# INVESTIGATING THE PROTECTIVE ACTIVITY OF VINPOCETINE IN THE CEREBRUM OF WISTAR RATS EXPOSED TO NICKEL CHLORIDE

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**SUPERVISED BY:**

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## CERTIFICATION

This is to certify that this research was carried out by **NMON OLISE EMINENCE (BMS2001034)** in the Department of Anatomy, School of Basic Medical Science, University of Benin, Benin City, Nigeria. In partial fulfillment of the requirement for the award of Bachelor of Science Degree (B.Sc.) in Anatomy.

_____ _____

DR IDEMUDIA USMAN OSAGIE DATE

(SUPERVISOR)

_____ _____

ENOGIERU ADAZE BIJOU (PhD) DATE

(HEAD OF DEPARTMENT)

_____ _____

EXTERNAL SUPERVISOR DATE

## DEDICATION

This work is dedicated to God Almighty for grace and guidance throughout the process of this study.

## ACKNOWLEDGEMENTS

All praise be to the Almighty God for enabling me achieve this feat. I want to appreciate my parent, Mr. and Mrs. Nmon, for their unending support throughout this journey.

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## ABSTRACT

Exposure to neurotoxic substances like nickel chloride poses significant risks to neurological health. Vinpocetine, a synthetic derivative of the alkaloid vincamine acts as a phosphodiesterase-1 inhibitor, modulating intracellular signaling and enhancing cerebral metabolism. It improves cerebral blood flow and supports synaptic plasticity, by increasing cyclic GMP and reducing neuroinflammation and neurodegenerative damage by reducing oxidative stress. Accordingly, this study was aimed at investigating the possible neuroprotective activity of vinpocetine on

Nickel Chloride-induced neurotoxicity in adult Wistar rats. In this study, Fourty-two (42) Wistar rats was randomly divided into six (6) groups (n=7). Group A rats served as the control group to be given 1ml of distilled water. Group B rats was administered Nickel Chloride only at a dose of 5mg/kg body weight. Group C rats was administered 2.5mg/kg body weight of Vinpocetine and 5mg/kg body weight of Nickel Chloride. Group D rats was administered 5mg/Kg body weight of Vinpocetine and 5mg/kg body weight of Nickel Chloride. Group E rats was administered with 2.5mg/Kg body weight of Vinpocetine only and group F rats was administered with 5mg/Kg body weight of vinpocetine. Neurobehavioral activities were assessed 24 hours before the last administration, and at the end of the experimental period, the rats were weighed and sacrificed, and the body and brain weight changes, antioxidant enzymes activity, as well as histological assessment of the cerebrum. Results indicate that nickel chloride administration led to significant decrease on body weight, brain weight, neurobehavioral activity, and antioxidant enzymes levels, while increasing lipid peroxidation. However, pretreated rats with Vinpocetine mitigated these detrimental effects induced by nickel chloride. Vinpocetine supplementation was associated with improved body and brain weight, enhanced neurobehavioral performance, elevated antioxidant enzyme levels. This study sheds light on vinpocetine's potential as a neuroprotective agent against nickel chloride-induced neurotoxicity, suggesting its importance in preventing or mitigating neurological disorder caused by nickel chloride.

# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 Background of the study

Neurotoxicity refers to the adverse effects on the structure or function of the central and peripheral nervous systems caused by exposure to natural or synthetic toxic substances (Giordano and Costa, 2012; Teleanu *et al.,* 2019). These effects can lead to the disruption of normal neuronal activity, resulting in cognitive, sensory, motor, and behavioural deficits (Legradi *et al*., 2018; Teleanu *et al.,* 2019). Neurotoxic agents can induce neuronal cell death through various mechanisms, 0including oxidative stress, inflammation, apoptosis, and disruption of neuronal signalling (Teleanu *et al.,* 2019; Behl *et al.,* 2021; Karvandi *et al.,* 2023). Also neurotoxicity" is the capacity of chemical, biologic, or physical agents to cause adverse functional or structural change in the nervous system. We use the term "environmental neurotoxicity" to refer broadly to adverse neural responses to exposures to all external, extragenetic factors (e.g., occupational exposures, lifestyle factors, and exposures to pharmaceuticals, foods, and radiation); it does not refer merely to the toxic effects of chemicals that are present in the environment as contaminants of air, water, and soil. Possible effects of chemical toxicants on the nervous system are varied. Neurotoxicity can occur at any time in the life cycle, from gestation through senescence, and its manifestations can change with age. The developing nervous system appears to be particularly vulnerable to some kinds of damage (Cushner, 1981; Blair *et al.,* 1984; Pearson and Dietrich, 1985; Annau and Eccles, 1986; Hill and Tennyson, 1986; Silbergeld, 1986), but the results of some early injuries may become evident only as the nervous system matures and ages (Rodier *et al.,* 1975). Common sources of neurotoxins include pesticides, industrial chemicals, some pharmaceuticals and heavy metals like

Nickel. The environment has been implicated to be a strong determinant of brain health with higher risk of neurodegeneration. The drastic rise in the prevalence of neurodegenerative diseases (NDDs) including Alzheimer's disease (AD), Parkinson's disease (PD), autism spectrum disorder (ASD), multiple sclerosis (MS) etc., supports the idea that environmental factors may 2play a major role in NDDs aetiology. Nickel is one of the listed environmental metals reported to pose a serious threat to human health. This project reported available studies on nickel level in NDDs covering both animal and human studies. Different databases were searched for articles reporting the main neurotoxicity mechanisms and the concentration of nickel in fluids and tissues of NDDs patients compared to controls. Data were extracted and synthesized by ensuring the articles were related to nickel and NDDs. Various mechanisms were reported as oxidative stress, disturbances in mitochondrial membrane potential, trace elements homeostasis destabilization, etc. Nickel was found elevated in biological fluids as blood, serum/plasma and CSF and in the brain of NDDs, as a consequence of unintentional exposure thorough nickel-contaminated air, food, water, and skin contact. In addition, after exposure to nickel, the concentration of markers of lipid peroxidation were increased, while some antioxidant defence systems decreased. Thus, the reduction in the exposure to nickel contaminant may hold a promise in reducing the incidence of NDDs.

Nickel is a prominent neurotoxic agent that has garnered attention due to its widespread use and significant health impacts. Heavy metals seem to be multi-organ toxicants involving central nervous system (CNS), peripheral nervous system (PNS), hematopoietic, respiratory, endocrine, reproductive, renal and cardiovascular systems (Luo *et al*., 2020). Moreover, they are not biodegradable and persist in the environment with an established possibility of entry into the human body by inhalation, dermal and ingestion routes (Okoye *et al.,* 2021, Amadi *et al.,* 2021,

Orisakwe *et al*., 2012). Nickel is a hard, ductile, silvery-white transition metal and abundant natural element that is 28th element in the periodic table. It may exist in several oxidative states (from −1 to +4); nevertheless, the + 2-oxidation state (Ni2+) is the most widespread in the environment and biological systems (Muñoz and Costa, 2012). Nickel forms alloys with iron, copper, chromium, and zinc used to make coins, stainless steel, jewellery, etc. Nickel can also combine with other elements such as chlorine, sulphur, and oxygen to form nickel compounds which dissolve appreciably in water. Nickel is a major player in modern metallurgies where it is used in a broad variety of metallurgical processes, such as alloy production, electroplating, in the production of nickel-cadmium batteries and as a catalyst in chemical and food industry (ATSDR, 2005, Genchi *et al.,* 2020). Nickel nanoparticles (NPs) are among the nanomaterials mostly employed in many fields, such as catalysts, magnetic materials, biological medicines, conductive pastes, and additives to lubricant (Sharma *et al.,* 2018).

The brain is the most complex organ, and the hallmark of this complexity is the vast number of synapses which are also highly complex at the molecular level, with > 1000 genes encoding postsynaptic proteins in excitatory synapses (Kaizuka and Takumi, 2018). The brain is particularly vulnerable to assault during development hence the focus point is the knowledge of the possible toxicants that could impair normal brain development.

Neurodegenerative diseases (NDDs) refer to a heterogeneous group of neurological disorders that affect distinct subsets of neurons in specific anatomical locations, and a number of studies have examined the relationship between exposure to environmental harmful substances and this abnormal development (Michalska and León, 2020, Brown *et al.,* 2005). The symptoms of NDDs include deficit in behaviour, cognition, communication and sometimes motor skills. Notwithstanding there may be different types of NDDs, the most notable include Alzheimer's

disease (AD), Parkinson's disease (PD), Huntington's disease (HD), multiple sclerosis (MS), autism spectrum disorder (ASD), attention deficit disorder (ADD) along with less common diseases such as frontotemporal dementia (FTD) (Mullin *et al.,* 2013, Young *et al.,* 2018). Neurodegenerations involve diminution of neurons and their associated processes namely axons, dendrites, synapses with complementary progressive impairment in neuronal function (Jack *et al*., 2015), characterized by protein aggregation disorders (Hampel *et al.,* 2017). Usually, NDDs are mediated by impaired autophagy, protein aggregation, inflammation, oxidative damage, genetic and epigenetic traits, derangement in mitochondrial processes, apoptosis, diminished growth factor and loss of synaptic plasticity (Jellinger, 2010). The major contributing factors to the noxious effects of excessive exposure to metals are their persistence, non-biodegradable and bioaccumulation (Ullah *et al.,* 2020). The outcome and possible underlying mechanisms associated with exposure to metals and their mixtures is a topical area of research because systemic interactions of metals may cause either antagonistic or synergistic effect (Wu *et al*., 2016, Martin *et al*., 2021). The huge increase in neurodevelopmental disorders has stimulated interest in the evaluation of environmental risk factors in their aetiogenesis, and the exposure to neurotoxic metals (e.g., lead, mercury, cadmium, nickel and manganese) influenced some neurodevelopmental disorder such as autism (Ijomone *et al*., 2021; De Felice *et al*., 2015). High level of unsaturated fatty acids, high consumption of oxygen per unit weight and the relatively low level of antioxidant enzymes predisposes the brain to oxidative damage (Lewis *et al*., 2021). Metals upregulate amyloid precursor protein (APP), interleukin (IL)− 1β, IL-6, and tumor necrosis factor (TNF)-α, acetylcholinesterase (AChE) and monoamine oxidase-A (MAO) genes in NDDs (Lukiw *et al.*, 2021, Cao *et al*., 2021), and in particular, the nervous system is profoundly vulnerable to nickel assault (Das *et.al*. 2008, Wu *et al.,* 2016).

Nickel was implicated in cognitive and behavioural deficits in rats with elevated levels of exposure to the metal (Lamtai *et.al.,* 2020). Nickel can result in distinct neurological effects, with different brain targets and modes of action, and it can disrupt presynaptic neurotransmission. Nickel chloride (NiCl2) is a highly soluble salt, appearing as yellow to green crystals. It is used in various industrial applications, including electroplating, as a catalyst in chemical reactions, and the production of nickel-cadmium batteries (Binkowski, 2019). Nickel chloride is also used in the synthesis of other nickel compounds and laboratory research. Due to its solubility and widespread use, nickel chloride can easily enter the environment, leading to potential human exposure through inhalation, ingestion, and dermal contact (Gonnelli and Renella, 2013; Binkowski, 2019; Klein and Costa, 2022).The toxic effects of nickel chloride are well-documented, affecting multiple organ systems (Genchi *et al.,* 2020; Wu and Kong, 2020). Acute exposure can lead to gastrointestinal distress (Magrone *et al.,* 2020), respiratory problems (Prueitt *et al.,* 2020), and skin irritation (Klein and Costa, 2022). Chronic exposure is associated with more severe health issues, including carcinogenicity, immunotoxicity, and organ damage (Guo *et al*., 2020; Costa, 2023). However, its neurotoxic effects are particularly concerning due to the potential for long-term neurological impairment. Nickel chloride exposure has been shown to generate reactive oxygen species (ROS), leading to oxidative stress, which is a key factor in the development of neurodegenerative diseases (Rizvi *et al.,* 2020; Genchi *et al.,* 2020). The oxidative damage affects lipids, proteins, and DNA within neuronal cells, disrupting their normal function (Genchi *et al.,* 2020). Chronic exposure to nickel chloride triggers inflammatory responses in the brain, contributing to neuronal damage and loss (Salimi *et al.,* 2020; Magrone *et al*., 2020). Prolonged exposure results in neuronal apoptosis and degeneration, manifesting as cognitive and motor deficits (Salimi *et al.,* 2020). Additionally, nickel chloride can interact with

DNA, causing mutations and chromosomal aberrations (Costa, 2023; Yılmaz *et al.,* 2023), further compounding its neurotoxic effects.

The cerebrum is the largest and most important part of the brain responsible for functions such as cognition, memory, and learning. Following exposure, Ni mainly accumulates in the cerebral cortex (He *et al.,* 2013) where it induces neurotoxicity (Das *et al.,* 2008); thus resulting in several neurological symptoms such as headaches, tiredness, lethargy, and giddiness (Das *et al.,* 2008; Slotkin and Seidler, 2009a; Xu *et al.,* 2010). Studies have revealed that Nickel (Ni) exposure induced apoptosis of olfactory sensory and cerebral cortex neurons, behavioral deficiencies and disrupts neurotransmitters in rats (Jia *et al.,* 2010; Song *et al.,* 2017).

Vinpocetine, also known as cavinton or ethyl apovincaminate , a semi-synthetic derivative of the Vinca minor (common periwinkle) plant alkaloid, vincamine. (Vas and Gulyas. (2005), Zhou *et al.* (2003), Li *et al*. (2008)). Vinpocetine, is chemically known as ethyl apovincaminate, it has a molecular formula of $C_{22}H_{26}N_2O_2$ and also a molecular weight of 350.462 g/mol. Its complex structure includes multiple fused rings, prominently featuring a benzene ring and a piperidine ring, with it's core structure derived from the vincamine molecule, where specific modifications have been made to enhance its bioavailability and efficacy. A significant structural component of vinpocetine is the ethyl ester group attached to the apovincaminic acid moiety, This modification increases its lipophilicity, allowing better penetration of the blood-brain barrier. Vinpocetine contains several functional groups, including an ester linkage, which plays a crucial role in its pharmacokinetics and biological activity (Dong, ZC., *et al*. 2024). Vinpocetine has been initially developed for the treatment of neurological diseases associated with cerebrovascular disorders such as stroke and dementia that are often caused by ischemia or other cognitive deficits. A number of studies have reported the protective effects of vinpocetine after ischemic injury of the

brain in rodents (Jincai *et al.,* 2014, Rischke and Krieglstein, 1991, Sauer *et al.,* 1988) and humans (Bonoczk *et al.,* 2002, Szilagyi *et al.,* 2005, Szobor and Klein, 1976, Vas and Gulyas, 2005, Vas *et al.,* 2002, Zhang *et al*., 2016). In addition, vinpocetine appears to be also beneficial for degenerative neuronal disorders such as Parkinson's disease (PD) (Medina, 2011, Sharma and Deshmukh, 2015), Huntington's disease (HD) (Gupta and Sharma, 2014), and Alzheimer's disease (AD) (Heckman *et al*., 2015, Medina, 2011). In the brain, vinpocetine improves brain blood flow by acting as a cerebral vasodilator (Bonoczk *et al.,* 2000, Bonoczk *et al*., 2002, Patyar *et al.,* 2011, Szilagyi *et al.,* Vas *et al.,* r 2002, Zhang and Yang, 2015); and enhances cerebral metabolism by increasing oxygen and glucose uptake and stimulating neuronal ATP production (Bonoczk *et al.,* 2000, Bonoczk *et al.,* 2002, Patyar *et al.,* 2011, Szilagyi *et al.,* 2005, Zhang and Yang, 2015). In a number of neuronal cells or nerve terminals, vinpocetine has also been shown to function as an antioxidant (Deshmukh *et al.,* 2009, Herrera-Mundo and Sitges, 2013, Horvath *et al.,* 2002, Pereira *et al.,* 2000, Santos *et al.,* 2000, Solanki *et al.,* 2011), and prevent neurotoxic calcium and sodium elevation (Sitges *et al.,* 2005, Sitges and Nekrassov, 1999, Tretter and Adam-Vizi, 1998). It is thus evident that multiple mechanistic actions of vinpocetine through different molecular targets contribute to the neuroprotective effects of vinpocetine. In addition, vinpocetine also elicits protective effects in other ischemia-related conditions, such as retina (Nivison-Smith *et al.,* 2014, Nivison-Smith *et al.,* 2017, Nivison-Smith *et al.,* 2015), liver (Abdel Salam *et al.,* 2007, Zaki and Abdelsalam, 2013), kidney (Fattori *et al*., 2017), and skin (Xiao-Xiao *et al.,* 2013).

Wistar rat which refers to a laboratory rat strain that has longer and thicker whiskers than other strains. These rats are often used in neuroscience research because of their highly sensitive whiskers, which allow them to detect very subtle changes in their environment.

Given the protective effects of vinpocetine on, it is reasonable to explore it's effect on Nickel chloride induced neurotoxicity . In this study, we aim to investigate the potential protective effects of vinpocetine on against Nickel chloride induced neurotoxicity in the cerebrum of adult wistar rats.

### 1.2 Aim and objectives of the study

The aim of this study is to investigate the possible neuroprotective activity of vinpocetine on nickel chloride-induced neurotoxicity in adult Wistar rats.

**The specific objectives of the study are to investigate the activity of vinpocetine on**

- The brain and body weight changes in rats treated with or without nickel chloride
- The neuro-behavioural activity of rats treated with or without nickel chloride treated with or without nickel chloride
- The lipid peroxidation (malondialdehyde) activity in the brain of rats treated with or without nickel chloride .
- The histology of the cerebrum, cerebellum, and hippocampus of rats treated with or without Nickel Chloride.

## 1.3 JUSTIFICATION OF THE STUDY

Nickel chloride is widely used in various industrial processes, including electroplating, battery manufacturing, and as a catalyst in chemical reactions (Gonnelli and Renella, 2013; Binkowski, 2019). Consequently, occupational exposure and environmental contamination are prevalent, raising significant health concerns. Exposure to nickel chloride has been shown to generate reactive oxygen species (ROS), which cause oxidative damage to lipids, proteins, and DNA (Genchi *et al.,* 2020; Salimi *et al.,* 2020; Costa, 2023). This oxidative stress is a key factor in the development of neurodegenerative diseases (Olufunmilayo *et al.,* 2023). Chronic exposure to nickel chloride triggers inflammatory responses in the brain (Gorantla *et al.,* 2020), leading to neuronal damage and loss. Prolonged exposure results in neuronal apoptosis and degeneration, manifesting as cognitive and motor deficits (Lamtai *et al.,* 2020). Additionally, nickel chloride can interact with DNA, causing mutations and chromosomal aberrations, further exacerbating its neurotoxic effects (Goel *et al.,* 2023). Given these detrimental effects, addressing nickel chloride-induced neurotoxicity is imperative to prevent long-term neurological damage and associated health complications.

Current treatment options for nickel chloride-induced neurotoxicity are primarily supportive and symptomatic, lacking specificity in targeting the underlying pathophysiological mechanisms. This gap necessitates the exploration of novel therapeutic agents with robust neuroprotective and antioxidant properties. Vinpocetine, also known as cavinton or ethyl apovincaminate, a semi-synthetic derivative of the Vinca minor (common periwinkle) plant alkaloid, vincamine, is a promising candidate for combating nickel chloride-induced neurotoxicity due to its reported antioxidant, anti-inflammatory and neuroprotective properties, (Deshmukh *et al.,* 2009, Herrera-Mundo and Sitges, 2013, Horvath *et al.,* 2002, Pereira *et al*., 2000, Santos *et al.,* 2000, Solanki *et*

*al.,* 2011). Also, its safety (which has been proven even over long term) and accessibility, further makes it a practical candidate for therapeutic intervention.

### 1.4 Expected contribution to knowledge

The study on vinpocetine on nickel chloride induced neurotoxicity in the cerebrum of adult wistar rats is expected to shed lights on the neuroprotective effects of vinpocetine on the cerebrum of an adult wistar rat that has been laced with nickel chloride. This research project, is expected to make meaningful contributions to the growing body of knowledge in neuroscience, toxicology, and pharmacology. By examining how vinpocetine might protect the brain specifically the cereberum from damage caused by nickel chloride, the study aims to shed light on both the harmful effects of environmental toxins and the potential of therapeutic agents to counteract them.

**Understanding How Nickel Affects the Brain:** Nickel is a trace element that the body needs in small amounts, but when exposure becomes excessive—especially in industrial settings—it can lead to serious health issues. Studies have shown that nickel compounds like nickel chloride can trigger oxidative stress, disrupt mitochondrial function, and even cause neuronal death (Das *et al.,* 2008; Flora *et al.,* 2012). Nickel has also been implicated in altering neurotransmitter levels and impairing synaptic transmission, which may contribute to cognitive and motor deficits (Sunderman, 2001; Wang *et al.,* 2015). While some research has explored these effects in general brain regions, the cerebellum hasn't received much attention. This study will focus specifically on how nickel chloride affects cerebellar tissue, using both histological and biochemical methods to assess structural damage and changes in key markers of neurotoxicity. By doing so, it hopes to fill a gap in our understanding of how environmental toxins target different parts of the brain.

**Investigating Vinpocetine's Protective Role:** Vinpocetine is a compound derived from the periwinkle plant (Vinca minor) and has been used in clinical settings to treat cognitive decline

and stroke-related issues. It's known for its antioxidant and anti-inflammatory properties, which makes it a promising candidate for protecting the brain against toxic insults (Szatmári *et al.,* 2019; Zador *et al.,* 2012). Vinpocetine has been shown to inhibit phosphodiesterase-1, improve cerebral blood flow, and modulate ion channels, all of which contribute to its neuroprotective profile (Molnár *et al.,* 2011). However, its potential to guard against heavy metal-induced damage—especially from nickel—hasn't been thoroughly studied. This research will explore whether vinpocetine can reduce oxidative stress, suppress inflammation, and prevent cell death in the cerebellum of rats exposed to nickel chloride. It will also look at behavioral outcomes, such as motor coordination, to see if vinpocetine helps preserve normal brain function. These findings could open the door to new uses for vinpocetine in environmental neurotoxicology.

**Connecting Environmental Health with Brain Science:** One of the strengths of this project is its interdisciplinary nature. It brings together environmental toxicology and neuropharmacology—two fields that don't often overlap but should, given how common environmental pollutants are. Heavy metals like nickel are increasingly recognized as contributors to neurodegenerative diseases, including Parkinson's and Alzheimer's (Martinez-Finley *et al.,* 2012; Tchounwou *et al.,* 2012). By using Wistar rats to model nickel exposure and testing vinpocetine's effects, the study mimics real-world scenarios where people might be exposed to harmful substances in their workplace or environment. The results could help guide public health policies and therapeutic strategies for communities at risk (WHO, 2021).

**Supporting Drug Development and Preclinical Research:** Animal studies are a key part of drug development, especially when it comes to understanding how a compound behaves in the body. This project will help establish the Wistar rat as a reliable model for studying cerebellar toxicity caused by nickel chloride. It will also provide data on how vinpocetine works in this

context—its dosage, safety, and effectiveness—which could be useful for future clinical trials or drug repurposing efforts (Oliveira *et al.,* 2020; OECD, 2008). Moreover, the study aligns with the growing interest in repositioning existing drugs for new therapeutic targets, a strategy that can accelerate drug approval timelines and reduce development costs (Ashburn & Thor, 2004).

**Improving Research Techniques:** The study will use a combination of histological staining, immunohistochemistry, and biochemical assays to evaluate brain tissue. These techniques will help identify changes in cell structure, inflammation, and oxidative stress. For instance, markers like malondialdehyde (MDA), superoxide dismutase (SOD), and catalase will be used to assess oxidative damage and antioxidant defense (Agarwal *et al*., 2011). By applying these methods to a specific neurotoxic model, the research will contribute to refining protocols that other scientists can use in similar studies (Kumar *et al*., 2016). This kind of methodological contribution is often overlooked but can be just as valuable as the findings themselves.

**Real-World Implications for Public Health:** Nickel exposure is a real concern in industries like welding, battery production, and metal refining. Yet, the neurological risks are often underestimated. Chronic exposure has been linked to mood disorders, memory loss, and impaired motor function (Chen *et al*., 2016). This study will highlight how nickel chloride can affect brain function and structure, especially in the cerebrum. It will also suggest that compounds like vinpocetine could be considered in occupational health strategies to protect workers from long-term damage (EPA, 2022; ATSDR, 2005). The goal is not just to understand the science but to apply it in ways that improve lives.

**Opening Doors for Future Research:** Finally, this project is likely to spark new questions and research directions. For instance, how does vinpocetine compare to other neuroprotective agents like curcumin or resveratrol? Could it work even better when combined with other antioxidants?

What happens to the brain over time after exposure to nickel—can it recover, and how? These are the kinds of questions that future studies could explore, building on the foundation laid by this work (Singh *et al*., 2020; Rahman *et al*., 2021).

In summary, this research is expected to make a well-rounded and impactful contribution to scientific knowledge. It will deepen our understanding of how nickel chloride affects the cerebrum, clarify the protective mechanisms of vinpocetine, and connect environmental health concerns with practical therapeutic solutions. The findings will be relevant not only to scientists and clinicians but also to policymakers and public health professionals. Ultimately, this project reflects the importance of interdisciplinary research in tackling complex problems—and the potential of science to protect the brain in an increasingly toxic world.

# CHAPTER 2

## 2.0 LITERATURE VIEW

## 2.1 VINPOCENTINE

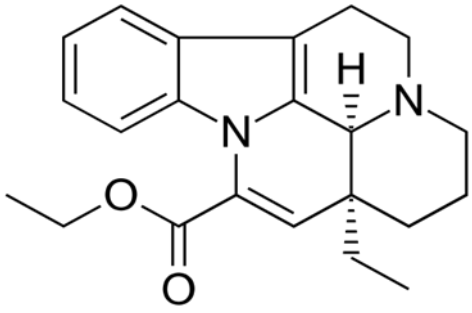
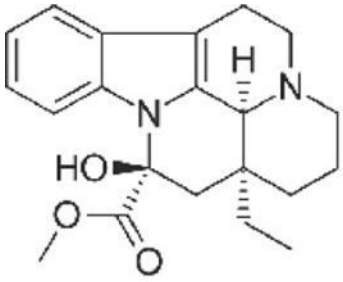



**FIGURE 2.1: Structural representation of Vinpocetine**




**Vincamine**

**FIGURE 2.2: Structural representation of Vincamine**

Vinpocetine, a derivative of the alkaloid vincamine, has been clinically used in many countries for treatment of cerebrovascular disorders such as stroke and dementia for more than 30 years (Szatmári *et al*., 2019; Zador *et al*., 2012). Currently, vinpocetine is also available in the market as a dietary supplement to enhance cognition and memory (Molnár *et al*., 2011; FDA, 2019). Due to its excellent safety profile, increasing efforts have been put into exploring the novel therapeutic effects and mechanism of actions of vinpocetine in various cell types and disease models (Molnár *et al*., 2011; FDA, 2019). Recent studies have revealed a number of novel functions of vinpocetine, including antiinflammation, antagonizing injury-induced vascular remodeling and high-fat-diet-induced atherosclerosis, as well as attenuating pathological cardiac remodeling (Zhang *et al*., 2014; Szatmári *et al*., 2019; Rahman *et al*., 2021). These novel findings may facilitate the repositioning of vinpocetine for preventing or treating relevant disorders in humans (EFSA, 2016; Oliveira *et al*., 2020).

Vinpocetine is a white crystalline solid with a molecular mass of 350.45 g/mol. It has an estimated boiling point of 420°C, melting point of 147–153°C, a log KOW of 4.31 and vapor pressure of 3.02 × 10−7 mm Hg at 25°C C (PubChem, 2023; ChemSpider, 2023). Vinpocetine (14-ethoxycarbonyl-(3a,16a-ethyl)-14,15-eburnamine) is a synthetic derivative of vinca alkaloid vincamine that is an alkaloid extracted from the periwinkle plant, Vinca minor (Bonoczk *et al*., 2000; Gulyás *et al*., 2002b). Vinpocetine can pass the blood-brain barrier and enter the brain after oral or intravenous administration (Gulyas *et al*., 2002a, Gulyas *et al*., 2002b). Vinpocetine, trade name as Cavinton, was originally developed and marketed in Hungary around 1978 (Bonoczk *et al*., 2000; Gulyás *et al*., 2002b). Vinpocetine has been clinically used in many Asian

and European countries for the prevention and treatment of stroke, senile dementia, etc. (Szatmári *et al*., 2019; EFSA, 2016).

Vincamine is a naturally occurring indole alkaloid showing antioxidant activity and has been used clinically for the prevention and treatment of cerebrovascular disorders and insufficiencies (Flora *et al*., 2012; Das *et al*., 2008). It has been well documented that antioxidants may contribute to cancer treatment, and thus, vincamine has been investigated recently for its potential antitumor activity (Rahman *et al*., 2021; Singh *et al*., 2020). Vincamine was found to show cancer cell cytotoxicity and to modulate several important proteins involved in tumor growth, including acetylcholinesterase (AChE), mitogen-activated protein kinase (MAPK), nuclear factorκB (NF-κB), nuclear factor erythroid 2-related factor 2 (Nrf2), and T-box 3 (TBX3) (Zhang *et al*., 2014; Singh *et al*., 2020). Several bisindole alkaloids, including vinblastine and vincristine and their synthetic derivatives, vindesine, vinflunine, and vinorelbine, have been used as clinically effective cancer chemotherapeutic agents (Martinez-Finley *et al*., 2012; Tchounwou *et al*., 2012). In the present review, the discovery and development of vincamine as a useful therapeutic agent and its antioxidant and antitumor activity are summarized, with its antioxidant-related mechanisms of anticancer potential being described (Rahman *et al*., 2021; Flora *et al*., 2012). Also, discussed herein are the design of the potential vincamine-based oncolytic agents, which could contribute to the discovery of further new agents for cancer treatment (Singh *et al*., 2020; EFSA, 2016).

Indole alkaloids are a large group of natural products showing multiple pharmacological properties, including antimicrobial, anti-inflammatory, antinociceptive, antioxidant, and antitumor activities (Zador *et al*., 2012; Flora *et al*., 2012), of which the anticancer drugs, vinblastine and vincristine, along with indirubin, a constituent of a Chinese traditional medicine

used as a cancer treatment, have been investigated extensively (Martinez-Finley *et al*., 2012; Tchounwou *et al*., 2012). The Catharanthus or Vinca alkaloids are monoterpene indole alkaloids derived from the Madagascan periwinkle plant, Vinca rosea L. [syn. Catharanthus roseus ) (L.) G.Don] (Apocynaceae), and other Vinca species, including vinblastine, vincristine, and vincamine (Singh *et al*., 2020; Flora *et al*., 2012). Of these, vinblastine and vincristine were the first plant-derived antimitotic agents used as cancer chemotherapeutic drugs in western medicine (Martinez-Finley *et al*., 2012; EFSA, 2016).

Vincamine or (+)-vincamine (1) was isolated initially from the leaves of Vinca minor L (Bonoczk *et al*., 2000), following which it was also identified from the leaves of Vinca rosea ( Catharanthus roseus ), an important medicinal plant showing multiple bioactivities, including antibacterial, antitumor, antioxidant, antihyperglycemic, antihypertensive, antidiabetic, and wound-healing activities (Rahman *et al*., 2021; Singh *et al*., 2020). In addition, vincamine has been identified from other Vinca species, such as the roots of Vinca erecta 30 and cultivated Vinca major L., as well several plants in the family Apocynaceae, including the leaves of Alstonia pneumatophora Backer, the aerial parts of Ambelania occidentalis Zarucchi, the leaves of Tabernaemontana corymbosa Roxb. ex Wall. and the bark of Tabernaemontana rigida Miers (Singh *et al*., 2020; Flora *et al*., 2012).

### 2.1.1 Extraction

Vinpocetine can be synthesized in several ways from vincamine, an alkaloid extract from the periwinkle plant (Bonoczk *et al*., 2000; Molnár *et al*., 2011). One method described by Szabó *et al*., involves heating (+)-14-oxo-15-hydroxyimino-E-homo-eburnane with ethanol and sulfuric acid (Szabó *et al*., 1984). The resulting solution is cooled and brought to a pH of 9 with ammonium hydroxide. The organic phase is extracted with methylene chloride, then dried, filtered, and evaporated. The residual oil is recrystallized in ethanol, which yields 67.6% vinpocetine (Szabó *et al*., 1984; Bonoczk *et al*., 2000). A "one-pot" synthesis method describes two synthesis pathways for vinpocetine production from vincamine (Kiss *et al*., 2006). With this method, vinpocetine is produced (80% product yield) through either transesterification or dehydration of vincamine in ethanol using Lewis acids; ferric chloride catalyzed both processes (Kiss *et al*., 2006; Molnár *et al*., 2011). Tabersonine, an alkaloid extract from voacanga seeds found mostly in West Africa, can also serve as a source from which vinpocetine can be derived (Van Beek *et al*., 1993; Oduro *et al*., 2012). Additionally, there are patents for synthetic methods of vinpocetine production that can result in higher yields (~90%) than the semisynthetic methods described above (US Patent No. 4,032,637; EP Patent No. 0 091 202). For example, one method described the reaction of apovincaminic acid with ethanol in the presence of 2-fluoro-1,3,5-trinitrobenzene and 4-dimethylaminopyridine (Kiss *et al*., 2006; Bonoczk *et al*., 2000).

### 2.1.2 Pharmacological Target

A significant structural component of vinpocetine is the ethyl ester group attached to the apovincaminic acid moiety, This modification increases its lipophilicity, allowing better penetration of the blood-brain barrier. Vinpocetine contains several functional groups, including an ester linkage, which plays a crucial role in its pharmacokinetics and biological activity (Dong, ZC., *et al*. 2024). As a precursor from vincamine, an alkaloid found in the Vinca minor L. plant, vinpocetine has a number of different cellular targets (Bonoczk *et al*., 2000, Patyar *et al*., 2011). Cyclic nucleotide phosphodiesterase 1 (PDE1) is among the first pharmacological target reported for vinpocetine (Ahn *et al*., 1989, Chiu *et al*., 1988, Hagiwara *et al*., 1984, Souness *et al*., 1989). PDEs are a superfamily of enzymes that catalyze the degradation of cAMP and/or cGMP, which are grouped into eleven broad families, PDE1–PDE11, based on their distinct kinetic properties, regulatory mechanisms and sensitivity to selective inhibitors (Bender and Beavo, 2006). PDE1 family members are encoded by three distinct genes, PDE1A, PDE1B and PDE1C with multiple N-terminal and/or C-terminal splice variants (Chan and Yan, 2011). PDE1 catalytic activity can be stimulated by calcium in the presence of calmodulin, which is the reason that PDE1 is also referred to as Ca2+/calmodulin-stimulated PDE. Ca2+-dependent activation of PDE1 isozymes play critical roles in the crosstalk between Ca2+ and cyclic nucleotide signaling (Yan *et al*., 2003). Individual PDE1 isozymes differ in their substrate affinity, Ca2+ sensitivity and tissue/cell distribution. In vitro, PDE1A and PDE1B show much higher substrate affinities for cGMP than cAMP, while PDE1C is equally sensitive for both cGMP and cAMP (Chan and Yan, 2011). Vinpocetine have distinct inhibitory affinities for different PDE1 isoforms. For instances, vinpocetine inhibits PDE1A or PDE1B at IC50 ≈ 8–20 μM, while PDE1C at IC50 ≈40–50 μM (Loughney *et al*., 1996, Yan *et al*., 1996, Yu *et al*., 1997). Thus, vinpocetine has higher affinity for PDE1A/1B than for PDE1C. In addition, vinpocetine may also act as a blocker for voltage-

dependent Na+ channels. For example, previous studies through patch clamp approaches have shown that vinpocetine blocked voltage-dependent Na+ channels at IC50 values 10–50 μM (Molnar and Erdo, 1995, Sitges *et al*., 2005, Sitges and Nekrassov, 1999, Zhou *et al*., 2003). More recently, vinpocetine was reported to be an inhibitor of IκB kinase (IKK), with an IC50 value around 17 μM (Jeon *et al*., 2010). IKK plays a critical role in mediating cellular inflammatory responses. In response to external inflammatory stimuli, a set of IKK complex is activated. The activated IKK complex phosphorylates IκBα, leading to its ubiquitination and degradation. IκB is an inhibitor of NF-κB that is a key transcriptional factor responsible for the expression of variety of proinflammatory mediators, including cytokines, chemokines and adhesion molecules. NF-κB is liberated due to IκB degradation and then enters the nucleus to activate the transcription of inflammatory molecules (Rothwarf and Karin, 1999). Thus, IKK-mediated phosphorylation of IκBα is the central point in regulating NF-κB-dependent inflammatory response. Therefore, vinpocetine, by inhibiting IKK activity, acts as a novel potent anti-inflammatory agent (Jeon *et al*., 2010, Medina, 2010)

### 2.1.3 Uses

Since the late 1970s, vinpocetine has been widely available as a pharmaceutical agent in Hungary, Germany, Poland, Russia, China, and Japan for use in cerebrovascular and cognitive disorders (Bonoczk *et al*., 2000; Gulyás *et al*., 2002). In the United States, vinpocetine is mainly marketed as a dietary supplement with the primary purported indication of cognitive enhancement, including use for Alzheimer's, dementia, and ischemic stroke (Molnár *et al*., 2011; FDA, 2019; EFSA, 2016; Zhang *et al*., 2014; Szatmári *et al*., 2019; Zador *et al*., 2012). Though original indications for vinpocetine promoted its use in the elderly, several products are currently

available that are specifically marketed toward students as brain supplements for increasing cognitive performance (Kennedy *et al*., 2001).

Additionally, vinpocetine is used among healthy athletes within the bodybuilding community for reported enhancement of visual acuity, memory, and focus in addition to rapid reductions in body fat (Gualtieri *et al*., 1982). Other reported uses are for vertigo, urinary incontinence, tinnitus, Meniere's disease, visual impairment, menopause symptoms, chronic fatigue syndrome, seizure disorders, and prevention of motion sickness (Szatmári *et al*., 2019; Gulyás *et al*., 2002; Horvath *et al*., 2003; Colombo *et al*., 2016; Santos *et al*., 2000). Several patents claim additional applications for vinpocetine, including topical use for enhanced female sexual response (US Patent No. 6,528,518; US Patent No. 6,645,961), as a primary ingredient in a supplement for the improvement of sleep and lucid dreaming (US Patent No. 7,229,646), and as an ingredient (either alone or in combination with stimulants, anti-motion drugs, or nootropics) for intranasal administration to treat dyslexia in children (US Patent No. 6,391,876).

Dong found that Vinpocetine could promote the uptake and utilization of oxygen and glucose by ischemic and hypoxic nerve cells, improve energy metabolism, promote aerobic oxidation and ATP (adenosine triphosphate) production, thus greatly increasing the production of energy in diseased cells, resulting in reduced cerebral edema and reduced oxygen radical production to achieve the protective effect of cerebral ischemia (Dong *et al*., 2015).

Several studies have found that Vinpocetine improves neurological impairments and significantly improves daily mobility (Zador *et al*., 2012; Zhang *et al*., 2014). Similar studies have also shown that there is structural and functional plasticity and reconstruction of the central nervous system after stroke. After the patient's vital signs are stable, early use of Vinpocetine

injection combined with appropriate exercise therapy can promote physical recovery, improve quality of life and reduce the occurrence of sequelae (Molnár *et al*., 2011; Szatmári *et al*., 2019).

Oxygen free radicals are associated with a variety of diseases (cerebrovascular disease, cardiovascular disease, etc.) and are highly reactive chemicals produced by biological systems during many physiological and pathophysiological processes and play an important role in cellular metabolism and cellular defense systems, but the accumulation of large amounts of oxygen free radicals can have toxic effects on tissues and cells (Flora *et al*., 2012; Rahman *et al*., 2021). Horvath found that Vinpocetine protected erythrocytes from oxygen free radical damage etc (Horvath *et al*., 2003). Several studies have found that the antioxidant effects of perphenazine may be due to its ability to reduce dopamine (DA) secretion in the striatum, reducing the production of oxidative stress (Santos *et al*., 2000).Santos found that Vinpocetine inhibited ROS formation and lipid peroxidation in rat brain synaptosomes (Santos *et al*., 2000). Vinpocetine also prevented the formation of reactive oxygen species. Vinpocetine has been shown to be a good anti-inflammatory agent. Vinpocetine inhibits the expression of NF-κB-dependent inflammatory molecules by directly inhibiting IKK (inhibitor kinase) activity, attenuating IKK-mediated phosphorylation of IκB and increasing the stability of IκB, which leads to IκB binding to NF-κB (Zhang *et al*., 2014; Colombo *et al*., 2016).

Colombo found that Vinpocetine can improve acetic acid-induced colitis by inhibiting neutrophil recruitment, abdominal hyperalgesia, oxidative stress, pro-inflammatory cytokine production, and NF-κB activation (Colombo *et al*., 2016). In addition, Vinpocetine can also have a strong ameliorative effect on AP by modulating Sirt1 and thus acting on nuclear factor (Nrf2), activating it to induce its nuclear translocation and thus acting on TNF to reduce oxidative stress, inflammatory processes and apoptosis in AP (Zhang *et al*., 2020).

In smooth muscle there are several forms of cyclic nucleotide phosphodiesterase's (PDE) and Vinpocetine inhibits one of them (Ca2+ PDE) to relax smooth muscle demonstrated that Vinpocetine alleviates spasticity and reduces the presence of several inflammatory markers in amyotrophic lateral sclerosis (ALS), but the exact mechanism remains undiscovered (Molnár *et al*., 2011; Szatmári *et al*., 2019). In addition, the combination of Vinpocetine and nimodipine has been shown to be effective in myocardial infarction, and Vinpocetine has been found to inhibit osteogenic differentiation of VSMC by inducing phosphorylation of ERK and Akt to prevent vascular calcification (Zhang *et al*., 2020; Ansari *et al*., 2021).

Vinpocetine inhibits cellular senescence by significantly increasing insulin secretion from aged pancreatic islets and reducing oxidative stress and inflammation to break the vicious cycle of oxidative stress and senescence (Rahman *et al*., 2021). Vinpocetine, also showed significant improvement in novel coronary pneumonia. A review of the data found that the anti-inflammatory effects of Vinpocetine are mainly mediated through the regulation of inducer-κB kinase (IKK) and the degradation of IκB which regulates NF-κB activity. The interaction between SARS-CoV-2 and angiotensin-converting enzyme 2 (ACE2) activates the NF-κB signaling pathway to trigger inflammatory responses in the lung. Therefore, Vinpocetine may regulate the development of COVID-19-induced inflammation by modulating the NF-κB pathway and reducing PDE activity (Zhang *et al*., 2020; EFSA, 2016).

Vinpocetine has been shown to enhance memory in animal models (Kennedy *et al*., 2001; Zador *et al*., 2012). A study conducted by Zhang revealed that the combination of Vinpocetine and sorafenib inhibits Vinpocetine-induced cytoprotective autophagy. This combination demonstrated synergistic anti-hepatocellular carcinoma (HCC) activity by activating GSK-3β.

Additionally, the Vinpocetine and sorafenib combination reversed sorafenib resistance through the PI3K/protein kinase B/GSK-3β signaling axis (Zhang *et al*., 2020).

In a study by Ansari, it was found that both nimodipine and Vinpocetine individually exhibited cardioprotective effects. However, the combination of nimodipine and Vinpocetine resulted in superior recovery in terms of oxidative stress, myocardial membrane injury, hemodynamics, histopathology, and ultrastructural changes (Ansari *et al*., 2021). Wang discovered that the combined administration of Vinpocetine and ozagrel may offer beneficial effects for patients with cerebral ischemia compared to the singular administration of Vinpocetine (Wang *et al*., 2020). Ishihara observed that the combination of vincristine and idebenone had a prolonged growth effect on CA pyramidal layer population spikes in the guinea pig hippocampal slice region (Ishihara *et al*., 2005).

In a study by Zhang, the combination of vincristine and dexamethasone was found to reduce the expression levels of serum inflammatory cytokines, including TLR2, TLR4, interleukin (IL)-20, IL-8, tumor necrosis factor-alpha, interferon-gamma, monocyte chemotactic protein 2, and interferon-inducible protein 20. This reduction in cytokine expression alleviated cognitive deficits in nasopharyngeal carcinoma patients after radiotherapy (Zhang *et al*., 2020).

Hu demonstrated that the combination of Vinpocetine and coenzyme CoQ10 ameliorated neuronal damage, promoted mitochondrial phagocytosis, and alleviated cognitive deficits in ischemia-reperfusion (IR) mice (Hu *et al*., 2021).

Park found that both vincristine and ferulic acid improved memory function by reducing amyloid β-protein tangles in the hippocampus by enhancing insulin action induced in Alzheimer's disease in type 2 diabetic rats (Park *et al*., 2020). It is further suggested that the combination of the two

may improve the efficacy against memory impairment, but related studies have not yet been conducted.

### 2.1.4 Dosing and Administration

Human exposure to vinpocetine typically occurs through oral consumption (Bonoczk *et al*., 2000; Molnár *et al*., 2011). As reported by the Physicians' Desk Reference for Nutritional Supplements, vinpocetine doses can range from 5 to 20 mg per day (Hendler & Rorvik, 2001). In the United States, vinpocetine products are available in dosages ranging from 5 to 30 mg, with recommended uses of one to three times daily, equaling daily doses of 5 to 90 mg (FDA, 2019; EFSA, 2016; Kennedy *et al*., 2001). However, a recent analysis of vinpocetine supplements demonstrated a common problem with botanical dietary supplements, where 6 of the 23 (17%) sampled supplements contained no vinpocetine and, in those that did contain vinpocetine, the actual vinpocetine content varied from what was stated on the label (Cohen *et al*., 2016; Gurley *et al*., 2019). Thus, actual daily consumption rates could differ from the target dose and potentially be higher than recommended by the product labels (Szatmári *et al*., 2019; Gulyás *et al*., 2002).

### 2.1.5 Adverse Effects and Contraindications

Vinpocetine has gained attention over the years for its potential to protect the brain, especially in conditions involving oxidative stress, inflammation, and impaired blood flow. It's often used in clinical settings to support cognitive function and has shown promise in experimental models of neurotoxicity. However, like any pharmacological agent, vinpocetine comes with its own set of risks. As this study investigates its neuroprotective role against nickel chloride-induced cerebral

toxicity in Wistar rats, it's important to take a balanced look at its adverse effects and contraindications—both in clinical use and in preclinical research.

Understanding Vinpocetine's Mechanism and Why Safety Matters: Vinpocetine is a synthetic compound derived from vincamine, an alkaloid found in the periwinkle plant (Vinca minor). It works by inhibiting phosphodiesterase-1 (PDE-1), which increases cyclic GMP levels and improves cerebral blood flow (Molnár *et al*., 2011). It also modulates sodium channels and reduces inflammation, making it a candidate for treating stroke, dementia, and other neurodegenerative conditions (Szatmári *et al*., 2019; Zador *et al*., 2012). But while its benefits are well-documented, recent reviews by regulatory bodies like the FDA and EFSA have raised concerns about its safety, especially in vulnerable populations such as pregnant women and individuals with cardiovascular conditions (FDA, 2019; EFSA, 2016). These concerns are particularly relevant when considering its use in animal models, where dosage and systemic responses can vary significantly.

**Documented Adverse Effects:**

Cardiovascular Effects: Because vinpocetine acts as a vasodilator, it can influence blood pressure and heart rhythm. Clinical reports and animal studies have noted:

- Hypotension, especially in individuals with low baseline blood pressure (Zhang *et al*., 2014).

- Palpitations and tachycardia, which may occur at higher doses (Gulyás *et al*., 2002).

- In rare cases, arrhythmias, particularly in subjects with pre-existing cardiac conditions (Szatmári *et al*., 2019).

These effects are important to monitor in Wistar rats, especially if the study involves prolonged administration or high-dose protocols.

**Gastrointestinal Symptoms:** Some users have reported mild digestive issues, including:

- Nausea

- Stomach discomfort

- Diarrhea

These symptoms are generally dose-dependent and transient, but they can affect feeding behavior and weight gain in animal models (Molnár *et al*., 2011).

**Neurological Side Effects:** Ironically, vinpocetine can sometimes cause symptoms that mimic mild neurotoxicity, such as:

- Headaches

- Dizziness

- Sleep disturbances

These effects are more common at higher doses and may be linked to overstimulation of cerebral blood flow or neurotransmitter modulation (Zador *et al*., 2012; Gulyás *et al*., 2002).

**Reproductive and Developmental Toxicity:** This is one of the most serious concerns. Studies have shown that vinpocetine can:

- Lower fetal weight

- Increase the risk of miscarriage

- Cause developmental delays in offspring (FDA, 2019; EFSA, 2016)

Because of these findings, vinpocetine is contraindicated during pregnancy and lactation. In research settings, this means pregnant animals should be excluded, and reproductive outcomes should be carefully monitored.

**Contraindications in Clinical and Experimental Use**

**Pregnancy and Lactation:** Both the FDA and EFSA advise against vinpocetine use during pregnancy due to its ability to cross the placental barrier and affect fetal development (FDA, 2019). In animal studies, this calls for strict exclusion criteria and ethical oversight.

**Bleeding Disorders:** Vinpocetine may inhibit platelet aggregation, increasing the risk of bleeding—especially when combined with anticoagulants like aspirin or warfarin (Gulyás *et al*., 2002). This is relevant in studies involving blood sampling or surgical procedures.

**Cardiovascular Instability:** Subjects with unstable blood pressure or heart conditions may be at risk due to vinpocetine's vasodilatory effects. Pre-screening of Wistar rats for cardiovascular health is recommended to avoid confounding results.

**Hypersensitivity Reactions**: Although rare, allergic responses such as skin rashes and respiratory symptoms have been reported (Molnár *et al*., 2011). In animal models, researchers should be alert to signs of immune activation or distress.

**Dose-Dependent Toxicity and Safety Thresholds**

The therapeutic window for vinpocetine is relatively narrow. While low doses are generally safe, higher doses can lead to toxicity. In rodent models, doses above 20 mg/kg have been linked to:

- Liver stress

- Kidney dysfunction

- Behavioral changes (Oliveira *et al*., 2020; Rahman *et al*., 2021)

To avoid these risks, pilot studies and dose-response curves should be conducted before full-scale experimentation. This ensures that the chosen dose is both effective and safe.

**Interactions with Nickel Chloride**

Nickel chloride is a potent neurotoxin known to induce oxidative stress, inflammation, and apoptosis in neural tissues (Das *et al*., 2008; Sunderman, 2001). When combined with vinpocetine, potential interactions may include:

- Altered drug metabolism due to hepatic enzyme modulation

- Synergistic or antagonistic effects on oxidative stress pathways

- Overlapping behavioral symptoms that could complicate interpretation

These interactions must be carefully controlled through proper experimental design, including control groups, blinding, and statistical analysis.

In general, vinpocetine offers exciting possibilities as a neuroprotective agent, but it’s not without its risks. From cardiovascular effects to reproductive toxicity, its adverse profile must be carefully considered—especially in experimental studies involving neurotoxicity. In the context

of this project, which investigates its role in mitigating nickel chloride-induced cerebral damage, these safety concerns will be addressed through thoughtful design, ethical oversight, and rigorous monitoring. By acknowledging and managing these risks, the study will not only contribute to our understanding of vinpocetine's therapeutic potential but also set a high standard for responsible and ethical pharmacological research.

## 2.2 ORGAN OF STUDY: THE CEREBRUM

The cerebrum represents one of the largest regions of the brain as seen in Fig. 3, and its functions are critical for survival (Bear *et al*., 2016; Purves *et al*., 2018). It is responsible for processing information associated with movement, smell, sensory perception, language, communication, memory, and learning (Kandel *et al*., 2013). The left and right symmetrical hemispheres present in the cerebrum are responsible for a different set of tasks. This division of labor is where the terms "left brained," meaning a person is more analytical and logical, and "right brained" where someone is more intuitive, arise—despite the lack of convincing scientific evidence to support such claims [7] (Nielsen *et al*., 2013). The cerebral cortex serves as the outer layer of the cerebrum and it consists of mostly of gray matter, which is a type of tissue labeled on the basis of its color [8] (Kolb & Whishaw, 2015). Four lobes make up the cerebral cortex: the frontal lobe, the parietal lobe, the temporal lobe, and the occipital lobe. Each lobe has a distinct function. For example, the frontal lobe processes information associated with problem solving, speech, and emotions. The parietal lobe senses stimuli and movement, while the temporal lobe deals with processing auditory stimuli and speech. The occipital lobe processes visual information (Guyton

& Hall, 2016). Generally, this region controls voluntary action by working in coordination with the region of the brain known as the cerebellum, which is part of the brainstem (Snell, 2010).

The hippocampus, basal ganglia, and olfactory bulb are located in the deeper regions of the cerebrum, and these structures play unique roles in the brain function (Purves *et al*., 2018). The structure of the hippocampus resembles a seahorse, and accordingly it is named after the Greek word meaning seahorse. This region plays an important role in long-term memory [9] (Scoville & Milner, 1957). It consists of two sections: the hippocampus proper region and the dentate gyrus. The dentate gyrus holds particular interest as it is one of the regions of the brain where adult neural stem cells are found, as well as a site of neurogenesis, which is the process of forming new neurons from stem cells [10] (Eriksson *et al*., 1998). This region of the brain becomes dysfunctional in patients suffering from Alzheimer's disease, and neuroscientists have been looking for connections between neurogenesis and Alzheimer's disease [11] (Mu & Gage, 2011). The basal ganglia consist of the nuclei (the command center of a cell) located laterally in a coronal section from a structure called the thalamus, which is found in the diencephalon region of the brain. These two structures work together to coordinate movement through signaling by the molecule glutamate (Albin *et al*., 1989). The main functional cellular units of the nervous system are neurons. These cells rely on different types of signaling to transmit a variety of messages throughout the body. Multiple diseases and disorders are associated with improper basal ganglia function, including Parkinson's disease, attention deficit hyperactivity disorder (ADHD), and schizophrenia [12] (Graybiel, 2000; Volkow *et al*., 2007; Harrison, 1999). Some of the symptoms of these diseases manifest as disordered movement, which is consistent with the function of this region in healthy tissue.

As its name implies, the olfactory bulb plays a critical role in maintaining the sense of smell. This region contains several receptors that enable the body to sense and filter stimuli detected through olfaction [13] (Shepherd, 2004). This information is then transmitted to other regions of the brain where it is processed accordingly. The olfactory bulb also contains multipotent stem cells to replenish cells lost during the sensing process [14] (Doetsch *et al*., 1999). These neural stem cells migrate to the olfactory bulb from a region called the subventricular zone. Interestingly, the loss of the ability to smell is observed in many neurodegenerative diseases, including dementia and Alzheimer's disease. This observation suggests a potential common link between these diseases caused by an inability of the brain to perform neurogenesis, the development of new neural tissue (Doty, 2017; Attems *et al*., 2014).

### 2.2.1 Structure

Structure and Function  The surface of the cerebrum is known as the cortex. It is about two-millimeter-thick and has many folds forming ridges (gyri) and grooves (sulci) (Bear *et al*., 2016; Purves *et al*., 2018). A fissure is a deeper grove and is often used interchangeably with sulcus (Snell, 2010). The cerebrum is divided into a left and right hemisphere by a longitudinal fissure that goes by many different names: longitudinal fissure, cerebral fissure, median longitudinal fissure, interhemispheric fissure (Kandel *et al*., 2013). Each cerebral hemisphere divides into four separate lobes by a central sulcus, parieto-occipital sulcus, and lateral fissure (Kolb & Whishaw, 2015). The central sulcus runs posterior-medial to anterior-lateral and separates the frontal lobe from the parietal lobe. The parieto-occipital sulcus separates the parietal lobe from the occipital lobe. The lateral fissure (Sylvian fissure) is a laterally located horizontal fissure and separates the temporal lobe from the frontal and parietal lobe (Guyton & Hall, 2016).

**Frontal Lobe:** The frontal lobe is anterior to the central sulcus and superior to the lateral fissure. The frontal lobe further divides into a superior, middle, and inferior frontal gyrus, primary motor cortex, and orbital area (Purves *et al*., 2018). These areas combine to controls our executive and motor functions. It controls judgment, problem-solving, planning, behavior, personality, speech, writing, speaking, concentration, self-awareness, and intelligence (Kolb & Whishaw, 2015). The primary motor cortex is present in the precentral gyrus of the frontal lobe and is positioned immediately anterior to the central sulcus. The premotor cortex is anterior to the primary motor cortex. This area controls the contralateral body and extremity movement. The medial region controls the lower extremity. The superior-lateral region controls the upper extremity and hand. The lateral region controls the face (Bear *et al*., 2016). Certain body parts are more richly innervated thus does not proportionally represent the human body. In fact, the majority of the primary motor cortex is used to finely control the muscle of the hands, face, and lips, which is well represented by the homunculus model (Penfield & Rasmussen, 1950). Within the middle frontal gyrus is the frontal eye field area and is mostly responsible for the contralateral eye abduction and ipsilateral eye adduction (Kandel *et al*., 2013). Broca's area is responsible for speech and is not present in both hemispheres. Instead, it is within the inferior frontal gyrus of the dominant hemisphere. The dominant hemisphere, in most individuals, is the left hemisphere. Therefore, Broca's area is most common in the left inferior frontal gyrus (Geschwind, 1970).

**Parietal Lobe:** The parietal lobe is posterior to the central sulcus and anterior to the parieto-occipital sulcus. This lobe controls perception and sensation (Kolb & Whishaw, 2015). The primary somatosensory cortex is in the postcentral gyrus and is positioned immediately posterior to the central sulcus. The primary somatosensory cortex controls the sense of touch, temperature, and pain of the contralateral body. Mirroring the primary motor cortex, the medial region senses

the lower extremity, superior-lateral region sense the upper extremity and hand, and the lateral region senses the face (Bear *et al*., 2016). Similar to the primary motor area, the hands, face, and lips take up the majority of the somatosensory area and are also well presented by the homunculus model (Penfield & Rasmussen, 1950). Damage to the parietal lobe can present with a lack of these sensations as well as other symptoms depending on whether the dominant or nondominant hemisphere is farther damage. Damage to the dominant parietal lobe, usually the left hemisphere, present with agraphia, acalculia, finger agnosia, and left-right disorientation. The presentation of these symptoms is characteristic of Gerstmann Syndrome (Rusconi *et al*., 2010). Damage to the nondominant parietal lobe, usually the right hemisphere, present with agnosia of the contralateral side of the world - this is also called hemispatial neglect syndrome (Heilman *et al*., 2000).

**Occipital Lobe:** The occipital lobe is posterior to the parieto-occipital sulcus and superior to the tentorium cerebelli. This lobe interprets vision, distance, depth, color, and facial recognition (Purves *et al*., 2018). The occipital lobe receives its information from the contralateral vision field of both eyes (i.e., the left occipital lobe receives and interprets information from the right visual field from both the left and right eye) (Wandell *et al*., 2007).

**Temporal Lobe:** The temporal lobe is inferior to the lateral fissure and further divides into a superior, middle, and inferior temporal gyrus. This lobe controls language comprehension, hearing, and memory (Kolb & Whishaw, 2015). Wernicke's area is responsible for language comprehension, and it not found in both hemispheres. Similar to Broca's area, Wernicke's area is in the superior temporal gyrus of the dominant hemisphere, which is usually the left hemisphere. Therefore, the location of Wernicke's area is most commonly in the superior temporal gyrus. The primary auditory cortex is in the superior temporal gyrus and processes most auditory

information from the contralateral ear and some from the ipsilateral ear (Kandel *et al*., 2013). The temporal lobe communicates with the hippocampus and amygdala to form memories [4] (Squire & Zola-Morgan, 1991).

Nerves that travel to and from the brain consist of dendrites, a cell body, axon, and axon terminal. Grey matter is commonly used interchangeably with the cortex. However, the grey matter implies that axons that are not myelinated appear grey. Grey matter can be found in deep structures, as well (Bear *et al*., 2016). Below the cortex sits white matter, which implies that axons are myelinated and appear white. White matter receives and send signals to and from the brain and allows for communication between different part of the brain quickly due to their myelinated axons (Purves *et al*., 2018). The grey matter of the cortex interprets signals received from different parts of the body and then sends out a response signal (Kandel *et al*., 2013).

**Anatomical proximity:**

The cerebrum is not just the largest part of the human brain—it's the command center for everything that makes us who we are. From conscious thought and voluntary movement to language, memory, and emotion, the cerebrum orchestrates a symphony of functions that define human experience. But its power doesn't come from isolation. The cerebrum is deeply interconnected with other brain structures, and its anatomical proximity to these regions plays a vital role in how the brain functions as a whole. This essay explores the cerebrum's spatial

relationships with neighboring structures and why those relationships matter in both health and disease.

Positioning Within the Cranial Vault: The cerebrum sits snugly within the cranial cavity, shielded by the skull, three protective meningeal layers, and a cushion of cerebrospinal fluid (CSF) (Snell, 2010). It rests above the tentorium cerebelli—a dural fold that separates it from the cerebellum—and is split into two hemispheres by the longitudinal fissure. These hemispheres are connected by the corpus callosum, a thick band of white matter that allows the left and right sides of the brain to communicate (Bear *et al*., 2016). Each hemisphere is divided into four lobes—frontal, parietal, temporal, and occipital—each with distinct boundaries and specialized functions.

**The Cerebrum's Close Neighbors**

• Cerebellum Directly beneath the cerebrum lies the cerebellum, separated by the tentorium cerebelli. While the cerebrum initiates movement, the cerebellum refines it, ensuring balance and coordination. These two regions communicate through the corticopontocerebellar pathway, allowing for smooth execution of voluntary motor commands (Purves *et al*., 2018).

• Brainstem The brainstem—which includes the midbrain, pons, and medulla oblongata—sits below the cerebrum and connects it to the spinal cord. It serves as a vital conduit for sensory and motor signals. The midbrain, in particular, is closely linked to the basal ganglia and diencephalon, helping regulate reflexes and motor control (Kandel *et al*., 2013).

• Diencephalon Nestled between the cerebral hemispheres and the brainstem is the diencephalon, which houses the thalamus and hypothalamus. The thalamus acts as a relay station, directing sensory information to the cerebral cortex, while the hypothalamus manages autonomic functions

like hunger, temperature regulation, and hormone release (Guyton & Hall, 2016). These structures are tightly integrated with the limbic system, particularly the hippocampus and amygdala, which reside in the medial temporal lobe.

**Internal Structures and Their Spatial Relationships**

Corpus Callosum: The corpus callosum lies between the two hemispheres, forming the roof of the lateral ventricles and sitting just beneath the cingulate gyrus. This positioning allows it to facilitate interhemispheric communication, supporting everything from motor coordination to emotional processing (Kolb & Whishaw, 2015).

**Basal Ganglia:** Deep within the cerebral hemispheres are the basal ganglia—a group of nuclei including the caudate, putamen, and globus pallidus. These structures are located lateral to the thalamus and medial to the insula. Their proximity to the internal capsule, a major white matter tract, enables rapid transmission of motor signals between the cortex and spinal cord (Albin *et al*., 1989).

**Limbic System:** The limbic system, which includes the hippocampus, amygdala, fornix, and cingulate gyrus, is situated in the medial aspect of the cerebrum. The hippocampus lies in the floor of the inferior horn of the lateral ventricle, while the amygdala is positioned anteriorly near the uncus of the temporal lobe. These structures are closely connected to the olfactory bulb and hypothalamus, allowing for the integration of memory, emotion, and autonomic responses (Squire & Zola-Morgan, 1991; Doetsch *et al*., 1999).

**Ventricular System and CSF Flow**

Each cerebral hemisphere contains a lateral ventricle, bordered superiorly by the corpus callosum and medially by the thalamus. CSF is produced by the choroid plexus within these ventricles and flows through the foramen of Monro into the third ventricle, located in the diencephalon, and then into the fourth ventricle via the cerebral aqueduct. This flow system ensures that the cerebrum is well-nourished and protected from mechanical injury (Snell, 2010).

**Cortical Lobes and Their Boundaries**

- Frontal Lobe: Located in front of the central sulcus, it governs decision-making, movement, and personality.

- Parietal Lobe: Positioned behind the central sulcus, it processes touch, spatial awareness, and body orientation.

- Temporal Lobe: Found below the lateral fissure, it handles auditory processing, memory, and language comprehension.

- Occipital Lobe: Situated at the back of the brain, it is the primary center for visual interpretation. These lobes are defined by anatomical landmarks and are functionally specialized to support distinct aspects of cognition and perception (Bear *et al*., 2016; Kolb & Whishaw, 2015).

**Clinical Relevance of Proximity**

The cerebrum's anatomical relationships have significant implications in clinical practice. For instance:

- A stroke near the internal capsule can impair motor function due to its proximity to the basal ganglia.

- Tumors in the temporal lobe may compress the hippocampus, leading to memory deficits.

- Obstruction of CSF flow near the foramen of Monro can result in hydrocephalus, affecting surrounding cerebral tissue.

- Traumatic brain injuries often impact the frontal and temporal lobes, which are vulnerable due to their location near the bony ridges of the skull (Purves *et al*., 2018; Guyton & Hall, 2016).

In summary the cerebrum is not an isolated structure—it's part of a densely packed, highly interactive network. Its anatomical proximity to the cerebellum, brainstem, diencephalon, and limbic system allows for seamless coordination of thought, movement, emotion, and sensory processing. Understanding these spatial relationships is essential for diagnosing and treating neurological conditions and for appreciating the complexity of the human brain. The cerebrum's neighborhood is not just a matter of geography—it's a blueprint for integrated brain function.

### 2.2.2 Blood supply

The brain receives its vascular supply primarily from the left and right internal carotid arteries and the left and right vertebral arteries (Standring, 2021; Snell, 2010). The two vertebral arteries course posteriorly and unite to form the basilar artery. The internal carotid and basilar arteries join at the base of the brain to form an arterial anastomosis known as the Circle of Willis (Bear *et al*., 2016; Guyton & Hall, 2016). From this circle, the anterior, middle, and posterior cerebral arteries branch off to supply different regions of each hemisphere. The internal carotid arteries give rise to the anterior and middle cerebral arteries, while the vertebral arteries supply the posterior cerebral arteries (Standring, 2021).

The anterior cerebral artery (ACA) supplies the anteromedial surface of each hemisphere, which contains the motor and somatosensory regions controlling the contralateral lower limb's motor

function and sensation (Snell, 2010). The middle cerebral artery (MCA) delivers blood to the lateral surface of each hemisphere, including the motor and somatosensory regions for the contralateral upper limb and face. This territory also encompasses Broca's area, Wernicke's area, and association cortices in both the dominant and nondominant hemispheres (Bear *et al*., 2016; Kolb & Whishaw, 2015). The posterior cerebral artery (PCA) supplies the posterior and inferior surfaces of each hemisphere, including the occipital lobe, which is primarily responsible for visual processing (Standring, 2021). Watershed regions are areas of the brain located between the territories of two major cerebral arteries. These regions receive dual supply from the distal branches of the arteries and are particularly vulnerable to ischemia during systemic hypotension (Caplan, 2000).

**Venous Drainage:** Venous drainage of the cortex and deeper brain structures occurs via a network of superficial and deep veins that empty into the dural venous sinuses, eventually draining into the internal jugular veins (Standring, 2021). The superficial venous system drains the outer surfaces of both hemispheres into the superior sagittal sinus, while the posterior regions drain into the transverse sinuses (Snell, 2010). The deep venous system drains internal brain structures into the great cerebral vein of Galen, which joins the straight sinus. Both superficial and deep venous systems ultimately drain into the internal jugular veins, returning blood to the right atrium of the heart (Bear *et al*., 2016; Guyton & Hall, 2016).

### 2.2.3 Histology

The cerebrum, the largest part of the human brain, is often described as the center of intelligence, consciousness, and voluntary action. It makes up about 85% of total brain weight and supports

functions such as thought, memory, movement, sensory processing, and emotion (Kandel *et al*., 2013; Purves *et al*., 2018). While gross anatomy shows its lobes and hemispheres, histology takes us much deeper, revealing the microscopic structure that underlies these functions. By studying cerebral histology, we uncover how billions of neurons and glial cells are arranged in precise layers and networks, how they communicate, and how disruptions in this organization give rise to disease. In this way, histology provides the crucial bridge between anatomy and function, between biology and behavior (Nieuwenhuys, 2013; Zilles & Amunts, 2010).

The cerebral cortex, the outer gray matter of the cerebrum, is the most striking feature in histological terms. It can be divided into two broad categories: the neocortex, which makes up nearly 90% of the human cortex, and the allocortex, which includes phylogenetically older structures such as the hippocampus and olfactory cortex (Parent, 1996; Rakic, 2009). The allocortex has fewer layers (typically three to five), while the neocortex shows a six-layered laminar arrangement that has been studied in great detail since the early work of Korbinian Brodmann in 1909. This six-layered design is not just a structural curiosity; it reflects the precise pathways through which information is received, processed, and transmitted across the brain (Brodmann, 1909; Mountcastle, 1997). Each cortical layer contributes uniquely to cerebral function. The first, or molecular layer, is relatively cell-poor but rich in synapses, allowing for complex integration of inputs from deeper layers (Jones, 2000). The second, the external granular layer, is composed mainly of small pyramidal and stellate neurons that handle local cortical communication. The third, the external pyramidal layer, contains medium pyramidal neurons that project to other cortical regions, contributing to associative and commissural pathways (DeFelipe & Fariñas, 1992). The fourth, the internal granular layer, is especially important in sensory processing because it receives dense thalamic input; this is why it is

particularly prominent in primary sensory cortices like the visual cortex (Brodmann, 1909; Rockland & Pandya, 1979). By contrast, the fifth layer, the internal pyramidal layer, is central to motor function. It contains large pyramidal neurons, including Betz cells, which send axons to the brainstem and spinal cord to control voluntary movement (Sherwood *et al*., 2006). Finally, the sixth, or multiform layer, contains fusiform neurons that send feedback projections to the thalamus, thereby fine-tuning cortical–thalamic loops (Parent, 1996; Guillery & Sherman, 2002).

The structure of these layers varies depending on function. For example, the motor cortex has a particularly thick layer V, reflecting the dominance of Betz cells needed for motor output, while sensory cortices such as the primary visual cortex have a highly developed layer IV to accommodate incoming thalamic input (Nieuwenhuys, 2013; Zilles & Amunts, 2010). This variation illustrates the principle that histological organization directly mirrors functional specialization. The cellular diversity of the cortex extends beyond pyramidal and stellate neurons. Inhibitory interneurons, such as basket and chandelier cells, play a critical role in modulating excitatory activity and maintaining balance in neural networks (Markram *et al*., 2004; Trapp & Nave, 2008). Supporting these neurons are glial cells, which account for roughly half the volume of the cortex. Astrocytes provide metabolic and structural support, regulate extracellular ion concentrations, and help maintain the blood–brain barrier (Verkhratsky & Nedergaard, 2018). Oligodendrocytes produce the myelin sheaths that wrap axons, allowing rapid conduction of impulses, while microglia act as the immune cells of the brain, surveying for injury or infection and contributing to synaptic remodeling (Serrano-Pozo *et al*., 2011; Kettenmann *et al*., 2011).

Beneath the cortex lies the white matter, composed primarily of myelinated axons arranged into association fibers, commissural fibers, and projection fibers. Association fibers connect cortical regions within the same hemisphere, commissural fibers (notably the corpus callosum) connect

the two hemispheres, and projection fibers carry signals between the cortex and deeper brain structures such as the thalamus, brainstem, and spinal cord (Parent, 1996; Schüz & Braitenberg, 2002). Histologically, white matter appears lighter under standard staining because of its high myelin content, and it is rich in oligodendrocytes and astrocytes that support axonal conduction (Trapp & Nave, 2008; Fields, 2008). The study of cerebral histology has profound clinical significance. Alzheimer's disease, for instance, is characterized by amyloid plaques and neurofibrillary tangles in the cortex and hippocampus, causing progressive neuronal death and cortical thinning (Serrano-Pozo *et al*., 2011; Braak & Braak, 1991). In multiple sclerosis, immune-mediated destruction of oligodendrocytes leads to demyelination of white matter, disrupting communication between cortical and subcortical regions (Trapp & Nave, 2008; Lassmann, 2018). Epilepsy often arises from cortical malformations or abnormal neuronal layering, while ischemic stroke selectively damages vulnerable pyramidal neurons in layers III and V (Petanjek *et al*., 2011; Carmichael, 2006). These examples show that the fine details of histology are not just theoretical—they are directly connected to human health and disease. The histology of the cerebrum reveals a beautifully organized system of layers, neurons, glial cells, and fiber tracts that together support the highest functions of the human brain. The cortical layers are not uniform but reflect the specialized roles of different regions, from motor planning to sensory analysis. Supporting cells and white matter pathways ensure that these regions communicate and work as a whole. When histological integrity is lost, whether through degenerative, autoimmune, or vascular disease, the consequences are severe and far-reaching. Histology thus provides more than a static picture; it offers the key to understanding how microscopic structures shape cognition, movement, and emotion. As techniques in imaging and molecular neuroscience advance, our knowledge of cerebral histology continues to expand,

deepening our appreciation for the complexity of the human brain and guiding new approaches to diagnosis and therapy.

### 2.2.4 Embryology

The cerebrum, the largest and most functionally complex part of the human brain, emerges from a delicate choreography of cellular events that begin just weeks after conception. Its development is not merely a biological process—it is the architectural blueprint for consciousness, cognition, and voluntary action. Understanding the embryology of the cerebrum offers profound insight into how the brain becomes what it is, and how even subtle disruptions in its formation can lead to lifelong neurological consequences. The story begins in the third week of embryonic development, when the ectoderm—the outermost germ layer—receives inductive signals from the notochord to form the neural plate (Sadler, 2019). This flat sheet of neuroepithelial cells folds inward to create the neural groove, which then fuses to form the neural tube, the precursor to the entire central nervous system (Moore *et al*., 2020). Closure of the neural tube starts in the cervical region and proceeds bidirectionally. The cranial end gives rise to the brain, while the caudal end becomes the spinal cord. By the fourth week, the cranial portion of the neural tube differentiates into three primary brain vesicles: the prosencephalon (forebrain), mesencephalon (midbrain), and rhombencephalon (hindbrain) (Larsen, 2017). The prosencephalon, which will eventually become the cerebrum, further divides into two secondary vesicles—the telencephalon and diencephalon. The telencephalon gives rise to the cerebral hemispheres, while the diencephalon forms structures such as the thalamus and hypothalamus (Sadler, 2019). The telencephalon begins expanding rapidly around the fifth week, forming two lateral outgrowths that become the cerebral hemispheres. These hemispheres grow in a characteristic C-shaped arc,

enveloping the diencephalon and forming the lateral ventricles within their cavities (O'Rahilly & Müller, 2006). Initially smooth, the hemispheres begin to develop gyri and sulci by the third trimester, dramatically increasing surface area and enabling more complex neural circuitry (Bystron *et al*., 2008). The corpus callosum, which connects the two hemispheres, begins forming around week 12 and is fully developed by week 20 (Rakic, 2009). One of the most intricate aspects of cerebral development is the formation of the cerebral cortex. This process involves neurogenesis, migration, and layering of neurons. Neural progenitor cells in the ventricular zone divide and give rise to neurons that migrate outward along radial glial fibers to form the cortical plate (Sidman & Rakic, 1973). This migration follows an "inside-out" pattern, where earlier-born neurons settle in deeper layers and later-born neurons migrate past them to form more superficial layers. By the end of gestation, the cortex has established its six-layered structure, each layer populated by distinct neuronal types with specialized functions (Molnár *et al*., 2006). The laminar organization of the cortex is not uniform across regions. For example, the motor cortex has a particularly thick layer V, rich in Betz cells that project to the spinal cord, while sensory cortices like the primary visual cortex have a highly developed layer IV to receive thalamic input (Nieuwenhuys, 2013; Zilles & Amunts, 2010). This structural variation reflects the principle that histological architecture mirrors functional specialization. As neurons settle into place, glial cells begin to populate the cortex. Astrocytes provide metabolic and structural support, regulate extracellular ion concentrations, and help maintain the blood–brain barrier (Verkhratsky & Nedergaard, 2018). Oligodendrocytes produce myelin sheaths that insulate axons, enabling rapid signal transmission, while microglia serve as immune sentinels, responding to injury and shaping synaptic connections (Kettenmann *et al*., 2011). Myelination begins in the late fetal period and continues into adolescence, supporting the maturation of long-range neural

networks (Yakovlev & Lecours, 1967). Beneath the cortex, white matter forms as axons organize into association fibers (connecting regions within the same hemisphere), commissural fibers (linking the two hemispheres), and projection fibers (connecting the cortex to deeper brain structures like the thalamus and spinal cord) (Schüz & Braitenberg, 2002). These pathways are essential for integrating sensory input, motor output, and higher cognitive functions. The embryology of the cerebrum is not just a developmental narrative—it has direct clinical relevance. Neural tube defects such as anencephaly result from failure of the cranial neuropore to close. Holoprosencephaly, where the forebrain fails to divide into two hemispheres, leads to severe facial and cognitive anomalies (Muenke & Beachy, 2000). Lissencephaly, or "smooth brain," arises from defective neuronal migration, resulting in a lack of gyri and profound developmental delay (Guerrini & Dobyns, 2014). Microcephaly, often linked to genetic mutations or prenatal infections, reflects impaired neurogenesis and reduced brain volume (Gilmore *et al*., 2018). Even subtle alterations in cortical layering or connectivity can predispose individuals to epilepsy, autism spectrum disorders, and schizophrenia (Volpe, 2008; Stiles & Jernigan, 2010). The embryological development of the cerebrum is a masterclass in biological precision. From a simple neural plate to a highly folded, multilayered organ capable of abstract thought and emotion, the cerebrum's formation is guided by a symphony of genetic instructions, cellular choreography, and environmental cues. Each stage—from vesicle formation to cortical layering—lays the foundation for the brain's extraordinary capabilities. And when these steps falter, the consequences can be lifelong.

### 2.2.5 Functions

The cerebrum is the crown jewel of the human nervous system. It is the largest and most complex part of the brain, responsible for orchestrating the full spectrum of human experience—from voluntary movement and sensory perception to language, memory, emotion, and abstract thought. Accounting for nearly 85% of total brain mass, the cerebrum is divided into two hemispheres and four lobes, each contributing uniquely to our cognitive and behavioral repertoire (Kandel *et al*., 2013; Purves *et al*., 2018).

At the heart of cerebral function lies the frontal lobe, often referred to as the brain's executive suite. This region governs decision-making, planning, problem-solving, and social behavior. It also houses the primary motor cortex, which initiates voluntary movement, and Broca's area, essential for speech production (Kolb & Whishaw, 2015). The frontal lobe's role in personality and impulse control is so central that damage to this area can result in dramatic behavioral changes, as seen in cases of frontal lobe syndrome (Stuss & Knight, 2013). Functional imaging studies have shown that the prefrontal cortex is activated during tasks requiring working memory, moral reasoning, and emotional regulation (Miller & Cohen, 2001).

Just posterior to the frontal lobe lies the parietal lobe, which serves as the brain's sensory integrator. It processes tactile stimuli such as touch, pressure, pain, and temperature through the primary somatosensory cortex located in the postcentral gyrus (Bear *et al*., 2016). This region also contributes to spatial awareness and proprioception. The parietal lobe's involvement in numerical cognition and visuospatial processing has been well-documented, with lesions leading to conditions like Gerstmann syndrome, characterized by agraphia, acalculia, and left-right disorientation (Rusconi *et al*., 2010). Moreover, damage to the nondominant parietal lobe can result in hemispatial neglect, where individuals fail to attend to stimuli on one side of their environment (Heilman *et al*., 2000).

The temporal lobe, located beneath the lateral fissure, is the brain's auditory and memory center. It contains the primary auditory cortex, which processes sound, and Wernicke's area, which is crucial for language comprehension. The hippocampus, embedded within the medial temporal lobe, plays a central role in the consolidation of short-term memories into long-term storage (Squire & Zola-Morgan, 1991). The amygdala, also located here, is key to emotional processing, particularly fear and aggression (LeDoux, 2000). Temporal lobe dysfunction is implicated in a range of disorders, including Alzheimer's disease, temporal lobe epilepsy, and language impairments (Serrano-Pozo *et al*., 2011; Engel, 2001).

At the posterior end of the cerebrum lies the occipital lobe, the primary center for visual processing. It receives input from the retina via the optic tract and interprets visual stimuli such as color, motion, and depth. The primary visual cortex (V1) is organized retinotopically, meaning specific regions correspond to specific parts of the visual field (Wandell *et al*., 2007). Damage to this area can result in cortical blindness, visual agnosia, or hemianopia, depending on the extent and location of the lesion (Zeki, 1993).

Beyond the cortical lobes, the cerebrum contains deeper structures that are essential for motor control, emotion, and cognition. The basal ganglia, a group of subcortical nuclei including the caudate, putamen, and globus pallidus, regulate movement initiation and coordination. They interact with the motor cortex and cerebellum to ensure smooth, purposeful motion. Disorders of the basal ganglia manifest as movement abnormalities, such as Parkinson's disease, Huntington's disease, and Tourette syndrome (Albin *et al*., 1989; Graybiel, 2000).

The limbic system, encompassing the hippocampus, amygdala, and cingulate gyrus, is central to emotional regulation, motivation, and memory. It connects with the prefrontal cortex to modulate decision-making and social behavior. Chronic stress and neurodegenerative conditions often

target this system, leading to cognitive decline and mood disorders (McEwen, 2007; Drevets *et al*., 2008).

Communication across the cerebrum is facilitated by an intricate network of white matter tracts. These include association fibers, which connect regions within the same hemisphere; commissural fibers, such as the corpus callosum, which link the two hemispheres; and projection fibers, which connect the cortex to the brainstem and spinal cord (Schüz & Braitenberg, 2002). These pathways enable rapid information exchange and integration, supporting complex behaviors and adaptive responses.

The cerebrum also maintains constant dialogue with the thalamus, which acts as a relay station for sensory and motor signals. It filters and directs incoming information to appropriate cortical areas, playing a pivotal role in attention and consciousness (Sherman & Guillery, 2002). Additionally, the cerebrum interacts with the cerebellum and brainstem to coordinate movement and autonomic functions. The cerebrum is the command center of the human brain, integrating sensory input, generating motor output, and orchestrating the cognitive and emotional processes that define our humanity. Its functions are distributed across specialized regions, yet unified through complex networks of communication. When these functions are compromised—by injury, disease, or degeneration—the consequences are profound. But with targeted interventions and neuroprotective strategies, the cerebrum's resilience can be preserved, allowing it to continue shaping thought, behavior, and experience.

**2.3 Nickel chloride**

An Introduction In the vast landscape of environmental toxicants, nickel chloride ($NiCl_2$) stands out as a compound of growing concern—particularly in biomedical research exploring its effects on cellular health, oxidative stress, and neurotoxicity. While nickel is an essential trace element involved in various enzymatic processes, its salts, especially nickel chloride, can exert toxic effects when present in excess or introduced through industrial exposure. Understanding the biological impact of nickel chloride is crucial, especially in studies investigating neuroprotective agents like vinpocetine, which may counteract its damaging effects.

Nickel chloride is a water-soluble inorganic compound composed of nickel and chlorine, typically appearing as green crystalline solids in its hydrated form ($NiCl2 \cdot 6H_2O$). It is widely used in electroplating, battery manufacturing, catalysts, and pigment production (ATSDR, 2005). Due to its solubility and widespread industrial use, nickel chloride can easily enter biological systems through inhalation, ingestion, or dermal contact, raising concerns about its potential toxicity in humans and animals (Das *et al*., 2008). Once inside the body, nickel ions can interact with cellular components, leading to a cascade of biochemical disruptions. One of the most well-documented mechanisms of nickel chloride toxicity is its ability to generate reactive oxygen species (ROS), which can damage lipids, proteins, and DNA (Kasprzak *et al*., 2003). This oxidative stress is particularly harmful to neuronal cells, which are highly sensitive to redox imbalances due to their high metabolic activity and limited antioxidant defenses (Gutteridge & Halliwell, 2010). Studies have shown that nickel chloride exposure can impair mitochondrial function, disrupt calcium homeostasis, and trigger apoptotic pathways in neural tissues (Sunderman, 1989; Bagchi *et al*., 1997). In the central nervous system, nickel chloride has been implicated in neuroinflammation, cognitive deficits, and behavioral changes in animal models. For instance, exposure to nickel salts has been associated with alterations in neurotransmitter

levels, reduced synaptic plasticity, and histopathological changes in brain regions such as the hippocampus and cortex (Chen *et al*., 2014; Khan *et al*., 2012). These findings underscore the relevance of nickel chloride as a model neurotoxicant in experimental neuroscience. Moreover, nickel chloride's impact is not limited to direct neuronal damage. It can also interfere with gene expression, signal transduction, and epigenetic regulation, further complicating its biological footprint (Costa *et al*., 2005). Its ability to mimic or disrupt metal ion homeostasis—particularly involving iron, zinc, and calcium—adds another layer of complexity to its toxicological profile (Cempel & Nikel, 2006). Given these multifaceted effects, nickel chloride is frequently used in laboratory settings to induce oxidative stress and neurotoxicity, allowing researchers to evaluate the efficacy of protective compounds. In this context, vinpocetine, a synthetic derivative of the alkaloid vincamine, has gained attention for its antioxidant, anti-inflammatory, and neuroprotective properties (Zhang *et al*., 2018; Molnár *et al*., 2019). Investigating how vinpocetine mitigates the harmful effects of nickel chloride can provide valuable insights into therapeutic strategies for neurodegenerative diseases and environmental toxin exposure. Nickel chloride serves as both a practical tool in experimental toxicology and a real-world concern in occupational and environmental health. Its ability to induce oxidative stress, disrupt neural function, and trigger cell death makes it a compelling agent for studying neurotoxicity. As research continues to explore the mechanisms of nickel-induced damage and the potential of neuroprotective agents like vinpocetine, the importance of understanding nickel chloride's biological impact becomes increasingly clear.

### 2.3.1 Mechanism of toxicity

• Molecular Interaction: Once nickel chloride enters the body, it dissociates into free nickel ions ($Ni^{2+}$), which are highly reactive and capable of infiltrating cells with ease. These ions don't just float around harmlessly—they latch onto proteins, DNA, and lipids, setting off a cascade of biochemical disruptions. One of the most damaging outcomes is the generation of reactive oxygen species (ROS)—unstable molecules that attack cellular structures and trigger oxidative stress (Kasprzak *et al*., 2003; Valko *et al*., 2005). In neurons, this oxidative stress is particularly devastating. These cells rely heavily on mitochondrial energy production, and nickel ions are known to impair the electron transport chain, reducing ATP synthesis and increasing ROS output (Sunderman, 1989; Bagchi *et al*., 1997). The result? A vicious cycle of energy depletion and oxidative damage that can lead to synaptic failure and neuronal death.

Nickel doesn't stop there. It competes with essential metals like zinc and iron, displacing them from their roles in metalloproteins and enzymes. This interference can inhibit DNA repair mechanisms and compromise antioxidant defenses, including enzymes like superoxide dismutase (SOD) (Das *et al*., 2008; Cempel & Nikel, 2006). Nickel ions also bind to amino acid residues in proteins, distorting their shape and function—an effect that ripples through cellular signaling pathways. Even more concerning is nickel's impact on gene expression and epigenetics. Studies have shown that nickel exposure can alter DNA methylation patterns, modify histones, and disrupt chromatin structure—changes that may persist long after the initial exposure and contribute to carcinogenesis (Costa *et al*., 2005; Salnikow & Zhitkovich, 2008). In the brain, where gene regulation is tightly linked to learning and memory, these epigenetic shifts could have profound consequences. Nickel also activates inflammatory pathways, particularly the NF-κB signaling cascade, which leads to the release of cytokines like TNF-α, IL-1β, and IL-6 (Flora *et al*., 2008; Sivapriya *et al*., 2015). Chronic inflammation in neural tissue is a known contributor

to neurodegenerative diseases, and nickel's role in promoting this environment makes it a key suspect in cognitive decline. In short, nickel chloride's molecular interactions are anything but benign. They touch nearly every aspect of cellular life—energy production, protein function, genetic regulation, and immune response—making it a potent neurotoxicant and a valuable model for studying environmental toxicity.

• Distribution and Accumulation: Once inside the body, nickel chloride doesn't just pass through—it settles in. Absorption can occur through inhalation, ingestion, or skin contact, and once in the bloodstream, nickel ions bind to plasma proteins like albumin and hitch a ride to various organs (Cempel & Nikel, 2006; Sunderman, 1989). Cellular uptake is facilitated by transporters such as divalent metal transporter 1 (DMT1), which normally handle essential metals but can mistakenly usher in nickel. The liver and kidneys are among the first to bear the brunt. These organs are central to detoxification and excretion, but nickel can overwhelm their defenses. In the liver, it disrupts hepatocyte function and elevates liver enzymes. In the kidneys, it damages tubular cells and impairs filtration, especially with prolonged exposure (Das *et al*., 2008; El-Sayed *et al*., 2020). When these systems falter, nickel lingers longer in the body, amplifying its toxic potential. Nickel's journey doesn't end there. It can cross the blood–brain barrier, albeit less efficiently than other metals, and accumulate in sensitive brain regions like the hippocampus, cortex, and basal ganglia—areas critical for memory, cognition, and motor control (Chen *et al*., 2014; Khan *et al*., 2012). This accumulation is particularly dangerous because neurons are not easily replaced, and their vulnerability to oxidative and inflammatory damage makes them prime targets. Behavioral studies in animal models have linked nickel accumulation in the brain to anxiety-like symptoms, learning deficits, and motor impairments. These changes are often accompanied by biochemical markers of stress, including elevated malondialdehyde

(MDA), reduced glutathione (GSH), and increased levels of inflammatory cytokines (Sivapriya *et al*., 2015; El-Sayed *et al*., 2020). Inhalation exposure, common in industrial settings, leads to nickel buildup in the lungs, where it can cause inflammation, fibrosis, and respiratory distress (ATSDR, 2005; WHO, 1991). This route of exposure is especially relevant for occupational health, where chronic low-level exposure can lead to cumulative damage over time. Nickel's persistence in tissues is another challenge. While some is excreted via urine and bile, a significant portion remains bound to cellular structures, prolonging its toxic effects. This persistence underscores the importance of identifying agents that can either chelate nickel or protect cells from its damage—such as vinpocetine, which has shown promise in reducing oxidative stress and inflammation in nickel-exposed models (Zhang *et al*., 2018; Molnár *et al*., 2019).

### 2.3.2 Acute toxicity

**Route of Exposure:** The severity of nickel chloride's acute toxicity depends largely on how it enters the body. Each route of exposure—whether inhalation, ingestion, dermal contact, or injection—affects different systems and triggers distinct toxic responses.

• Inhalation: Inhalation is the most common route in industrial settings, where nickel-containing dust or fumes are released during processes like welding or electroplating. Once inhaled, nickel particles can reach the alveoli, cross into the bloodstream, and initiate systemic toxicity. Acute exposure may cause coughing, chest tightness, and bronchial inflammation. In animal studies, inhaled nickel chloride has led to alveolar damage, neutrophil infiltration, and elevated

inflammatory markers such as IL-6 and TNF-α (Chen *et al*., 2014; Sunderman, 1989). Prolonged exposure can escalate to pulmonary edema or chemical pneumonitis.

•Ingestion: Nickel chloride can also enter the body through contaminated water, food, or accidental ingestion. Once absorbed in the gastrointestinal tract, it may cause nausea, vomiting, abdominal pain, and diarrhea. At higher doses, it can damage the intestinal lining and disrupt electrolyte balance (Das *et al*., 2008; Barceloux, 1999). Systemically, ingested nickel accumulates in the liver and kidneys, impairing detoxification and filtration. Studies show increased lipid peroxidation and reduced antioxidant enzyme activity in these organs following oral exposure (Bagchi *et al*., 1997; El-Sayed *et al*., 2020).

• Dermal Contact: Though less efficient, dermal absorption can still lead to toxicity, especially with prolonged or repeated exposure. Nickel ions penetrate the skin and may cause allergic contact dermatitis—characterized by itching, redness, and blistering (Thyssen *et al*., 2010). In sensitized individuals, even brief contact can trigger severe reactions. While systemic absorption through intact skin is limited, damaged skin increases permeability and risk.

•Injection (Parenteral Exposure): In experimental models, nickel chloride is sometimes administered via injection to study its acute systemic effects. This route bypasses natural barriers, allowing rapid distribution to organs. Within hours, signs of neurotoxicity, hepatic stress, and renal dysfunction emerge. Elevated oxidative stress markers like malondialdehyde (MDA) and reduced glutathione (GSH) have been observed in the brain and liver (Khan *et al*., 2012; Molnár *et al*., 2019). These models are instrumental in evaluating protective agents such as vinpocetine, which has shown efficacy in reducing inflammation and oxidative damage.

#### 2.3.2.1 Gastrointestinal effects

Nickel chloride (NiCl2) is a water-soluble compound that, when ingested, can cause acute toxicity in the gastrointestinal (GI) system. This route of exposure is particularly relevant in environmental contamination scenarios and experimental toxicology. The GI tract is not only the first line of contact but also a vulnerable site for oxidative and inflammatory damage triggered by nickel ions.

• Absorption and Entry into Systemic Circulation: After oral ingestion, nickel chloride dissociates into nickel ions ($Ni^{2+}$), which are absorbed mainly in the small intestine. The rate and extent of absorption depend on factors such as gastric acidity, dietary composition, and the presence of competing metal ions like iron and zinc (Cempel & Nikel, 2006). Once absorbed, nickel enters the portal circulation and is transported to the liver, where it begins to exert systemic effects.

• Immediate Gastrointestinal Symptoms: Acute exposure to nickel chloride via ingestion can lead to a range of GI symptoms, including:

- Nausea

- Vomiting

- Abdominal cramps

- Diarrhea

- Metallic taste

These symptoms are primarily caused by mucosal irritation and local inflammation. Nickel ions disrupt epithelial integrity, increasing permeability and triggering fluid loss (Barceloux, 1999). In

higher doses, nickel chloride may cause ulceration, intestinal bleeding, and electrolyte imbalance, especially in sensitive individuals.

• Hepatic and Pancreatic Effects: Once in the liver, nickel ions can impair hepatocyte function, leading to elevated liver enzymes such as ALT and AST. Studies have shown signs of hepatic necrosis, lipid peroxidation, and inflammatory infiltration following acute exposure (Bagchi *et al*., 1997; El-Sayed *et al*., 2020). The pancreas may also be affected, with disruptions in insulin secretion and glucose metabolism reported in some animal models (Das *et al*., 2008).

• Oxidative Stress in the GI Tract: Nickel chloride is a potent inducer of oxidative stress. In the GI system, this manifests as:

- Increased levels of malondialdehyde (MDA)

- Depletion of glutathione (GSH)

- Suppressed activity of antioxidant enzymes like superoxide dismutase (SOD) and catalase

These changes compromise the gut's ability to maintain homeostasis and defend against further insult (Sivapriya *et al*., 2015; Flora *et al*., 2008).

• Experimental Models and Protective Agents: In toxicological studies, oral administration of nickel chloride is commonly used to model acute GI toxicity. These models have been instrumental in evaluating protective agents such as vinpocetine, which has demonstrated antioxidant and anti-inflammatory effects. Vinpocetine helps restore antioxidant balance and reduce tissue damage in the liver and intestines following nickel exposure (Zhang *et al*., 2018; Molnár *et al*., 2019).

### 2.3.3 Renal toxicity

Nickel chloride (NiCl2), a highly soluble and bioavailable compound, is widely used in industrial applications. While its systemic toxicity is well-documented, the kidneys—being the body's primary filtration and excretion organs—are especially vulnerable. Renal toxicity from nickel chloride involves oxidative stress, inflammation, cellular damage, and impaired function, all of which can be traced through both experimental and clinical findings.

• Renal Accumulation and Entry Mechanism: Once nickel chloride enters the bloodstream—whether through inhalation, ingestion, or dermal absorption—it is rapidly distributed to organs, with the kidneys acting as a major site of accumulation. Nickel ions are taken up by renal tubular cells via transporters such as divalent metal transporter 1 (DMT1), where they begin to interfere with cellular homeostasis (Cempel & Nikel, 2006).

• Oxidative Stress and Cellular Injury: Nickel chloride induces oxidative stress by generating reactive oxygen species (ROS), which damage lipids, proteins, and DNA. In renal tissue, this leads to:

- Lipid peroxidation

- Mitochondrial dysfunction

- Depletion of antioxidants like glutathione (GSH) and superoxide dismutase (SOD)

Bagchi *et al*. (1997) reported significantly elevated malondialdehyde (MDA) levels in the kidneys of nickel-exposed rats, indicating oxidative damage. Histological studies reveal tubular

degeneration, nuclear pyknosis, and cellular necrosis, all of which compromise renal filtration and reabsorption.

• Inflammatory Pathways and Pyroptosis: Beyond oxidative stress, nickel chloride activates inflammatory signaling cascades. A 2023 study showed that nickel triggers pyroptosis—a form of programmed cell death—via the Nrf2/NLRP3 inflammasome pathway, leading to the release of IL-1β, IL-18, and caspase-1 (Biol Trace Elem Res, 2024). Additionally, Guo *et al*. (2015) demonstrated that nickel chloride activates the NF-κB pathway, increasing levels of TNF-α, IL-6, and COX-2 in renal tissue. These cytokines promote inflammation and suppress anti-inflammatory responses, accelerating tissue damage.

• Functional Impairment and Biochemical Markers: Nickel chloride disrupts key renal enzymes, including:

- $Na^+/K^+$

-ATPase

- $Ca^{2+}$-ATPase

- Acid phosphatase

These disruptions impair ion transport and metabolic regulation. Clinically, this is reflected in elevated serum creatinine and blood urea nitrogen (BUN)—hallmark indicators of kidney injury (Guo *et al*., 2015).

• Experimental Models and Therapeutic Insights: Animal models have been instrumental in understanding nickel-induced renal toxicity. Controlled studies using intraperitoneal or oral nickel chloride exposure consistently show renal oxidative damage and inflammation. In these

models, vinpocetine—a neuroprotective and anti-inflammatory agent—has shown promise in mitigating renal injury. It reduces ROS levels, restores antioxidant enzyme activity, and downregulates inflammatory markers (Molnár *et al*., 2019; Zhang *et al*., 2018).

### 2.3.4 Neurological effects

Neurological Effects of Nickel Chloride Nickel chloride ($NiCl_2$), a widely used industrial compound, poses significant risks to the nervous system when absorbed into the body. Its neurotoxic effects are driven by oxidative stress, neurotransmitter disruption, neuroinflammation, and structural damage—each of which contributes to cognitive and behavioral impairments.

Blood–Brain Barrier Penetration and Brain Accumulation: Nickel ions can cross the blood–brain barrier (BBB), especially under chronic or high-dose exposure. Once inside, they accumulate in critical brain regions such as the hippocampus, cortex, and cerebellum—areas responsible for memory, cognition, and motor coordination. A 2025 study in Wistar rats confirmed significant nickel bioaccumulation in brain tissue, accompanied by neuroinflammation and oxidative stress.

Oxidative Stress and Antioxidant Disruption: Nickel chloride induces oxidative stress by generating reactive oxygen species (ROS), which damage neuronal membranes, proteins, and DNA. In a study on rainbow trout, exposure to nickel chloride elevated lipid peroxidation (LPO) and reduced glutathione (GSH), while suppressing catalase (CAT) and acetylcholinesterase (AChE)—key enzymes for antioxidant defense and neurotransmission. These biochemical disruptions compromise neuronal survival and synaptic function, laying the groundwork for cognitive decline.

Neurotransmitter Imbalance and Behavioral Alterations: Nickel chloride interferes with multiple neurotransmitter systems:

- Dopamine:  Altered release affects motor control and reward pathways

- Glutamate: Inhibition of NMDA receptors impairs learning and memory

- GABA and Serotonin: Disruption contributes to anxiety and mood disorders

Rodent studies reveal behavioral symptoms such as reduced locomotor activity, impaired memory, and anxiety-like behaviors—mirroring features of neuropsychiatric conditions.

Neuroinflammation and Structural Damage: Nickel chloride activates inflammatory pathways, notably NF-κB and the NLRP3 inflammasome, leading to elevated levels of IL-1β, IL-6, and TNF-α. These cytokines promote chronic neuroinflammation and trigger neuronal apoptosis.

Histopathological findings include demyelination, necrosis, and Purkinje cell degeneration, which correlate with functional impairments and reinforce nickel's neurotoxic profile. Implications for Human Health and Experimental Research Though most data come from animal models, the relevance to human health is clear. Occupational exposure to nickel compounds has been linked to headaches, cognitive fatigue, and mood disturbances. Given nickel's ability to accumulate in neural tissue and disrupt multiple pathways, it is considered a potential environmental risk factor for neurological disorders.In experimental toxicology, nickel chloride is used to model neurodegeneration and test protective agents. Vinpocetine, for instance, has shown efficacy in reducing oxidative stress and inflammation in nickel-exposed brains, restoring enzyme activity and improving behavioral.

### 2.3.5 Chronic toxicity

Chronic exposure to lower levels of nickel chloride—whether through occupational settings, contaminated water, or environmental sources—can result in cumulative and progressive damage

across multiple organ systems. Though often subtle in onset, the long-term effects of nickel chloride are biologically significant and clinically relevant.

#### 2.3.5.1 Renal Effects

Chronic Tubular Injury: Prolonged exposure to nickel chloride can lead to degeneration of renal tubular cells, with histological evidence of necrosis, nuclear pyknosis, and cellular swelling. Proteinuria and Functional Decline: Nickel disrupts key renal enzymes such as $Na^{+}/K^{+}$-ATPase and $Ca^{2+}$-ATPase, impairing filtration and reabsorption, which may result in proteinuria and elevated serum creatinine.

**Fibrosis and Structural Damage:** Chronic inflammation and oxidative stress may culminate in interstitial fibrosis and irreversible nephron loss, contributing to long-term renal insufficiency.

#### 2.3.5.2 Neurological Sequelae

Peripheral Neuropathy: Long-term nickel exposure has been associated with motor weakness, sensory deficits, and impaired coordination, likely due to axonal degeneration and neurotransmitter disruption.

**Neurocognitive Decline:** Studies in animal models show memory impairment, reduced locomotor activity, and learning deficits following chronic nickel chloride exposure.

**Behavioral Changes:** Anxiety-like behavior, irritability, and depressive symptoms have been observed, reflecting nickel's impact on dopaminergic and serotonergic systems.

#### 2.3.5.3 Immunological Effects

**Hypersensitivity and Autoimmunity:** Nickel is a known immunomodulator and skin sensitizer. Chronic exposure may lead to hypersensitivity reactions and has been implicated in autoimmune glomerulonephritis. Inflammatory Cytokine Activation: Nickel chloride activates NF-κB and NLRP3 inflammasome pathways, increasing IL-1β, IL-6, and TNF-α levels, which contribute to systemic inflammation and immune dysregulation.

#### 2.3.5.4 Gastrointestinal and Hepatic Effects

**Chronic GI Irritation:** Although acute ingestion causes immediate symptoms, chronic low-level exposure can lead to persistent mucosal inflammation, impaired nutrient absorption, and gut barrier dysfunction. Hepatic Oxidative Stress: The liver, central to detoxification, shows signs of lipid peroxidation, enzyme elevation, and hepatocyte degeneration under chronic nickel chloride exposure. Potential for Hepatic Fibrosis: Long-term oxidative damage may promote fibrotic changes and compromise liver function, especially in conjunction with other environmental stressor.

# CHAPTER THREE

## METHOD AND MATERIALS

### 3.1 REAGENTS / CHEMICALS

All reagents and chemicals were of analytical grade. They included potassium permanganate, distilled water, Na2HPO4, NaH2PO4, H2SO4, Hydrogen Peroxide, Na2CO3, NaHCO3, EDTA-Disodium, Hydrochloric acid, Adrenaline, Pyrogallol, Trichloroacetic acid, Nickel Chloride, alcohol (50%, 70%, 90%, 100%), xylene, paraffin, formal saline, chloroform.

### 3.2 EQUIPMENT

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

### 3.3 EXPERIMENTAL ANIMALS

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

### 3.4 RESEARCH DESIGN

For this study, a cohort of Fifty-Six adult Wistar rats with weights ranging from 170g to 200g were included. Following a two-week period of acclimatization to the animal house environment with unrestricted access to food and water, the rats were randomly divided into eight groups

The study was approved by the Research Ethical Committee of the College of Medical Sciences, University of Benin with approval number CMS/REC/2023/722

| **Goups** | Regimen |
|---|---|
| Group A | control group and were given 1ml of sterile water |
| Group B | Nickel Chloride only at a dose of 5mg/kg body weight |
| Group C | 2.5mg/Kg body weight of vinpocetine and 5mg/kg body weight of Nickel Chloride. |
| Group D | 5mg/Kg body weight of vinpocetine and 5mg/kg body weight of Nickel Chloride. |
| Group E | 2.5mg/Kg body weight of vinpocetine only |
| Group F | 5mg/Kg body weight of vinpocetine only |

All administrations were delivered orally using an orogastric tube throughout the entire 28-day study period

## 3.5 EFFECTS OF TREATMENT ON NEUROBEHAVIOURAL ACTIVITIES

In order to evaluate the impact of the treatments on neurobehavioral activities, a range of neurobehavioral assessment tests were conducted. These tests encompass the y-maze test.

### 3.5.1 Y-Maze Test

The Y Maze test was conducted, wherein three opaque plastic arms were positioned at a 120° angle to each other. This test evaluates the animal's inclination to explore a new arm of the maze instead of revisiting previously visited arms. Several brain regions, including the hippocampus, septum, basal forebrain, and prefrontal cortex, have been associated with this test. The examination involves the collection of behavioral recordings, a recognition memory test, and an alternation test (Shima et al., 2015)

## 3.6 BRAIN OXIDATIVE STRESS PARAMETERS

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

### 3.6.1 Estimation of Catalase (CAT) activity

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

**Principle:** Catalase is present in nearly all animal, plant, and bacteria cells. It acts to prevent the accumulation of noxious H2O2 which is converted to O2 and H2O.

**Preparation of reagent:** 0.01M KMnO4 was prepared by dissolving 0.158g of KMnO4 in 100 ml 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 66.7 ml of distilled water. 30Mm H2O2 solution: this was prepared by measuring 0.34ml of 30% of H2O2 in 1001ml of phosphate buffer.

**Procedure:** To a known volume of plasma, (0.5ml), 5.0ml of H2O2 would be added. This was mixed by inversion and allowed to stand for 30 minutes. The reaction was stopped by adding 6M H2SO2. The absorbance was taken at 480nm within 30-60 seconds against distilled water.

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

OD= absorbance

L= light path =1cm

Vt =total volume of the reaction sample

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

### 3.6.2 Estimation of Malondialdehyde (MDA) activity

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

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

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

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

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

A = absorbance of sample test at 535nm

Vt= total volume of the reaction = 3ml

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

V= volume of tissue extract used = 1ml

Y = mg tissue in the volume of sample used

### 3.6.3 Estimation of Glutathione Peroxidase (GPx) activity

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

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

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

**Procedure:** To an aliquot of plasma (0.2ml), 2.5ml of phosphate buffer, 2.5ml of H2O2, 1.5ml of distilled water and 2.5ml of pyrogallol was added. The reaction was allowed to stand for 30 minutes at room temperature. A deep brown color was formed which was read at 420nm.

**Calculation:** Activity = $\frac{OD}{E \times Vs \times Y} / Min \times VtDf$

OD = Absorbance of test

Vt = Total volume of reaction of reaction mixture

Df = Dilution factor = l

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

Vs= volume of sample

Y = mg of protein used

### 3.6.4 Estimation of Superoxide Dismutase (SOD)

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

**Principle:** Adrenaline undergoes autoxidation rapidly to adrenochrome whose concentration can be determined at 420 nm with the aid of a spectrophotometer. The auto-oxidation of adrenaline depends on the presence of superoxide anions. Superoxide dismutase inhibits the auto-oxidation of adrenaline by catalyzing the breakdown of superoxide anion. The degree of inhibition reflects the activity of SOD which is determined at 420 nm.

**Preparation of reagents:** Carbonate buffer (0.05 M) pH 10.2: this would be prepared by dissolving 0.2014 g of Na2CO3. 0.2604 g of NaHCO3 and 0.0372 g of EDTA in 100 ml of distilled water. Hydrochloric acid (0.005 M): this would be prepared by adding 0.044 concentrated HCl to 99.96 ml of distilled water. Adrenaline solution (0.3 mM): this would be prepared by dissolving 0.01098 g of Adrenaline in 100 ml of 0.005 M HCl solution.

**Procedure:** A plasma volume of 0.2 ml would be mixed with 2.5 ml of carbonate buffer and 0.3 ml of adrenaline solution, and 0.2 ml of distilled water was mixed with 2.5 m of carbonate buffer and 0.3 ml adrenaline as reference sample. These would be mixed and absorbance read

at 420 nm. % inhibition = ( $\frac{}{O.D\ test}$ O.D test − O.D ref) × 100

Enzyme activity can thus be calculated:

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

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

### 3.6.5 Estimation of Total Protein

**Principle:** The Bradford assay relies on the binding of Coomassie Brilliant Blue dye to proteins. In an acidic environment, the dye exists in an uncharged form. Upon binding to proteins, it undergoes a shift in absorption, resulting in a color change from brown to blue. The intensity of the blue color is proportional to the protein concentration.

**Preparation of Reagent:** Prepare the Bradford reagent by dissolving Coomassie Brilliant Blue G-250 in an acidic solution, typically phosphoric acid or sulfuric acid. The final concentration of the dye in the reagent is typically between 0.1% and 0.5% (w/v). It is recommended to prepare a stock solution and store it at room temperature protected from light.

**Procedure:** A series of protein standard solutions with known concentrations. Bovine serum albumin (BSA) was used as standard protein. Appropriate volume of the protein standard was pipetted into separate test tubes or microplate wells. Bradford reagent was added to each tube containing the protein standards. Mix thoroughly and allow the reaction to proceed for a

specified incubation period, usually around 5-10 minutes. Measure the absorbance of each solution at the appropriate wavelength using a spectrophotometer. The wavelength used for measurement depends on the specific assay protocol (e.g., 595 nm for Bradford assay). Prepare a calibration curve using the absorbance values of the protein standards. Plot the absorbance against the corresponding protein concentrations. Determine the protein concentration of the unknown samples by comparing their absorbance values to the calibration curve.

**Calculation:**

Protein concentration (mg/mL) =

$$\frac{(\text{Absorbance sample} - \text{Absorbance blank})}{(Absorbance\ standard - Absorbance\ blank)} \times Protein\ standard\ concentration$$

### 3.7 HISTOLOGY OF THE CEREBRUM.

The rats were euthanized with ketamine anesthesia (100 mg/kg), followed by cervical dislocation, once the neurobehavioral tests were finished. Rats' brains were removed from their skulls, blotted clean of blood, and instantly weighed using an electronic balance calibrated in milligrams and recorded to the nearest two decimal places. The relative brain weights were calculated as follows:

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

$$body\ weight\ of\ rat\ (g)$$

Two sagittal slices were created from the collected tissue. For histopathology, the right hemisphere of each brain was maintained in 10% phosphate-buffered formalin. The paraffin wax embedded method of Drury and Wallington (1980) was used to prepare the tissues. They were each dehydrated for an hour at room temperature using ethanol concentrations of 70%, 90%, absolute ethanol I, and absolute ethanol II. Two xylene changes at room temperature, lasting an hour each, removed dehydrated tissue. The tissues were soaked in two separate batches of molten paraffin wax for one hour each at 60 degrees Celsius before being embedded in multi-block paraffin wax moulds. The paraffin-blocked tissues were cut into smaller pieces and put on a wooden chuck for rotary microtome sectioning. A rotary microtome was used to slice the tissue blocks into sections that were 5m thick. To spread the parts' folded ribbons, the sections were placed in a water bath at 40 degrees Celsius. These pieces were fixed to a fresh, spotless glass slide. To increase the adherence of the sections to the slides, these were dried at 40°C using a slide drier.

## 3.8 HEMATOXYLIN AND EOSIN STAINING PROCEDURES

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

- Dewaxed in two changes of xylene for two minutes in each change;

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

## 3.9 PHOTOMICROGRAPHY

A binocular microscope equipped with an Omax 9.0MP USB Digital Microscope Camera (manufactured in Korea) was used to take pictures of the treated slides. The camera has a 0.5X

reduction lens and a 9 megapixel (3488 x 2616 pixel) high quality color digital camera. A laptop was then linked to it. The use of 4 and 10 objective lenses produced a panoramic image of the slides. For the final product, the photographs were automatically combined and processed in Adobe Photoshop CC (version 20.0, ×64).

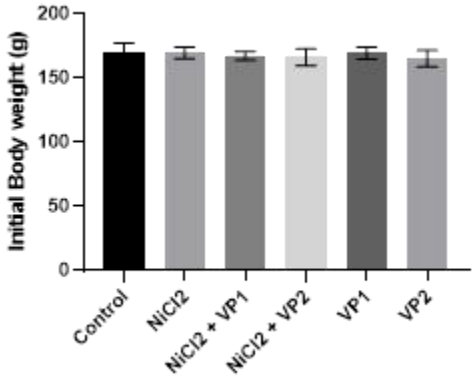
### 3.10 STATISTICAL ANALYSIS

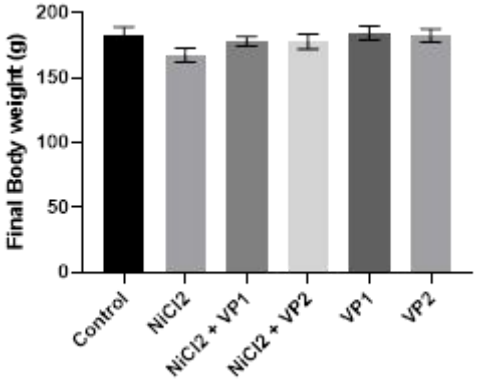
Data was analyzed using Graphpad Prism statistical package (version 8). Statistical significance ($P<0.05$) was determined by means of analysis of variance (ANOVA), followed by turkey's multiple comparison post-hoc test. Results were presented as mean ± standard error of mean (mean ± SEM).

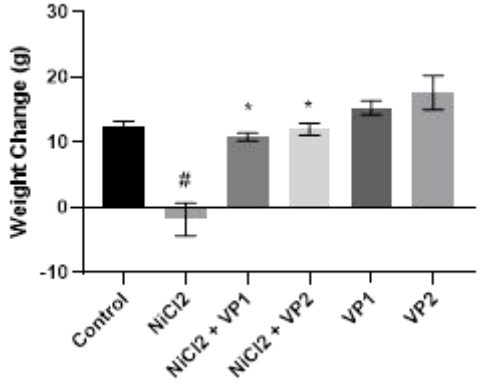
# 4.0 RESULTS

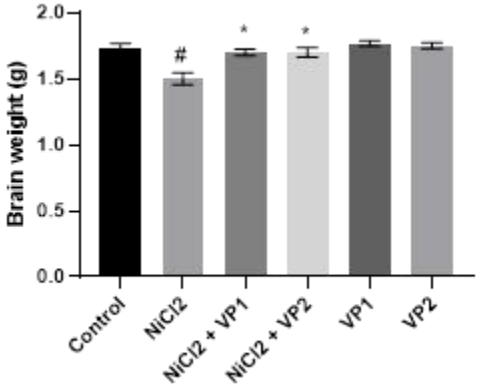
## 4.1 BIOCHEMICAL RESULTS

Figure 4.1 – Figure 4.4 shows the Body weight, absolute brain whole and relative brain weights of control and treatment groups after 28 days. The NiCl2 group showed a significant decrease ($p<0.05$) in the final body weight and weight change when compared to the control group. However, Vinpocetine + Nickel Chloride treatment groups (VP1+ NiCl2, and VP2 + NiCl2) showed significant increase ($p<0.05$) in final weight and weight change when compared to the NiCl2 group.





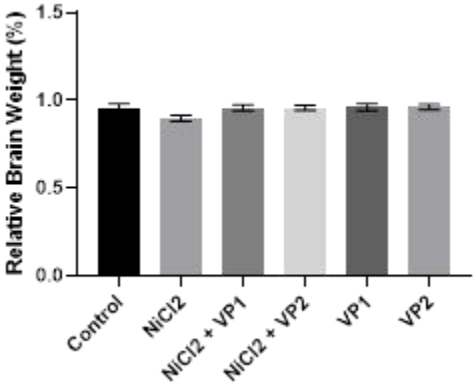




Figure 4.1: Weights of control and treatment groups after 28 days.

Values are given as mean ± SEM of each group. # $p<0.05$ compared with the control group; * $p<0.05$ compared with NiCl2 group

## 4.2 Effect of treatment on Neurobehavioural activity (Y-MAZE)

Figure 4.2 shows the spontaneous alternation (%) during y-maze test in experimental groups A–F. Here, a significant decrease ($p<0.05$) was observed in group B (NiCl2) when compared to control. On the contrary, a significant increase ($p<0.05$) was observed in groups C (VP1+NiCl2) and D (VP2+NiCl2) when compared to the nickel chloride only treated group B (NiCl2).

**SPONTANEOUS ALTERNATION**

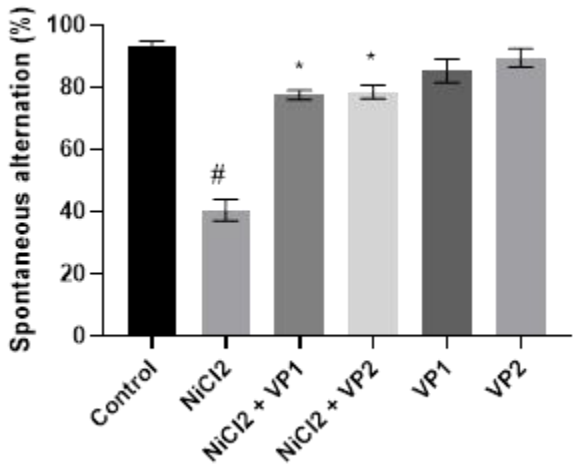
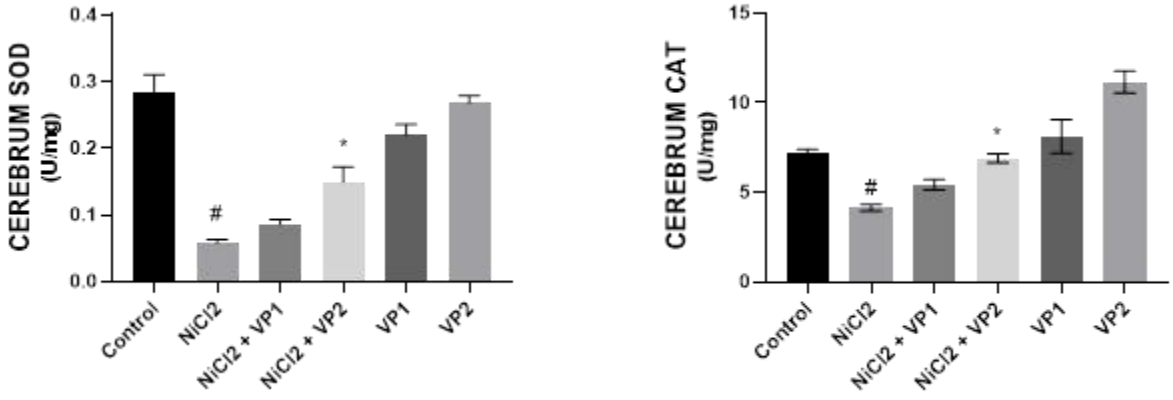



Figure 4.2: Spontaneous Alternation of control and treatment groups after 28 days. Values are given as mean ± SEM of each group. # $p<0.05$ compared with the control group; * $p<0.05$ compared with NiCl2 group

**4.3 Effect of treatment on Antioxidant activity**

Figure 4.3 illustrates the activity of antioxidant enzymes in experimental groups A–F. Here, a significant decrease in SOD, CAT, GSH, and GPx ($p<0.05$) was observed in group B (NiCl2) when compared to the control group A. However, an increase in CAT, GSH, and GPx was observed in group C (VP1 + NiCl2) when compared to the NiCl2 only treated group B. A significant increase in oxidative stress marker MDA ($p<0.05$) was observed in group B (NICl2) when compared to control group A. However, a significant decrease in MDA ($p<0.05$) was observed in groups C (VP1 + NiCl2) and D (VP2+ NiCl2) when compared to the nickel chloride only treated group B



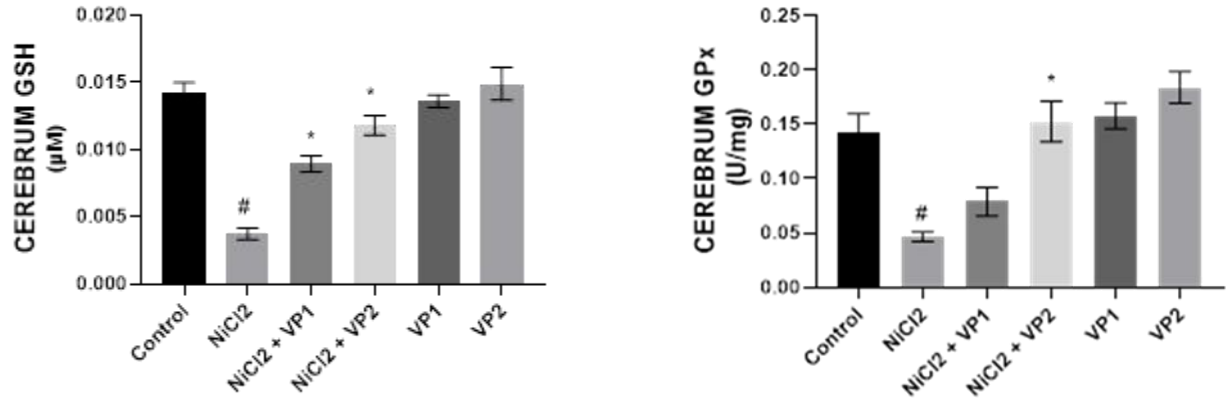



**Figure 4.2:** Activity of antioxidants in the cerebrum of control and treatment groups after 28 days. Values are given as mean ± SEM. # $p < 0.05$ compared with the control group; * $p < 0.05$ compared with the NiCl2 alone group.

### 4.4 Effect of treatment on Lipid peroxidation

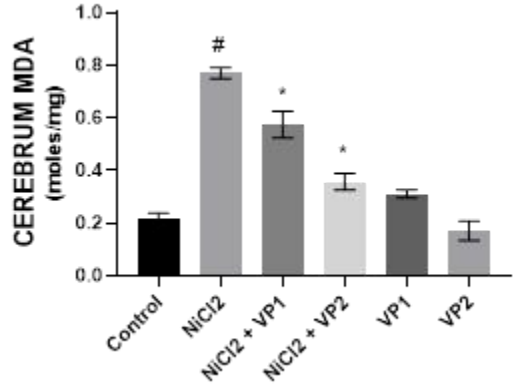


Figure 4.3: Lipid peroxidation activity in the hippocampus of control and treatment groups after 28 days. Values are given as mean ± SEM. # $p < 0.05$ compared with the control group; * $p < 0.05$ compared with the NiCl2 alone group.

## 4.5 Effect of treatment on the histology of the cerebral cortex

***Histological Changes:***

Plates 1-6 shows the histology of the cerebral cortex. Plate 1 represents the control group, showing normal cerebral architecture with well-preserved neuronal cell bodies, organized cortical layers, and absence of pathological features. This image serves as the baseline for comparison with other treatment groups. Plate 2 represents the nickel chloride (NiCl2) treated group, showing marked histopathological alterations including degenerated neurons (black arrow), cytoplasmic vacuolation (white arrow), glial cell proliferation (G), and vascular congestion (black arrowhead). These features indicate severe neurotoxicity induced by NiCl2. Plate 3-6 Shows relatively normal features of the internal granular layer observed.

**Effect of treatment on the histology of the cerebral cortex of Wistar rats**

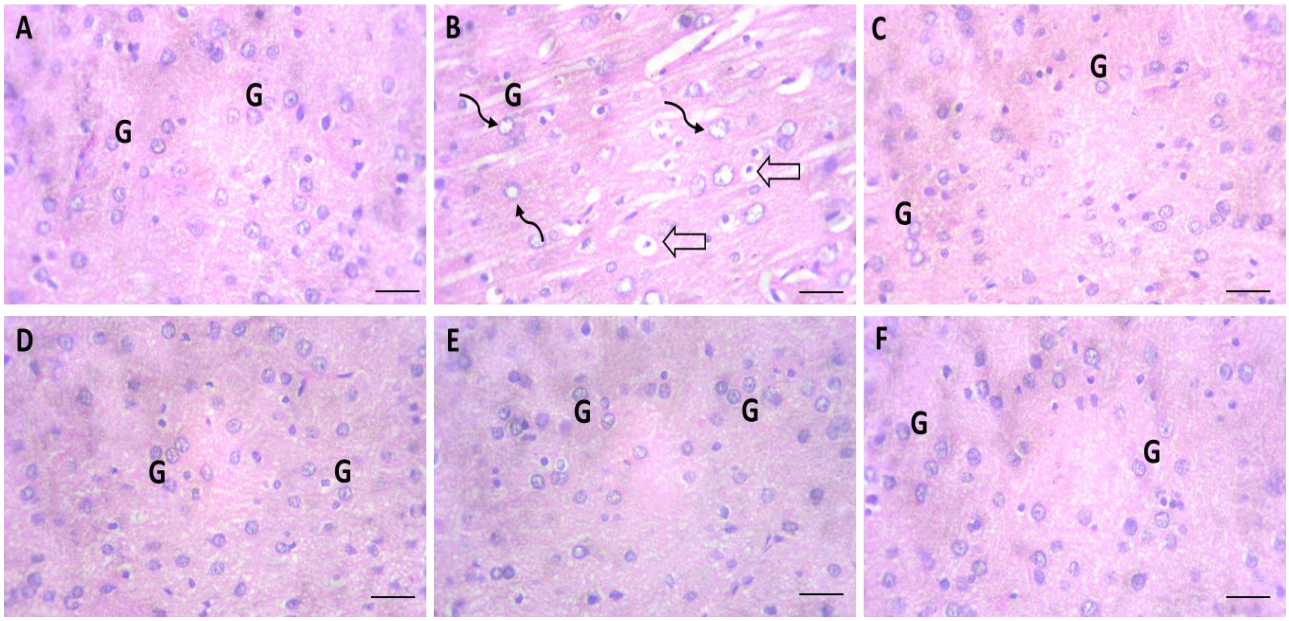



**PLATE 1: Representative histology of the cerebral cortex (internal granular layer, IV) in control and treatment rats. (A) Control group showing normal granular (G) cells. (B) Vacuolization and pyknotic nuclei observed (arrows); Cytoplasmic vacuolization is also observed (curved arrows) (C-F) Relatively normal features of the internal granular layer observed. (H&E; Scale bar: 25µm)**

# CHAPTER FIVE

## 5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

### 5.1 DISCUSSION

The presented results from a 28-day study involving different treatment groups of rats provide a comprehensive view of the effects of Nickel Chloride and Vinpocetine on body weight, brain weight, relative brain weight, cognitive function, antioxidant activity, and histological changes in the cerebral cortex.

One of the earliest and most telling signs of systemic toxicity was the significant reduction in body weight observed in rats exposed to NiCl2. This decline is not merely a superficial marker, it reflects deeper metabolic disruptions, likely stemming from impaired nutrient absorption and oxidative stress, as previously reported in studies on heavy metal exposure (Kumar *et al*., 2014). Interestingly, rats pretreated with 2.5mg/kg and 5mg/kg of Vinpocetine showed a dose-dependent recovery in body weight, food intake, and relative organ weight. These improvements suggest that vinpocetine may help restore metabolic balance, possibly by counteracting the inflammatory and oxidative cascades triggered by nickel toxicity. The relative brain weight, expressed as a percentage of body weight, remained relatively consistent across groups, suggesting that alterations in body weight were paralleled by changes in brain weight.

Beyond physical health, the study delved into cognitive function using the Y-MAZE test, a well-established measure of spatial working memory. Rats in the NiCl2 group exhibited a marked decline in spontaneous alternation behavior, this aligns with earlier findings that nickel disrupts cholinergic signaling and induces oxidative damage in brain regions critical for memory and learning (Sunderman, 1989). Spontaneous Alternation Behavior describes the tendency of animals, mainly rodents, to alternate in their pursuit of different stimuli in consecutive trials despite a lack of training or reinforcement (Harvey and Bovell, 2006). This behavior emerged from experiments using animals who naturally demonstrated the behavioral pattern when placed in previously unexplored maze shapes (Harvey and Bovell, 2006).

Spontaneous Alternation testing is a behavioral assessment method derived from Spontaneous Alternation Behavior. It is used to investigate exploratory behavior and cognitive function (related to spatial learning and memory) (Harvey and Bovell, 2006). Notably, pretreated rats with 2.5mg/kg and 5mg/kg of Vinpocetine, showed significant improvements in alternation behavior.

These results echo the neuroenhancing effects of Vinpocetine documented in prior research, where it was shown to improve cerebral blood flow and modulate intracellular signaling pathways (Zhang *et al.,* 2018; Molnár *et al.,* 1999).

According to a study by Chen *et al.* (2003), the cellular antioxidant defense regulates the potentially detrimental effects of free radicals produced by nickel chloride. The study demonstrated that acute exposure to NiCl2 significantly increased intracellular reactive oxygen species (ROS), lipid peroxidation, and hydroxyl radical levels, leading to reduced lymphocyte viability. However, the presence of antioxidants was shown to counteract these effects, highlighting their protective role against nickel-induced oxidative damage. According to Boujbiha *et al.* (2009), the superoxide dismutase enzyme catalyzes the conversion of superoxide radicals into hydrogen peroxide and molecule oxygen. In order to prevent oxidative cell damage, catalase transforms H2O2 to H2O and oxygen (Renugadevi and Prabu, 2010). Glutathione reductase (GSH) is a free radical scavenger in the brain (Cárdenas-Rodríguez *et al.,* 2014). A decrease in the GSH levels can elevate cellular velnurability towards oxidative stress characterized by accumulating reactive oxygen species (Cárdenas-Rodríguez *et al.,* 2014). Glutathione peroxidase (GPx) is an enzyme that plays a crucial role in promoting hydrogen peroxidase metabolism and protecting cell membrane structure and function from oxidative damage (Zhang *et al.,* 2020). These damages may include oxidative stress (Zhang *et al.,* 2020), neurological effects and impaired immune response (WHO, 2017).

The results of this investigation showed that Nickel Chloride caused oxidative stress by increasing lipid peroxidation, as indicated by a decline in cerebral SOD, CAT, GPx, and GSH activities. Pretreatment with 2.5mg/kg and 5mg/kg of Vinpocetine resulted in increased levels of antioxidants, particularly CAT, GSH, and GPx, indicating a potential protective effect against

oxidative damage. Malondialdehyde, a product of lipid peroxidation was significantly increased in the cerebrum upon exposure to Nickel Chloride thus indicating oxidative damage. An increase in MDA levels suggest an increase in oxidative stress and damage within the cells (Durak *et al.,* 2010). Similar to our findings, research shows that Nickel Chloride alone can increase the level of malondialdehyde (Wu *et al.,* 2013). Pretreatment with 2.5mg/kg and 5mg/kg of Vinpocetine resulted in decreased levels of malondialdehyde, indicating a potential protective effect against oxidative damage.

Histological analysis provided a visual confirmation of the biochemical and behavioral findings. The cerebral cortex of NiCl2 treated rats revealed extensive damage degeneration of pyramidal cells, cytoplasmic vacuolation, necrosis, and vascular congestion. These pathological features are hallmarks of metal-induced neurodegeneration and mirror the oxidative stress observed in the biochemical assays. In contrast, rats which where pretreated with 2.5mg/kg and 5mg/kg of Vinpocetine, displayed markedly improved cortical architecture. The damage was notably milder, with reduced vacuolation and preserved neuronal structure. These histological improvements reinforce the idea that vinpocetine exerts a protective effect at both the cellular and tissue levels, likely through its anti-apoptotic and vasodilatory properties (Molnár *et al.,* 1999).

Vinpocetine, especially at higher doses, demonstrated comparable efficacy in restoring biochemical balance and preserving histological integrity. This positions vinpocetine as a potential adjunct or alternative therapy in the management of heavy metal-induced neurotoxicity. Its multifaceted mechanism combining antioxidant, anti-inflammatory, and neurovascular actions makes it a compelling candidate for further exploration.

## 5.2 CONCLUSION

The potential neurotoxic effects of Nickel Chloride on rats are highlighted in this study, including weight loss, cognitive decline, oxidative stress, and histological damage to the prefrontal cortex of the cerebrum. It is significant to note that Vinpocetine pretreatment of Nickel Chloride exposed rats appears to offer protection against these adverse effects, highlighting the potential as a therapeutic agent in mitigating Nickel Chloride-induced toxicity.

## 5.3 RECOMMENDATION

To clarify the underlying processes of this protection and to evaluate the efficacy and safety of Folic acid, mechanistic investigations are necessary.

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