EFFECT OF CARBON TETRACHLORIDE (CCL_4) IN THE CEREBRUM OF WISTAR RATS.

BY

ODEDEJI TAIWO JOSHUA

BMS2009052

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THE DEPARTMENT OF ANATOMY,

SCHOOL OF BASIC MEDICAL SCIENCES,

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UNIVERSITY OF BENIN.

SUPERVISED BY: MRS AKPORUFUOMA O.

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CERTIFICATION

This is to certify that this research was carried out by **ODEDEJI TAIWO JOSHUA** matriculation number **BMS2009052** in the department of anatomy school of basic medical sciences, university of Benin Benin city, Nigeria. In partial fulfillment for the award of bachelor of science (B.Sc)degree.

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(PROJECT SUPERVISOR)	
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(HEAD OF DEPARTMENT)	
______________________	______________________
(EXTERNAL EXAMINER)	**DATE**

DEDICATION

I dedicate this work to God and my family who was with me through it all. I dedicate this project research to my supervisor Mrs. Ufuoma whose guidance brought about the success of this research.

ACKNOWLEDGEMENT

I am most grateful to God, it's all been God keeping me from day one. My appreciation goes to my family, my dad **Amos Odedeji** who has always been supportive, my big sister and big brother; **Sis. Dolapo** and **Bro. Flourish** their love and care was beyond me. To my twin **Caleb Odedeji** thank you for pushing me to be the best and to my little brother **Stephen**. My warmest regards goes to my supervisor, **Mrs Ufuoma** who beyond a doubt was a torch lit to my path, her guidance played key role.

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ABSTRACT

Carbon tetrachloride is a colorless, highly volatile liquid with a sweetish odor similar to chloroform used in refrigerants, propellants and industrial solvents. CCl_4 is rapidly absorbed via oral, inhalation and dermal routes, distributing to the brain and other organs. It has been reported that CCl_4 can metabolize to give out free radicals inducing oxidative stress and lipid peroxidation which can ultimately alter brain structure, and impair learning and memory, even at low doses. This study was aimed at investigating the effect of CCl_4 on the cerebrum of adult Wistar rats. Eighteen adult Wistar rats weighing 140 g to 150 g were used in this study. They were randomized into three (3) groups of six (6) rats each. Group A served as the control and received 1ml of distilled water daily to compensate for stress of administration, whereas, rats in group B received 1.5mg/kg body weight of CCl_4 and group C received 3mg/kg body weight of CCl_4. All administration intraperitoneally lasted for a period of 28 days. The body weights of the rats were recorded daily. After the end of the experimental period, the rats were sacrificed by cervical dislocation and the organ (cerebrum) weight was recorded. The parameters accessed include cerebral antioxidant enzymes (SOD, CAT, GPx and GSH), MDA concentration and the histology of the cerebrum using Haematoxylin and Eosin staining technique. Data was analyzed using SPSS/IBM statistical package version 20. Results obtained showed no significant change ($p>0.05$) in the initial body weight of rats across experimental groups. However, a significant decrease ($p<0.05$) in final body weight and weight change of rats in group B (1.5 mg/kg bw CCl_4) and C (3 mg/kg bw CCl_4) when compared to control. No significant change ($p>0.05$) was observed in the cerebral weight of rats across experimental groups. However, a significant increase ($p<0.05$) was observed in relative cerebral weight of rats in group B (1.5 mg/kg bw CCl_4) and C (3 mg/kg bw CCl_4) when compared to control. A significant decrease ($p<0.05$) was observed in cerebral SOD, CAT, GPx and GSH activity of rats in group C (3 mg/kg bw CCl_4) when compared to control. A significant increase ($p<0.05$) was observed in MDA concentration of rats in group B (1.5 mg/kg bw CCl_4) and C (3 mg/kg bw CCl_4) when compared to control. Histological findings revealed normal archictecture of the cerebrum in group A, whereas cytoplasmic vacoulizaion were seen in the granular cells of rats in group B and C. In conclusion, findings from this study shows that CCl_4 induced neurotoxic effect on the cerebrum via inducing oxidative stress and altering the architectural integrity of the cerebrum.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

A toxicant is any toxic substance, whether artificial or naturally occurring (Francisco *et al.,* 2011). Carbon tetrachloride (CCl_4) is a colorless, highly volatile liquid with a sweetish (ethereal) odor similar to chloroform. It decomposes to highly toxic fumes of phosgene upon heating (Suryanarayana *et al.,* 2017). The main effects of carbon tetrachloride in humans are on the liver, kidneys, and central nervous system (CNS. (National Institute for Occupational Safety and Health (NIOSH) 2020.

Carbon tetrachloride was produced in large quantities to make refrigerants and propellants for aerosol cans, as a solvent for oils, fats, lacquers, varnishes, rubber waxes, and resins, and as a grain fumigant and a dry cleaning agent. Consumer and fumigant uses have been discontinued and only industrial uses remain (United States Environmental Protection Agency (EPA), 2020.

Carbon tetrachloride is rapidly absorbed via oral (gastrointestinal tract) and inhalation (lungs) routes, as well as, to a lesser extent, the dermal (skin) route, both in humans and animals. Following absorption, carbon tetrachloride rapidly diffuses via the blood to different internal organs (e.g., liver, kidney, brain), with peak concentrations reached within 1–6 hours depending on the duration of exposure and concentration (Suryanarayana *et al.,* 2017). A fraction of the chemical accumulates in the adipose tissue (Sanzgiri *et al.,* 1997).

The earliest as well as most severe aftermaths of carbon tetrachloride poisoning are derangements of the nervous system, including headache, ataxia, vertigo, blurred vision, lethargy, coma, convulsions, optic neuritis, and polyneuritis.(Sarah *et al.,* 1967).

The damage-causing mechanism of CCl4 in tissues can be explained as oxidative damage caused by lipid peroxidation which starts after the conversion of CCl4 to free radicals of highly toxic trichloromethyl radicals ($•CCl_3$) and trichloromethyl peroxyl radical (CCl_3O_2) via cytochrome P450 enzyme (Velid *et al.,* 2020) Complete disruption of lipids (i.e., peroxidation) is the hallmark of oxidative damage.

CCl4-induced lipid peroxidation is the cause of oxidative stress, mitochondrial stress, and endoplasmic reticulum stress. Free radicals such as CCl4 play a role in various pathological conditions including Pulmonary disease, ischemia / reperfusion rheumatological diseases, autoimmune disorders, cardiovascular diseases, cancer, kidney diseases, hypertension, eye diseases, neurological disorders, diabetes and aging. Free radicals are antagonized by antioxidants and quenched (Velid *et al.,* 2020). The brain is the command center that controls the nervous system (Lauren, 2023). It functions in the regulation of various physiological processes, such as movement, sensation, perception, and cognition (Kandel *et al.,* 2013). The cerebrum, also called the telencephalon, refers to the two cerebral hemispheres (right and left) which form the largest part of the brain. It sits mainly in the anterior and middle cranial fossae of the skull (Edwin, 2024).

1.2 AIM OF STUDY

The aim of this study is to investigate the effects of carbon tetrachloride (CCl4) on the cerebrum of wister rats.

1.3 SPECIFIC OBJECTIVES

To investigate the effects of CCl_4 on;

i. The body and cerebral weight changes in rats treated with or without CCl_4

ii. Antioxidant enzymes (CAT, GPx and SOD) activity in the cerebrum of rats treated with or without CCl_4.

iii. MDA concentration (lipid peroxidation) in the cerebrum of rats treated with or without CCl_4.

iv. Histology of the cerebrum of rats treated with or without CCl_4.

1.4 JUSTIFICATION OF STUDY

The study will enhance understanding of the toxicity mechanisms of CCl_4 exposure, focusing on its specific impact on the cerebrum, thus contributing to the broader field of neurotoxicology.

By examining cellular changes like inflammation, oxidative stress, and neuronal cell death, the research could provide valuable insights into the development of neurodegenerative diseases.

The study aims to identify and characterize structural and functional damage in the cerebrum, helping to clarify how chemical exposure, like CCl_4, impacts brain health.

The findings could inform public health strategies by highlighting the potential risks of CCl_4 exposure to the brain, offering guidance for safer chemical management and preventive measures.

1.5 EXPECTED CONTRIBUTION TO KNOWLEDGE

This study will provide additional information to the neurotoxic effects of CCl4.

CHAPTER TWO

LITERATURE REVIEW

2.0 A REVIEW OF CCL4

2.1.1 DESCRIPTION

Carbon tetrachloride (CCl4 or CTC) is an ozone-depleting substance whose emissive uses are controlled and practically banned by the Montreal Protocol (MP) (David *et al*., 2018). Carbon tetrachloride is an organic compound of which chemical formula is CCl_4. It is very toxic to the kidney, testicle, brain, heart, lung, other tissues, and particularly in the liver(Velid *et al.,* 2020). Although banned, the use of CCl4 continues to provide an important service today as a model substance to elucidate the mechanisms of action of hepatotoxic effects such as fatty degeneration, fibrosis, hepatocellular death, and carcinogenicity(Lutz *et al.,* 2003).

CCl4 was manufactured in 1839 by the reaction of chlorine with carbon disulfide or with methane, the process with methane became dominant in the United States in the 1950s, however; the process with carbon disulfide remains important in countries where natural gas (the principal source of methane) is not plentiful, Carbon tetrachloride boils at 77° C (171° F) and freezes at -23° C (-9° F); it is much denser than water, in which it is practically insoluble (The Editors of Encyclopaedia Britannica, 2024).

2.1.2 HUMAN EXPOSURE TO CCl_4 AND ITS USE

Carbon tetrachloride (CCl_4) is a non-flammable, volatile, and colorless liquid that has been used widely in industry and commerce (ATSDR, 2015). Humans can be exposed to CCl_4 via multiple routes, including inhalation, ingestion, and skin contact (EPA, 2019).

One of the significant sources of exposure to CCl_4 is contact with products that have the chemical, such as cleaning agents, degrease-rs, and pesticides (ATSDR, 2015). For example, CCl_4 was used as a solvent in the production of refrigerants, propellants, and pharmaceuticals (EPA, 2019). However, its utilization has diminished significantly since the 1980s due to its toxicity and environmental persistence issues (UNEP, 2018).

Occupational exposure to CCl_4 is also of great concern, particularly in workplaces involving the manufacture, use, or disposal of the chemical (NIOSH, 2016). Workers in these workplaces may be exposed to CCl_4 through inhalation of vapor or dermal contact with contaminated surfaces (OSHA, 2020).

Other than occupational exposure, CCl_4 can also contaminate soil, water, and air, leading to environmental exposure (EPA, 2019). For example, CCl_4 was detected in groundwater and surface water near industrial plants and waste disposal sites (ATSDR, 2015).

CCl_4 is also deemed a probable human carcinogen by the International Agency for Research on Cancer (IARC, 2019). Exposure to CCl_4 has been linked to a variety of health effects, including liver and kidney damage, neurological damage, and cancer (EPA, 2019).

2.1.3 TOXIC EFFECTS OF CCl_4

The human body is composed of various systems and organs that work together to maintain homeostasis and overall health. For example, the nervous system, which controls and coordinates the body's activities, is comprised of the central nervous system (CNS) and the peripheral nervous system (PNS) (Bear, Connors, & Paradiso, 2018).

The CNS includes the brain and spinal cord, while the PNS consists of nerves that transmit messages from the CNS to the body (Carlson, 2019). The brain contains several areas, including the cerebrum, cerebellum, and brainstem, which have specialized functions (Moore, Persaud, & Torchia, 2016).

The circulatory system, also known as the cardiovascular system, supplies oxygen and nutrients to the body's cells and organs and removes waste products (Guyton & Hall, 2016). The system consists of the heart, arteries, veins, and blood vessels and is controlled by the autonomic nervous system (ANS) (Costanzo, 2018).

The respiratory system is responsible for the entry of oxygen into, and removal of carbon dioxide from, the body through the process of respiration (West, 2017). The system includes the lungs, airways, and respiratory muscles, and is controlled by the ANS (Costanzo, 2018).

The digestive system converts food into usable and absorbable nutrients for the body (Guyton & Hall, 2016). The system consists of the mouth, esophagus, stomach, small intestine, and large intestine, and is controlled by the ANS (Costanzo, 2018).

The urinary system, or renal system, removes waste and excess water from the body through the process of urination (Guyton & Hall, 2016). The system consists of the kidneys, ureters, bladder, and urethra, and is controlled by the ANS (Costanzo, 2018).

The integumentary system safeguards the body from external damage, maintains body temperature, and aids in the production of vitamin D (Guyton & Hall, 2016). The system consists of the skin, hair, nails, and associated glands and is controlled by the ANS (Costanzo, 2018).

2.2 THE ORGAN STUDY

2.2.1 THE CEREBRUM

The cerebrum is the largest part of the brain, responsible for processing sensory information, controlling movement, and facilitating thought, emotion, and memory (Kandel *et al.,* 2013). It is divided into two hemispheres: the left hemisphere, which is involved in language processing, logical reasoning, and analytical thinking; and the right hemisphere, which is involved in spatial reasoning, pattern recognition, and emotional processing (Springer and Deutsch, 2016). The cerebrum is composed of several distinct structures, including the cerebral cortex, basal ganglia, and limbic system. The cerebral cortex is the outermost layer of the cerebrum, responsible for processing sensory information and controlling movement (Purves *et al.,* 2018). The basal ganglia are a group of structures involved in movement control and cognition (Guyton and Hall, 2016). The limbic system is a network of structures involved in emotion, motivation, and memory (Bear *et al.,* 2018). The cerebral cortex is divided into four lobes: the frontal lobe, parietal lobe, temporal lobe, and occipital lobe. Each lobe is responsible for processing different types of sensory information and controlling different types of movement. The frontal lobe is responsible for processing information related to movement, language, and decision-making, It is divided into several distinct regions, including the primary motor cortex, premotor cortex, and prefrontal cortex (Blumenfeld, 2010). The parietal lobe is responsible for processing information related to touch, temperature, and spatial awareness, It is divided into several distinct regions, including the primary somatosensory cortex and secondary somatosensory cortex (Kolb and Whishaw, 2011).

The temporal lobe is responsible for processing information related to hearing, memory, and language, It is divided into several distinct regions, including the primary auditory cortex and secondary auditory cortex (Blumenfeld, 2010). The occipital lobe is responsible for processing information related to vision, It is divided into several distinct regions, including the primary visual cortex and secondary visual cortex (Bear *et al*., 2018).

CLINICAL SIGNIFICANCE

Damage to the cerebrum can result in a range of cognitive and motor deficits, including aphasia, apraxia, and hemiparesis (Kolb and Whishaw, 2011). Neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease also affect the cerebrum, leading to cognitive decline and motor impairment (Selkoe, 2011).

2.2.2 HISTOLOGY OF THE CEREBRUM

The histology of the cerebrum is complex, with multiple layers and types of cells working together to enable its various functions (Crossman and Neary, 2019).

Layers of the cerebrum

The cerebrum is composed of several distinct layers, each with its own unique characteristics and functions.

1. Molecular Layer: The molecular layer is the most superficial layer of the cerebrum, composed of a dense network of fibers and a few scattered neurons (Purves *et al.,* 2018).

2. External Granular Layer: The external granular layer is a layer of small neurons and neuroglial cells, located just beneath the molecular layer (Kolb and Whishaw, 2011).

3. External Pyramidal Layer: The external pyramidal layer is a layer of pyramidal neurons, which are involved in motor and sensory functions (Damasio, 2004).

4. Internal Granular Layer: The internal granular layer is a layer of small neurons and neuroglial cells, located just beneath the external pyramidal layer (Kolb and Whishaw, 2011).

5. Internal Pyramidal Layer: The internal pyramidal layer is a layer of pyramidal neurons, which are involved in motor and sensory functions (Damasio, 2004).

6. Polymorphic Layer: The polymorphic layer is the deepest layer of the cerebrum, composed of neurons and neuroglial cells (Purves *et al.*, 2018).

Cell Types of the Cerebrum

The cerebrum is composed of multiple cell types, each with its own unique characteristics and functions.

1. Pyramidal Neurons: Pyramidal neurons are the primary excitatory neurons of the cerebrum, involved in motor and sensory functions (Damasio, 2004).

2. Granule Cells: Granule cells are small, inhibitory neurons that play a critical role in regulating the activity of pyramidal neurons (Kolb and Whishaw, 2011).

3. Neuroglial Cells: Neuroglial cells, including astrocytes and oligodendrocytes, provide support and maintenance functions for neurons (Purves *et al.,* 2018).

Blood Supply of the Cerebrum

The cerebrum receives its blood supply from several major arteries, including the anterior cerebral artery, middle cerebral artery, and posterior cerebral artery (Kandel *et al.*, 2013).

2.2.3 DEVELOPMENT OF THE CEREBRUM

The development of the cerebrum is a complex process that involves the coordinated action of multiple cell types and tissues.

Early Embryonic Development

The development of the cerebrum begins during the third week of embryonic development, when the neural plate forms, the neural plate is a layer of cells that will eventually give rise to the central nervous system (Sadler and Langman, 2012).

Neural Tube Formation

During the fourth week of embryonic development, the neural plate folds in on itself to form the neural tube, the neural tube is a hollow structure that will eventually give rise to the brain and spinal cord (Carlson, 2019).

Brain Vesicle Formation

During the fifth week of embryonic development, the neural tube expands and differentiates into three primary brain vesicles: the prosencephalon (forebrain), mesencephalon (midbrain), and rhombencephalon (hindbrain) (Schoenwolf *et al.,* 2015).

Cerebral Hemisphere Formation

During the sixth week of embryonic development, the prosencephalon divides into two cerebral hemispheres, the cerebral hemispheres will eventually give rise to the cerebrum (Moore *et al*., 2016).

Cortical Layer Formation

During the seventh week of embryonic development, the cerebral hemispheres begin to differentiate into distinct cortical layers (Carlson, 2019). The cortical layers will eventually give rise to the different cell types and tissues of the cerebrum (Schoenwolf *et al.,* 2019).

Neurogenesis and Migration

During the eighth week of embryonic development, neurogenesis (the process of neuron formation) begins in the cerebral hemispheres, newly formed neurons migrate to their final positions in the cortical layers (Moore *et al.,* 2016).

Synaptogenesis and Myelination

During the ninth week of embryonic development, synaptogenesis (the process of synapse formation) begins in the cerebral hemispheres, myelination (the process of forming a myelin sheath around axons) also begins during this time (Moore *et al.,* 2016).

Fetal Development

During fetal development, the cerebrum continues to grow and differentiate, the cerebral hemispheres expand and fold, forming the characteristic gyri and sulci of the adult brain (Moore and Persaud, 2016).

Postnatal Development

After birth, the cerebrum continues to develop and mature, synaptogenesis and myelination continue, and the cerebral hemispheres continue to expand and fold (Carlson, 2019).

2.2.4 FUNCTION OF CEREBRUM

Motor Function

The cerebrum plays a critical role in motor function, including the planning and execution of movement. The primary motor cortex, located in the precentral gyrus, is responsible for transmitting signals to the spinal cord and other motor centers to execute movement (Bear *et al.*, 2018).

Sensory Function

The cerebrum is also responsible for processing sensory information from the environment, including visual, auditory, tactile, olfactory, and gustatory information, the primary sensory cortices, located in the postcentral gyrus, are responsible for receiving and processing sensory information from the thalamus (Bear *et al.*, 2018).

Cognitive Function

The cerebrum plays a critical role in cognitive function, including attention, perception, memory, language, and problem-solving, the prefrontal cortex, located in the frontal lobe, is responsible for executive function, including decision-making, planning, and working memory (Bear *et al.*, 2018).

Emotional Function

The cerebrum is also involved in emotional processing, including the recognition and expression of emotions, the amygdala, located in the temporal lobe, is responsible for processing emotional information and triggering emotional responses (Bear *et al.*, 2018).

Language Function

The cerebrum plays a critical role in language processing, including speech production and comprehension, the left hemisphere, particularly the left inferior frontal gyrus, is involved in language processing (Moore *et al.*, 2016).

Memory Function

The cerebrum is involved in memory formation and storage, including short-term and long-term memory, the hippocampus, located in the temporal lobe, is responsible for forming new memories (Bear *et al.,* 2018).

CHAPTER THREE

MATERIALS AND METHODS

3.0 ANIMALS AND MANAGEMENT

In this study, eighteen adult Wistar rats with weights ranging from 140g to 150g were used. The rats were bred in the animal House of the Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State. They were housed in polypropylene cages under ambient room temperature conditions. Throughout the experiment the animals were fed with Topline Grower Mash and provided with unrestricted access to water. At the start of the experiment and throughout its duration, the animals weights were monitored daily using a digital scale calibrated in grams and recorded to the nearest whole number. All procedures adhered to the guidelines for the care and use of laboratory animals (National Research Council of the National Academics, 2011).

3.1 COLLECTION AND IDENTIFICATION OF COMPOUND

Tetrachloromethane (CCl_4) was brought at PYREX chemicals situated No 65 first east circular road, Benin, Nigeria. 50ml of CCl_4 was mixed with 50ml of olive oil.

3.2 REGENT/CHEMICALS

All regents and chemicals were of analytical grade. They include distilled water, olive oil, Tetrachloromethane (CCl_4), methylated spirit, alcohol (50%, 70%, 90%, 100%), xylene, paraffin, normal saline, buffered saline,

3.3 EQUIPMENT

Surgical latex gloves,weighing balance, measuring cylinder, conical flask, volumetric flask, polypropylene cages, refrigerator, oven, sample bottles, water baths, paraffin dispenser, dissecting sets, glass rods, rotary microtome, binocular microscope.

3.4 PREPARATION

50ml of tetrachloromethane was mixed with 50ml of olive oil, making the volume ratio of olive oil to tetrachloromethane 50:50.

3.5 EXPERIMENTAL DESIGN

A total of eighteen (18) adult wistar rats with weights ranging from 140g to 150g were used in this study, they were randomly divided into three (3) groups of six (6) rats each, after acclimatization to the animal house conditions for two weeks with free access to feed and water, the administration was carried out for twenty-eight (28) days and it was done intraperitoneally via a syringe.

Table 3.1: Experimental design

GROUPS	DOSAGE
GROUP A	1ml of distilled water
GROUP B	1.5ml/kg body weight of CCl_4
GROUP C	3ml/kg body weight of CCl_4

3.6 SACRIFICE OF ANIMALS AND SAMPLE COLLECTION

At the end of the twenty-eight days administration, the rats were weighed and sacrificed through cervical dislocation, after which the head was cut open to reveal the skull and the brain was harvested. The weight of the entire brain and cerebrum was taken, and the organ of study; the cerebrum, was detached from the entire brain and put in a sample bottle, fixed in buffered formalin and sent to the laboratory for histological processing.

3.7 METHODOLOGY FOR OXIDATIVE PARAMETERS

The concentration of MDA was determined according to the method of Buege and Aust (1978). The principle that underlies this assay is that MDA – a product of lipid peroxidation when heated with thiobarbituric acid (TBA), in the presence of an acid, forms a pink or reddish complex that is measured spectrophotometrically at 532 nm. The table below clearly illustrates the procedure adopted in the determination of the level of malondialdehyde.

Assay Procedure

An aliquot of the liver homogenate was added to 3.0 mL of TCA – TBA – HCl reagent and mixed thoroughly by swirling. The solution was heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate was removed via centrifugation at 1000 g for 10 min. The absorbance of the clear supernatant was measured against a reference blank at 535 nm.

Calculation

The MDA concentration of each sample was calculated as follows:

O.D × Vt × 1000

a × V × L × Y

where,

O. D = Absorbance of sample test at 535 nm

Vt = Total volume of the reaction mixture = 3.6 mL

a = Molar extinction coefficient of product = 1.56 × 105 M-1cm-1

L = Light path = 1.0 cm

V = Volume of sample homogenate used = 0.6 mL

Y = mg of tissue in the sample used

The unit of MDA is moles/mg wet tissue

Determination of Superoxide Dismutase (SOD) Activity

Principle

The activity of SOD was assessed based on the method of Misra and Fridovich (1972). Adrenaline auto-oxidizes rapidly in aqueous solution to adrenochrome whose concentration can be determined spectrophotometrically at 420 nm. The auto-oxidation depends on the presence of superoxide anions (O2-). Superoxide dismutase (SOD) inhibits this auto-oxidation by catalyzing the breakdown of superoxide anions. The degree of inhibition is thus a measure of SOD activity. The amount of enzyme producing 50 % inhibition is defined as one unit of the enzyme activity.

Assay Procedure

Sample homogenate (0.2 mL) was added to 2.5 mL of 0.05 M carbonate buffer (pH 10.2) and allowed to equilibrate. The reaction was initiated by the addition of 0.3 mL of freshly prepared 0.03 mM adrenaline as substrate. The solution was mixed by inversion. The reference tube contained 2.7 mL of carbonate buffer and 0.3 mL of adrenaline, while the blank contained 2.5

mL of carbonate buffer, 0.2 mL of distilled water and 0.3 mL of 0.03 mM adrenaline. The increase in absorbance at 420 nm due to the formation of adrenochrome was monitored every 30 sec for 120 sec. One unit of SOD activity was taken as the amount of SOD necessary to cause 50 % inhibition of the oxidation of adrenaline to adrenochrome within 120 sec.

Calculation

$$\% \text{ Inhibition} = \frac{\text{O.Dtest} - \text{O.Dreference}}{\text{O.Dtest}} \times 100$$

$$\text{Enzyme Activity (units/mg protein)} = \frac{\% \text{ inhibition}}{50 \times Y}$$

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

A unit of SOD activity was taken as the amount of SOD required to cause 50 % inhibition of the auto-oxidation of adrenaline to adrenochrome per minute.

Determination of Catalase Activity

Principle

This is based on the method of Cohen *et al.,* (1970). This estimation is based on the measurement of the rate of decomposition of hydrogen peroxide (H2O2), after the addition of the material containing the enzyme.

Catalase catalyses the reaction: → $2H_2O_2$ $2H_2O + O_2$

The quantity of hydrogen peroxide decomposed is directly proportional to the concentration of the enzyme in the sample. The hydrogen peroxide produced in tissues is measured by reacting it with excess potassium permanganate ($KMNO_4$) and then measuring the residual $KMNO_4$ spectrophotometrically at 480 nm.

Assay Procedure

Sample homogenate (0.5 mL) was placed in ice – cold test tubes, the blank contained 0.5 mL distilled water. Cold phosphate-buffered H_2O_2 (30 mM, 5 mL) was added to both blank and sample tubes at fixed intervals, and were mixed by inversion. After 3 min, the reaction was stopped by rapid addition of 1 mL of 6 M H_2SO_4. The tubes were mixed thoroughly by inversion after which 7 mL of 0.01 M $KMNO_4$ was added. Absorbance was read at 480 nm within 3 min.

Calculation

The activity of catalase in each sample is calculated thus:

$$\frac{O.D/min \times Vt \times 1000}{M \times V \times L \times Y}$$

where,

O.D = Absorbance of sample test at 480 nm

Vt = Total volume of the reaction mixture = 13.5 mL

M = Molar extinction coefficient of H_2O_2 = 43.6M-1 cm-1

L = Light path = 1.0 cm

V = Volume of sample homogenate used = 0.5 mL

Y = mg of protein in tissue used

Determination of Glutathione Peroxidase Activity

Glutathione peroxidase (GPx) activity was measured according to the method described by Nyman (1959).

Principle

This is based on the oxidation of pyrogallol to purpuragallin by peroxidase, resulting to a deep brown colouration, which is read at 430 nm.

Procedure

To an aliquot of sample (0.2 mL), 5 mL of phosphate-buffered H2O2, and 1.5 mL of pyrogallol were added. The reaction mixture was allowed to stand for 30 min at room temperature. A deep colour was formed, which was read at 430 nm.

Calculation

$$\text{Enzym Activity} = \frac{\text{OD/min} \times Vt \times Df}{E \times Vs \times Y}$$

where OD = Absorbance of test

Vt = Total volume of reaction mixture

Df = Dilution factor

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

Vs = Volume of sample

Y = mg of protein used

Determination of Concentration of Reduced Glutathione

The 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 sample, 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

$$\text{Concentration of GSH} = \frac{A_{test} \times \text{Conc. of Standard}}{A_{standard}}$$

3.8 HISTOLOGICAL PROCEDURES

3.8.1 Paraffin Tissue Processing of Drury and Wallington (1980)

Following fixation in buffered formalin , the tissues were processed as follows.

Dehydration in ascending grades of ethanol, from 70% ethanol to 90% ethanol and then 100% absolute ethanol for the duration of one hour each.

The dehydrated tissues were cleared in three changes of xylene for one hour each.

Infiltration of tissues was done in three changes of paraffin wax at 60°C for one hour each.

Paraffin wax was used in embedding the tissues, the paraffin blocked tissues were trimmed and mounted on wooden block for rotary microtome.

3.8.2 Haematoxylin and Eosin Staining Method of Drury and Wallington (1980).

Sections were dewaxed in two changes of xylene for two minutes in each change.

Sections were rehydrated in decreasing grades of alcohol, from 100%, to 90% and 70% and then transferred to water.

Staining of sections was carried out in Iron Haematoxylin for 15-20 minutes.

Excess stains were removed by washing under tap water.

Differentiated in acid alcohol (0.5% HCl in 70% ethanol) for two to three minutes.

Rinse well in running water for 10 to 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 microwscope ;

Dehydrated in ascending grades of alcohol (from 50% to absolute alcohol)

Cleared in xylene and mounted in Distrene plasticizer and xylene (DPX).

3.9 PHOTOMICROGRAPHY

A binocular microscope equipped with an Omax 9.0MP USB Digital Microscope Camera was used to take pictures of the treated slides. The camera has a 0.5X reduction lens and a 9 megapixel (3488 x 2616 pixel) high quality color digital camera. A laptop was then linked to it. The use of 4 and 10 objective lenses produced a panoramic image of the slides. For the final product , the photographs were automatically combined and processed in Adobe Photoshop CC (Version 20.0, x 64).

3.10 STATISTICAL ANALYSIS

Data was analysed 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).

CHAPTER FOUR

RESULTS

4.1 BODY WEIGHT:

No significant change ($p>0.05$) was observed in the initial body weight, final body weight, or weight change among the experimental groups

4.2 CEREBRAL WEIGHT:

No significant changes ($p>0.05$) were observed in the cerebral weight of rats in all experimental groups

4.3 RELATIVE CEREBRAL WEIGHT:

a significant change ($p<0.05$) in the Relative cerebral weight of rats in group B and C when compared to control

4.4 ANTIOXIDANT ENZYMES

A significant decrease ($p<0.05$) in the activities of cerebral superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and reduced glutathione (GSH) was noted in rats administered 0.4ml/kg body weight of CCl_4(Group C), compared to the control group

4.5 MALONDIALDEHYDE (MDA) CONCENTRATION:

A significant increase ($p<0.05$) in MDA concentration was observed in rats from both group B (1.5ml/kg CCl_4) and group C (0.4ml/kg CCl_4) compared to the control group.

4.6 HISTOLOGICAL OBSERVATIONS:

Control rats displayed normal cerebral cortex architecture, well organized layers and cells

Rats in group B (1.5ml/kg CCl_4) and group C (0.4ml/kg CCl_4) exhibited histopathological changes, including:

Shrunken neuronal cell bodies and cytoplasmic vacoulization in the granular cells of the inner granular layer.

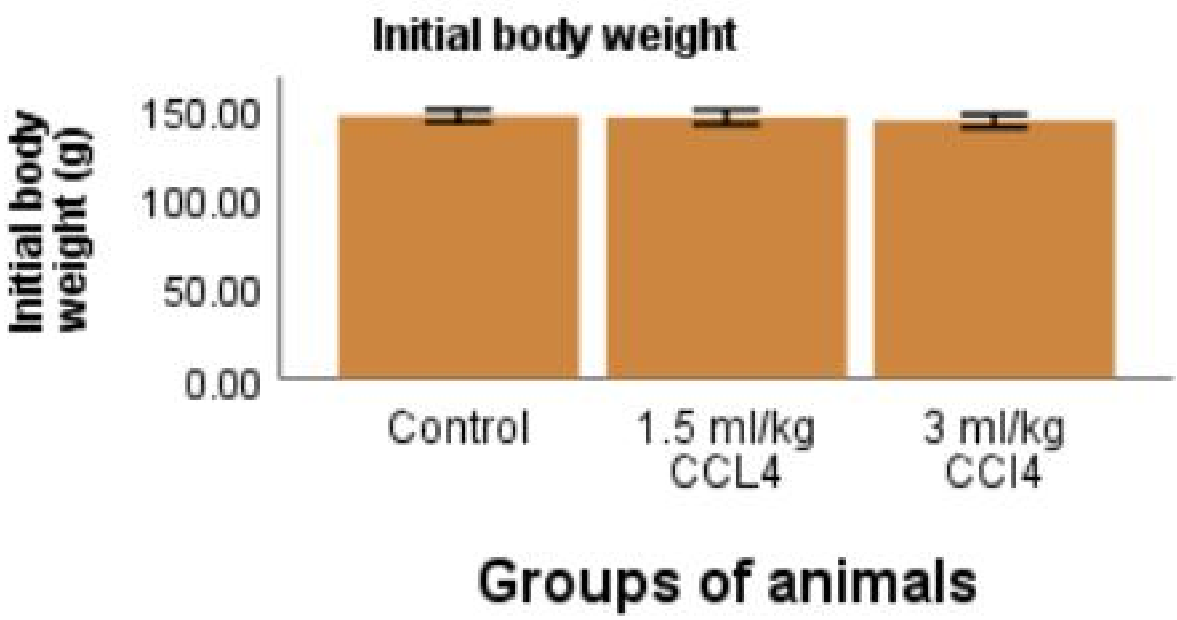

Figure 4.0.1: initial body weight of rat across experimental groups.

Results obtained showed that CCl_4 does not significant differences($P>0.05$) in the initial body weight of rat across experimental groups.

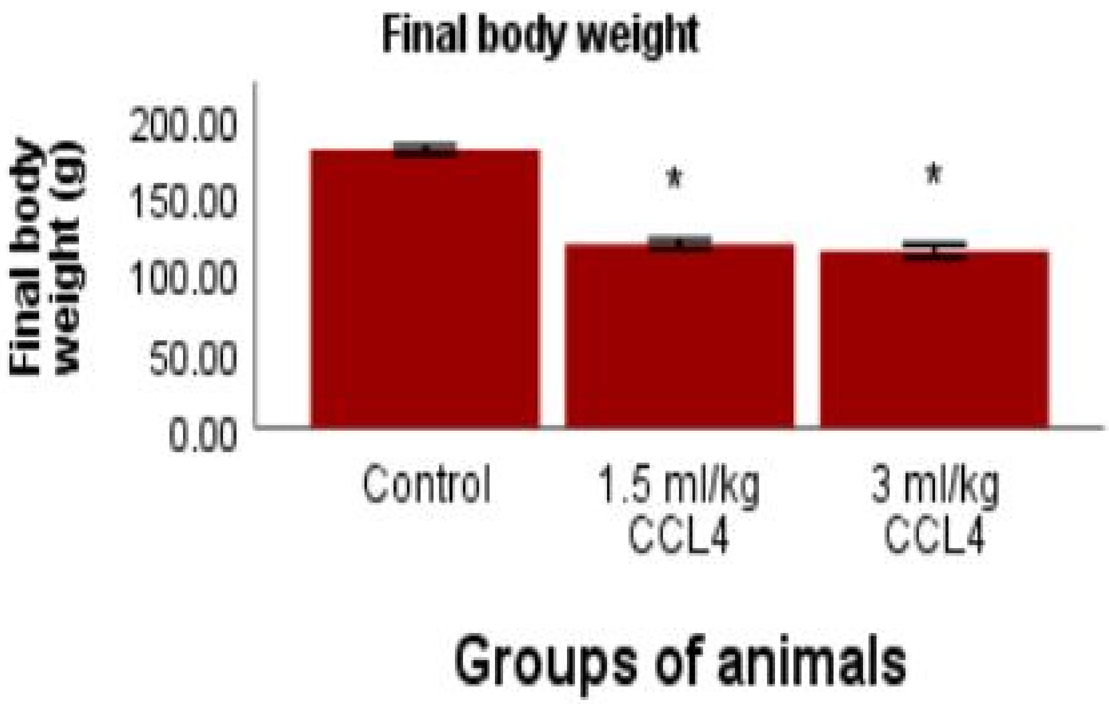

Figure 4.0.2: Final body weight of rats in experimental groups

Results obtained shows that CCl_4 cause a significant difference(P<0.05) in the final body weight among treated groups(group B and C) when compared to control.

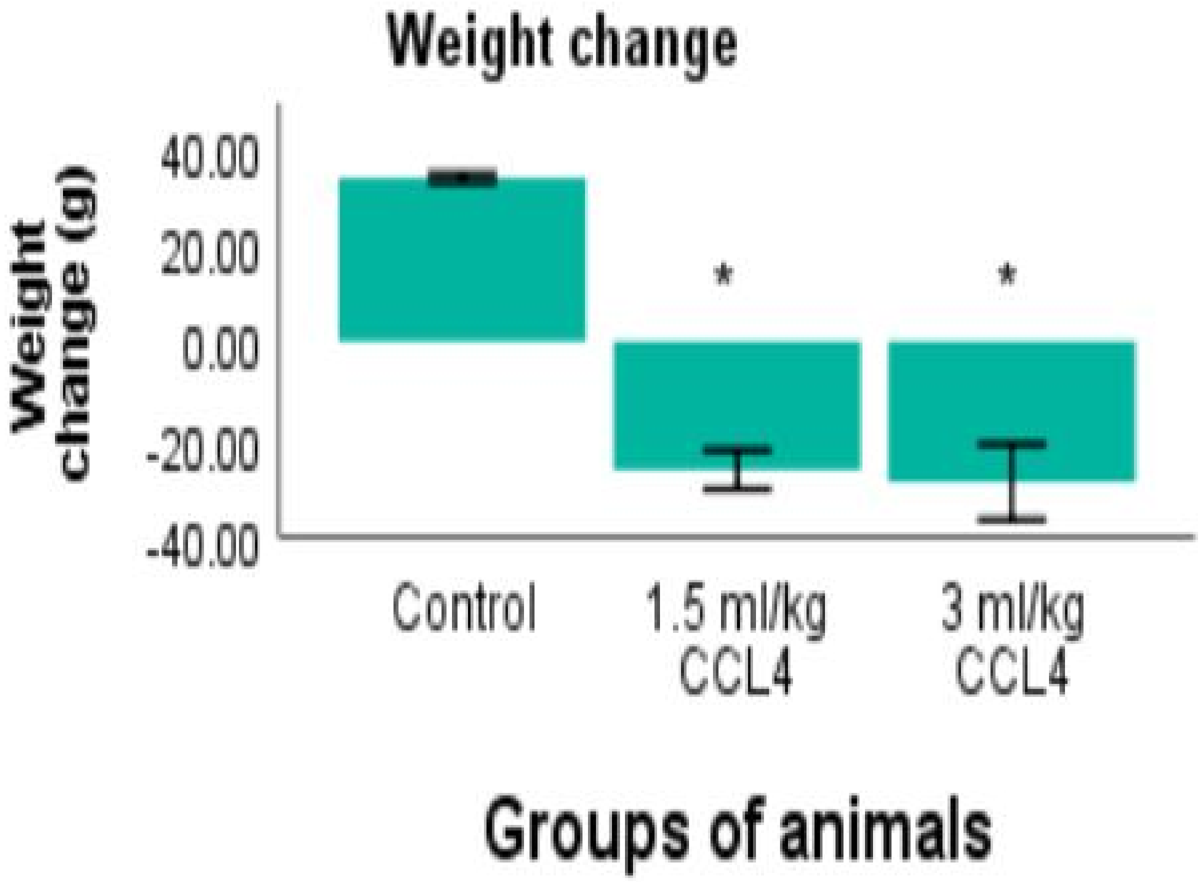

Figure 4.0.3: body weight change in experimental groups.

Results obtained showed a significant decrease ($P<0.05$) in change in body weight among treated groups (group B and C) respectively when compared to control.

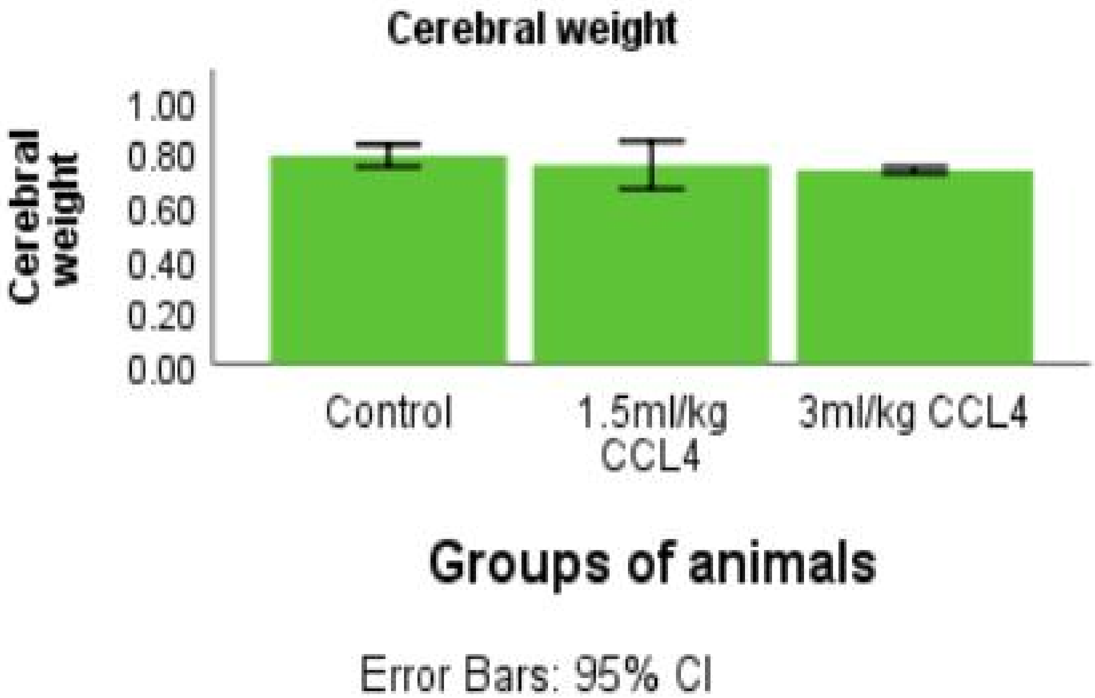

Figure 4.0.3: Cerebral weights of rats in group across experimental.

Results obtained shows that CCL4 did not cause any significant difference ($P>0.05$) in the cerebral weights of rats in group across experimental groups.

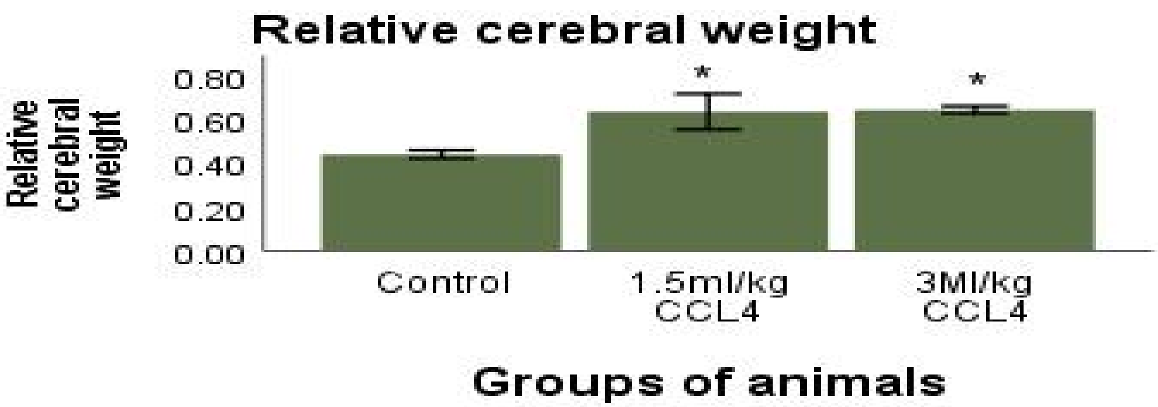

Figure 4.0.4: The Relative cerebral weight of rats in Experimental groups.

Results Obtained shows that CCL4 caused a significant change($p<0.05$) in the Relative cerebral weight of rats in group B and C when compared to control

ANTIOXIDANTS

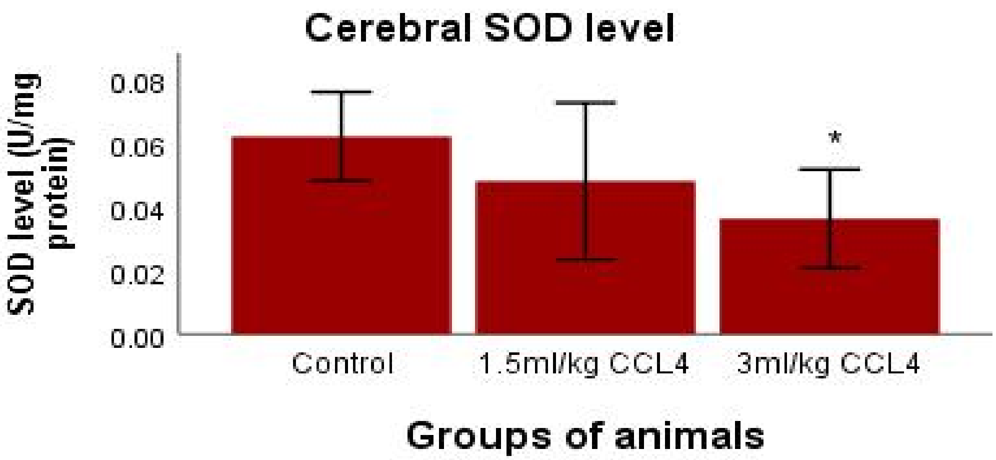

Figure 4.0.5: Cerebrum superoxide dismutase (SOD) activity in control and treatment groups.

Shows superoxide dismutase (SOD) activity in control rats and rats administered with 1.5ml/kg CCl4 and 0.4ml/kg CCl4 for 28 days. Values are represented as mean +_ SEM for each group; n=3/group. *: Control compared to CCl4.

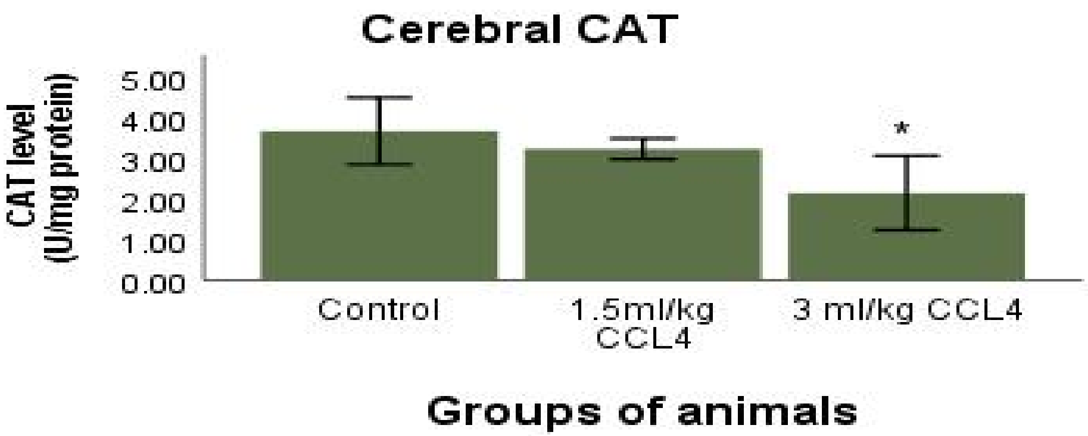

Figure 4.0.6: Cerebrum catalase (CAT) activity in control and treatment groups.

Shows catalase (CAT) in control rats and rats administered with 1.5ml/kg CCl4 and 0.4ml/kg CCl4 for 28 days. Values are represented as mean +_ SEM for each group; n=3/group. *: Control compared to CCl

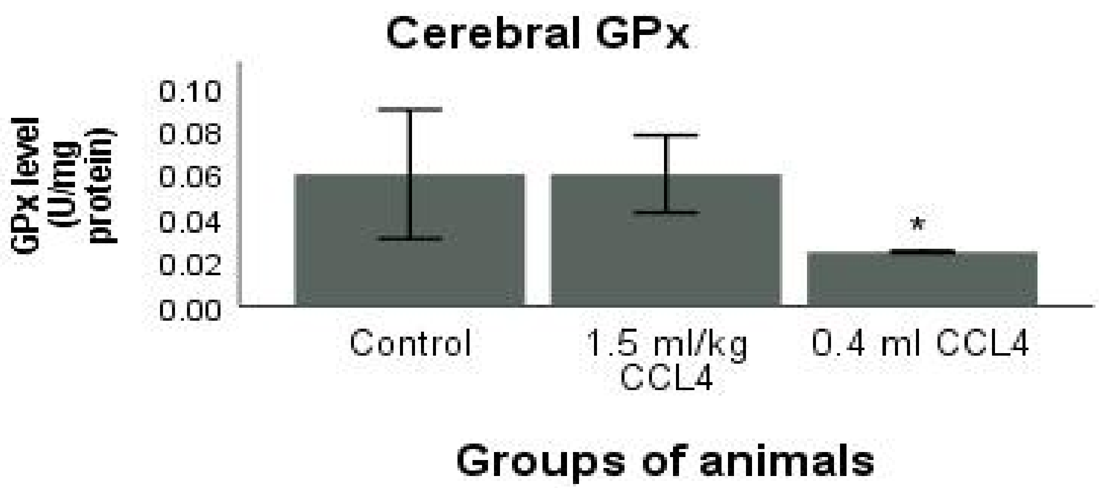

Figure 4.0.7: Cerebrum glutathione peroxidase (GPx) activity in control and treatment group. Shows glutathione peroxidase (GPx) activity in control rats and rats administered with 1.5ml/kg CCl4 and 0.4ml/kg CCl4 for 28 days. Values are represented as mean +_ SEM for each group; n=3/group. *: Control compared to CCl4.

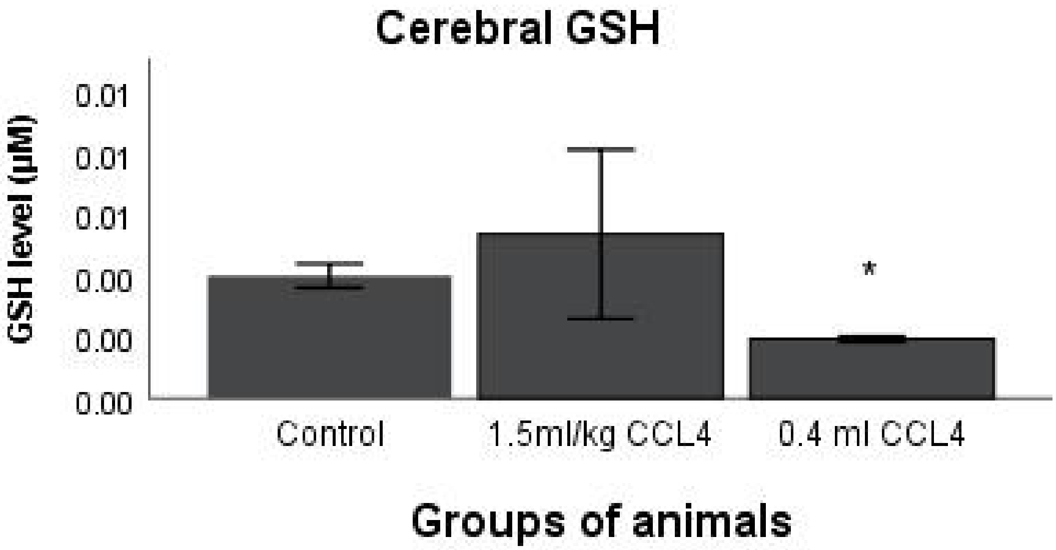

Fig4.0.8: Cerebrum GSH concentration in control and treatment group

Shows concentration of reduced glutathione (GSH) in control rats and rats administered with 1.5ml/kg CCl4 and 0.4ml/kg CCl4 for 28 days. Values are represented as mean +_ SEM for each group; n=3/group. *: Control compared to CCl4.

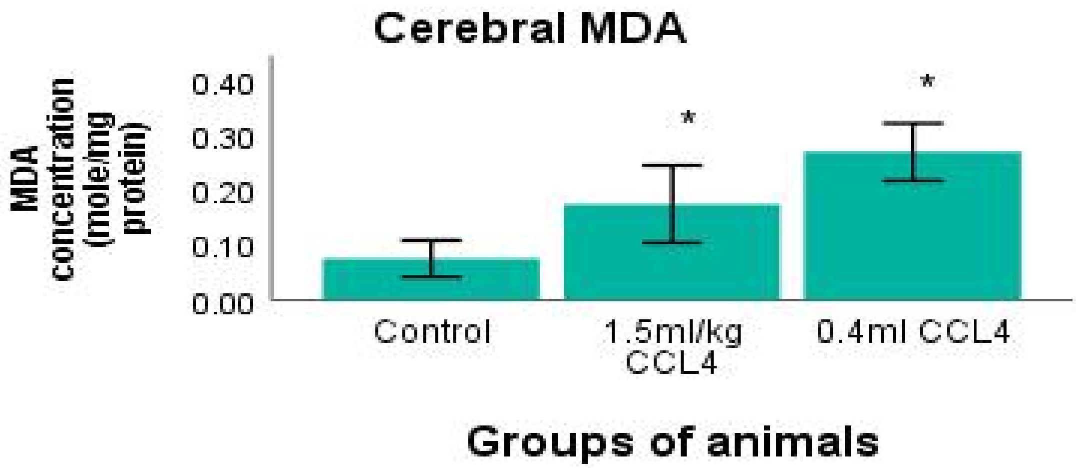

Fig4.0.9: Shows malondialdehyde (MDA) concentration in control rats and rats administered with 1.5ml/kg CCl4 and 0.4ml/kg CCl4 for 28 days. Values are represented as mean +_ SEM for each group; n=3/group. *: Control compared to CCl4.

HISTOLOGICAL RESULTS

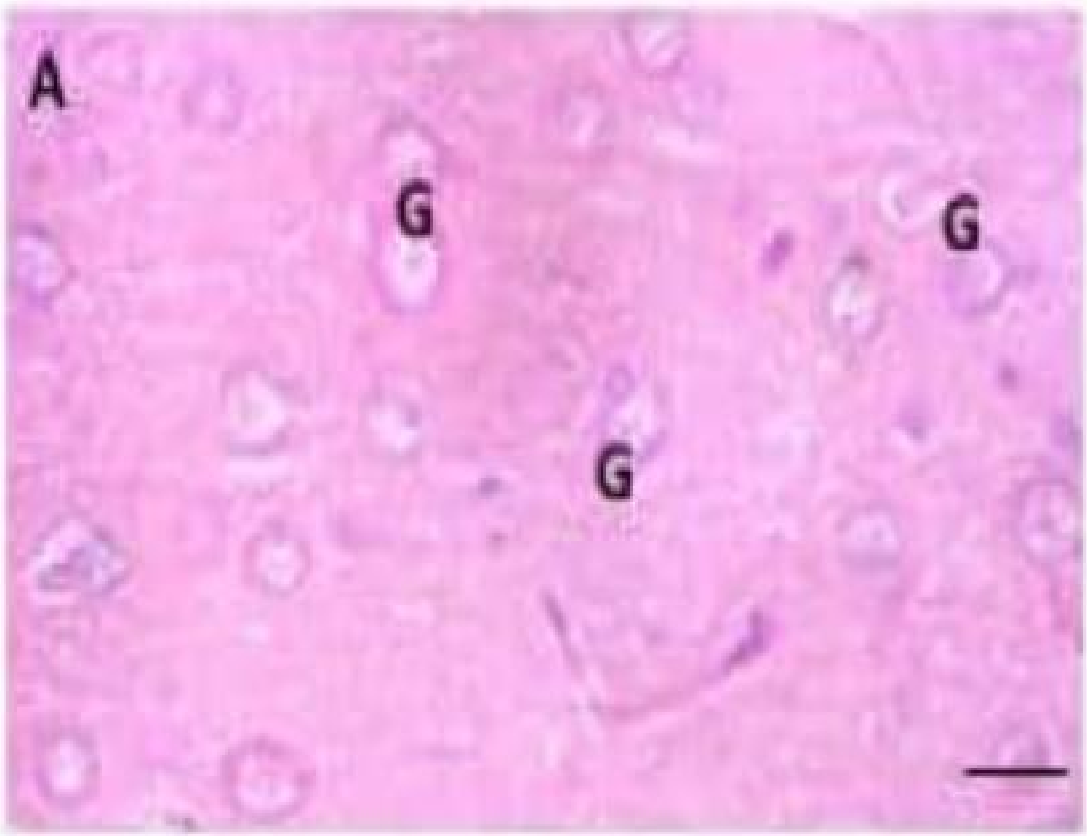

PLATE 1: Representative histology of the cerebral cortex (internal granular layer, IV) in control and treated rats showing normal granular (G) cells. (H&E; Scale bar: 25µm).

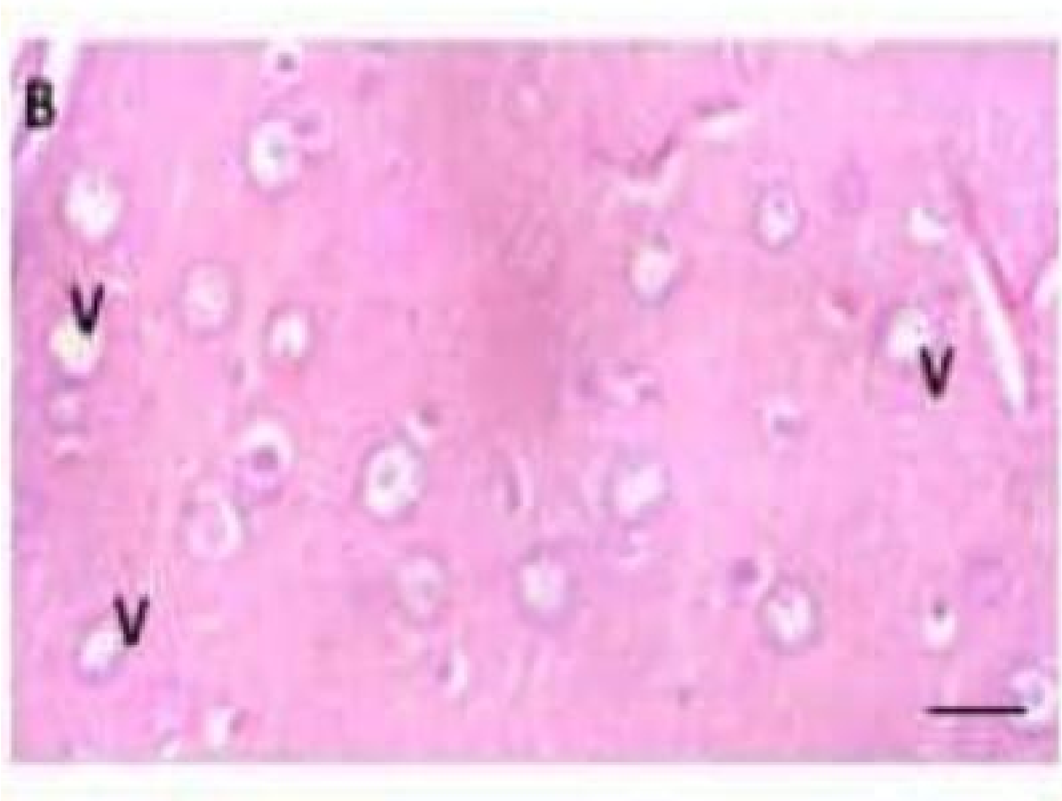

PLATE 2: Representative histology of the cerebral cortex (internal granular layer, IV) in control and treatment rats. (B) Cytoplasmic Vacoulization (V) and dark shrunken neuronal cell bodies (H&E; Scale bar: 25µm)

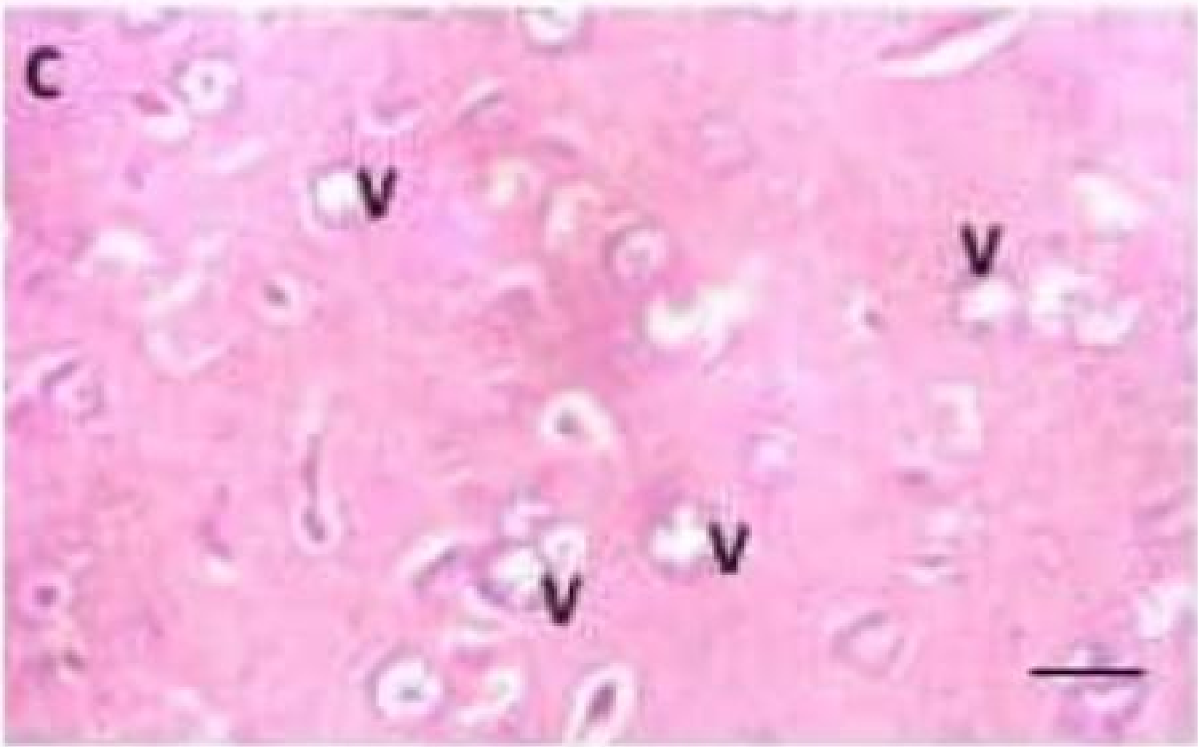

PLATE 3: Representative histology of the cerebral cortex (internal granular layer, IV) in control and treatment rats. (C) Cytoplasmic Vacoulization (V) and dark shrunken neuronal cell bodies (H&E; Scale bar: 25µm)

CHAPTER FIVE

DISCUSSION

5.1 DISCUSSION

Findings from this study showed no significant difference in the initial body weight of rats across experimental groups. However, there was a significant difference in the final body weight among treated groups (Group B and C) compared to the control group. In addition, there was a significant decrease in body weight change among treated groups compared to the control group. These findings suggest that CCl_4 administration affects body weight, particularly in the treated groups.

The study also found no significant difference in cerebral weight among experimental groups. However, there was a significant change in relative cerebral weight in Group B and C compared to the control group. This suggests that CCl4 administration affects the brain-to-body weight ratio.

In antioxidant enzymes study showed a significant decrease in antioxidant enzyme activities (SOD, CAT, GPx) in treated groups compared to the control group, which suggests that CCl4 administration leads to oxidative stress, which can damage cellular components. Findings in this study was consistent with previous studies that have shown that CCl4 is a potent neurotoxicant that can cause damage to the brain and nervous system (Weber *et al*., 2003; Sánchez-Bayo *et al*., 2011).

It was observed in the analysis that there was a significant decrease in GSH concentration in treated groups compared to the control group. GSH is an antioxidant that helps protect cells from oxidative damage.

There was a significant increase in MDA concentration in treated groups compared to the control group. MDA is a marker of lipid peroxidation, which can indicate cellular damage.

The study found histopathological changes in the cerebral cortex of treated rats, including cytoplasmic vacuolization and dark shrunken neuronal cell bodies (Plates 1-3). These changes suggest that CCl_4 administration causes neuronal damage. This is in agreement with the research work by Kumar *et al.,* 2014.

5.2 CONCLUSION

In conclusion, findings from this study shows that carbon tetrachloride induced neurotoxic effects on the cerebrum via inducing oxidative stress and altering the architectural integrity of the cerebrum.

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