax 9.0MP USB Digital Miscroscope Camera (made in Korea). The camera features 9 megapixels (3488 × 2616 pixel) high resolution color digital camera and 0.5X reduction lens which would be connected to a laptop. A panoramic view of the slides were captured using ×4 and ×10 objective lenses. The images would be immerged and processed automatically with Adobe Photoshop CS6 (version 13.0, ×64) for final output.

3.11 MOLECULAR DOCKING

For Inflammations, Tumor necrotic factor-alpha (TNF-alpha), Nuclear factor-Kappa B (NF-Kb), Interleukin – 6 (IL-6) (PDB ID: 6rmj, 8yhw, and 1alu respectively), structures were obtained from the Protein Data Bank. The 3D structure of the ligands was provided in SDF format and was downloaded from PubChem database. These SDF files were then converted to PDB files using Pymol software (DeLano, 2002). Preparation of the protein and ligands before molecular docking was performed using Auto Dock tools and this involved, the removal of co-crystallized ligands, the addition of charges, polar hydrogen, and grid set-up. Active site studies of target proteins were carried out using the Molecular Operating Environment (MOE, 2015). Molecular docking of the ligands against target protein (TNF-alpha, NF-kB, Tyrosine and IL-6) was performed using Auto Dock Vina Software (Trott and Olson, 2010), and the binding affinities/energy reported in Kcal/mol. The renderings for the 2D diagrams and 3D (surface) view of the interactions were computed using Discovery Studio software (Bovia, 2019) and

Pymol software (DeLano, 2002) respectively. The Physicochemical, Blood Brain Barrier (BBB), lipophilicity, solubility, pharmacokinetics, and Lipinski drug-likeness of studied compounds were determined using the SwissADME Server (Daina et al., 2017).

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

RESULTS

4.1 EFFECT OF TREATMENT ON BODY AND BRAIN WEIGHT

Figure 4.1 shows the initial body weight of rats treated with or without Nickel chlorides and the final body weight of rats treated with or without nickel chloride after 28 days. Figure 4.2 shows the general brain weight of rats treated with or without nickel chloride and the cerebellar weight of rats treated with or without nickel chloride. Values are given as mean ± SEM of each group. #$p<0.05$ compared with $NiCl_2$ group.

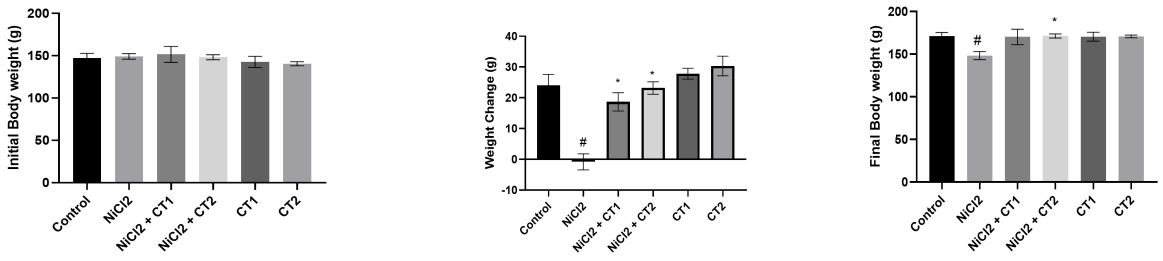


Figure 4.1: General body weight change of control and treatment groups after 28 days. Values are given as mean ± SEM of each group. #p<0.05 compared with $NiCl_2$ group. $^{\#}p$<0.05 compared with the control group, *p< 0.05 compared with the $NiCl_2$ group.

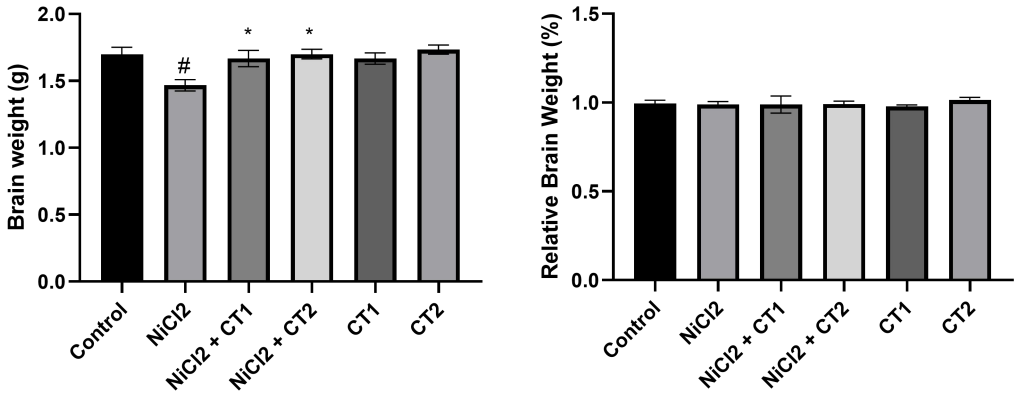
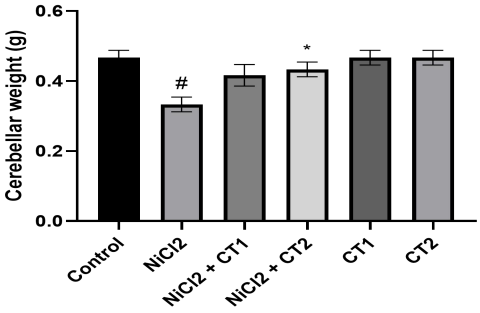


Figure 4.2: General brain and relative brain weight of control and treatment groups after 28 days. Values are given as mean ± SEM of each group. $^{\#}p<0.05$ compared with $NiCl_2$ group. #$p<0.05$ compared with the control group, *$p< 0.05$ compared with the $NiCl_2$ group.



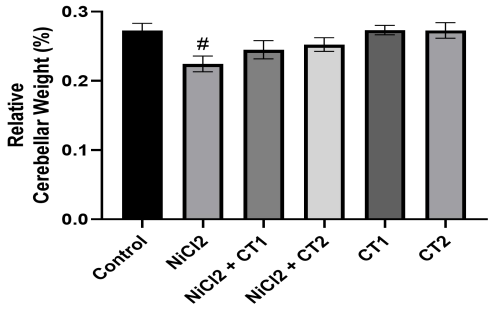


Figure 4.3: General cerebellar weight and relative cerebellar weight change of control and treatment groups after 28 days. Values are given as mean ± SEM of each group. #p<0.05 compared with NiCl2 group. #p<0.05 compared with the control group, *p< 0.05 compared with the NiCl2 group.

4.2 EFFECT OF TREATMENT ON NEUROBEHAVIOURAL ACTIVITY: Y-MAZE

Figure 4.3 shows the spontaneous alteration (%) during y-maze test in experimental groups A to F. Here a significant decrease ($p<0.05$) was observed in the nickel chloride only group when compared to the control group. A significant increase ($p<0.05$) was observed in groups C and D when compared to the nickel chloride only treated group.

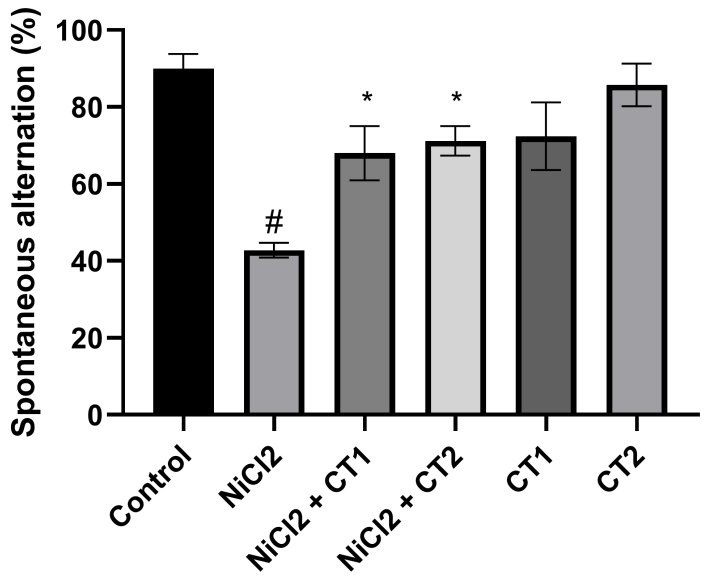
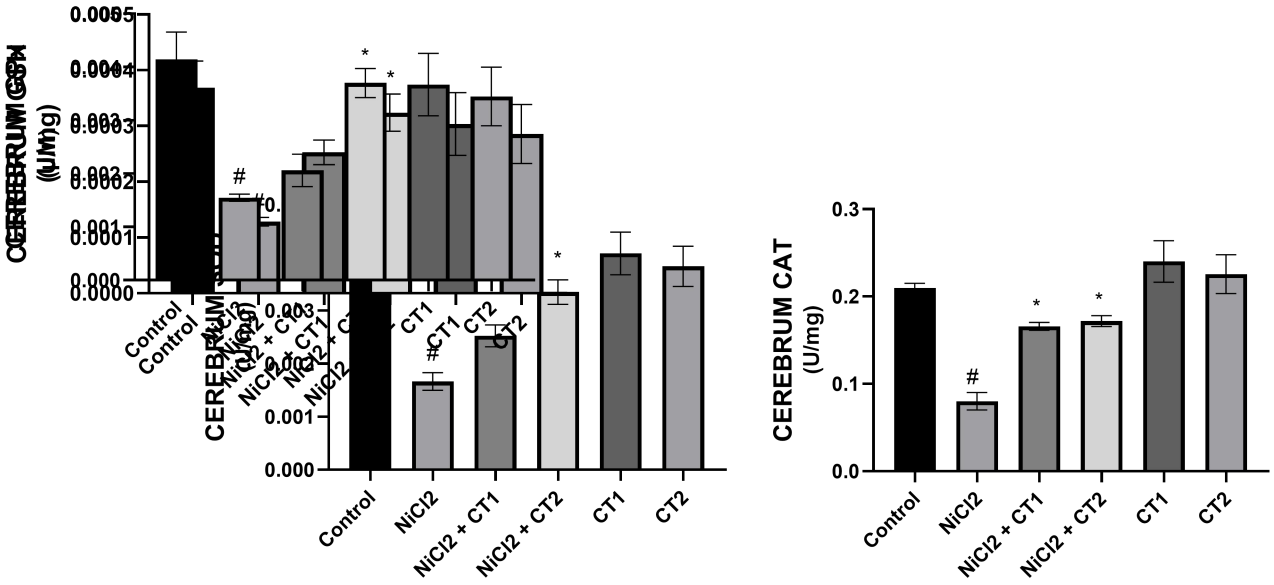


Figure 4.4: Spontaneous alternation of control and treatment groups after 28 days. Values are given as mean ± SEM of each group. $^{\#}$ $p<0.05$ compared with the control group; * $p<0.05$ compared with $NiCl_2$ group

4.3 EFFECT OF TREATMENT ON ANTIOXIDANT ACTIVITES

Figure 4.4 illustrates the activity of antioxidant enzymes in experimental groups A to F. Here, a significant decrease ($p<0.05$) in SOD, CAT, GPx and GSH was observed in group B when compared to the control group while a significant increase ($p<0.05$) was observed in groups C and D when compared to the nickel chloride only group.



4.5: Activity of SOD and CAT in the cerebrum 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 $NiCl_2$ group.

Figure 4.6: Activity of GPx and GSH in the cerebrum and treatment groups after 28 days. Values are given as mean ± SEM of each group. #p<0.05 compared with NiCl2 group. #p<0.05 compared with the control group, *p< 0.05 compared with the NiCl2 group.

4.4 EFFECT OF TREATMENT ON LIPID PEROXIDATION

Figure 4.5 illustrates the activity of antioxidant enzymes in experimental groups A to F. Here, a significant increase (p<0.05) in MDA was observed in group B when compared to the control group, while a significant decrease (p<0.05) was observed in groups C and D when compared to the nickel chloride only group.

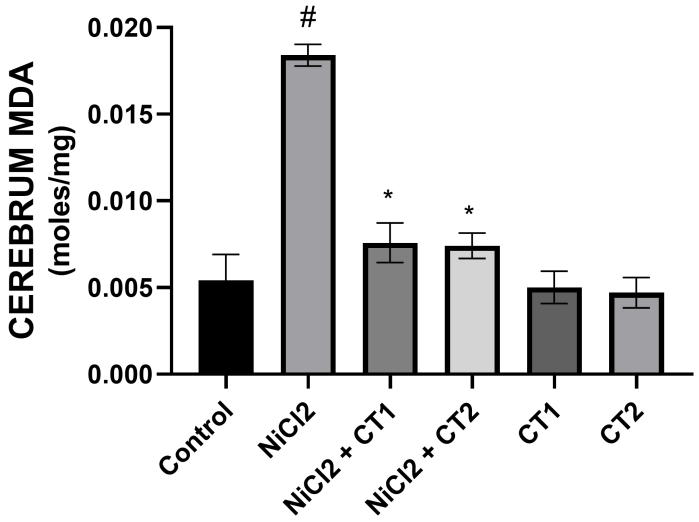


Figure 4.7: Lipid peroxidation concentration in the cerebrum of control and treatment groups after 28 days. Values are given as mean ± SEM of each group. $^{\#}$ $p<0.05$ compared with the control group; * $p<0.05$ compared with $NiCl_2$ group.

4.5 EFFECT OF TREATMENT ON THE HISTOLOGY

The plates show the histology of the cerebral cortex. Plate 4.1 shows the normal granular cells (G) in the control group. Plate 4.2 shows cytoplasmic vacuolization (V) in neuronal cell bodies observed (arrows). Plate 4.3, 4.4, 4.5 and 4.6 shows relatively normal features of the internal granular layer observed.

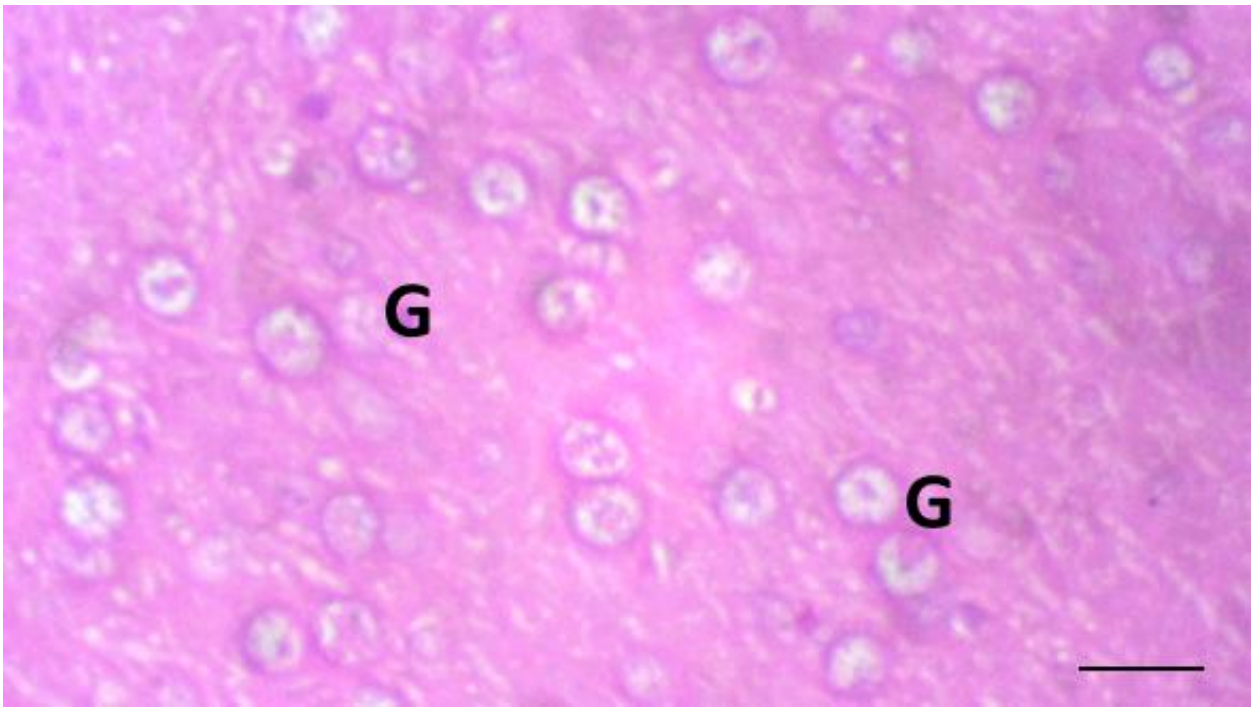


Plate 4.1 Representative histology of the cerebral cortex in control group revealing normal granular cells (G). (H&E; Scale bar: 25 μm)

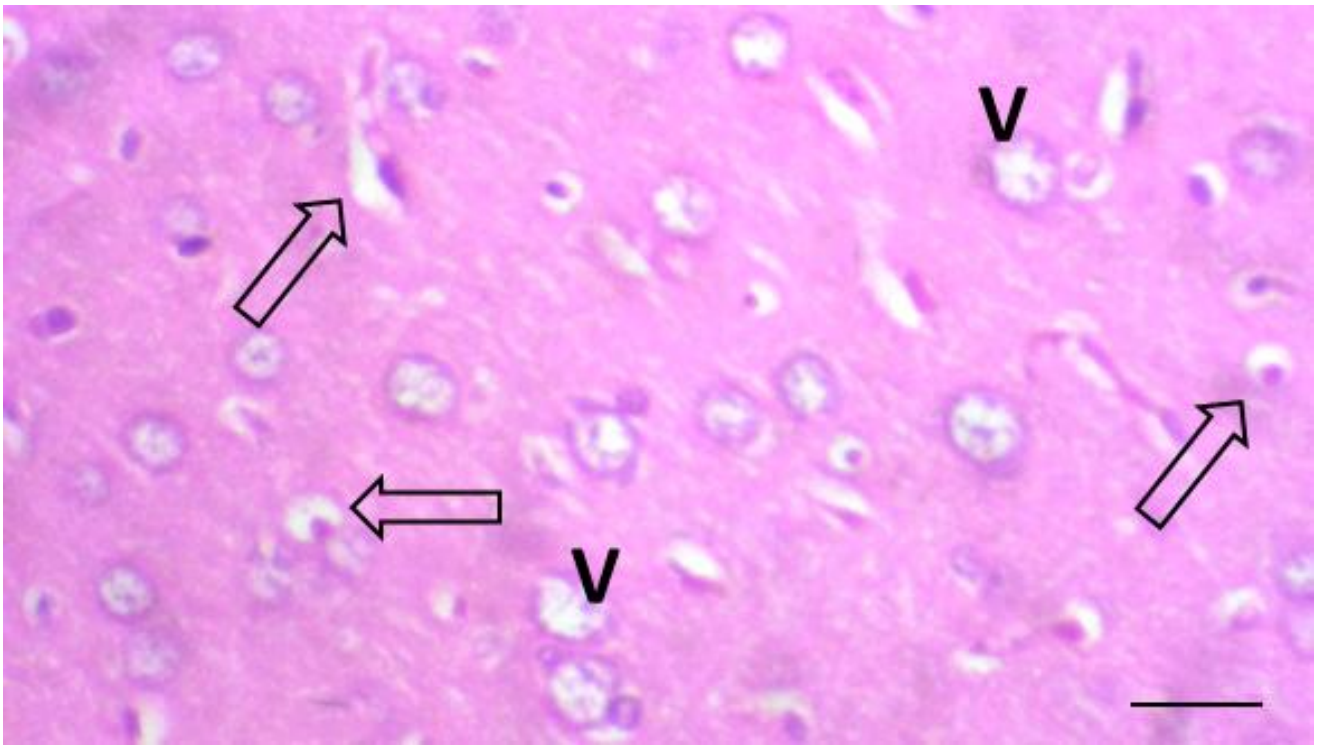


Plate 4.2 Representative histology of the cerebral cortex in group B. Showing cytoplasmic vacoulization (V) in neuronal cell bodies observed (arrows). (H&E; Scale bar: 25 μm)

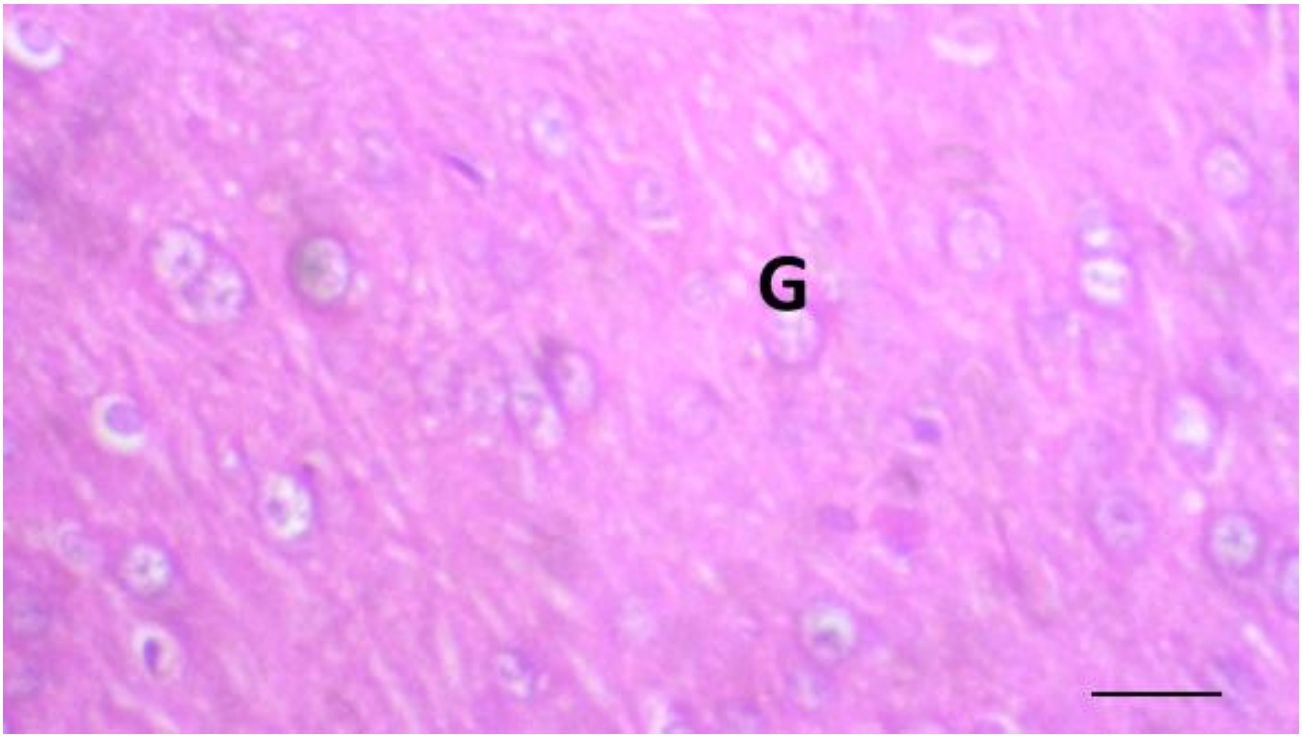


Plate 4.3 Representative histology of the cerebral cortex in group C. Showing relatively normal features of the internal granular layer observed. (H&E; Scale bar: 25 μm)

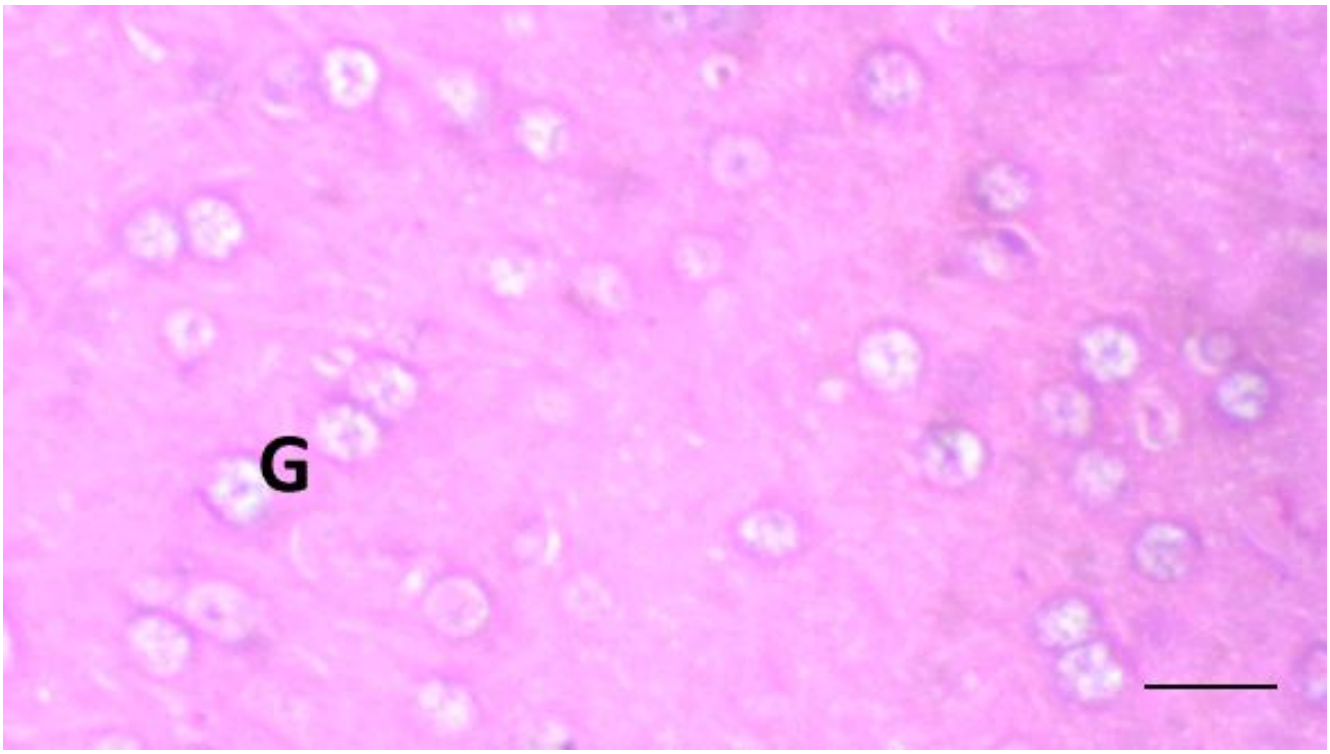


Plate 4.4 Representative histology of the cerebral cortex in group D. Showing relatively normal features of the internal granular layer observed. (H&E; Scale bar: 25 μm)

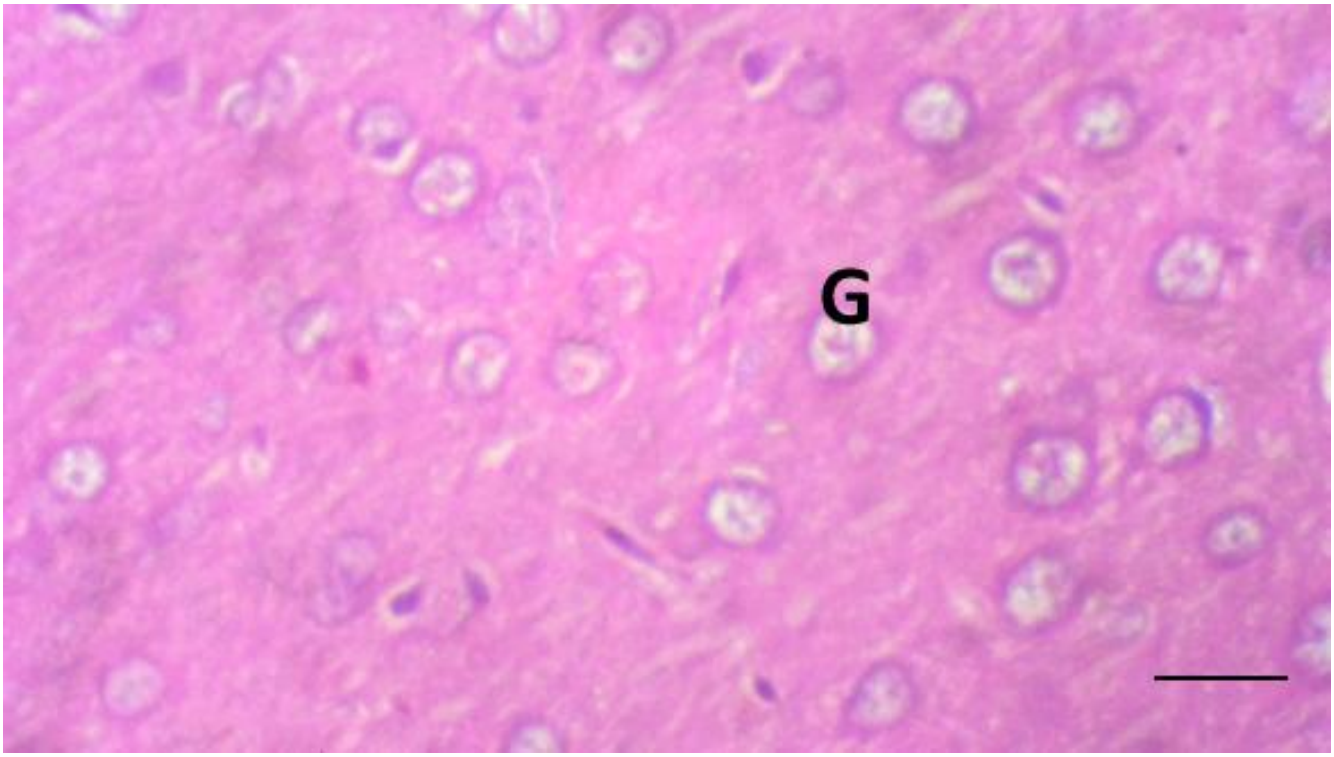


Plate 4.5 Representative histology of the cerebral cortex in group E. Showing relatively normal features of the internal granular layer observed. (H&E; Scale bar: 25 μm)

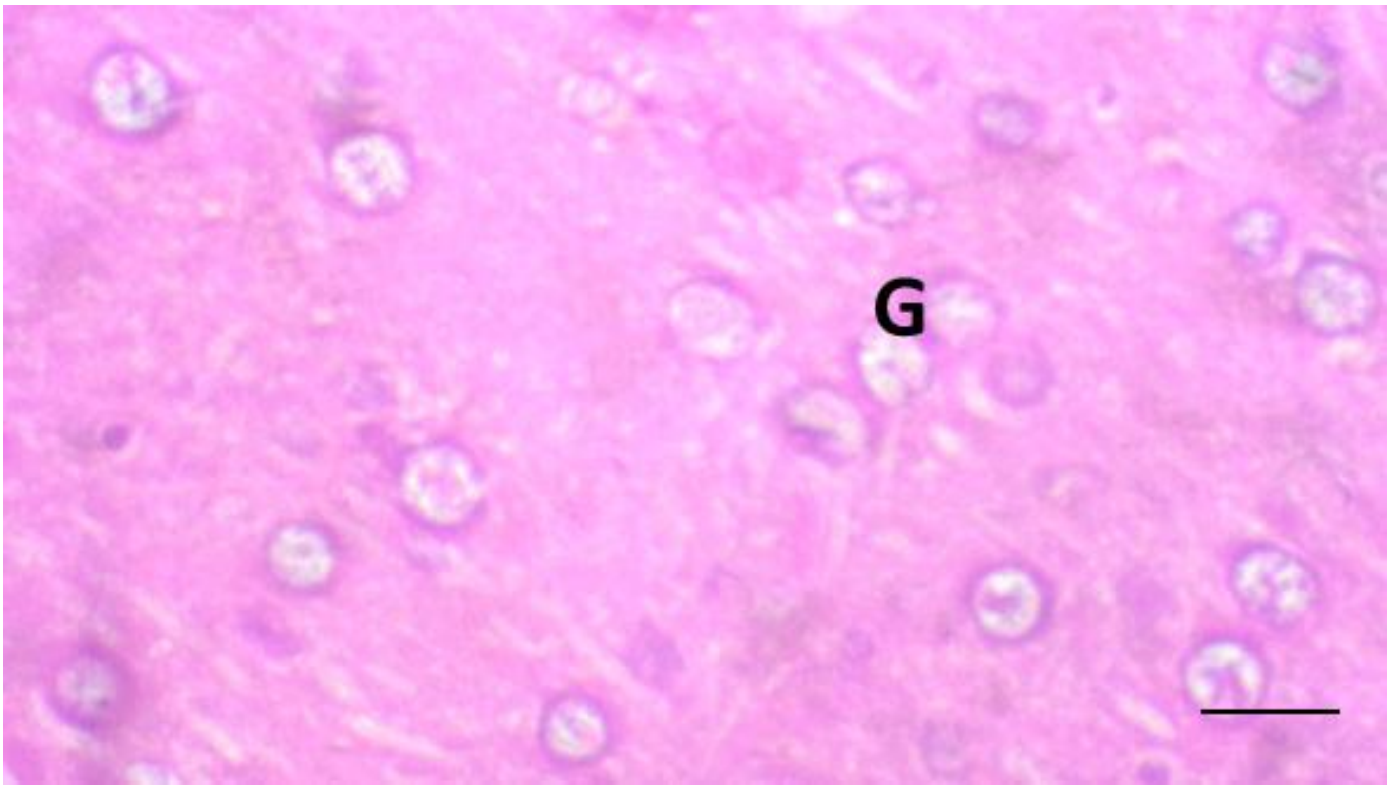


Plate 4.6 Representative histology of the cerebral cortex in group F. Showing relatively normal features of the internal granular layer observed. (H&E; Scale bar: 25 μm)

4.2 MOLECULAR DOCKING

Table 4.1 shows the binding energies of citicoline against NF-kB for inflammatory activities. Figure 4.6 shows the 2D active site view of compounds against NF-kB. Here, citicoline and donepezil were both bounded to NF-kB. The results shows that citicoline has a higher binding energy compared to donepezil.

INFLAMMATORY [Nuclear factor-kappa B] NF-kB

Binding energies of compounds against NF-kB for Inflammatory

Table 4.1 Binding energies of compounds against NF-Kb for inflammatory

Compounds	NF-Kb (Kcal/mol)
Citicoline	-7.1
STANDARDS	
Donepezil	-6.6

Citicoline

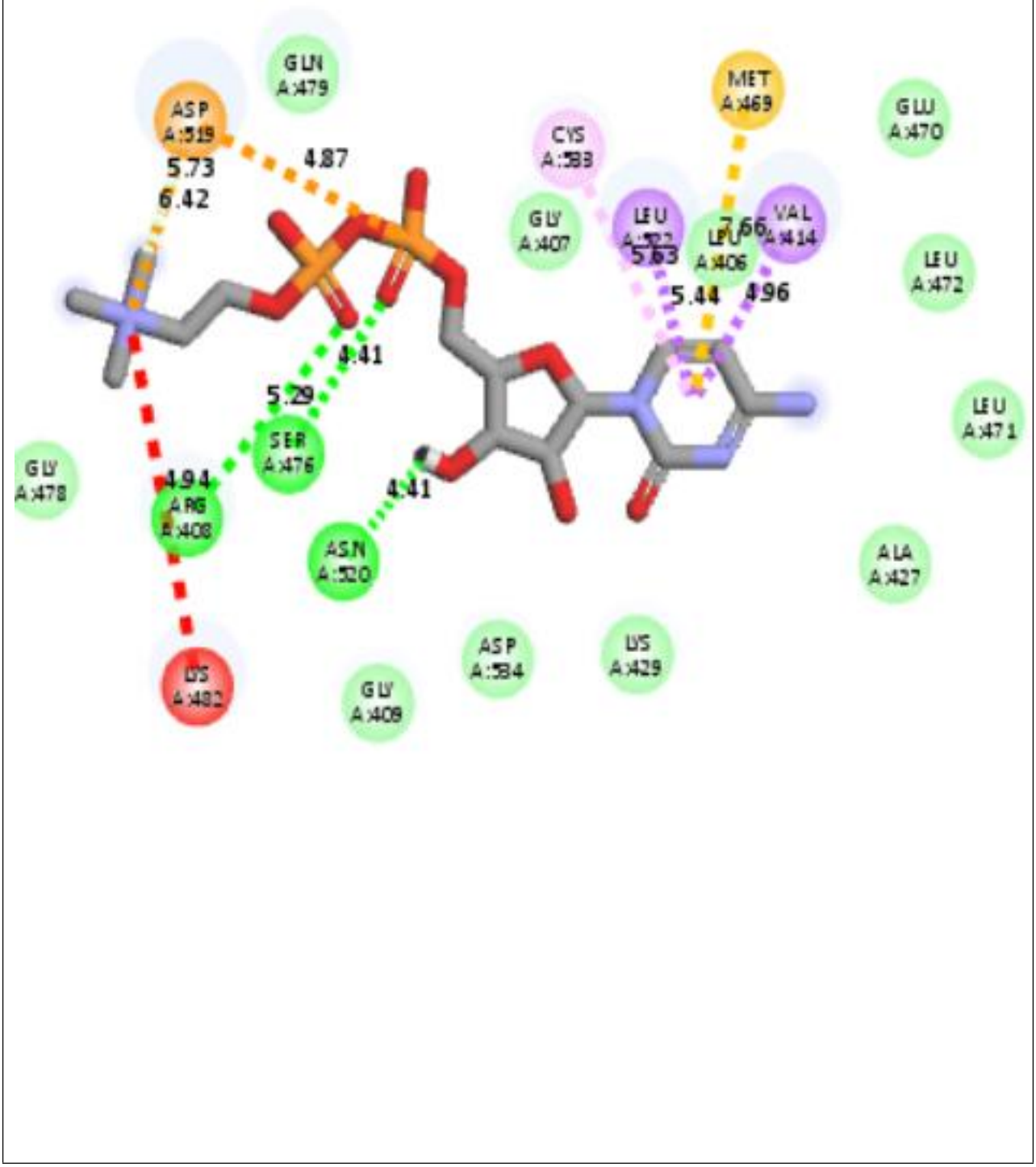


Figure 4.8 2D active site view of compounds against NF-kB

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 DISCUSSION

Nickel chloride toxicity in humans has increased overtime due to its abundance and has become a public health concern. However, studies have shown that this effect can be ameliorated by antioxidants (Salah *et al.,* 2021; Das *et al.,* 2020; Mouloud *et al.,* 2020). In this study, the protective activity of Citicoline on nickel chloride induced cerebral toxicity was investigated.

The findings show a decrease in the body weight of Nickel chloride only treated groups in contrast to the control group. This finding is consistent with other studies which reported that nickel chloride exposure in rats reduces body weight (El-Sharaky *et al.,* 2007; Das *et al.,* 2008). Similarly, Sivaprasad *et al.* (2004) found that adminstration of nickel chloride led to weight loss in rats, possibly due to oxidative stress and alternations in nutrient metabolism. The results of this study also showed a significant decrease in brain weight of rats treated with nickel chloride and is consistent with previous studies (Ijomone *et al.,* 2018). A study showed significant decrease in mice pup brain and body weight alongside brain anomalities after nickel chloride exposure (Mahmood *et al.,* 2013). However, Citicoline is observed to have attenuated these effects in rats, thus demonstrating it's protective activity against nickel-induced body and brain weight loss.

The cerebrum is involved in memory and learning and is vulnerable to neurotoxicity. The Y-maze test measures cognitive impairment (learning and memory) by using the animals spontaneous alternations as a proxy for cognitive performance and is a spatial working memory test (Ben-Azu *et al.,* 2016). The results of this study showed a significant decrease in the number

of total arm entries, number of alternations and spontaneous alteration in the Nickel chloride treated rats when compared to control, indicating signs of cognitive dysfunction. This is in line with previous studies that showed imparied cognitive and motor behavior following nickel chloride exposure (El Brouzi *et al.,* 2021; Lamtai *et al.,* 2018). Conversely, a significant increase in spontaneous alternations was observed in rats pretreated with Citicoline when compared to the nickel chloride only treated rats. This suggested that Citicoline has the ability to improve cognition and repair the cognitive impairment caused by nickel chloride. This corresponds to a study by Hassan *et al.,* (2016) which showed that by modulating oxidative stress in mice, repeated treatment of Citicoline attenuates behavioural changes caused by sodium arsenite.

The cerebrum, which is crucial for emotion regulation, learning and memory processes, is highly susceptible to oxidative stress because of it's high polyunsaturated lipid content, inadequate antioxidant defense and massive oxygen consumption. Exposure to nickel chloride increases the production of reactive oxygen species (ROS), which leads to oxidative damage by disrupting the lipid bilayer, damaging nucleic acids and denaturing proteins. Free radicals are scavenged by antioxidants from cells within the body, preventing or reducing the damage brought on by oxidation. Large concentrations of free radicals, such as hydroxyl radical (OH) and superoxide anions (O^2), can directly modify cellular lipids, which may result in neuronal damage and numerous degenerative disorders in the cerebral cortex. SOD and CAT enzymes shield cells from oxidative damage and stop lipid peroxidation by eliminating free radicals (Gupta *et al.,* 2009). Results from this study showed a significant decrease in SOD, CAT GPx and GSH in the cerebral cortex of rats treated with nickel chloride only when compared to the control group. This indicates that nickel chloride disrupts the antioxidant system. This is consistent with previous studies (Ahmed *et al.,* 2011; Mourelle *et al.,* 2004, Lamtai *et al.,* 2018). However,

citicoline was observed to have protected against the disruptiion of these enzymes by nickel chloride, thus demonstrating it's protective activity against nickel-induced alterations in the antioxidant activities of experimental rats. Malondialdehyde (MDA) is a persistent byproduct of lipid peroxidation which can be utilized as an estimate for lipid peroxidation levels. In this study, MDA was significantly increased in rats treated with nickel chloride when compared to the control group. This is consistent with previous studies (Gal *et al.,* 2017; Sahin *et al.,* 2009; El-Sayyad *et al.,* 2011). Citicoline pretreatment of Nickel chloride exposed rats was observed to have decreased MDA concentration in the cerebral cortex, thus indicating the lipid peroxidation-attenuating activity of Citicoline.

The histological analysis and the biochemical changes in this investigation indicated a strong correlation. The granular cells of the cerebral cortex serves as a sensory processor that processes sensory information from the thalamus (Jones, 2007) and information transmitter (Douglas & Martin, 2004). They are crucial for plasticity and learning (Abbott & Nelson, 2000) and regulation of cortical activity (Traub *et al.,* 2005). The histology of the cerebral cortex showed cytoplasmic vacuolization in neuronal cell bodies in nickel chloride treated rats when compared to the control. This indicated neuronal loss in the cerebral cortex which can be linked to memory loss, cognitive dysfunction and learning impairment. This results aligns with previous studies showing neurodegeneration and impairment in memory, learning and cognition following nickel chloride exposure (Omamuyovwi *et al.,* 2018; Mouloud *et al.,* 2020; Ijomone, 2021). Citicoline pretreated rats, however showed relatively normal features of the internal granular layer of the cerebral cortex, thus indicating that Citicoline was able to mitigate the toxic effect of the nickel chloride in the cerebrum of the experimental rats.

Molecular docking is a computational method used to predict the binding of a small molecule to a protein. In this study, molecular docking was used to compare the binding of Citicoline and donepezil to the NF-kB enzyme, which is a key target for the treatment of Alzheimer's disease. NF-kB (Nuclear Factor kappa-light-chain-enhancer of activated B cells) is a protein complex that controls DNA transcription and plays a crucial role in regulating the immune response, inflammation and cell survival. Citicoline may inhibit the activation of NF-kB, which can lead to a decrease in the expression of pro-inflammatory genes and a reduction in inflammation (Secades, & Frontera (1995). By binding to NF-kB, citicoline may exert anti-inflammatory effects by reducing the production of pro-inflammatory cytokines and mediators (Savci, et al., 2003). NF-kB plays a role in the regulation of neuronal survival and death. Binding citicoline to NF-kB may provide neuroprotection by inhibiting the expression of pro-apoptotic genes and promoting the expression of anti-apoptotic genes (Wang et al., 2013). Citicoline may modulate the immune response by binding to NF-kB and regulating the expression of immune-related genes (Grieb, et al., 2015). NF-kB is involved in the regulation of antioxidant defenses. Binding citicoline to NF-kB may enhance antioxidant defenses and reduce oxidative stress (Hurtado et al., 2007). NF-kB is often constitutively activated in cancer cells, promoting their survival and proliferation. Binding citicoline to NF-kB may inhibit cancer cell growth and induce apoptosis (Zhang, et al., 2017). Overall, the binding of citicoline to NF-kB can have therapeutic implications for various diseases, including neurodegenerative disorders, cancer, and inflammatory diseases. The results showed that Citicoline had a higher binding energy compared to donepezil, a drug used for treatment of Alzheimer's disease. This is consistent with previous studies which showed that both citicoline and donepezil form hydrogen bonds with the p65 subunit of NF-kB, which is a key component of the NF-kB complex (Lee *et al.,* 2018).

5.2 CONCLUSION

The findings from this suggests that Citicoline attenuated nickel chloride-induced weight alterations, cognitive and memory deficits, oxidative stress and histological changes in experimental rats.

5.3 RECOMMENDATIONS

Following the findings of this study, it is recommended that other mechanisms underlying citicoline's protective benefits against nickel chloride-induced toxcity, as well as the possible synergistic effects of its combination with other antioxidants or neuroprotective agents, be investigated further

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