# ROLE OF RUTIN IN THE ATTENUATION OF LEAD-INDUCED HIPPOCAMPAL TOXICITY IN WISTAR RATS

**BY**

**OKWUBUASI MARK CHUKWUMEKA**
**BMS1902071**

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

**OCTOBER, 2023.**

# DECLARATION

I declare that:

1. This project report is based on the experimental work undertaken by me in the Department of Anatomy, University of Benin, under the supervision of ADAZE. B. ENOGIERU (PhD).

2. This work has not been previously submitted for the award of a degree elsewhere.

3. All ideas and views are essentially based on this research and where the views of others have been expressed, such words were duly acknowledged.

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**OKWUBUASI MARK CHUKWUEMEKA**

# CERTIFICATION

This is to certify that this project work titled 'Role of Rutin In The Attenuation Of Lead-induced Hippocampal Toxicity In Wistar Rats' was carried out by OKWUBUASI MARK CHUKWUEMEKA (BMS1902071) in the Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin.

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| **ADAZE. B. ENOGIERU (PhD).** **(Project supervisor)** | **DATE** |
| **DR. S. O INNIH** **(H.O.D.)** | **DATE** |
| **EXTERNAL EXAMINER** | **DATE** |

# DEDICATION

I dedicate this work to Amighty God for his infinite favour and Grace upon my life. I also dedicate this to my mother for her unwavering support and my late father whose memory and wisdom continue to inspire me.

## ACKNOWLEDGEMENT

I am profoundly grateful to the God Almighty for making this study project a reality.

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## ABSTRACT

Lead exposure is thought to be harmful and has been linked to behavioral abnormalities, hearing deficiencies, neuromuscular weakness, and decreased cognitive abilities in humans. Flavonoids have beneficial biological activities such as antioxidant, anti-inflammatory, anti-allergic, antiviral, anticarcinogenic effects. Flavonoids are the most recognised phytochemicals that function as antioxidants. Flavonoids' antioxidant activity includes suppressing ROS generation by inhibiting enzymes, scavenging free radicals, and regulating antioxidant defenses. Rutin is a typical dietary flavonoid that is nontoxic and naturally derived. It has a variety of beneficial biological properties including anti-cancer, antioxidant, antidiabetic, anti-inflammatory, anti-bacterial, anti-fungal, neuroprotective, cardioprotective, hepatoprotective, nephroprotective, haematoprotective, anti-arthritis, anthelmintic effects. Accordingly, this study was designed to investigate the possible attenuative effects of Rutin on lead-induced neurotoxicity in Wistar rats. After purchase and acclimatization, the Wistar rats were weighed and divided into six equal groups (control and treatment groups). Group A (Control) was administered 1 ml $dH_2O$/day. Group B (Pb) was administered 100 mg/kg body weight (BW) of Pb acetate only. Group C (RUT1 + Pb) was administered 50 mg/kg BW of Rutin and 100 mg/kg BW of Pb acetate. Group D (RUT2 + Pb) was administered 100 mg/kg BW of Rutin and 100mg/kg of Pb acetate. Group E (RUT1) was administered 50 mg/kg BW of Rutin only and Group F (RUT2) was administered 100mg/kg BW of Rutin only. The administration, via an orogastric tube, lasted for 28 days and rats were fed with standard rat chow and had free access to water throughout the entire study period. All Rutin administration pre-treatment were done one hour before Lead. Animals were weighed and neurobehavioral activity (Novel object recognition test) was evaluated. The rats were then sacrificed for sample collection, and the hippocampus was harvested for assessment of antioxidant activity and histological alterations . The findings showed that the Pb group showed a significant decrease ($p<0.05$) in final body weight (FBW) compared to the control and Rutin treated groups, which showed a greater FBW. Neurobehavioral findings revealed that rats in the Pb group had significantly lower neurobehavioral function when compared to Control and Rutin treated groups. The Pb alone groups demonstrated oxidative stress (low antioxidant activity and increased lipid peroxidation), whereas the Control and Rutin treated groups had significant increase ($p<0.05$) in antioxidant activity. Histological findings shows altered morphology with the presence of vacuoles and pyknotic nuclei in the CA1 region of the Pb treated group, however the pretreated groups showed a healthier tissue architecture when compared to lead only treated group. In conclusion, the findings showed that Rutin was not toxic to the animals and protected against Pb toxicity.

# CHAPTER ONE

# INTRODUCTION

## 1.1 BACKGROUND OF THE STUDY

Metals are a significant class of hazardous substances present in a variety of occupational and environmental settings. Due to the prevalence of exposure, there is currently a great deal of interest in the effects of these hazardous substances on human health (Mahurpawar, 2015). These heavy metals are dispersed in the environment by a number of natural processes, including volcanic eruptions, spring waters, erosion, and bacterial activity, as well as by anthropogenic activities, such as burning fossil fuels, using industrial processes, farming, and feeding (Engwa *et al.,* 2019). When heavy metals build up in soft tissues and are not metabolized by the body, they become poisonous (Masindi *et al.,* 2018). Children have a higher risk of lead poisoning because their bones do not absorb lead like they do in adults. Instead, excess lead is absorbed by their various soft tissues. People who are deficient in calcium, zinc, or iron can easily absorb lead because it functions as an analog of calcium. Male and female reproductive systems are both impacted by lead (Collin *et al.,* 2022). Ninety-nine percent of the lead that is ingested is maintained in the blood for around 30 to 35 days after absorption, and over the subsequent four to six weeks it is distributed and accumulates in different tissues, including the liver, kidney cortex, aorta, brain, lungs, spleen, teeth, and bones (Gillis *et al*., 2012). Lead-associated deficits have been documented in most fields including verbal intelligence quotient (IQ), performance IQ, academic skills such as reading and mathematics, visual/spatial skills, problem-solving skills, executive functions, fine and gross motor skills as well as memory and language skills. Numerous neurologic problems can develop in the brain as a result of lead-induced damage to

the prefrontal cerebral cortex, hippocampus, and cerebellum (Sanders *et al.,* 2009). Lead can interfere with biological systems through changing cell signaling, molecular interactions, and eventually cellular function (Lakshmi *et al*., 2013). It is recognized that the production of reactive oxygen species (ROS), such as superoxide radicals ($O^{2-}$), hydrogen peroxide ($H_2O_2$), hydroxyl radicals (.OH), and lipid peroxides, caused by Lead ions, can harm a variety of cellular components (Bandyopadhyay *et al.,* 2014). Given that lead causes oxidative stress, enhancing the body's antioxidant defense may assist in the protection against lead toxicity, particularly for those who are susceptible to exposure.

Due to their capacity to donate electrons, which neutralize radicals without creating another, antioxidants are beneficial in decreasing and avoiding damage from free radical reactions (Anwar *et al*., 2018). Over 8000 distinct compounds known as flavonoids are phenolic components that have been identified from a wide variety of vascular plants. They function in plants as light filters, photoreceptors, visual attractants, feeding repellants, antioxidants, antimicrobials, and photoreceptors. Numerous studies have revealed that flavonoids have biological acticities such as antiviral, anti-inflammatory, antiallergenic, and vasodilating properties (Pietta, 2000). The antioxidant activity of flavonoids, however, has drawn the greatest attention because of their capacity to both prevent the creation of free radicals and scavenge existing ones. (Pietta, 2000). One of the frequent secondary metabolites of plants is rutin, a naturally occurring flavonoid glycoside also known as rutoside, quercetin-3-O-rutinoside, sophorin, or vitamin P (Semwal *et al.,* 2021). Chemically, it is referred to as 2-(3,4-dihydroxyphenyl).-5,7-dihydroxy-3-[α-l-rhamnopyranosyl-(1→6)-β-d-glucopyranosyloxy]-4H-chromen-4-one. It has a molar mass of 610.521 g/mol and is a yellowish powder. It rapidly dissolves in pyridine but is poorly soluble in water. The most significant species that contain

rutin as one of the principal ingredients include *Carpobrotus edulis, Ruta graveolens, Fagopyrum esculentum, Camellia sinensis, Citrus sinensis, Malus domestica, Ficus carica, and Sophora japonica*. The earliest commercial source of rutin was green buckwheat (Fagopyrum esculentum), which has a rutin content of 3-5% (Semwal *et al.,* 2021).

Rutin works as a sedative, anticonvulsant, analgesic and anti-arthritic agent, and has benefits against diabetes and hypercholesterolemia in addition to boosting neural crest cell survival, thyroid uptake, and reducing neuro-inflammation (Singh *et al.,* 2020). In addition to these additional protective roles, it also has anticancer effects, chemotherapeutic activity, antibacterial, antifungal, antimycobacterial, larvicidal, antimalarial, antiretroviral, antiviral, neuroprotective activity, hepatoprotective activity, nephroprotective activity, and wound healing activity (Singh *et al.,* 2020).

## 1.2 AIM AND OBJECTIVES OF THE STUDY

### AIM

This study investigated the protective activity of Rutin on lead acetate-induced Hippocampal neurotoxicity in Wistar rats.

### SPECIFIC OBJECTIVES

The specific objectives of the study were to investigate the protective activity of Rutin on:

1. The brain and body weight changes in rats treated with and without lead acetate.
2. The neurobehavioral activity (Novel object recognition test) of rats treated with and without lead acetate.

3. The antioxidant activities (Superoxide dismutase (SOD), Catalase (CAT), Reduced Glutathione (GSH) , Glutathione peroxidase (GPx) ) in rats treated with and without lead acetate.
4. The Pb concentration in rats treated with and without Lead acetate.
5. Lipid peroxidation (MDA) in rats treated with and without Lead acetate.
6. The histology of the Hippocampus in rats treated with and without lead acetate.

## 1.3 STATEMENT OF RESEARCH PROBLEM

The toxicity of heavy metals, which are substantial environmental contaminants, poses a serious threat to the ecological, evolutionary, nutritional, and environmental balances. Lead is an extremely poisonous metal, and regular exposure has led to excessive environmental deterioration and health issues in many regions of the world (Bhat *et al.,* 2019). Chronic Lead exposure can cause short-term memory loss, exhaustion, sleep issues, depression, nausea, abdominal pain, loss of coordination, and anemia, among other symptoms. In the case of children, lead intoxication is more likely to have an impact on their growing central nervous system (Słota *et al.,* 2022). Numerous physiological processes, such as neurotransmission and the maintenance of a constant blood pressure, depend heavily on calcium. When lead enters the body, it partially replaces the calcium needed for these vital processes. This causes a variety of problems, including neurological and cardiovascular damage (Clay *et al.,* 2023). Lethargy, abdominal pain, loss of appetite, and irritability are the earliest symptoms of severe lead poisoning, which later results in seizures and coma. Low-dose lead exposure has been linked to neurological problems, anemia, impaired heme synthesis, elevated auditory feedback threshold, and low blood levels of vitamin D. In Nigeria, between 2010-2013, more than Seventeen (17,

000) villagers were poisoned and 400-500 young children died in Zamfara State , as a result of lead poisoning (Tirima, 2014). The economic losses from lead-related IQ decline in Africa are approximately 4% of GDP, while these losses in Asia are approximately 2% of GDP (Fuller *et al.,* 2022). According to an update from World Health Organization (WHO) in 2021, nearly half of the Two (2) million lives lost in 2019 were caused by lead exposure. Lead exposure is estimated to account for 21.7 million years lost to disability and death worldwide due to long-term effects on health, including 30% of the global burden of idiopathic intellectual disability, 4.6% of the global burden of cardiovascular disease and 3% of the global burden of chronic kidney diseases (WHO, 2022).

## 1.4 JUSTIFICATION OF STUDY

Chelation therapy, which enhances Lead elimination, is the most often employed therapeutic approach for Lead (Pb) toxicity. However, chelators for Pb poisoning are said to have a variety of safety and effectiveness issues. Due to the adverse reactions they cause, none of the chelation therapies (CaNa2EDTA and meso-2,3-dimercaptosuccinic acid) for Pb poisoning have yet been licensed for clinical use (Zhai *et al.,* 2014).

Rutin, commonly known as vitamin P, is one of the most bioactive flavonoids and has anti-oxidant and anti-inflammatory activity . It binds to the iron ion ($Fe^{2+}$) in humans, preventing it from connecting to hydrogen peroxide and producing a highly reactive free radical that can damage cells (Medvidović-Kosanović *et al.,* 2010). Several studies have found that Rutin may have neuroprotective effects in a variety of neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease and ischemic stroke (Habtemariam, 2016; Moshahid Khan *et al.,* 2012; Cordeiro *et al.,* 2022; Rana *et al.,* 2023). Rutin has also been seen to attenuate toxicity induced by heavy metals like mecury, cadmium, managnese (Caglayan *et al.,*

2019; Oboh *et al.,* 2020; Nkpaa *et al*., 2020). However, there is a scarcity of scientific evidence on the role of Rutin in the prevention of lead-induced Hippocampal toxicity. As a result, the purpose of this study is to investigate into the potential neuroprotective action of Rutin against lead-induced Hippocampal toxicity in Wistar rats. The findings of this study can provide additional information on the neuroprotective effect of Rutin, which can be useful for the development of novel neuroprotective drugs for the treatment of Lead toxicity.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 COMPOUND OF STUDY: (RUTIN)

Medicinal plants have long been used in traditional medicine. In the field of drug development, phytochemicals have proved crucial in uncovering novel potential medicines and compounds. (Grdina et al., 2002, Weiss and Landauer, 2003, Arora *et al.,* 2005).

Flavonoids, a major class of plant-based compounds, are polyphenolic substances with a benzopyrone structure. Approximately 4,000 flavonoid variants have been identified in various plant sources (Guardia *et al.,* 2001).

Rutin, also known as 3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside (depicted in Fig. 2.1), is a flavonol compound that is widely distributed in various plants like passion flower, buckwheat, tea, and apples. It serves as an essential nutritional element in these food sources (Harborne, 1986). Rutin, alternatively known as rutoside, quercetin-3-rutinoside, and sophorin, is a flavonoid glycoside commonly present in buckwheat (Kreft *et al.,* 1997).

Rutin is named after the plant Ruta graveolens, which also contains this compound as well. It is a glycoside that combines the flavonolic aglycone quercetin with the disaccharide rutinose. Rutin has been demonstrated to have antioxidant, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective, and cardioprotective activities (Javed *et al.,* 2012).

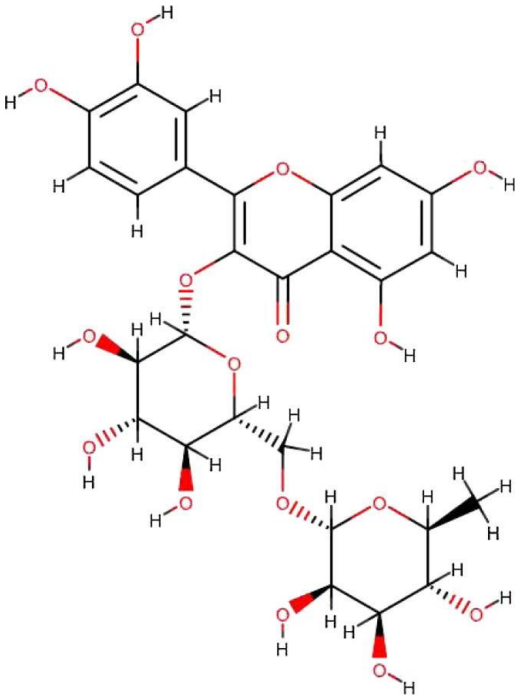

Figure 2.1 showing Chemical structure of Rutin obtained from *https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5355559/figure/f0005/* , accesssed on October 5th , 2023.

### 2.1.1 TRADITIONAL USES OF RUTIN

Historically, rutin has been employed to support blood circulation, believed to enhance the resilience and flexibility of blood vessels, including arteries and capillaries. It has also been used in various cultures to manage cholesterol, heart attacks, strokes, and in the management of arthritis pain (Wilson and Gotten, 2017).

### 2.1.2 PHARMACOLOGICAL ACTIVITIES OF RUTIN

#### 2.1.2.1 Neuro protective effects of Rutin

Neuronal degeneration is caused by pathologic processes such as oxidative stress, inflammation, mitochondrial dysfunction, apoptosis, and genetic factors. The central nervous system, which has

a high concentration of polyunsaturated fatty acids, is significantly affected by peroxidation processes. Because the brain has lesser antioxidant activity than other organs, neural cells are more prone to oxidative harm (Singh *et al.,*2019).

Rutin's antioxidant action prevents neurotoxicity. Previous research discovered that rutin's neuroprotective impact in rat brain ischemia was due to its capacity to lower TBARS (Thiobarbituric acid reactive substances), $H_2O_2$, and GSH in the hippocampus and frontal cortex during middle cerebral artery occlusion. In addition to its capacity to decrease p53 expression and increase antioxidant enzymatic activity (Almutairi *et al.,* 2017). It binds to the iron ion (Fe2+) in humans, preventing it from connecting to hydrogen peroxide, which would otherwise produce a highly reactive free radical that can damage cells (Afanas'av *et al.,* 1989).

#### 2.1.2.2 Anti-neuroinflammatory effects

Rutin has been demonstrated to protect the brain from neuroinflammation and ischemia-related problems. Rutin minimizes the occurrence of 'ischemic neuronal apoptosis' by suppressing p53 expression and lipid peroxidation while enhancing the activity of 'endogenous antioxidant defense enzymes' when administered (Khan *et al.,* 2009). Rutin has also been shown to be efficacious in hypoxia, glutamate exposure, and oxidative stress (Pu *et al.,* 2007). Furthermore, it has been discovered that rutin treatment reduces 'neuroinflammation' in a rat model of'sporadic dementia of Alzheimer's type' (Javed *et al.,* 2012), and it exhibits neuroprotective effects in 'dexamethasone-treated mice' (Tongjaroenbuangam *et al.,* 2011).

#### 2.1.2.3 Sedative effects

The hole board test, thiopental-induced sleeping time test, and locomotor activity test in mice were used to assess the effects of Rutin on the CNS and behavior. Rutin had a CNS depressive

effect when delivered intraperitoneally. However, research has shown that Rutin's CNS depressing effect is most likely unrelated to the activation of GABAA receptors (Fernández *et al.,* 2006).

#### 2.1.2.4 Anticonvulsant effects

Rutin also has anticonvulsant properties and appears to be a safe option for individuals with epilepsy since it neither affects the effectiveness of prescribed antiepileptic medications nor shows any negative side effects (Nieoczym *et al.,* 2014).

#### 2.1.2.5 Anti-Alzeheimer activity and treatment of hyperkinetic movement disorders

Rutin has been found to reduce the activity of proinflammatory cytokines like TNF-α and IL-1β in microglia, which suggests its potential utility in Alzheimer's disease treatment. This effect is evident from its ability to protect against the harmful effects of β-amyloid oligomers (Wang *et al.,* 2012). Rutin also diminishes inflammation induced by streptozotocin by lowering the levels of glial fibrillary acidic protein, interleukin-8, cyclooxygenase-2, inducible nitric oxide synthase, and nuclear factor-kB, thus preventing significant structural changes in the rat hippocampus. This property could be valuable in preventing cognitive decline and proving beneficial in the treatment of 'sporadic dementia of Alzheimer's type' (Javed *et al.,* 2012).

#### 2.1.2.6 Antidepressant effects

Forced swimming test and tail suspension test in mice were utilized to analyze ‘antidepressant-like effects’ of Rutin isolated from Schinus molle. There was a reduction in the immobility time in the tail suspension test. There was no alteration in locomotor activity. Studies demonstrated the antidepressant-like effect of rutin mediated due to increasing the availability of serotonin and noradrenaline in the synaptic cleft (Machado *et al.,* 2008).

#### 2.1.2.7 Stroke recovery

Stroke is a significant global public health concern, contributing substantially to both mortality and adult disability (Writing Group Members *et al.,* 2009). Following ischemic brain injury, two critical processes that occur are oxidative stress and inflammation (Deb *et al.,* 2010). Researchers investigated the protective effects of rutin in an animal model of focal cortical ischemia induced by thermocoagulation of superficial blood vessels in the primary motor (M1) and somatosensory (S1) cortices. Rutin administration notably enhanced the recovery of sensorimotor deficits by reducing neurodegeneration around the site of cortical injury (Ortolani *et al.,* 1995).

#### 2.1.2.8 Analgesic and Nocioceptive property

The pain-relieving properties of Rutin were investigated using the hot plate test in Swiss albino mice, and it was established that rutin indeed has an analgesic effect (Rylski et al., 1979). Additionally, it was confirmed that Rutin displayed both peripheral and central antinociceptive activities (Selvaraj *et al.,* 2014).

#### 2.1.2.9 Anti-athritic effects

Animals treated with Rutin exhibited a significant reduction in both rheumatoid arthritis and Fanconi anemia by curbing the excessive production of oxygen radicals (Ostrakhovitch and Afanas'ev, 2001). In a rat model of adjuvant arthritis, Rutin effectively inhibited inflammation during both the acute and chronic phases, with its highest efficacy observed in the chronic stage (Guardia *et al.,* 2001). Rutin's antifungal and anti-arthritic properties make it a valuable therapeutic option for septic arthritis caused by Candida albicans (Han, 2009). Additionally, an independent study found that Rutin slowed down the expression of inflammatory and cartilage-degrading markers in osteoarthritic lesions in Hartley guinea pigs (Horcajada *et al.,* 2014).

#### 2.1.2.10 Antidiabetic effects

Streptozotocin is a hazardous substance that is known to lower insulin levels by destroying pancreatic islets. It specifically targets pancreatic -cells, producing free radicals of oxygen and nitrogen monoxide while lowering NAD and NADP levels. This causes excessive glucose synthesis and decreased use by tissues, resulting in hyperglycemia (Chattopadhyay, 1993). Chronic administration of rutin to diabetic rats induced by streptozotocin resulted in lower blood glucose levels, increased insulin levels, and the restoration of glycogen content and glycolytic enzymes. Rutin-treated diabetic rats also had significantly better pancreatic islet health and less fat infiltration (Prince and Kamalakkannan, 2006, Srinivasan *et al.,* 2005). Rutin treatment further resulted in reduced fasting plasma glucose, glycosylated hemoglobin, C-peptide, and malondialdehyde levels in streptozotocin-induced diabetic rats (Kamalakkannan and Prince, 2006). Rutin protected against streptozotocin-induced hepatic (Fernandes et al., 2010) and cardiac toxicity (Krishna et al., 2005), as demonstrated by lower levels of enzymes such as ALT, AST, and LDH in serum, liver, and heart. Rutin also impacted matrix metalloproteinase activity and protected the kidney from streptozotocin-induced damage (Kamalakkannan and Prince, 2006). Furthermore, Rutin increased glucose absorption in the soleus muscle, most likely by activating extracellular calcium and calcium-calmodulin-dependent protein kinase II. Rutin was also engaged in boosting intracellular calcium levels, which helped to activate DNA (Kappel *et al.,* 2013). For glycemic control, rutin enhanced insulin receptor kinase activity, promoting the insulin signaling pathway, leading to increased GLUT4 translocation and improved glucose uptake (Hsu *et al.,* 2014).

#### 2.1.2.11 Anti-hypercholesterolemic effects

Rutin is a safe and specific regulator for managing hypercholesterolemia. In a study involving a diet-induced hypercholesterolemic Golden Syrian hamster model, rutin effectively decreased plasma triglyceride levels in the test animals (Kanashiro *et al.,* 2009). Additionally, Rutin led to a reduction in both total cholesterol and HDL cholesterol levels (Silva *et al.,* 2001).

#### 2.1.2.12 Antihypertensive effects

Buckwheat, which contains a significant amount of rutin, has been shown to protect aortic endothelial cells from oxidative damage by lowering nitrotyrosine levels. Buckwheat sprout extract is also antihypertensive and may protect arterial endothelial cells from the damaging effects of oxidative stress (Kim *et al.,* 2009). The restoration of decreased baroreflex sensitivity and vascular reactivity in hypertensive rats is mostly due to the reduction in oxidative stress caused by oral rutin treatment (Mendes-Junior *et al.,* 2013). Rutin enhances nitric oxide (NO) production in human endothelial cells, thereby improving endothelial function (Ugusman *et al.,* 2014).

#### 2.1.2.13 Anti-coagulation effects

Chan et al. (2009) investigated how rutin impacts the anticoagulant effect of oral warfarin, how it interacts with proteins, and the pharmacokinetics of its enantiomers in rats. In laboratory testing, Rutin boosted the binding of both S- and R-warfarin to blood proteins. Rutin therapy reduced the elimination half-life of S-warfarin by 37%, owing to a 69% increase in the clearance of the unbound S-enantiomer. To summarize, coadministration of Rutin could decrease the anticoagulant effect of racemic warfarin (Chan *et al.,* 2009).

#### 2.1.2.14 Antiplatelet aggregatory effects

In a laboratory setting, rutin demonstrated a dose-dependent ability to inhibit washed rabbit platelet aggregation induced by platelet activating factor. Additionally, Rutin effectively reduced the increase in intra-platelet free calcium concentration triggered by platelet activating factor in a manner that correlated with the dosage used (Chen *et al.,* 2002).

#### 2.1.2.15 Antiulcer effects

A peptic ulcer is a health condition that affects a significant portion of the global population. Ulcers develop when there is an imbalance between factors that can harm the stomach lining, known as 'aggressive' factors, and those that protect it. Aggressive factors include substances like stomach acid (HCl), pepsins, nonsteroidal anti-inflammatory drugs, Helicobacter pylori infection, bile acids, lack of blood supply (ischemia), oxygen deficiency (hypoxia), smoking, and alcohol consumption. On the other hand, protective factors consist of bicarbonate, a layer of mucus, adequate blood flow to the stomach lining, prostaglandins (PGs), and growth factors (Harold *et al.,* 2007).

Ethanol is a well-known substance that can harm the lining of the stomach, as shown in both animal and clinical studies. When ethanol is present in concentrations exceeding 400 ml/l, it leads to significant changes in the appearance of the stomach, characterized by mucosal redness, tissue damage, swelling, and bleeding either in the mucosal or submucosal layers (Oates and Hakkinen, 1988 ; Szabo and Goldberg, 1990). These injuries are likely caused by the formation of oxygen-derived free radicals (Pihan et al., 1987 ; Szelenyi and Brune, 1988). However, pretreatment with rutin before administering ethanol provides considerable protection against this damage, including the restoration of glutathione peroxidase levels and an 'anti-lipoperoxidant effect' (La Casa *et al.,* 2000).

In a similar vein, in a model of ulcers induced by indomethacin, rats that were pretreated with rutin showed improvements in oxidative stress and biochemical parameters. This improvement is possibly attributed to reduced neutrophil infiltration, suppression of oxidative stress, and the restoration of nitrite/nitrate levels. These protective effects were also confirmed through histopathological examinations (Abdel-Raheem, 2010).

Furthermore, another study delved into how rutin affects gastric proton pumps at a molecular level. Rutin exhibited concentration-dependent inhibition of goat gastric ATPase, with an IC50 value of 36 μg/ml. This suggests that rutin exerts its antiulcer effect by inhibiting gastric proton pumps (Dubey *et al.,* 2013).

#### 2.1.2.16 Anti-asthmatic effects

Researchers investigated the effectiveness of Rutin as an antiasthmatic agent in conscious guinea pigs that had been sensitized to ovalbumin and then exposed to aerosolized ovalbumin to induce airway resistance during both the immediate and late phases of their response. The study found that Rutin significantly reduced specific airway resistance and mitigated the immediate-phase response. It also showed a decrease in histamine, phospholipase A2, and eosinophil peroxidase levels, along with a reduction in the recruitment of neutrophils and eosinophils into the lungs (Jung *et al.,* 2007). There is historical evidence suggesting the use of Rutin, in combination with vitamins C and K, in the management of whooping cough (De Sarmento and Kimura, 1957). Moreover, Rutin has been effectively employed in the treatment of idiopathic chylothorax in cats and whippets, as supported by various studies (Kopko, 2005 ; Gould, 2004; Schuller *et al.,* 2011).

#### 2.1.2.17 Antiosteoporotic and antiosteopenic properties

Osteoporosis is a condition that is characterized by decreased bone strength, which increases the risk of fractures (Bhutani and Gupta, 2013). It's becoming a major health concern, particularly among the elderly. Osteoporosis develops when bone breakdown by osteoclasts outpaces bone synthesis by osteoblasts (Lau and Guo, 2011, Roux, 2010). Most treatments for osteoporosis focus on inhibiting bone resorption by osteoclasts. Parathyroid hormones are one class of agents that promote bone formation (Hodsman *et al.,* 2005).

In experiments related to bone health, rutin has been found to promote the growth and differentiation of human osteoblast-like MG-63 cells. It also increases the activity of alkaline phosphatase, the expression of collagen type I, and the mineralization of bone (Hyun *et al.,* 2014). Similar positive effects have been observed with rat calvarial osteoblast cells (Yang *et al.,* 2006). Rutin inhibits the formation of osteoclasts by reducing oxidative stress and TNF-alpha production through the inhibition of NF-kappaB activation (Kyung *et al.,* 2008). Additionally, rutin slows down bone loss induced by ovariectomy in rats by decreasing resorption and increasing osteoblastic activity (Horcajada-Molteni *et al.,* 2000). Consequently, rutin can also be considered a substance that stimulates osteoblast activity.

#### 2.1.2.18 Anticataract and opthalmic effects

The development of cataracts, a complication of diabetes, is connected to the formation of advanced glycation end products (AGE). Therefore, suppressing this glycation process may be a useful technique for avoiding such consequences. In one study, Rutin was discovered to successfully reduce protein glycation, bind to metal complexes, and partially inhibit post-Amadori formation (Muthenna *et al.,* 2010). Another study employed selenium to induce cataracts in Wistar rat pups and looked at the preventive effects of rutin on cataract development.

Rutin treatment resulted in the restoration of antioxidant enzymes in the ocular lenses of rats, as well as a decrease in the formation of malondialdehyde. The findings imply that rutin may help prevent cataract formation, presumably through antioxidant mechanisms (Isai *et al.,* 2009). Rutin has been shown to significantly decrease intraocular pressure when administered orally (Vetrugno *et al.,* 2012). The combination of vitamins B1, B2, forskolin, and rutin has a protective effect on the ocular surface, helping to restore the normal balance of the tear film that has been upset by pollutants (Nebbioso et al., 2013).

#### 2.1.2.19 Diuretic effects

The vascular endothelium is impacted by quercetin, a chemical derived from rutin that is commonly present in Hibiscus sabdariffa Linn. It triggers nitric oxide release, which then improves renal vasorelaxation and finally results in an increase in kidney filtration (Alarcón-Alonso *et al.,* 2012).

#### 2.1.2.20 Antifungal effects

Candida gattii growth has been demonstrated to be inhibited by rutin, with a minimum inhibitory dose of 60 g/ml (Johann *et al.,* 2011). It has been suggested that altering rutin chemically by including replacement groups could alter its physical and chemical characteristics, such as its electron density, hydrophobicity, and steric strain, potentially increasing its antifungal potency. Rutin has also been suggested as a potential treatment for septic arthritis brought on by C. Albicans (Han, 2009).

#### 2.1.2.21 Antimycobacterial effect

A study reported that a flavonoid-rich extract, containing Rutin, had antimycobacterial properties against Mycobacterium smegmatis (da Cruz *et al.,* 2012).

## 2.2 LEAD ACETATE

Numerous chemicals in the environment and workplaces can negatively affect reproductive health and fertility, as noted by (Cherry *et al.,* 2008 ; Taskinen *et al.,* 2011). Among these, lead is a prominent environmental and occupational hazard. This heavy metal is found naturally in the environment and is emitted through activities like burning fossil fuels, mining, and manufacturing, as mentioned by Gabby (2006). It is also used in various domestic and industrial applications, including ammunition production, cosmetics, glass pigments, lead-acid batteries, solder and pipes, paint pigments, and X-ray shielding devices (Gabby, 2006). Lead contamination can arise from sources like tainted food, lead-containing water pipes, improper food preservation, industrial emissions, traffic pollution, paint, cosmetics, and drinking water (López-Carrillo *et al.,* 1996).

### 2.2.1 LEAD EXPOSURE

One of the most significant environmental toxins is lead (Pb), and numerous studies have shown that exposure to lead can lead to health issues. Natural properties such as softness, high malleability, ductility, and a low melting point make Lead a serious hazard to human health in several ways. Lead is a component of the earth's crust. Lead is a metal that can be found in the air, water, and soil. It is principally derived from a wide range of produced items, including leaded gasoline, paints, ceramics, solders, water pipes, hair color, cosmetics, airplanes, farm equipment, shielding for x-ray machines, etc (Boskabady *et al.,* 2018). Due to a lack of information about lead's safe handling, many employees in developing nations continue to be exposed to its hazardous effects; particular populations in the general population, such as pregnant women and their fetuses, are still at risk from lead exposure. In this context, lead exposure and occupational and environmental contamination continue to be a major threat to

human health. The primary exposure pathways, particularly for subjects who are exposed at work, are inhalation and ingestion. Lead is absorbed, dispersed, and accumulates in the blood, bones, and soft tissues. Depending on the degree and length of exposure, lead exposure in humans can result in a variety of biological effects, including harmful effects on the hematological, cardiovascular, nervous, and reproductive systems (Fenga *et al.,* 2020). Early-life cognitive impairment has been strongly linked to childhood exposure to environmental Lead. This harmful substance has been recognized as a blatant inhibitor of early-life neurodevelopment. Elderly cognitive deterioration is also correlated with lead exposure. The onset of cognitive decline is an important intermediary for the development of neurodegenerative diseases, specifically Alzheimer's disease (Eid and Zawia , 2016).

### 2.2.2 TOXICOKINETICS OF LEAD

The pharmacokinetics of lead (Pb) in humans is complex. Humans are in a state of positive lead balance from birth.Humans are in a state of positive lead balance from birth.Data from the 1976–1980 cycle of the National Health and Nutrition Examination Survey (NHANES) indicated that an estimated 88% of children aged 1–5 years had BLLs (Blood lead levels) ≥ 10 μg/dL. Since then, the percentage has fallen sharply, to 4.4% during 1991–1994, to 1.6% during 1999–2002, and to 0.8% during 2007–2010. National estimates of the geometric mean (GM) BLL for children aged 1–5 years declined significantly over time, from a 1976–1980 estimated GM BLL of 15 μg/dL to a 2015–2016 estimated GM BLL 0.82 μg/dL (Raymond *et al.,*2014 ; Roux,2010).

#### 2.2.2.1 Absorption

##### 2.2.2.1.1 Gastrointestinal absorption

The amount of lead absorbed in the gastrointestinal (GI) tract varies. When compared to adults, children are at a larger risk of lead absorption. The physical shape of lead, the size of the

particles swallowed, the time it takes for items to transit through the GI system, and the individual's nutritional status are all factors that influence lead absorption.

As particle size grows, lead absorption reduces; smaller particles result in more complete absorption. As a result, exposure to lead dust causes more absorption than exposure to a comparable amount of lead from lead-based paint chips..

Lead absorption is exacerbated when iron, zinc, and/or calcium deficiencies exist. Malnutrition also increases lead absorption, whereas a diet rich in phosphorus, riboflavin, vitamin C, and vitamin E decreases it. A high-fat diet and a low-calorie diet have been associated to higher lead absorption.

Age has an inverse relationship with lead absorption. Children typically absorb 30-50% of ingested lead, whereas adults absorb just about 10% (Holstege *et al.,* 2013).

#### 2.2.2.1.2 Dermal absorption

Lead does not penetrate through intact skin in its elemental or inorganic forms. Organic lead compounds, such as tetraethyl lead, can, nonetheless, be absorbed via the skin. (Holstege *et al.,* 2013).

#### 2.2.2.1.3 Inhalational absorption

When lead is inhaled in fine particles, it can be absorbed directly through the lungs or transported to the neck by the mucociliary system, where it is ingested and absorbed by the GI system. The degree of absorption through the respiratory system is determined by characteristics such as particle size, lung capacity, amount of deposition, and mucociliary clearance of breathed lead. Almost all of the lead ingested as fumes is directly absorbed by the lungs (Holstege *et al.,* 2013).

#### 2.2.2.2 Distribution

Lead is distributed in the blood, bone, and soft tissues. Only 1% of the blood's lead concentration, which is 99% attached to red blood cells, is present in the plasma and can be exchanged for lead found in other organs. In those with normal renal function, the half-life of lead in the blood is around 30 days, while it is greater in people with renal insufficiency.

The half-life of lead in bones is decades, making up more than 95% of the body's total lead content. As a result, the body's primary storage location for this element is bone (Vaziri, 2008).

#### 2.2.2.3 Excretion

Lead ingested into the body is not readily digested, therefore excretion is minimal and the urinary system is the main route of excretion. Additionally, chelating agents can make it easier for the body to eliminate lead through the urine. Due of the little amounts of lead that are eliminated through the gastrointestinal tract, sweat, and nails, these pathways are not given much weight (Kabeer *et al.,* 2019).

### 2.2.3 MECHANISM OF LEAD POISONING

Lead's extensive historical use, driven by its adaptable qualities like malleability, ductility, low conductivity, softness, and resistance to corrosion, has made it widely prevalent for over 6000 years (D'Souza *et al.,* 2011). The primary cause of lead poisoning occurs when the generation of free radicals surpasses the body's capacity to effectively eliminate them, leading to cellular damage (Flora, 2002). There are two simultaneous pathways of oxidative stress:

(a) the production of reactive oxygen species (ROS) such as hydroperoxides (HOO•), singlet oxygen, and hydrogen peroxide ($H_2O_2$), and

(b) the depletion of the body's natural antioxidant reserves (Flora, 2002).

Lead's strong tendency to share electrons plays a crucial role in forming covalent bonds. These covalent bonds are established between lead and sulfhydryl groups found in antioxidant enzymes, making these enzymes particularly susceptible to lead-induced inactivation (Hultberg *et al.,* 2001). Additionally, lead binds to the sulfhydryl groups of reduced glutathione (GSH), rendering it ineffective. Lead also disrupts the activity of various enzymes, including δ-aminolevulinic acid dehydratase (ALAD), glutathione reductase, glutathione peroxidase, and GST (Glutathione-S-transferase), leading to a decrease in GSH levels (Ahamed *et al.,* 2007). Furthermore, lead interferes with the function of antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT) by replacing zinc ions, which serve as cofactors for these enzymes. Lead targets the sulfhydryl groups of these enzymes as well. This reduction in SOD concentration impairs the removal of superoxide radicals, while decreased CAT activity hinders the scavenging of superoxide radicals ($O_2$ $^{-\bullet}$) (Flora *et al.,* 2007).

Exposure to lead causes alterations in the blood system, leading to hematological changes (Assi et al., 2016). These changes occur primarily due to the poisoning of three enzymes involved in the heme biosynthesis pathway: ALAD, ferrochelatase, and δ-aminolevulinic acid synthetase (ALAS) (CDC, 2011). Additionally, lead disrupts the integrity of red blood cells (RBCs) by damaging their cell membranes, making them more fragile (White *et al.,* 2007).

The organ most sensitive to lead exposure is the brain (Cleveland *et al.,* 2008). Lead can directly or indirectly harm neurons by causing the accumulation of heme precursors like ALA, primarily through its impact on ALAD (Fujita *et al.,* 2002). Furthermore, lead interferes with the production of neurotransmitters and the organization of ion channels (Casaret *et al.,* 2007). The fundamental cellular processes, including intra and intercellular signaling, cell adhesion, protein folding, maturation, apoptosis, ionic transport, enzyme regulation, and neurotransmitter release,

are significantly affected by lead's ionic mechanism of action (Garza *et al.,* 2006). In this mechanism, lead replaces bivalent cations like $Ca^{2+}$, $Mg^{2+}$, and $Fe^{2+}$, as well as monovalent cations like Na+ (Lidsky *et al.,* 2003), primarily contributing to neurological deficits. Lead can cross the blood-brain barrier (BBB) at a notable rate by substituting calcium ions and being taken up by the calcium ATPase pump (Ara *et al.,* 2003). Inside the brain, it accumulates in astroglial cells, which contain lead-binding proteins. However, its toxic effects are more pronounced in immature astroglial cells, particularly in the developing nervous system, where these proteins are lacking. This leads to damage to astroglial cells and inhibits the formation of the myelin sheath, both of which play roles in the development of the BBB (Assi *et al.,* 2016).

The primary focus of lead toxicity is believed to be on N-methyl-D-aspartate receptors, as lead acts by blocking these receptors and reduces the expression of genes associated with them (Brent et al., 2006). Lead exposure can also impact protein kinase C, a crucial neurotransmitter, by substituting calcium ions, which it normally relies on to regulate long-term neural excitation and memory storage (Bressler *et al.,* 1999). Additionally, lead affects sodium ion levels, disrupting essential processes dependent on sodium, such as the generation of action potentials in excitable tissues for cell-to-cell communication, the uptake of neurotransmitters like choline, dopamine, and GABA, and the regulation of calcium uptake and retention by synaptosomes (Bressler *et al.,* 1999).

Lead exposure affects both the central and peripheral nervous systems, but its impact differs between children and adults. In children, the central nervous system is more heavily affected, particularly in terms of synapse formation in the cerebral cortex (Ara *et al.,* 2015). Morphological investigation reveals that developmental lead exposure reduces the length of the dendritic field and the number of dendritic branches of hippocampus dentate granule cells. It has

been found that oxidative damage to synapses in the rat cerebral cortex and hippocampus contributes to cognitive function deficits (Soleimani *et al.,* 2016).Adults who were exposed to elevated lead levels during childhood, as observed through magnetic resonance imaging, display reduced brain volume, especially in the prefrontal cortex (Cleveland *et al.,* 2008).

In the peripheral nervous system, lead exposure leads to peripheral neuropathy by diminishing motor activity due to the loss of the myelin sheath that normally insulates nerves. This damage severely impairs the transmission of nerve impulses, resulting in a deficiency of muscular coordination, fatig ue, and muscular weakness, particularly in the external muscles (Sanders *et al.,* 2009). Furthermore, lead poisoning reduces the number of neurons and hampers neuronal growth (Pearson *et al.,* 2003).

### 2.2.4 PATHOPHYSIOLOGY AND CLINICAL MANIFESTATION OF LEAD POISONING

Lead interacts with human physiology in two ways. First, it has a considerable affinity for sulfhydryl groups and electron donor groups, which allows it to attach to a diverse spectrum of proteins. Because of its similarity to other divalent cations such as calcium and zinc, it disturbs several cellular processes regulated by these cations. Given the abundance of electron donor groups and divalent cations in the human body, the pathophysiology of lead toxicity is complex and affects practically every organ system (Mitra *et al.,* 2017).

In terms of neurological impacts, lead is thought to interfere with the normal synaptic pruning process in developing brains, perhaps leading to cognitive and behavioral changes reported in young children exposed to high levels of lead (Lidsky and Schneider, 2003). Chronic lead toxicity in adults frequently presents as peripheral neuropathy, while the precise mechanism underlying its development is unknown. Acute lead encephalopathy causes the most severe

neurological symptoms, such as seizures and coma, due in part to lead-induced cerebral microvascular alterations that cause cerebral edema and elevated intracranial pressure (De Souza *et al.,* 2013).

In terms of hematological effects, Lead causes anemia by interfering with the function of enzymes involved in heme production and enzymes essential for maintaining erythrocyte membrane integrity. This disturbance reduces erythrocyte production while increasing erythrocyte destruction (Mitra *et al.,* 2017). The characteristic basophilic stippling look is most likely caused by aggregated degraded RNA, which is normally removed by an enzyme known as pyrimidine-5'-nucleotidase, which is blocked by lead lead (Valentine *et al.*, 1976).

Lead can cause proximal tubule malfunction, which is similar to Fanconi syndrome. Furthermore, it competes with uric acid for excretion in the distal tubule, leading in increased blood urate concentrations, which can lead to urate crystal formation in joints, culminating in "saturnine gout." Lead is also linked to hypertension and consequent cardiovascular disease, most likely due to a combination of variables such as increased serum renin concentrations and the development of peripheral autonomic nervous system neuropathy (Mitra *et al.,* 2017; Nolan and Shaikh, 1992).

Despite contradicting evidence, it has been stated that lead buildup affects the majority of the endocrine glands. It appears to have an effect on the hypothalamic-pituitary axis in particular, resulting in decreased TSH, GH, and FSH/LH responses to TRH, GHRH, and GnRH stimulation, respectively. Suppressed GH release has been recorded, which is most likely due to decreased GHRH synthesis, suppression of GHRH release, or decreased somatotrope responsiveness. High LH and FSH levels are frequently associated with normal testosterone levels in lead-exposed persons, whereas low testosterone levels do not cause high LH and FSH levels in long-term

exposed individuals (Doumouchtsis *et al.,* 2009). According to some studies, lead can cause functional deterioration of the pituitary-thyroid axis, altering thyroid physiology, and chronic lead exposure can cause anatomic-histological changes in the thyroid gland, such as a decrease in the size of thyroid follicles and an alteration of the follicle cell nucleus. Several studies in recent years have found that lead has an effect on thyroid function, causing a decrease in the generation of tetraiodothyronine (T4) and an increase in thyroid-stimulating hormone (TSH) (Rivera-Buse *et al.,* 2023).

## 2.3 ORGAN OF STUDY: THE HIPPOCAMPUS

Hippocampus is a complex brain structure embedded deep into temporal lobe. It has a major role in learning and memory. It is a plastic and vulnerable structure that gets damaged by a variety of stimuli. Hippocampus is an extension of temporal part of cerebral cortex (Gilbert *et al.,*2009) . It can be distinguished externally as a layer of densely packed neurons, which curls into S-shaped structure on the edge of temporal lobe. Therefore, it is known as a part of limbic lobe (limbic means border).

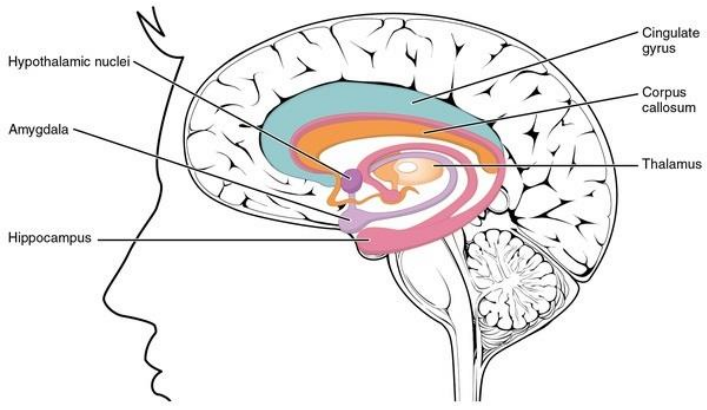


Fig 2.2 showing the Hippocampus obtained from https://anatomyinfo.com/hippocampus-anatomy/ , accessed October 7th , 2023.

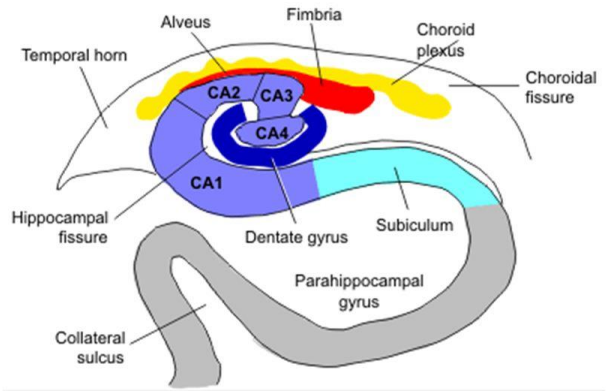


Fig 2.3 showing the Hippocampus obtained from https://www.researchgate.net/publication/348479617_An_in-vitro_investigation_to_determine_the_neuroinflammatory_response_of_CNS_cells_to_oral_bacteria_and_their_virulence_factors , accessed October 7th , 2023.

### 2.3.1 DEVELOPMENT OF HIPPOCAMPUS

The hippocampus, which lies within the fifth limbic lobe of the brain on the medial surface of the temporal hemisphere, originates from the isocortex. It's also considered to be a part of the olfactory cortex. The fornix, a string of fibers, draws it to the temporal lobe. The choroid fissure allows the choroid plexus to invade the lateral ventricle (Fogwe *et al.,*2018).

The whole hippocampus formation is evident on the medial surface of the cerebral hemisphere around 13 weeks, prior to the formation of the corpus callosum . The inner limbic arch, which includes the hippocampal formation, runs from the olfactory tract to the temporal lobe. The hippocampal sulcus stretches from the frontal lobe to the temporal pole; it is broad and noticeable on the hemisphere's medial surface.The temporal lobe neocortex and the uncus are both tiny. The neocortical outer limbic arch, which includes the cingulate gyrus and the

parahippocampal gyrus, can now be detected at 16 weeks. The fornix and hippocampus formation are viewed as independent structures in the temporal region, and the hippocampal sulcus is still substantial and prominent. However, in the frontal and parietal regions, the supracallosal hippocampus has shrunk and has become the indusium griseum. The corpus callosum is present and obscures the indusium griseum. The hippocampus sulcus in the frontal and parietal regions develops into the corpus callosum sulcus, which is associated with corpus callosum development.

The neocortical outer limbic arch, which includes the subcallosal region, cingulate gyrus, and parahippocampus gyrus, is more pronounced at 18 weeks. The hippocampal formation is found deep to the medial surface of the hemisphere and begins to fade from view as a result of substantial expansion of the neocortex in the region of the parahippocampal gyrus and uncus. After 18 weeks, the hippocampus structure and sulcus are further obscured by the ongoing growth of temporal lobe neocortex (Kier *et al.,* 1995).

### 2.3.2 FUNCTIONS OF HIPPOCAMPUS

#### 2.3.2.1 HIPPOCAMPUS AND MEMORY

The hippocampus is divided into three regions: CA1, CA2, and CA3, together forming the trilaminar loop, which is essential for long-term memory processing. Long-term potentiation (LTP), a type of neural plasticity, occurs in the hippocampus and plays a crucial role in memory storage. The complex processes of memory encoding in the hippocampus and retrieval in the frontal lobe involve two primary pathways: the polysynaptic and direct pathways.

In the polysynaptic pathway, the hippocampus receives input from the entorhinal cortex through axons that terminate in the dentate gyrus. From there, dentate gyrus neurons send mossy fibers to

CA3 pyramidal cells, which further split into two branches. One branch extends to the opposite side of the hippocampus via the corpus callosum, while the other connects to CA1 through Schaffer collateral pathways. These projections eventually leave the hippocampus to reach the inferior temporal cortex, temporal pole, and prefrontal cortex. The polysynaptic pathway primarily supports semantic memory, which involves facts and concepts.

On the other hand, the direct pathway is crucial for episodic memory, which is the recollection of specific events, and spatial memory, which relates to recognizing spatial information (Sanchari, 2023).

#### 2.3.2.2 HIPPOCAMPUS AND LEARNING

Pyramidal cells within the hippocampus have a crucial role in classical eye blink conditioning, which serves as a fundamental model for studying associative learning. Research on delay eye blink conditioning has demonstrated that these pyramidal cells establish a predictive pattern for the timing and intensity of the learned behavioral response. The speed at which this learning occurs can be influenced by various manipulations of the hippocampus, either impairing or enhancing it. In this type of learning, the hippocampus is particularly important in trace conditioning, where a short time gap exists between the conditioned and unconditioned stimuli. Following eye blink conditioning, the hippocampus undergoes long-lasting neuronal plasticity, which is essential for the learning process involved in trace eye blink conditioning (Sanchari, 2023).

#### 2.3.2.3 HIPPOCAMPUS AND SPATIAL NAVIGATION

A primary function of the hippocampus is the creation of a cognitive map, which is a mental representation used for acquiring, encoding, storing, recalling, and interpreting information about

relative locations within a specific environment. Place cells, a specific type of pyramidal cell, play a central role in the hippocampus-mediated process of spatial navigation. These cells become active when an animal enters a specific location within its environment, known as a "place field." However, they remain inactive when the animal moves outside of this designated place field. Additionally, the firing rate of place cells is influenced by factors such as the direction of movement, the intended destination, or other task-related aspects (Sanchari, 2023).

#### 2.3.2.4 HIPPOCAMPUS AND BEHAVIOR

The hippocampus plays a critical role in facilitating adaptable and goal-driven behavior. An intact hippocampus is essential for creating and reassembling relational memories, which are crucial for remembering arbitrary connections between objects or events, enabling flexible thinking and social interactions. Numerous studies have demonstrated that any damage to the hippocampus can hinder the flexible use of information and result in maladaptive behavior.

Furthermore, the hippocampus has a well-established role in behavioral inhibition. This connection between the hippocampus and inhibition is primarily based on two key observations: first, hippocampal damage leads to increased activity in animals, and second, it impairs their ability to inhibit responses they've previously learned.

The hippocampus serves as an evaluation center linked to behavioral inhibition, obsessive thinking, scanning, and the creation of spatial mental maps. However, once an experience is established, the hippocampus doesn't actively control behavior. External stress, which elevates corticosterone levels, ultimately reduces the firing rate of the hippocampus.

Recently, a novel function of the hippocampus has emerged. It has been discovered that low-frequency firing or activity in the hippocampus can influence the functional coordination

between distant regions in the cerebral cortex, resulting in heightened sensory responses like vision, hearing, and touch (Sanchari, 2023).

# CHAPTER THREE

# METHODOLOGY

## 3.1 REAGENT / CHEMICALS

All reagents and chemicals were of analytical grade. They include distilled water, lead acetate, antioxidant (Vitamin E), alcohol (50%, 70%, 90%, 100%), xylene, paraffin, normal saline, and 10% formalin.

## 3.2 EQUIPMENT

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

## 3.3 COMPUTER SOFTWARE

Graphpad prism statistical package, version 7 (manufactured by Graphpad software, Inc; released in 2016).

## 3.4 DETERMINATION OF DOSAGE

The dosage of Rutin was 50 mg/Kg body weight (Al-Naely *et al.,* 2022) and 100 mg/Kg body weight (Ziaee *et al.,* 2009). Lead Acetate dosage was 100 mg/Kg. This is the dose that is known to induce toxicity in Wistar rats (Mokhtari and Zanboori, 2011).

## 3.5 ANIMAL CARE AND MANAGEMENT

The animals for 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. The Wistar rats were kept in polypropylene cages at normal room temperature.

The animals were fed with Chikun Feed Grower Mash (Olam Agri Holdings Pte Ltd., Lagos State, Nigeria) and have free access to water throughout the entire study period of twenty-eight days. The animals were weighed weekly before commencement and throughout the duration of the experiment using a digital weighing scale calibrated in grams and recorded to the nearest whole number. Protocols for this experiment were in accordance with the guide for the care and use of laboratory animals (National Research Council of the National Academics, 2011).

The study was approved by the Research Ethical Committee of the College of Medical Sciences, University of Benin with approval number CMS/REC/2023/454.

### 3.6 EXPERIMENTAL DESIGN

A total of forty-eight Wistar rats weighing between 140g and 180g were used for this study. They were randomly assigned into Six groups (A, B, C, D, E and F) of eight rats each after acclimatization to animal house conditions for three weeks with free access to feed and water. Group A rats served as the control group and were given 1ml of distilled water. Group B rats were given 100 mg/kg body weight of Lead acetate only. Group C rats were administered 50 mg/kg body weight of Rutin and 100 mg/kg body weight of lead . Group D rats were administered 100 mg/Kg body weight of Rutin and 100 mg/kg body weight of Lead. Group E rats was administered 50 mg/Kg body weight of Rutin only and Group F rats were administered 100 mg/Kg body weight of Rutin only. All administrations were consumed orally with the help of an orogastric tube, throughout the entire study period of twenty-eight days.

## 3.7 NEUROBEHAVIOURAL TESTS : NOVEL OBJECT RECOGNITION (NOR) TEST

The novel object recognition test (NORT) also known as the object recognition test (NORT), was performed as described by (Pitsikas *et al.,* 2001) with minor modifications.The NORT was conducted inside of an open box made of wood that measured (80 x 60 x 40 cm) .The objects to be distinguished were constructed of painted wood, about 10 cm tall, and hefty (so it won't be moved by the animals throughout the test), and were produced in two distinct shapes (triangle and cylindrical). In addition, these objects will have no genuine significance for the rats and will never be associated with reinforcement. Rats were given a 2-minute habituation session the day before testing to use the equipment.. The first 3-minute sample trial test (T1) began twenty-four hours later with the presentation of two comparable objects (designated as sample objects FO1 and FO2) at the corners of the box. After T1, all of the rats were returned to their home cages, and the inter-trial interval for T2 was set at 60 minutes One of the items (FO2) provided in the first 5-minute choice trial (T1) was swapped out for a different item (NO) in T2. Rats were exposed to the apparatus once again to assess the impact on long-term memory, and the amount of time they spent examining FO1 and NO were recorded. After each trial, the equipment and the objects were thoroughly cleaned to remove any smell trails. Exploration will be considered as directing the nose to the objects at a distance ≤ 2 cm to the objects and/or touching it with the nose. Rats' exploration times of two objects in T1 and T2 were recorded separately. A series of 32 variables were calculated: total time spent in exploring two identical objects in T1 and that spent in exploring two different objects in T2. The discrimination between the familiar and the novel object during T2 were measured by comparing the time spent in exploring the familiar

object with that spent in exploring the new object. To eliminate any bias in overall exploration levels, a discrimination index (D) was calculated; D = N−F/N + F.

**Figure 3.1:** Image showing the NORT apparatus

### 3.8 BRAIN OXIDATIVE STRESS PARAMETERS

Following brain harvesting, it was blotted clear of blood and instantly weighed using an electronic weighing balance calibrated in milligrams and recorded to the nearest two decimal places. The harvested and weighed brains was washed twice in cold phosphate buffered saline (PBS), homogenized using acid-washed sand and PBS in porcelain mortar and pestle. The homogenate was centrifuged at 10,000 g for 15 minutes at 4°C .The supernatant was collected in order to estimate the outcomes of different biochemical studies.

### 3.8.1 ESTIMATION OF CATALASE (CAT) ACTIVITY

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

- **Principle**

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

- **Preparation of reagent**

0.01M $KMnO_4$ was prepared by dissolving 0.158g of $KMnO_4$ in 100ml of distilled water

Phosphate buffer (pH 7.4): 0.426 of $NaHPO_4$ $NaH_2PO_4$ was weighed and dissolved in 100ml of distilled water.

6M $H_2SO_4$: 32.3 ml of conc. $H_2SO_4$ was added to 66.7 ml of distilled water. 30Mm $H_2O_2$ solution: this will be prepared by measuring 0.34 ml of 30% of $H_2O_2$ in 1001 ml of phosphate buffer.

- **Procedure**

To a known volume of plasma (0.5ml), 5.0ml of $H_2O_2$ was added. This was mixed by inversion and allowed to stand for 30 minutes. The reaction was stopped by adding 6M $H_2SO_2$.

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

- **Calculation**

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

OD = absorbance

L = light path =1cm

Vt = total volume of reaction sample

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

### 3.8.2 ESTIMATION OF MALONDIALDEHYDE (MDA) ACTIVITY

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

- **Principle**

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

- **Preparation of Reagent**

Stock TCA-TCB-HCL was prepared by mixing 15g of trichloroacetic acid, 0.375g of thiobarbituric acid and 0.25N hydrochloric acid. This solution was 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 was heated for 15 minutes in a boiling water bath. After cooling, the flocculent precipitate was 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:

$$\text{MDA (unit/mg protein)} = \frac{(A \times Vt \times 1000)}{(M \times V \times 1 \times Y)}$$

A = absorbance of sample test at 535nm

Vt= total volume of the reaction = 3ml

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

V= volume of tissue extract used = 1ml

Y = mg tissue in the volume of sample used

### 3.8.3 ESTIMATION OF GLUTATHIONE PEROXIDASE (GPx) ACTIVITY

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

- **Principle**

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

- **Preparation of reagent**

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

- **Procedure**

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

- **Calculation**

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

OD = Absorbance of test

Vt = Total volume of reaction of reaction mixture Df = Dilution factor = l

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

Vs= volume of sample

Y = mg of protein used

### 3.8.4 ESTIMATION OF SUPEROXIDE DISMUTASE (SOD)

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

- **Principle**

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

- **Preparation of reagents**

Carbonate buffer (0.05 M) pH 10.2: this was prepared by dissolving 0.2014 g of $Na_2CO_3$. 0.2604 g of NaHCO3 and 0.0372 g of EDTA in 100 ml of distilled water. Hydrochloric acid (0.005 M): this was prepared by adding 0.044 concentrated HCl to 99.96 ml of distilled water. Adrenaline solution (0.3 mM): this was prepared by dissolving 0.01098 g of Adrenaline in 100 ml of 0.005 M HCl solution.

- **Procedure**

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 would be 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 = (O.D test − O.D ref) × 100 $O.D$ test

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.

### 3.8.5 ESTIMATION OF REDUCED GLUTATHIONE CONCENTRATION (GSH)

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

- **Reagents**

5, 51-dithiobis-2-nitrobenzoic acid (DTNB): sodium citrate, and trichloroacetic acid (TCA)

- **Procedure**

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

- **Calculation**

$$\text{Concentration of GSH} = \frac{A_{test} \text{ x Conc. of Standard}}{A_{standard}} \quad \text{..... (50)}$$

$$\% \text{ Glutathione Reduced} = \frac{(A_0 - A_1) \times 100}{A_0} \quad \text{..... (51)}$$

where A0 = Absorbance of reference sample

A1 = Absorbance of sample

## 3.9 HISTOLOGY OF THE HIPPOCAMPUS

The rats were euthanized with ketamine anesthesia (100 mg/kg), followed by cervical dislocation, once the neurobehavioral tests are finished. The rats Hippocampus was 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 will be calculated as follows:

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

For histopathology, the extracted Hippocampus tissue was stored in 10% formalin. The tissues were processed via paraffin wax embedded method of Drury and Wallington (1980).

**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 was used. Procedures of Haematoxylin and Eosin adopted on the sections was described by Drury and Wallington (1980). The sections were:

- Dewaxed in two changes of xylene for two minutes in each change;
- Rehydrated in descending grades of alcohol (absolute II, absolute I, 95%, 90%, 70% and 50% ethanol) for two minutes each;
- Rinsed in distilled water for three minutes
- Stained in hematoxylin for 15-20 minutes
- Excess hematoxylin stain was removed by rinsing well in running tap water for two to three minutes (sections were 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.
- Counter stained in 1% aqueous eosin for two to four minutes;
- Excess stain was washed off in running water and examined under microscope;
- Dehydrated rapidly in ascending grades of ethanol (50% through absolute ethanol): cleared in Xylene and mounted in a synthetic resin medium (DPX).

### 3.10 PHOTOMICROGRAPHY

The processed slides were captured with a binocular microscope on which will be mounted an Omax 9.0MP USB Digital Miscroscope Camera (made in Korea). The camera features 9 megapixels (3488 × 2616 pixel) high resolution color digital camera and 0.5X reduction lens which would be connected to a laptop. A panoramic view of the slides were captured using ×4 and ×10 objective lenses.

### 3.11 STATISTICAL ANALYSIS

Data were analyzed using Graphpad prism statistical package (version 7). Statistical significance ($P<0.05$) was determined by means of analysis of variance (ANOVA), followed by turkey's multiple comparison post-hoc test. Results were presented as mean ± standard error of mean (mean ± SEM).

# CHAPTER FOUR

# RESULTS

## 4.1 EFFECT OF TREATMENT ON BODY AND BRAIN WEIGHT

Table 4.1 shows the body and organ weight of control and treatment groups after 28 days. For whole brain weight, group B (Pb) showed a significant decrease ($p<0.05$) when compared to control. However, a significant increase ($p<0.05$) was observed in the whole brain weight of groups D (RUT1 + Pb) and E (RUT2 + Pb) when compared to Lead-only treated group B. For the relative brain weight, a significant increase ($p<0.05$) was observed in group D (RUT2+ Pb) when compared to Lead-only treated group.

**Table 4.1**: Body weight, absolute whole brain and relative brain weights of control and treatmentgroups after 28 days.

| Groups | Initial BW (g) | Final BW (g) | Weight Change (g) | Whole brain weight (g) | Relative brain weight (%) |
|---|---|---|---|---|---|
| **Control** | 127.0 ± 2.507 | 172.2 ± 4.398 | 44.2 ± 5.380 | 1.66 ± 0.075 | 0.964 ± 0.024 |
| **Pb** | 126.7 ± 2.265 | 152.8 ± 1.908 | 25.2 ± 2.478 | 1.34 ± 0.051 # | 0.880 ± 0.028 |
| **RUT1 + Pb** | 128.8 ± 2.272 | 168.4 ± 4.226 | 40.0 ± 5.683 | 1.66 ± 0.060 * | 0.986 ± 0.036 |
| **RUT2 + Pb** | 125.9 ± 3.528 | 162.8 ± 6.304 | 40.8 ± 6.996 | 1.70 ± 0.032 * | 1.050 ± 0.041 * |
| **RUT1** | 129.7 ± 3.293 | 167.8 ± 3.967 | 41.0 ± 3.782 | 1.64 ± 0.068 | 0.978 ± 0.025 |
| **RUT2** | 127.6 ± 6.873 | 175.8 ± 9.631 | 45.5 ± 2.754 | 1.65 ± 0.043 | 0.948 ± 0.042 |

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

## 4.2 EFFECT OF TREATMENT ON NEUROBEHAVIOURAL ACTIVITY (NOR)

Figure 4.2 shows the mean exploration time(s) of the Novel object (NO) during NOR test in experimental groups A-F. A significant decrease ($p< 0.05$) in mean exploration times for the novel object (NO) was observed in rats treated with lead alone (Pb) when compared to control. However, significant increase ($p< 0.05$) was observed in pretreated rats (RUT1+ Pb) and (RUT2 + Pb) when compared to Lead-only treated rats (Pb).

Figure 4.4 shows the discrimination index (DI) during NOR test in experimental groups. A significant decrease ($p< 0.05$) was observed in rats treated with lead alone (Pb) when compared to control. However, a significant increase ($p< 0.05$) was observed in pretreated rats (RUT1 + Pb) and (RUT2 + Pb) when compared to Lead-only treated rats (Pb).

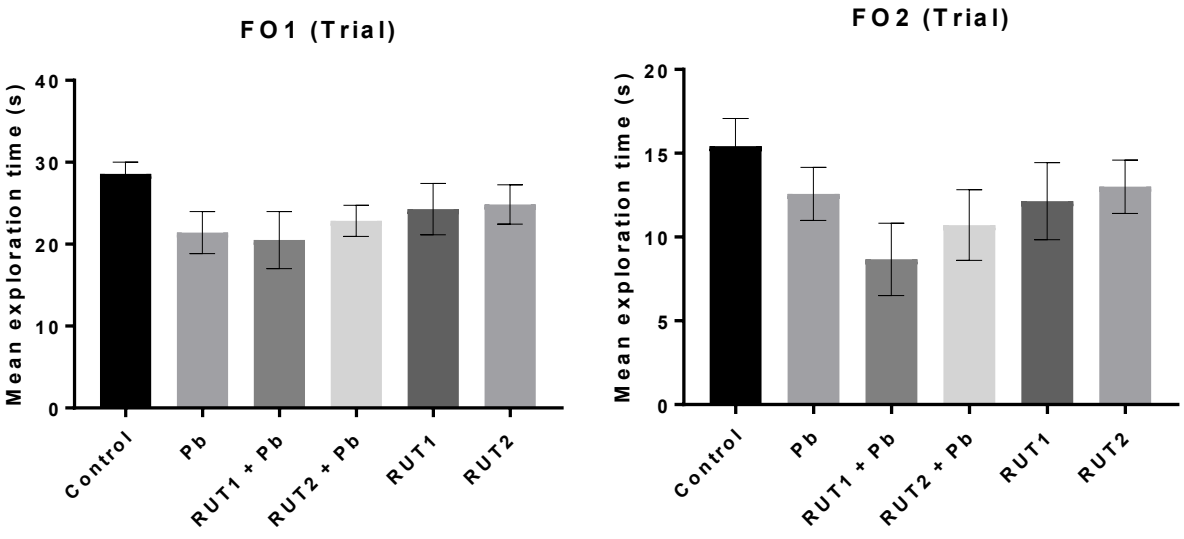


**Figure 4.1:** Trial novel object recognition test of control and treatment groups after 28 days. (FO1 – Familiar object 1; FO2 – Familiar object 2). Values are given as mean ± SEM.

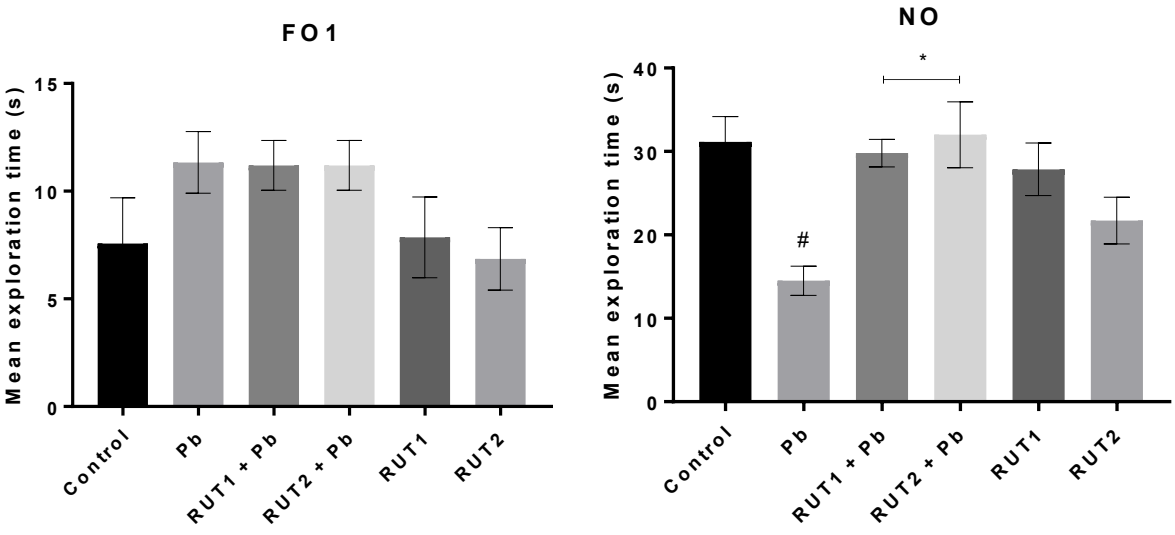


**Figure 4.2:** Real novel object recognition test of control and treatment groups after 28 days (FO1 – Familiar object 1; NO – Novel object). Values are given as mean ± SEM. $^{\#}$ $p< 0.05$ compared with the control group; * $p< 0.05$ compared with the Pb-alone group.

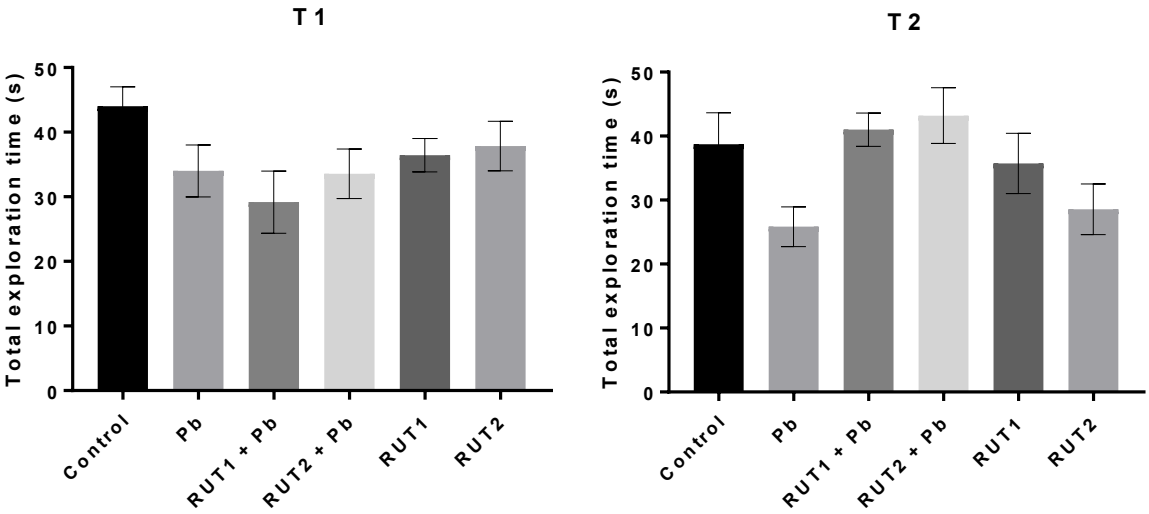


**Figure 4.3:** Total exploration times (NOR) of control and treatment groups after 28 days. Values are given as mean ± SEM. $^{\#}$ $p< 0.05$ compared with the control group.

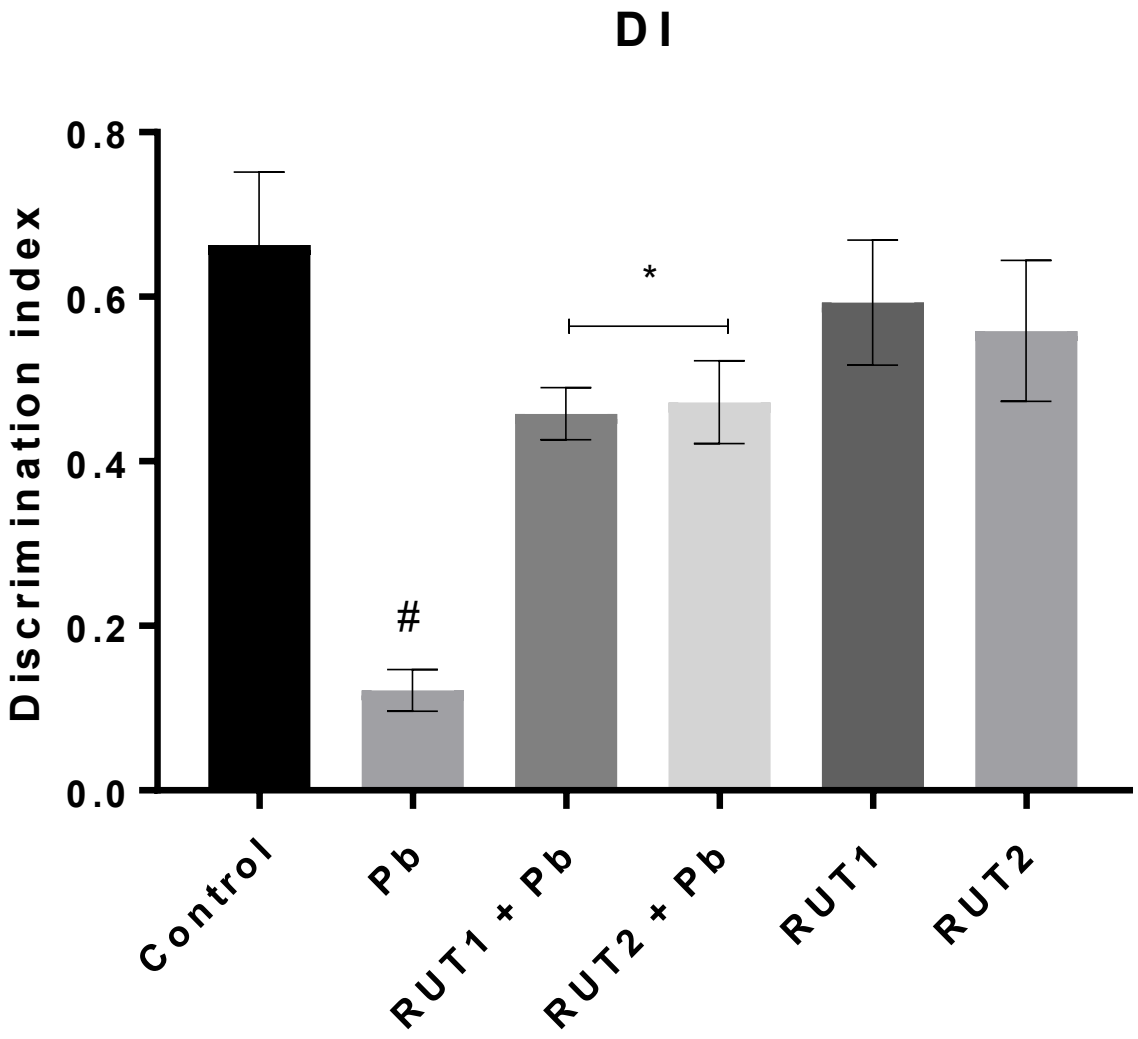


**Figure 4.4:** Discrimination index (NOR) of control and treatment groups after 28 days. Values are given as mean ± SEM. # $p< 0.05$ compared with the control group; * $p< 0.05$ compared with the Pb-alone group.

## 4.3 EFFECT OF TREATMENT ON ANTIOXIDANT ACTIVITY

Figure 4.5 illustrates the activity of antioxidant enzymes in control and treatment groups. Here, a significant decrease in SOD, CAT, GSH and GPx ($p<0.05$) was observed in group B (Pb) when compared to control. However, a significant increase ($p<0.05$) in SOD (RUT1+ Pb) and (RUT2+ Pb), CAT (RUT2+ Pb), GSH (RUT2+ Pb) , GPx (RUT2 + Pb) was observed when compared to Lead-only treated rats.

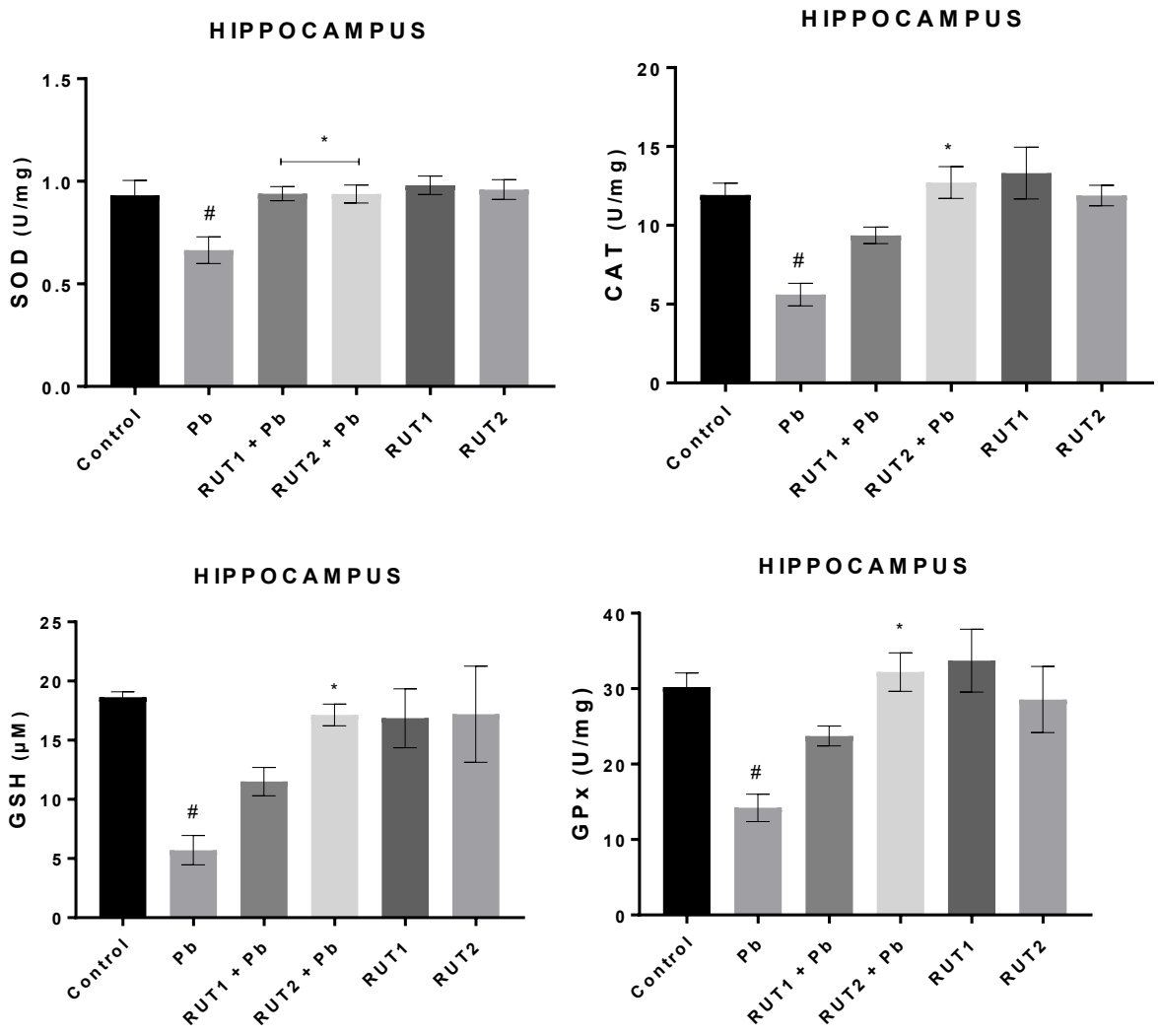


**Figure 4.5:** Activity of antioxidants in the hippocampus of control and treatment groups after 28

days. Values are given as mean ± SEM. $^{\#}$ $p< 0.05$ compared with the control group; * $p< 0.05$ compared with the Pb-alone group.

## 4.3 EFFECT OF TREATMENT ON Pb CONCENTRATION

Figure 4.6 illustrates the Lead (Pb concentration) in control and treatment groups. Here, a significant increase ($p<0.05$) in Pb concentration (ppm) was observed in group B (Pb) when compared to control. Also, a significant decrease ($p<0.05$) in Pb was observed in group C (Pb+RUT1) and group D ( Pb+RUT2) when compared to Lead only treated (group B).

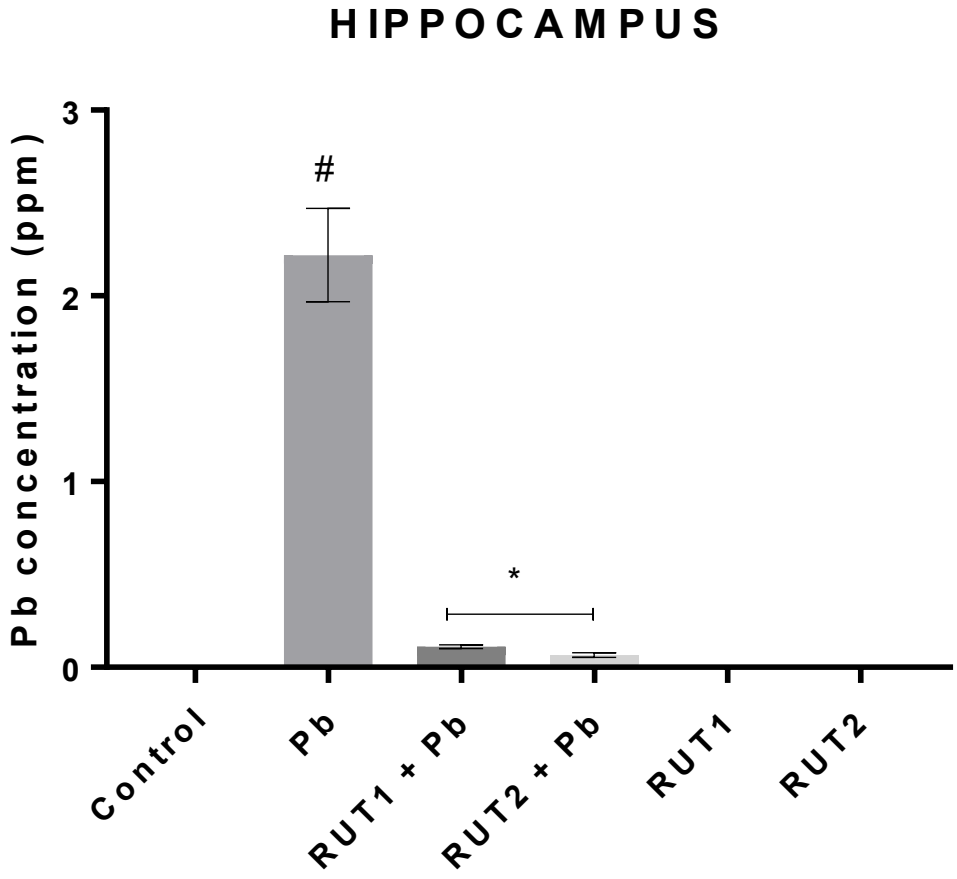


**Figure 4.6:** Pb concentration in the hippocampus of control and treatment groups after 28 days. Values are given as mean ± SEM. # $p< 0.05$ compared with the control group; * $p< 0.05$ compared with the Pb-alone group.

## 4.4 EFFECT OF TREATMENT ON LIPID PEROXIDATION

Figure 4.5 illustrates the Lipid peroxidation (MDA) in control and treatment groups. Here, a significant increase ($p<0.05$) was observed in group B (Pb) when compared to control. Also, a significant decrease ($p<0.05$) in MDA was observed in group C (RUT1+ Pb) and group D (RUT2+ Pb) when compared to Lead only treated (group B).

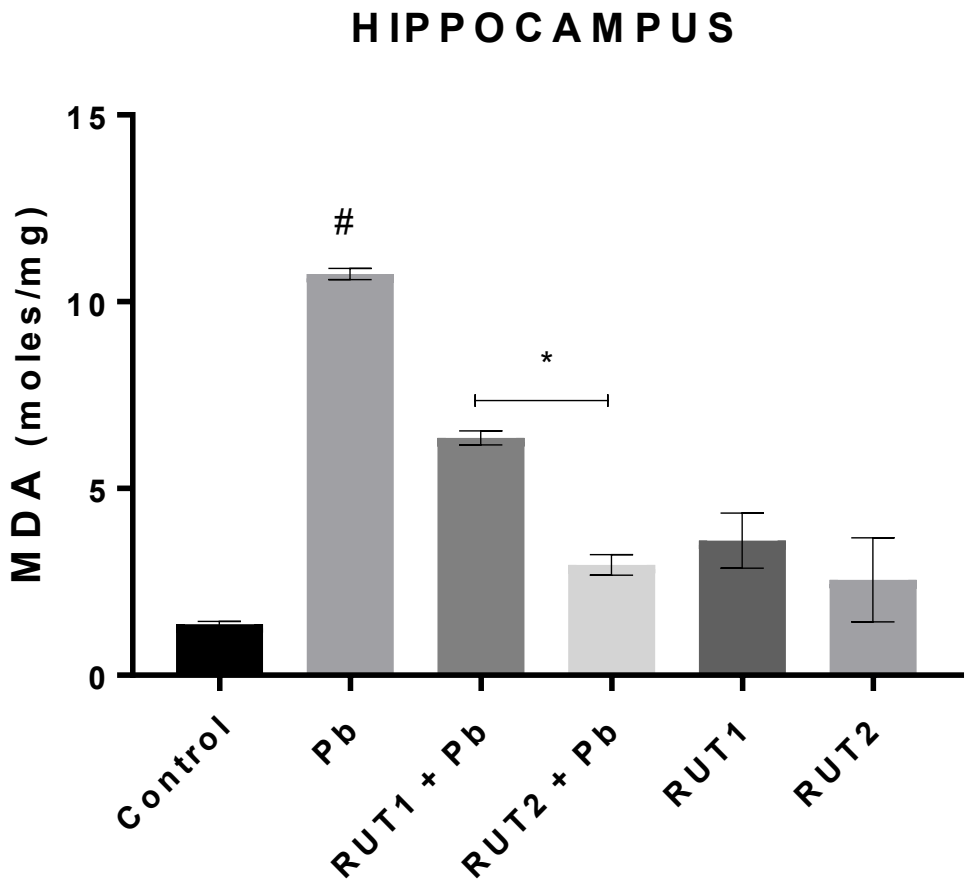


**Figure 4.7:** Lipid peroxidation activity in the hippocampus of control and treatment groups after 28 days. Values are given as mean ± SEM. $^{\#}$ $p< 0.05$ compared with the control group; * $p< 0.05$ compared with the Pb-alone group.

## 4.5 EFFECT OF TREATMENT ON THE HISTOLOGY

Plate 4.1 – 4.6 shows the representative histology of the hippocampus CA1 in control and treatment rats. Plate 4.1 (Control group) revealed normal structure of pyramidal cells (arrows) and astrocytes (circles). Plate 4.2 (Pb treated) showed atrophy and vacuolated pyramidal cells (arrows) and astrocytes (thick arrows). Plates 4.3 and 4.4 (RUT1+ Pb) and (RUT2 + Pb) groups showed fewer vacuoations and normal pyramidal cells. Plates 4.5 and 4.6 (RUT1) and (RUT2) groups showed normal pyramidal cells and astrocytes.

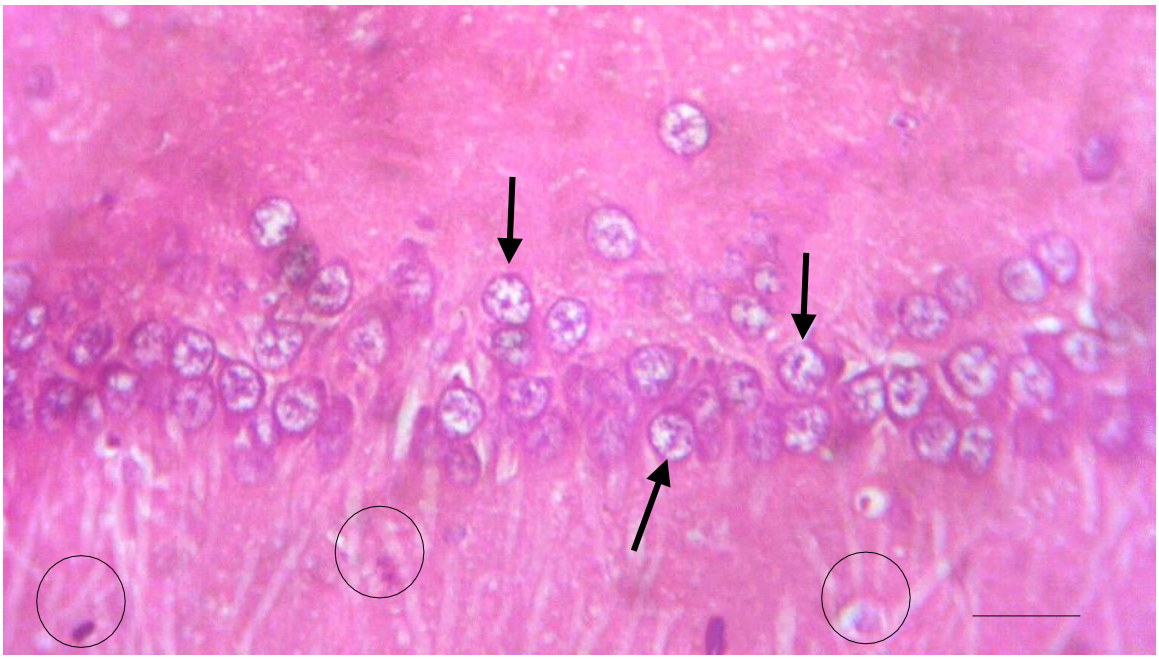

**Plate 4.1:** Representative histology of the Hippocampus in control rats showing normal structure of pyramidal cells and astrocytes.

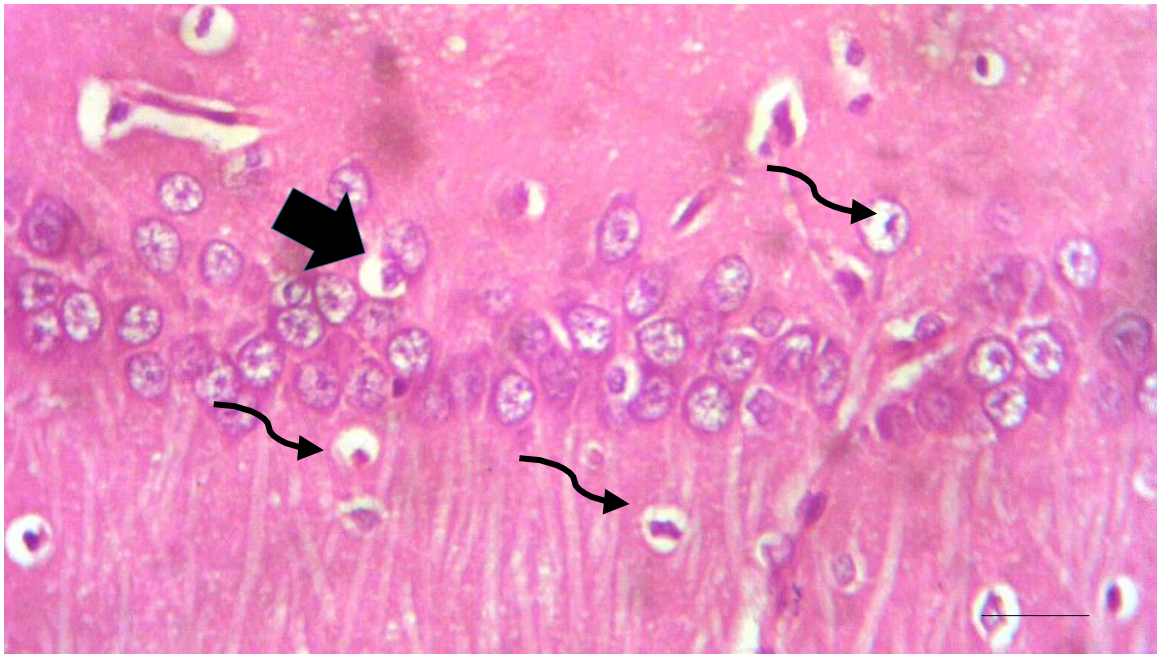

**Plate 4.2:** Representative histology of the Hippocampus in group B (Pb) rats showing atrophy and vacuolated pyramidal cells and astrocytes.

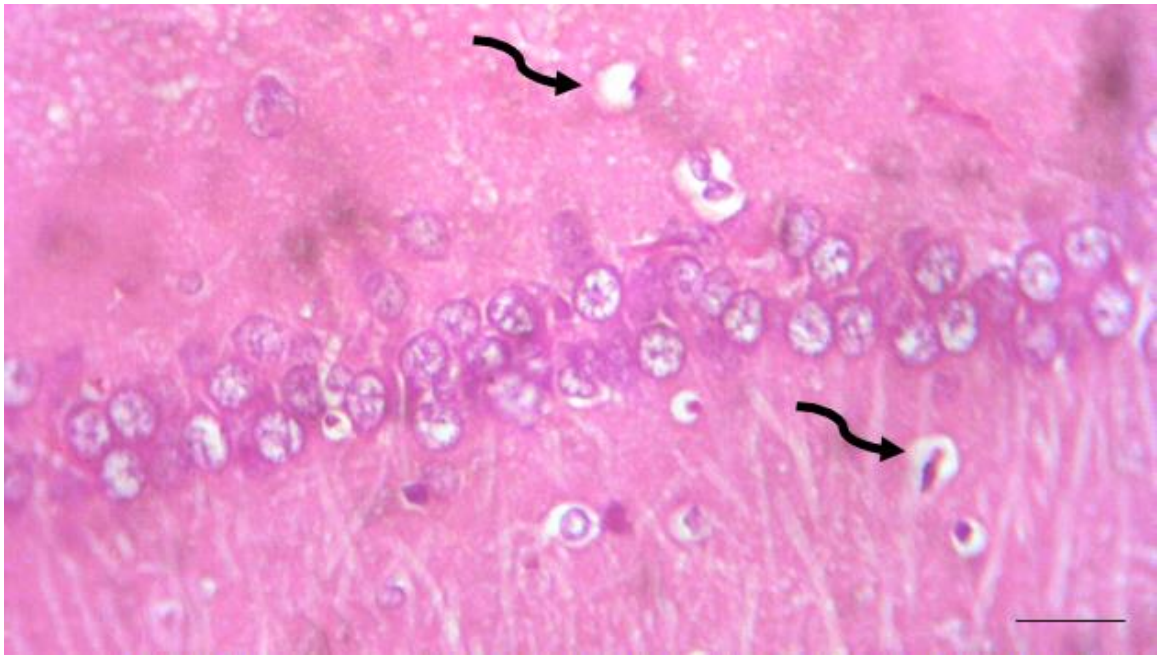

**Plate 4.3:** Representative histology of the Hippocampus in group C (RUT1+ Pb) rats showing fewer vacuoations and normal pyramidal cells.

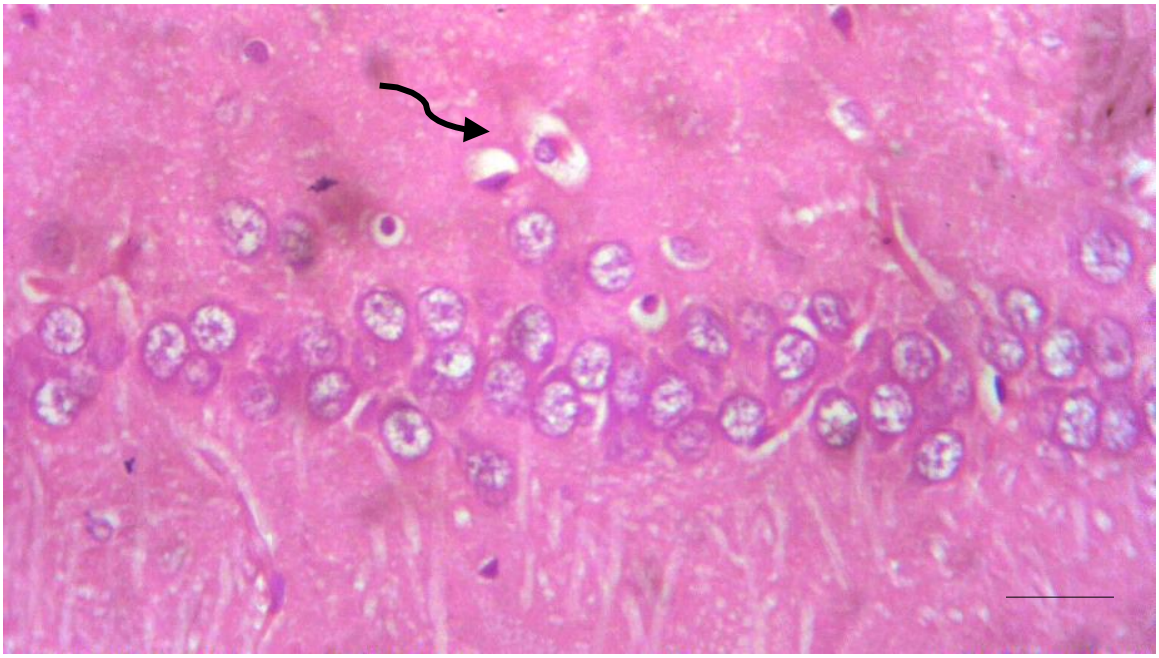

**Plate 4.4**: Representative histology of the Hippocampus in group D (RUT2 + Pb) ratsshowing fewer vacuoations and normal pyramidal cells.

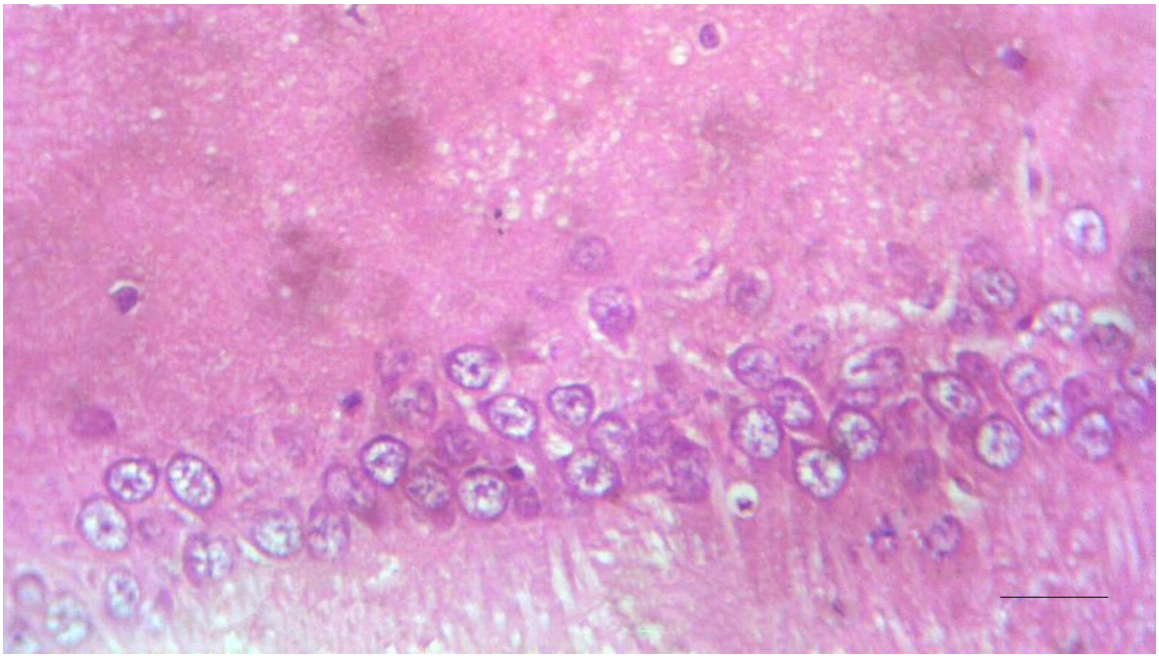

**Plate 4.5:** Representative histology of the Hippocampus in group E (RUT1) rats showing normal pyramidal cells and astrocytes.

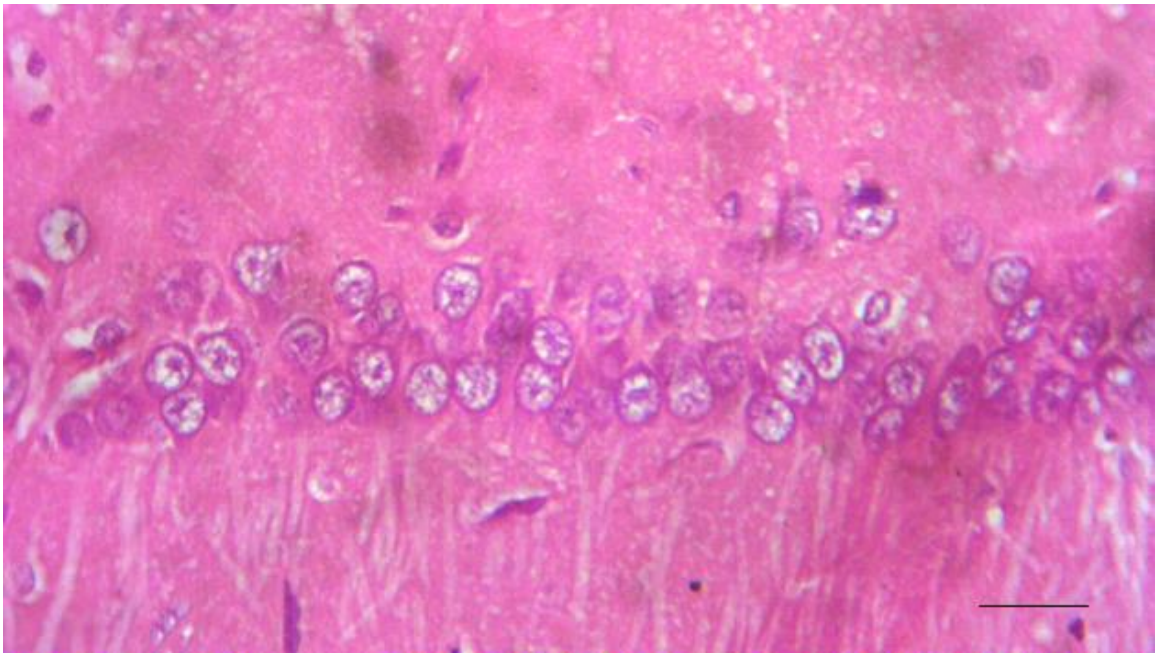

**Plate 4.6**: Representative histology of the Hippocampus in group F (RUT2) rats showing normal pyramidal cells and astrocytes.

# CHAPTER FIVE

# DISCUSSION, CONCLUSION AND RECOMMENDATION

## 5.1 DISCUSSION

Numerous studies have shown that exposure to lead may cause a variety of biochemical and physiological dysfunctions in both humans and animals (Gargouri *et al*., 2013). The organ system most susceptible to lead-induced poisoning is the nervous system (Kim *et al.,* 2015). The blood-brain barrier is easily breached by Lead, which then accumulates in the brain and causes neurotoxicity (Fang *et al.,* 2021). It triggers an increase in free radical production and reduces the availability of endogenous antioxidant reserves (Glutathione, Glutathione peroxidase, Superoxide dismutase, and catalase), which are necessary for scavenging ROS produced in lead-exposed individuals (Sani and Amanabo, 2021). Direct scavenging of ROS is possible with flavonoids. By giving an atom of hydrogen or by transferring a single electron, flavonoids can chelate free radicals right away (Banjarnahor and Artanti, 2014). Hence, this study aims to evaluate the protective activity of a flavonoid, Rutin, on lead-induced Hippocampal toxicity.

The findings show a decrease in the final body weight of the Lead-exposed rats when compared to control. This is consistent with other studies demonstrating that exposure to Lead decreased rats' body and organ weights (Khalaf *et al.*, 2012; Shahandeh *et al.*, 2013). The observed loss in body and brain weight may be associated with loss of appetite that has been associated with Lead intake (Mamoun *et al.*, 2015; Li *et al.*, 2021). This increase in body and brain weight in pretreated rats suggests that rutin provides protection against the weight loss caused by lead.

These results are consistent with earlier studies showing that Rutin can mitigate and guard against toxin-induced weight loss in rats (Motamedshariaty *et al.,* 2014 ; Alhoshani *et al*., 2017).

In addition to its vital involvement in memory and spatial cognition, the hippocampus has recently been associated with several other processes, such as parts of vision and picturing hypothetical and future scenes (Maguire et al., 2016). The novel object recognition test (NOR) test measures recognition memory through a two-trial cognitive paradigm. NOR is often used in mice to investigate deficiencies in a variety of animal models of disorders that impair cognition (Grayson *et al.,* 2015). The NOR test evaluates the normal tendency of a rat to explore a novel versus familiar object. This test involves both memory recall and exploratory behavior, therefore an animal needs to have thoroughly explored the familiar object during T1 in order to identify it from a novel object in T2. The results of this study revealed that lead-treated rats significantly reduced their exploration of the Novel object . In this study, lead-treated rats spent less time exploring overall during T1 than control animals. This is consistent with earlier studies indicating that lead-treated rats exhibit significantly reduced exploratory behavior and a significantly reduced discrimination index during the NOR test (Moosavirad *et al.,* 2016 ; Shvachiy *et al.,* 2022). This also supports the study of Nava-Ruiz *et al*., (2012) demonstrating that memory impairment is a characteristic of lead-associated encephalopathies. Conversely, there was a significant increase in the exploratory behavior and discrimination index of the Rutin pretreated rats when compared to lead-only treated rats. This infers that Rutin

was able to produce a cognition-enhancing effect, being able to reverse lead-induced cognitive deficit. This agrees with previous studies demonstrating that treatment with Rutin improves cognitive impairments (Oboh *et al.,* 2020).

Lead exposure causes excessive ROS generation, which causes structural damage to cells, proteins, nucleic acids and membranes, as well as lipid peroxidation (Jaishankar *et al.,* 2014). Antioxidants operate as scavengers to prevent cell and tissue damage as a result of oxidation (Sen *et al.,* 2010). Results from this study show that there was a significant decrease in SOD, CAT , GSH and GPx activities in the Hippocampus of Lead-only treated rats when compared to control. This demonstrates that Lead can cause oxidative stress by inhibiting antioxidant activity and agrees with previous findings that Lead inhibits antioxidant activities (Elsheikh *et al*., 2020; Shaban *et al.,* 2021). However, a significant increase was observed in the antioxidant activities of the Rutin pretreated rats when compared to Lead-only treated rats, thus demonstrating a protective activity against lead. These findings correspond to previous studies reporting that Rutin is capable of increasing antioxidant activities (Xu *et al.,* 2014; Annapurna *et al.,* 2013; Lins *et al.,* 2017; Gelen *et al*., 2017 ).

Lead accumulates in the body and can damage the human nervous system (Long *et al.,* 2022). In this study, a significant increase in Pb concentration (ppm) in the Hippocampus tissues was observed in group B (Pb) when compared to control . This is line with previous reports demonstrating that administration of Lead increases it's concentration in body tissues (El-Khadragy *et al.,* 2020 ; Moneim *et al.,* 2011) . However, a significant decrease was observed in the Pb concentration

of of Rutin pretreated rats when compared to Lead-only treated rats , thus showing a metal chelating property against Lead.

Lipid peroxidation can be induced by the interaction of ROS and polyunsaturated fatty acids of lipid membranes. Malondialdehyde (MDA) is an end product of lipid peroxidation and it is one of the most popular and reliable biomarkers for Lipid peroxidation (Ayala *et al.,* 2014). The results showed a significant increase in MDA level in the Hippocampus of Lead-only treated rats when compared to control rats indicating high level of lipid peroxidation. This is in line with previous findings that Lead increases MDA levels (Prasanthi *et al.,* 2010 ; Sudjarwo and Sudjarwo, 2017). Conversely, a significant decrease was observed in Hippocampal MDA level of the Rutin pretreated groups when compared to Lead-only treated rats. This corresponds to previous reports demonstrating that Rutin decreases MDA levels (Wei *et al*., 2011 ; Kandemir *et al.*, 2020), thus highlighting Rutin's ability to protect against Lead-induced oxidative stress.

Histological findings of control group showed normal histology of the Hippocampus CA1, however , Lead treated rats showed atrophy and vacuolation of pyramidal cells and astrocytes in the Hippocampus. These alterations in the Hippocampus ultimately disrupt normal functioning and cause cognitive impairment and it agrees with previous neuropathological findings on lead-induced toxicity and its effects on Hippocampal histology (Elisha *et al.,* 2023; Barkur *et al.,* 2023). In Rutin pretreated rats, the histology of the Hippocampus shows fewer vacuoations and normal pyramidal cells when compared to that of the Lead-only treated rats. The administration of Rutin was able to mitigate the atrophy and

vacuolation in pyramidal cells and astrocytes, thus indicating protective activity. The results also show that the rats treated with Rutin alone had similar appearance when compared to that of control , indicating that Rutin was not toxic to the animals.

## 5.2 CONCLUSION

In conclusion, results from this study showed that Rutin protected against Lead-induced Hippocampal toxicity in Wistar rats. This therefore provides the first research evidence of the protective activity of Rutin against lead-induced Hippocampal toxicity in Wistar rats.

## 5.3 RECOMMENDATIONS

Additional mechanistic investigations are needed to corroborate these findings and establish the exact mechanisms of action . These are crucial for the development of novel neuroprotective drugs that might be useful in the treatment of Lead toxicity and it's related neurological disorders.

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