EVALUATING THE PROTECTIVE ACTIVITY OF ETHANOLIC *CURCUMA LONGA* ROOT EXTRACT ON CADMIUM-INDUCED HIPPOCAMPAL DAMAGE IN ADULT WISTAR RATS.

BY

EGHOMWANRE ABIEUWA JENNIFER

BMS1601623

DEPARTMENT OF ANATOMY

SCHOOL OF BASIC MEDICAL SCIENCES

UNIVERSITY OF BENIN

BENIN CITY.

JULY, 2021.

EVALUATING THE PROTECTIVE ACTIVITY OF ETHANOLIC *CURCUMA LONGA* ROOT EXTRACT ON CADMIUM-INDUCED HIPPOCAMPAL DAMAGE IN ADULT WISTAR RATS.

BY

EGHOMWANRE ABIEUWA JENNIFER

BMS1601623

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

JULY, 2021.

CERTIFICATION

I certify that this project work titled “Evaluating the protective activity of ethanolic root extract of *Curcuma longa* root extract on cadmium-induced hippocampal damage in adult wistar rats" was carried out by **EGHOMWANRE ABIEUWA JENNIFER (BMS1601623)** in the Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin.

……………………………….. ………………………

EGHOMWANRE ABIEUWA JENNIFER Date

(Student)

………………………………….. ……………….........

DR. ADAZE. B. ENOGIERU Date

(Project Supervisor)

…………………………………….. …………………….

DR. C. L SAKPA Date

(Head of Department)

……………………………………... ……………………

External Supervisor Date

DEDICATION

This work is dedicated to the Almighty GOD for his infinite mercy and also for the strength and knowledge for this research work.

ACKNOWLEDGEMENTS

All thanks to God Almighty for giving me the capability to achieve this feat. My inestimable gratitude goes to my parents, Mr and Mrs Sunday Osarugue Eghomwanre for their financial and moral support throughout my stay in this University, especially during the course of this research work. To my aunt, Mrs Walter for your advice, and to my younger brother, Emmanuel for his prayers and encouragement.

My sincere appreciation goes to my project supervisor, Dr Adaze, B. Enogieru for his immense contribution and advice which led to the successful completion of this research work. I also thank all my lecturers of the Department of Anatomy for their support.

I wish to show my gratitude to my project partners, Omotara, Gabriel, Radiance and Nathaniel. The list is endless though, but it cannot do without my lovely friends, Gina, Samantha, Emeka, Merit, Jethson, Precious, whose love and help has contributed immensely to the success of this research work. I pray that the Lord will bless you all in return.

ABSTRACT

Many different forms of exposure to Cadmium have been shown over the past century with Cadmium been present in the environment as a result of many human activities. The constant sources of Cadmium contamination are related to it's application in industry as a corrosive reagent, as well as it's use as a stabilizer in PVC products, color pigments and Ni-Cd batteries. In areas with contaminated soils, house dust is a potential route for cadmium exposure. Cadmium exposure causes pathological conditions such as neurodegenerative diseases, Alzheimer's disease and Parkinson's disease. Medicinal drugs have been administered to fight the effect on humans. Due to this, many plants have been discovered for the treatment of the disease caused by Cadmium. Turmeric is one of the medicinal plants which contains anti-oxidant and anti-inflammatory properties which helps to fight against oxidative stress caused by Cadmium. It helps fight against neurodegenerative diseases. Accordingly, this research was designed to evaluate the protective activity of the ethanolic Curcuma longa root extract on cadmium-induced hippocampal damage in adult Wistar rats. For this study period of twenty-one days, thirty adult Wistar rats were randomized into six groups(A-F) consisting of five rats each. The rats were left to acclimatize for two weeks after which their weights were taken and recorded as initial weights while feeding then with standard rat chow and free access to water throughout the study. All administration, given orally, started with Group A serving as the control group and received 1ml of water. Group B was given 5mg/kg body weight of Cadmium only. Groups C and D were given 100mg and 200mg/kg body weight of ethanolic Curcuma longa root extract and 5mg/kg body weight of cadmium respectively. Groups E and F were given only 100mg and 200mg/kg body weight of ethanolic Curcuma longa root extract, respectively. At the end of the experiment, neurobehavioural activity (y-maze test) was evaluated, recorded, and the rats were then sacrificed for sample collection. Following appropriate harvesting of the hippocampus, the antioxidant activity and histological alterations were investigated. Results showed a significant decrease in the brain and body weight of rats treated with cadmium. For the Y-maze test, Curcuma longa demonstrated significant inhibition of Cadmium-induced cognitive dysfunction. Curcuma longa produced a significant decrease in MDA level and increased SOD, CAT, GPx enzymes as compared to the Cadmium treated group. The histology of rats pre-treated with ethanolic *Curcuma longa* root extract showed considerable retention of the normal histological morphology of the hippocampus. In conclusion, the findings of the study validated the toxicity of cadmium and provided the first research evidence of the protective activity of *Curcuma longa* against cadmium toxicity in adult Wistar rats.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF PROJECT

Cadmium is a chemical element with the symbol Cd and atomic number 48. It is a soft, silvery-white bivalent metallic element which has been used in nickel-cadmium battery, as a pigment in paint production, in electroplating and producing polyvinyl chloride plastic. Furthermore, cadmium is present in most foodstuffs, and depending on dietary habits, its level varies greatly (Meepun et al., 2014). As a result of human activities, such as the use of fossil fuels, metal combustion and waste burning, cadmium considerably exists in environment. It can also exist through leaking sewage sludge to agricultural soils which is absorbed by plants, consumed and accumulated in various organs in Man. Also, the other great source of cadmium exposure is cigarette smoke (Fresquez et al., 2013). When cadmium was measured in smokers' blood samples, it showed that they had 4-5 times Cd levels in blood higher than the non-smokers. Exposure to cadmium affects neurobiological processes which lead to oxidative stress.

Cadmium can induce oxidative stress by generating reactive oxygen species (ROS) (Romero-Puertas et al., 2007). Oxidative stress is a phenomenon caused by an imbalance between production and accumulation of reactive oxygen species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products. It has lead to great chemical reactions in the body. It has been linked to several neurological diseases such as Parkinson disease, Alzheimer's disease, multiple sclerosis, depression and memory loss. ROS are considered to be unavoidable by-products of normal aerobic metabolism. Much stress can disrupt cellular homeostasis, thus enhancing the production of ROS (Mittler, 2002). These ROS reacts with lipids, proteins, pigments causing lipid peroxidation, membrane damage, enzyme inactivation, thus affecting cellular metabolism. (Romero-Puertas et al., 2007).

In Alzheimer's disease, several experimental and clinical researches report that oxidative stress plays a pivotal role in neuron loss and progress to dementia. It is impossible to completely avoid free radical exposure and oxidative stress. However, it can be managed by increasing the levels of antioxidants and decreasing the formation of free radicals.

Recently, natural remedies have been used to treat diseases caused by oxidative stress. They include garlic, onions and turmeric. *Curcuma longa* is a member of the ginger family (*Zingiberaceae*). Its rhizomes (underground stems) are the source of a bright yellow spice and dye. *Curcuma longa* is sterile (does not produce seed, but it does grow vigorously from the rhizomes). It is thought to have arisen by selection and vegetative propagation of a hybrid between wild *Curcuma aromatica*, native to India, Sri Lanka and the eastern Himalayas, and some other closely related species. (Sirirugsa et al., 2007).

The exact origin of *Curcuma longa* is not known, but it is known to originate from South or Southeast Asia, most probably from Vietnam, China or western India. It is only known as a domesticated plant and not found in the wild. India is the world's largest producer, consumer and exporter of *Curcuma longa*. *Curcuma longa* is also cultivated extensively in Bangladesh, China, Thailand, Cambodia, Malaysia, Indonesia and the Philippines. It is an upright, perennial herb to about 1 m tall. The rhizome (underground stem) is thick and ringed with the bases of old leaves. *Curcuma longa* only reproduces via its rhizomes. Its leaves are large, oblong, up to 1 m long, dark green on upper surface, pale green beneath. Each leafy shoot (pseudostem) bearing 8-12 leaves. Its flowers are yellow-white, borne on a spike-like stalk 10-15 cm long. They are sterile and do not produce viable seed. The seeds are small, ovoid, and brown. (Leong-Skornickova et al., 2008). *Curcuma longa* has been used for many conditions in traditional medicine in India, Pakistan and Bangladesh. The rhizome is the part that is most widely used. It can be prepared in various ways and is reputed to alleviate asthma and coughs. Many of its traditional uses are supported by scientific evidence.

Hot water extracts of the dried rhizome have been taken orally in Ayurvedic medicine to reduce inflammation. *Curcuma longa* is also regarded as a 'rasayana' herb, which is a branch of Ayurvedic medicine and is used to counteract ageing processes. In Unani medicine, *Curcuma longa* has been used for conditions such as liver obstruction and jaundice and has been applied externally for ulcers and inflammation. Roasted turmeric has been used as an ingredient of a preparation used to treat dysentery. *Curcuma longa* has also been used in tooth powder or paste. Studies show that the rhizomes contain compounds that may have therapeutic effects. *Curcuma longa* has been shown to have anti-bacterial, anti-fungal, antioxidant and anti-inflammatory effects, which is used in the treatment of Human Immunodeficiency Virus (HIV) and Acquired Immunodeficiency Syndrome (AIDs). (Tang et al., 2009). It also possess anti-ulcer, wound-healing, liver-protective and anti-cancer properties. It contains yellow pigments called curcuminoids. One example of a curcuminoid is curcumin. Some studies suggest that components of the essential oil, such as *ar* -tumerone, have anti-snake venom activity. The essential oil is also reported to have some insect repellent and insecticidal activity.

1.2 AIMS AND OBJECTIVES

1.2.1 AIMS

The purpose of this study is to evaluate the protective activity of the ethanolic root extract of *Curcuma longa* on cadmium-induced damage to the hippocampus of adult Wistar rats.

1.2.2 SPECIFIC OBJECTIVES

The specific objective of this study is to evaluate the protective activity of *Curcuma longa* extract on:

- The neurobehavioral activity(NOR) of rats treated with or without cadmium,
- The antioxidant activities (superoxide dismutase, catalase, malondialdehyde, glutathione peroxidase) in rats treated with or without cadmium,
- The histology of the Hippocampus of rats treated with or without cadmium.

1.3 JUSTIFICATION OF STUDY

Cadmium is a well-recognized environmental pollutant with numerous adverse health effects. It is introduced into the environment as a result of the rapid development of industries and modern technologies. (Soisungwan, 2018). Chronic cadmium exposure affects different organs and tissues such as the central nervous system and its deleterious effects can be linked to reactive oxygen species (ROS) generation. ROS play important roles in the regulation of physiological processes (Wang, 2016).When ROS production overwhelms cellular antioxidant defenses, the intracellular redox can be altered and oxidative stress responsible for the harmful effect of cadmium occurs. (Jomova et al, 2011).

Many drugs have been used in combating the effects of oxidative stress caused by cadmium. However, they have been reported to have side effects, and consequently the use of medicinal herbs has been encouraged. One of which is *Curcuma longa.*

Curcuma longa is a perennial plant from *Zingiberaceae* family. It is highly regarded as a universal panacea in herbal medicine with a wide spectrum of pharmacological activities. It is an essential spice all over the world with distinguished human use particularly among the Eastern people (Ravindran et al., 2007). Apart from its use as a spice, it is used for traditional medicine because of its numerous beneficial properties (Chattopadhyay et al., 2004).

Extensive researches have proven that most of the turmeric activities are due to curcumin. Recent studies have authenticated turmeric as anti-cancer, anti-diabetic, anti-fungal, anti-oxidant and anti-inflammatory. It is also reported to possess anti-HIV activity to combat AIDs (Tang et al., 2009). These medicinal properties of turmeric are responsible for its status as a spice with multifunctional medicinal properties.

Although, there are numerous beneficial properties of *Curcuma longa*, to the best of our knowledge there is no experimental evidence to demonstrate the protective activity of turmeric on cadmium-induced neurotoxicity in adult Wistar rats. The result from this study will provide the first evidence of the neuroprotective activity of *Curcuma longa* in rats induced with cadmium.

CHAPTER TWO

LITERATURE REVIEW

2.1 PLANT OF STUDY:

Figure 2.1**:** Image showing *Curcuma longa*
Source: (https://images.app.goo.gl/oW427MvFRcNczES49)

Figure 2.2**:** Image showing powdered *Curcuma longa*
Source: (https://images.app.goo.gl/pWMH6gxe92ZWvJ2aA)

Curcuma longa is a flowering plant of the ginger family, *Zingiberaceae*, the roots of which are used in cooking. (Priyadarsini, 2014).The plant is a perennial, rhizomatous, herbaceous plant native to the Indian subcontinent and Southeast Asia. It requires temperatures between 20 and 30°C (68 and 86°F) and a considerable amount of annual rainfall to thrive. Plants are gathered each year for their rhizomes, some for propagation in the following season and some for consumption. The rhizomes are used fresh, or boiled in water and dried, after which they are ground into a deep orange-yellow powder, which is known as Curcumin. It is commonly used as a coloring and flavoring agent in many Asian cuisines, especially as curries. It is also used for dyeing. Powdered *Curcuma longa* has a warm, bitter, black pepper like flavor and earthy, mustard-like aroma. (Brennan, 2008). Curcumin is a bright yellow, chemical produced by the *Curcuma longa* plant, it is approved as a food additive by the World Health Organization, European Parliament, and United States Food and Drug Administration.

2.1.1 History:

Curcuma longa has been used in Asia for centuries and is a major part of Ayurveda, Siddha medicine, traditional Chinese medicine, Unani, and the animistic rituals of Austronesia peoples (Kikusawa et al., 2007). It was first used as a dye, and then later for its supposed properties in folk medicine (Nelson et al., 2017). From India, it spread to Southeast Asia along with Hinduism and Buddhism, as the yellow dye is used to color the robes of monks and priests. *Curcuma longa* has also been found in Tahiti, Hawaii and Easter Island before European contact. (Pickersgill et al., 2005). There is linguistic and circumstantial evidence of the spread and use of turmeric by the Austronesia peoples into Oceania and Madagascar. The populations in Polynesia and Micronesia, in particular, never came into contact with India, but use turmeric widely for both food and dye. *Curcuma longa* was found in Farmana, dating to between 2600 and 2200BCE, and in a merchant's tomb in Megiddo, Israel. (Scott et al., 2020). It was noted as a dye plant in the Assyrians Cuneiform medical texts from Ashurbanipal’s library at Nineveh from 7th century BCE. (Pickersgill et al., 2005). In Medieval Europe, turmeric was called "Indian saffron. (Pickersgill et al., 2005).

2.1.2 Origin and distribution:

The greatest diversity of Curcuma species by number alone is in India, at around 40 to 45 species. Thailand has a comparable 30 to 40 species. Other countries in tropical Asia also have numerous wild species of Curcuma.

2.1.3 Etymology:

The name possibly derives from Middle English or Early Modern English as turmeryte or tarmaret. It may be of Latin origin, *terra merita* ("meritorious earth"). (Dictionary.com.2013).

2.1.4 TAXONOMY AND NOMENCLARURE:

Kingdom-Plantae

Taxonomic rank-Species

Synonym-Curcuma domestica

Common name-Turmeric (English)

2.1.5 Taxonomic Hierarchy

Kingdom-Plantae

Sub-Kingdom-Viridiplantae

InfraKingdom-Streptophyta

Superdivision-Embryophyta

Division-Tracheophyta

Subdivision – Spermatophyta

Class-Magnoliopsida

Superorder-Lilianae

Order-Zingiberales

Family-Zingiberaceae

Genus-Curcuma

Species-Curcuma longa

National Plant center (1996), The Plant data base.

2.1.6 PHARMACOLOGICAL ACTIVITIES OF *CURCUMA LONGA*

2.1.6.1 Benefits of *Curcuma longa* on the Brain:

Besides curcumin, *Curcuma longa* also contains a compound called aromatic turmerone. This compound has been found through clinical research to promote the growth of the building blocks called Stem cells within the brain. The Stem cells are responsible for the formation of new brain cells that can help hold information, improve memory and enhance concentration. Infact, it is believed that by increasing the formation of stem cells, conditions such as Alzheimer's disease and stroke can either be prevented or treated a lot more rapidly.

Not just that, Curcumin is also believed to help improve memory and can be beneficial to those patients suffering from Alzheimer's disease (Limet al., 2010). Many individuals have a spoonful of turmeric and a glass of water every morning. This may be quite helpful when it comes to enhancing the power of the mind.

2.1.6.2 *Curcuma longa* Increase the Antioxidant capacity of the Body:

Oxidative Stress is believed to be one of the mechanisms behind aging and many diseases. It involves free radicals, highly reactive molecules with unpaired electrons. Free radical tends to react with important organic substances, such as fatty acids, proteins or DNA.

The main reasons antioxidant are so beneficial is that they protect the body from free radicals. *Curcuma longa* is a potent antioxidant that can neutralize free radicals due to its chemical structure. (Barclay et al., 2000).

2.1.6.3 *Curcuma longa* possesses anti-inflammatory Compound:

Inflammation is incredibly important. It helps your body fights foreign invaders and also has a role in repairing damage. Without inflammation, pathogens like bacteria could easily take over your body and kill you. Although, acute, short-term inflammation is beneficial, it can become a major problem when it becomes chronic, and inappropriately attacks your body own tissues. Scientists now believe that chronic, low-level inflammation plays a major role in almost every chronic Western disease 'This includes heart disease, metabolic Syndromes, and various degenerative conditions. (Menon et al., 2007). Therefore, anything that can help fight chronic inflammation is of potential importance in preventing and even treating these diseases. *Curcuma longa* is strongly anti-inflammatory. In fact, it's so powerful that it matches the effectiveness of some anti-inflammatory drugs, without the side effects (Jurenka, 2009).

Curcuma longa boosts levels of the brain-derived neurotrophic factor, and lower the risk of brain disease:

Neurons are capable of forming new connections, but in certain areas of the brain they can also multiply and increase in number. One of the main drivers of this process is brain-derived neurotrophic factor (BDNF), which is a type of growth hormone that functions in your brain. Many common brain disorders have been linked to decreased levels of this hormone, including depression and Alzheimer’s disease. Interestingly, *Curcuma longa* can increase the level of BDNF. By doing this, it delays or even reverses many brain diseases and age related decreases in brain function (Sarraf et al., 2019).

2.1.6.4 *Curcuma longa* possesses hepatoprotective effects:

Several agents, such as alcohol, drugs, pollutants, parasites, and dietary components, among others, can trigger acute and chronic liver injuries, including liver fibrosis, non-alcoholic liver disease, and even cirrhosis. *Curcuma longa* has been extensively studied for its hepatoprotective effects. (Rahmani et al., 2016).

2.1.6.5 *Curcuma longa* helps to prevent and perhaps treat cancer:

Cancer is a terrible disease characterized by uncontrollable cell growth. There are many forms of cancer, which still have several things in common. *Curcuma longa* has been studied as a beneficial herb in cancer treatment and been found to affect cancer growth, development and spread (Cheah et al., 2009).

2.2 CHEMICAL OF STUDY

Heavy metals are natural components of the earth’s crust and are considered as constant environmental pollutants since they cannot be degraded or destroyed easily (Pinot et al., 2000). These metals are emitted into the atmosphere with composition of fine particles or in gaseous form and are transferred by atmospheric fluxes to the unwanted distances where they enter ecosystem of remote regions (Rusconi et al., 2010). Cadmium (Cd) is a toxic metal which was classified as a human carcinogen by the North Carolina National Toxicology Program (NTP, 2000). Cadmium deposits are found with zinc, copper, or lead, and the smelting processes of these metals releases cadmium as a by-product into the atmosphere, water bodies, and soil thereby contaminating the human environment (Robin, 2013).

Cadmium (group IIB of the periodic table of elements) is a heavy metal posing severe risks to human health. Up to this day, it could not be shown that cadmium has many physiological functions within the human body. Interest has therefore risen in its biohazardous potential. As first described by Friedrich Stromeyer (Göttingen, Germany) in 1817, cadmium intoxication can lead to kidney, bone, and pulmonary damages. It is regularly found in ores together with zinc, copper and lead. Therefore, volcanic activity is one natural reason for a temporary increase in environmental cadmium concentrations. Cadmium is widely used in industrial processes, for example as an anticorrosive agent, as a stabilizer in PVC products, as a color pigment, a neutron-absorber in nuclear power plants, and in the fabrication of nickel-cadmium batteries. Phosphate fertilizers also show a big cadmium load. Although some cadmium-containing products can be recycled, a large share of the general cadmium pollution is caused by dumping and incinerating cadmium-polluted waste [Jarup, 2003]. In Scandinavia for example, cadmium concentration in agricultural soil increases by 0.2% per year. Total global emission of cadmium amounts to 7000 t/year.

Cadmium generates free radicals such as superoxide radicals, hydroxyl radicals, and nitric oxide which cause tissue damage (Halliwell, 2003). Many organs including the brain are affected by free radical accumulation in animals (Kim et al., 2014; Mezynska et al., 2018). The nervous system is highly receptive to free radical damage as it is the site for many unsaturated fatty acids and iron. Many epidemiological studies also showed that Cd vulnerably effect renal, prostate, liver, hematopoietic system, urinary bladder, pancreatic, testis and even causes stomach ulcers (Joseph et al., 2001).

The oxidative reaction is one of the important mechanisms of cadmium-induced free radical damage, but this effect can be restricted by the supplementation of some antioxidants (Renugadevi et al., 2010). All the healthy foods such as vegetables, fruits, grain cereals, eggs, meat, legumes, and nuts are the sources of photochemical that behave as antioxidants and anti-inflammatory agents through different protective mechanisms (Slavin et al., 2012).

2.2.1 Cadmium Resorption:

Basically, there are some possible ways of cadmium resorption: Gastrointestinal and Pulmonary.

2.2.2 Digestive system:

The uptake through the human gastrointestinal is approximately 5% of an ingested amount of cadmium, depending on the exact dose and nutritional composition (Jin et al., 2002). An average German citizen has a daily intake of 30–35 µg cadmium; 95% of this taken up with food and drinks. An average smoker has an additional intake of 30 µg per day. Several factors can increase this amount, such as low intakes of vitamin D, calcium, and trace elements like zinc and copper.

2.2.3 Respiratory system:

The major source of inhalative cadmium intoxication is cigarette smoke. The human lung resorbs 40–60% of the cadmium in tobacco smoke. A 50 year-old average non-smoker has a cadmium body burden of 15 mg. While a comparable life-long smoker shows a value of 30 mg. Smokers generally have cadmium blood levels 4–5 times higher than those of non-smokers.

Workers exposed to cadmium-containing fumes have been reported to develop acute respiratory distress syndromes (ARDS). Inhalativly, resorbed cadmium reaches blood circulation usually in form of cadmium-cysteine complexes (Zalups, 2003).

2.2.4 Hazards to human health:

2.2.4.1 Acute intoxication

The respiratory system is affected severely by the inhalation of cadmium-contaminated air. Shortness of breath, lung edema and destruction of mucous membranes as part of cadmium-induced pneumonitis are described (Seidal et al., 2000). As already reported in 1942, intake of cadmium-contaminated food causes acute gastrointestinal effects (Nordberg, 2004).

2.2.4.2 Kidney damage

Kidney damage has long been described to be the main problem for patients chronically exposed to cadmium (Barbier et al., 2005). As mentioned above, cadmium reaches the kidney in form of cadmium-metallothionein (Cd-MT). Cd-MT is filtrated in the glomerulus, and subsequently reabsorbed in the proximal tubules. It then remains in the tubules cells and makes up for the major part of the cadmium body burden. The amount of cadmium in the kidney tubules cells increases during every person's life span. A perturbance of the phosphor and calcium metabolism as a result of this phenomenon is in discussion (Bernard et al., 2004). An increasing cadmium load in the kidney also result in a higher calcium excretion, thus leading to a higher risk of kidney stones.

2.3 ORGAN OF STUDY:

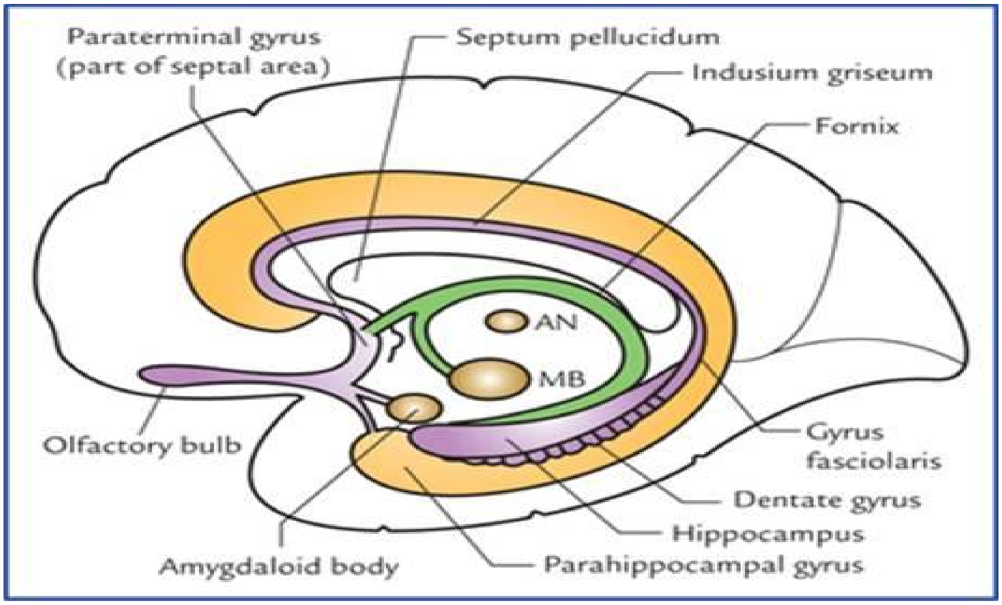

FIGURE 2.3: Image showing structures forming hippocampal formation (viz. hippocampus, dentate gyrus, gyrus fasciolaris and indusium griseum) and associated structures. (MB = mammillary body of hypothalamus, AN = anterior nucleus of thalamus).

Source: (Singh *et al*., 2014)

Hippocampus is a region of the brain that is associated primarily with memory. The name hippocampus is derived from the Greek hippokampus (hippos, meaning “horse,” and kampos, meaning “sea monster”), since the structure’s shape resembles that of a sea horse. The hippocampus, which is located in the inner (medial) region of the temporal lobe, forms part of the limbic system, which is particularly important in regulating emotional responses.

Humans and other mammals have 2 hippocampi, one in each side of the brain. It is located in the allocortex, with neural projections into the neocortexil in humans. (Amaral et al., 2007) .It contains two main interlocking parts; the hippocampus proper (also called Ammon's horn) and the dentate gyrus.

It is concerned with hunger motivation, sex drive, mood, pain, pleasure, appetite, memory; etc. The volume of hippocampus on each side of the brain is about 3-3.5cm3 as compared to 320-420cm3 for the volume of the neo cortex. Thus, hippocampus is 100 times smaller in volume compared to cerebral cortex.

Papez (1930) proposed that emotional response is organized in hippocampus and is expressed in cingulate gyrus via mammillary bodies. It has also been implicated in recollecting the past experience and imagining the future. (Addis et al., 2011).

2.3.1 Gross anatomy

2.3.1.1 Location

The hippocampus lies in the hippocampal sulcus immediately below the floor of the temporal horn of the lateral ventricle, and in cross section (coronal) has appearances that are reminiscent of a seahorse. It has a head (posterior to the amygdala), a body, and a tail (which follows the upwardly curving lateral ventricle).

Although there is a lack of consensus relating to terms describing the hippocampus and the adjacent cortex, the term hippocampal formation generally applies to the dentate gyrus, fields CA1-CA3 (CA4 is frequently called the hilus and considered part of the dentate gyrus), and the subiculum (parahippocampalgyrus). The hippocampus proper is made up of CA1, CA2 and CA3 fields.

NB: CA is an abbreviation of cornu Ammonis.

2.3.1.2 Arterial supply

Collateral branches of the posterior cerebral artery and anterior choroidal artery forming the deep intrahippocampal arteries.

2.3.1.3 Venous drainage

Intrahippocampal veins, draining in the superficial hippocampal veins, forming two arches, which ultimately drain into the basilar vein.

Posterior end: draining in the medial atrial vein.

Anterior end: inferior ventricular vein, draining in the medial atrial vein.

2.3.2 HISTOLOGY OF HIPPOCAMPUS:

The hippocampus is comprised primarily of pyramidal cells. Like all cells, pyramidal cells have afferent processes (dendrites) and efferent processes (axons). It should be noted that the dendrites of a pyramidal cell extend from both the apex and base. The basal dendrites extend toward the surface of the lateral ventricles; while the apical dendrites extend away from the lateral ventricles and toward the dentate gyrus.

The axons of pyramidal cells take information received by the hippocampus and send it to other structures in the brain.

The tissue comprising the hippocampus has many layers. From the ventricular surface to the dentate gyrus, the hippocampus is made up of:

- An external plexiform layer,
- A stratum oriens layer,

- A pyramidal cell layer,
- A stratum radiatum layer,
- A stratum lacunosum-moleculare layer.

The external plexiform layer is situated near the inferior horn of the lateral ventricle.

The stratum oriens layer contains two types of cells: basal dendrites and basket cells.

The pyramidal cell layer, consistent with its name, contains pyramidal cells of the hippocampus.

The stratum radiatum layer and stratum lacunosum moleculare layer bothcontainthe perforant pathway, which is composed of the apical dendrites of pyramidal cells and hippocampal afferent fibers coming from the entorhinal cortex.

The pyramidal cells in the hippocampus are arranged in a C-shaped pattern, and some of these cells also fit snugly into the C-shaped dentate gyrus.

The hippocampus is divided into different regions called fields. They are four in number namely;

CA1,

CA2,

CA3,

CA4.

The CA1 field, also known as Sommer's sector, contains the pyramidal cells located closest to the subiculum; whereas the CA4 field contains cells within the hilus of the dentate gyrus. The CA2 and CA3 fields are located in between.

One special feature of the CA3 field to note is that the collaterals of the axonal processes extending from the CA3 pyramidal cells are known as recurrent or Schaffer collaterals, and these fibers actually project back to the CA1 field.

2.3.2.1 Sources of input:

The entorhinal cortex is an important source of two different groups of afferent fibers delivering information to the hippocampal formation. The lateral perforant pathway arises from the lateral entorhinal cortex and extends into the molecular layer of the hippocampus. The medial perforant pathway arises from the medial entorhinal cortex, extends through the white matter from the subicular cortex, and enters the alveus of the hippocampus. Many of these fibers carry olfactory, visual, and auditory information to the hippocampus.

The diagonal band of Broca originates from the septal area, and acts as part of a feedback circuit to the hippocampus from the septal area. The other part of this feedback circuit is the precommissural fornix, which allows the septal area to receive feedback from the hippocampus.

2.3.2.2 Output from the hippocampus:

The efferent fibers of the hippocampal formation, sending signals from the hippocampus to other parts of the brain, come from the pyramidal cells of the hippocampus and subicular cortex. Fibers from the amygdala, situated anteriorly to the hippocampus, also travel largely in tandem with the hippocampal fibers. These fiber bundles arising from the hippocampus and amygdala pass postero-dorsally along the body of the lateral ventricle, around the posterior part of the thalamus, and then anteriorly along the inferior horn of the lateral ventricle.

The fiber bundle that arises from the hippocampus is called the fornix; it runs just inferiorly to the corpus callosum. The fiber bundle that arises from the amygdala is called the stria terminalis, and it runs parallel and ventromedial to the tail of the caudate nucleus. The fornix and stria terminalis fiber networks eventually terminate in different parts of the hypothalamus and septal area.

Fibers from the fornix that travel rostrally to the anterior commissure are called the precommissural fornix. These originate from both the hippocampus and subicular cortex and terminate in the septal area. The fibers of the precommissural fornix are topographically organized: this means that fibers near the anterior pole of the hippocampal formation project to the lateral region of the lateral septal nucleus, whereas fibers near the posterior part of the hippocampal formation project to more medial parts of the lateral septal nucleus.

Fibers from the fornix that travel ventrally behind the anterior commissure are called the postcommissural fornix. These fibers originate in the subicular cortex and terminate either in the anterior thalamic nucleus or in the mamillary bodies of the hypothalamus. The postcommissural fornix innervates parts of the diencephalon, including the anterior thalamic nucleus, mammillary bodies, and parts of the medial hypothalamus.

Neurons from the subiculum also send axons to the entorhinal cortex, cingulate cortex, and regions of the prefrontal cortex. The entorhinal cortex subsequently sends axons to the amygdala and parts of the temporal cortex. These networks of connections allow the hippocampal formation to send signals throughout the cerebral cortex, including to regions which receive and process different types of sensory information.

There is also a commissural component of the fornix: the purpose of this part of the fornix is to connect the hippocampi on either side of the brain to each other. The axons that do this arise predominantly from the CA3-CA4 parts of the hippocampus and synapse in the contralateral hippocampus. It is theorized that these connective fibers may provide the pathway by which seizures spread from their primary epileptogenic focus in one hippocampus to the contralateral hippocampus, ultimately allowing for a secondary epileptogenic focus to form in the contralateral hippocampus.

2.4 FUNCTIONS OF THE HIPPOCAMPUS

Hippocampus is a S-shaped structure within the medial aspect of the temporal lobe that can be identified as a layer of densely packed neurons.

Being an integral part of the limbic system, hippocampus plays a vital role in regulating learning, memory encoding, memory consolidation, and spatial navigation.

2.4.1 Hippocampus and memory:

Hippocampus is divided into three regions: CA1, CA2, and CA3. These regions form the trilaminar loop, which is the processing center of long-term memory. Long-term potentiation (LTP), which is a form of neural plasticity, occurs in the hippocampus, and LPT is a vital brain mechanism involved in memory storage. The complex processes of memory encoding in the hippocampus and retrieval of experiences from the frontal lobe involve two prominent pathways: polysynaptic and direct pathways. In the polysynaptic pathway, hippocampus receives afferent inputs via axons of the entorhinal cortex, which terminate in the dentate gyrus. From there, neurons of the dentate gyrus send mossy fibers to the neurons of pyramidal cells located in CA3. These pyramidal cell neurons are further divided into two branches: one branch reaches the opposite side of the hippocampus via corpus callosum; the other branch connects to CA1 through Schaffer collateral pathways. From there, the projections leave the hippocampus to return to the inferior temporal cortex, temporal pole, and prefrontal cortex. The polysynaptic pathway is important for the semantic memory (facts and concepts), and the direct pathway is important for the episodic (recollection of events) and spatial memory (recognition).

2.4.2 Hippocampus and learning:

Pyramidal cells of the hippocampus play an essential role in classical eye blink conditioning, which is a standard model for studying associative learning. Studies involving delay eye blink conditioning have revealed that pyramidal cells form a predictive paradigm of time-amplitude sequence of the learned behavioral response. The rate of acquisition can be impaired or increased by hippocampal manipulations. In this form of learning process, hippocampus plays an important role in trace conditioning wherein a short interval is imposed between the condition and unconditioned stimuli. After eye blink conditioning, a long-lasting neuronal plasticity is generated in the hippocampus, which is needed for the learning process in the trace eye blink conditioning.

2.4.3 Hippocampus and spatial navigation:

One of the major functions of hippocampus is forming cognitive map, which is a type of mental representation related to acquisition, coding, storing, recalling, and decoding of information on relative locations within a specific environment. Place cells, a type of pyramidal cell, are mainly involved in hippocampus-mediated spatial navigation. These cells are activated when an animal enters a particular place in its environment (place field); however, these cells remain silent when an animal is moving outside the place field. Besides place field, the firing rate of place cells depends on the direction of movement, direction of destination, or other task-related factors.

2.4.4 Hippocampus and behavior:

Hippocampus plays a vital role in flexible and goal-directed behavior. An intact hippocampal activity is required for forming and reconstructing relational memory (required for remembering arbitrary associations between objects or events) associated with flexible cognition and social behavior. Many studies have revealed that any damage to hippocampus can impair flexible use of information and produce maladaptive behavior.

The role of hippocampus in behavioral inhibition is also well-established in the literature. The link between hippocampus and inhibition has been derived mainly from two basic observations: 1st observation is that damage to hippocampus makes animals hyperactive; and 2nd observation is that damage to hippocampus reduces the learning ability of animals to inhibit responses that they have learned previously.

Hippocampus acts as an evaluation center associated with behavioral inhibition, obsessional thinking, scanning, and spatial map formation. However, the hippocampus does not actively participate in controlling behavior, once an experience is characterized. External stress-induced increased corticosterone level eventually reduces the firing rate of the hippocampus.

Recently, a new hippocampal function has been put forward. It has been found that low-frequency firing/activity in the hippocampus can influence the functional integration between spatially separated regions in the cerebral cortex, leading to increased sensory responses, such as vision, hearing, and touch.

CHAPTER THREE

3.0 METHODOLOGY

3.1 REAGENTS /CHEMICALS

All reagents and chemicals were of analytical grade. They would include potassium permanganate, Distilled water, Na_2HPO_4, NaH_2PO_4,H_2SO_4, Hydrogen Peroxide, Na_2CO_3, $NaHCO_3$, EDTA-Disodium, Hydrochloric acid, Adrenaline, Pyrogallol, Trichloroacetic acid, cadmium chloride, ethanol, alcohol(50%, 70%, 90%,100%), xylene, paraffin, normal saline, 10% formaldehyde.

3.2 EQUIPMENTS

Surgical latex gloves, orogastric tubes, sample bottles, plastic cages, open field maze, weighing balance, refrigerator, rotary microtome, binocular microscope, oven, water bath, paraffin dispenser, dissecting set, measuring cylinder, conical flask, volumetric flask, glass rods, freeze dryers, cheesecloth.

3.3 COMPUTER SOFTWARE

Adobe Photoshop CS6, version 13.0, x64 (manufactured by Adobe Systems Software Ireland Limited) and Graph pad prism software, version 7 (developed by Graphpad software, Inc.; Released in 2016)

3.4 PREPARATION OF ETHANOLIC ROOT EXTRACT OF *CURCUMA LONGA*

Turmeric was bought at a nearby market and identified. The identified roots were chopped into smaller pieces and air-dried on a slab in the laboratory. 3 kg of the sample was pulverized using an industrial grinding machine. The sample was soaked in 7.5 litres of absolute ethanol of analytical grade and kept in the dark cupboard for 72 hours with intermittent stirring before filtering it using cheesecloth. The filtrate obtained was freeze dried or lyophilized.

3.5 PHYTOCHEMISTRY OF *CURCUMA LONGA* RHIZOMES

Table 3.1 Preliminary Phytochemical Evaluation of *Curcuma longa* was carried out using standard methods specified by Trease and Evans, 1989 and Sofowora, 1993.

Test	**Observation**	**Inference**
Test for carbohydrates: 2 drops of the ethanolic extract of the sample + 2 drops of 10% alcoholic solution of naphtol + 2 drops of concentrated H2SO4 into the test tube inclined at 45 degrees.	A brown ring at the interphase between the acid and extract observed.	Carbohydrates present
Test for saponin: 2 drops of ethanolic extract + shake vigorously in a test tube.	Persistent frothing observed.	Saponin present
Test for phenolic: 2 drops of ethanolic extract + 1 drop of aqueous FeCl3.	Blue-black colouration observed.	Phenolic present.
Test for flavonoids: 2 drops of ethanolic extract + 1 drop of NaOH + 1 drop of dilute HCl.	The red coloured solution turned colourless.	Flavonoid present.
Test for steroids: 2 drops of the ethanolic extract + drops of chloroform	A reddish brown ring at the interphase between the acid and the chloroform layer	Steroids present.

+ 2 drops of concentrated H_2SO_4 carefully poured down the side of the test tube.	observed.	
Test for reducing sugar: 2 drops of the ethanolic extract + Fehling's solution A+B + boiling.	Persistent blue solution observed.	Reducing sugar present.
Test for alkaloids: 2 drops of ethanolic extract of the sample + 1 drop Dragendorff reagent. 2 drops of the ethanolic extract of the sample + 1 drop of Marquis' reagent. 2 drops of the ethanolic extract of the sample + 1 drop of picric acid solution	Red coloured precipitate observed. Yellow precipitate observed. Brown solution observed.	Alkaloids present.
Test for Tannins: 2 drops of the ethanolic extract of the sample + 5ml ferric ammonia citrate + 0.5g sodium acetate + boiled + cooled	A reddish bulky precipitate in hot water observed	Tannins observed

3.6 DOSAGE

The dose of *Curcuma longa* extract was used is 100mg/kg body weight and 200mg/kg body weight (Sudjarwo et al., 2017). The dose was chosen in line with previous studies that show its protective effects in the testis. Administration of the extract requires a medium for absorption, distilled water was served as the medium for this study. Cadmium was administered orally as cadmium chloride at a dose of 6mg/kg body weight. (Wong and Klaassen, 1982).

3.7 ANIMALS

The animals for this study were bred at the Animal House, Department of Anatomy, School of Basic Medical Sciences, College of Medical Science, University of Benin, Benin City, Edo State, Nigeria. They were kept in polypropylene cages under normal room temperature. They were fed with Top feeds Growers Mash (manufactured by Premier feed mills Co. Ltd, 1 Eagle Flour Road, Lagos/Ibadan expressway Toll point, Ibadan, Oyo State, Nigeria) They were weighed weekly before commencement and throughout the duration of the experiment using a digital weighing scale calibrated in gram and recorded to the nearest whole number. Protocols for this experiment would be in accordance with the guide for the care and use of laboratory animals (National Research Council of the National Academies, 2011).The study would be submitted for review and approval by the Ethical Committee of the College of Medical Sciences, University of Benin.

3.8 RESEARCH DESIGN

Table 3.2 Experimental Grouping and Dosage

GROUP A(Control)	1ml of water daily
Group B(Cd)	5g/kg body weight of cadmium
Group C (Cd + Cl1)	5g/kg body weight of cadmium and 100mg/kg body weight of *Curcuma longa* daily
Group D (Cd +Cl2)	5mg/kg body weight cadmium + 200mg/kg body weight of *Curcuma longa* daily
Group E (Cl1)	100mg/kg body weight of *Curcuma longa* daily
Group F (Cl2)	200mg/kg body weight of *Curcuma longa* daily

3.9 EFFECTS OF TREATMENT ON NEUROBEHAVIORAL ACTIVITIES

To assess the effects of treatments on neurobehavioral activities, the Y-maze test was used.

3.9.1 Y-MAZE TEST

The Y-maze test (YMT) is used to measure the spontaneous alternation performance, which allowed the evaluation of cognitive searching behavior, as an index for the cognitive dysfunction (Monte et al., 2013). Animals were gently placed individually in the Y-maze apparatus, which consisted of three identical arms (33×11×12cm each) in which the arms are symmetrically separated at 120° (Dall'igna et al., 2007). Specifically, each mouse was placed at the end of arm A, and allowed to explore all the three arms (labeled A, B, C) freely for 5 min, taking the following parameters: the number of arm visits and sequence (alternation) of arm visits visually. An alternation was defined as entries in all three arms on consecutive occasions (Monte et al., 2013). The percentage of alternation was calculated as total of alternations/ (total arm entries –2), as previously described (Yamada et al., 1996; Dall'igna et al., 2007). After each mice session, the observation chamber was cleaned with 10% ethanol to remove residual odor.

3.10 BIOCHEMICAL INVESTIGATION (ANTIOXIDANT ENZYMES)

3.10.1 Estimation of Catalase (CAT) activity

Catalase is an enzyme that brings about (catalyzes) the reaction by which hydrogen peroxide is decomposed to water and oxygen. It is found extensively in organisms that live in the presence of oxygen, catalase prevents the accumulation of and protects cellular organelles and tissues from damage by peroxide, which is continuously produced by numerous metabolic reactions. In mammals, catalase is found predominantly in the liver.

The determination of catalase was be carried out using the method of Cohen et al, 1970.

3.10.1.1 Principle

Catalase is present in nearly all animal, plant and bacteria cells. It acts to prevent the accumulation of noxious H_20_2 which is converted to O_2 and H_2O.

3.10.1.2 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 : 32.3ml of conc. H_2SO_4 was added uh 66.7ml of distilled water.
- 30Mm H_2O_2 solution: this was prepared by measuring 0.34ml of 30% of H_20_2 in 1001ml of phosphate buffer.

3.10.1.3 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 was taken at 480nm within 30 to 60secs against distilled water.

3.10.3.4 Calculation

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

OD=absorbance

L=Light path=1cm

Vt=total volume of reaction sample

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

3.10.2 Estimation of Superoxide Dismutase (SOD)

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

3.10.2.1 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 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.

3.10.2.2 Preparation of reagents

Carbonate buffer (0.05 M) pH 10.2: this would be prepared by dissolving 0.2014 g of Na_2CO_3. 0.2604 g of $NaHCO_3$ and 0.0372 g of EDTA in 100 ml of distilled water.

Hydrochloric acid (0.005 M): this would be prepared by adding 0.044 concentrated HCl to 99.96 ml of distilled water.

Adrenaline solution (0.3 mM): this would be prepared by dissolving 0.01098 g of Adrenaline in 100 ml of 0.005 M HCl solution.

3.10.2.3 Procedure

Plasma volume of 0.2 ml would be mixed with 2.5 ml of carbonate buffer and 0.3 ml of adrenaline solution, 0.2 ml of distilled water would be mixed with 2.5 m of carbonate buffer and 0.3 ml adrenaline as the reference sample. These would be mixed and absorbance read at 420 nm.

$$\% \text{ inhibition} = \frac{(\text{O. Dtest} - \text{O. Dref}) \times 100}{\text{O. Dtest}}$$

Enzyme activity can thus be calculated

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

3.10.3 Estimation of Malondialdehyde (MDA) activity

Malondialdehyde would be determined using the thiobarbituric acid assay (Buege and Aust, 1978)

3.10.3.1 Principle

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

3.10.3.2 Preparation of reagent

Stock TCA-TCB-HCL would be prepared by mixing 15g of trichloroacetic acid, 0.375g of thiobarbituric acid and 0.25N hydrochloric acid. This solution would be mildly heated to assist in the dissolution of the thiob0arbituric acid.

3.10.3.3 Procedure

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

The concentration MDA would be determined using the formula

M×V×I×Y

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

A = absorbance of sample test at 535nm

Vt= total volume of the reaction = 3ml

M = molar extinction co-efficient of product= $1.56 \times 10^{5} m^{-1}\ cm^{-1}$

l = light path= 1cm

V= volume of tissue extract used = 1ml

Y = mg tissue in the volume of sample used

3.10.4 Estimation of glutathione peroxidase (GPx) activity

This would be determined by the method of Nyman (1959).

3.10.4.1 Principle

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

3.10.4.2 Preparation of reagent

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

3.10.4.3 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 would be allowed to stand for 30 minutes at room temperature. A deep brown colour would be formed which would be read at 420nm.

3.10.4.4 Calculation

Activity = $\frac{\mathrm{OD/Min} \times \mathrm{Vt\ Df}}{\mathrm{E} \times \mathrm{Vs} \times \mathrm{Y}}$

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

3.11 HISTOLOGY OF THE HIPPOCAMPUS

On the completion of the neurobehavioral tests, the rats were sacrificed by cervical dislocation (100mg/kg). The skulls were opened and the brain of the rats were harvested, blotted free of blood and weighed immediately using an electronic weighing balance calibrated in milligram and recorded to the nearest two decimal places. The relative brain weights were calculated as follows:

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

The harvested tissue was preserved in 10% formalin for histopathology. The tissues were processed via paraffin wax embedded method of Drury and Wallington (1980). They would be dehydrated for one hour each at room temperature through ascending grades of ethanol; 70% ethanol, 90% ethanol, absolute ethanol I, Absolute ethanol II. Dehydrated tissue will be cleared at room temperature in two changes of xylene for 1 hour in each change. The tissues were infiltrated in two changed of molten paraffin wax at 60°C for one hour in each change and finally embedded in paraffin wax multi-block plastic embedded molds. The paraffin blocked tissues was and mounted on wooden chauck for sectioning on a rotary microtome. Sections of 5µm thickness was produced from the tissue blocks using a rotary microtome. The sections was then transferred into water bath (40°C) to allow spreading of the folded ribbons of sections. These sections was then mounted on new clean glass slide. They were then dried at 40°C on a slide drier to enhance adherence of the sections to the slides.

3.12 HAEMATOXYLIN 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 were used. Procedures of Haematoxylin and Eosin adopted on the sections would be described by Drury and Wallington (1980) and Scheehan and Hrapchak (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 haematoxylin for 15-20 minutes
- Excess haematoxylin stain would be removed by rinsing well in running tap water for two to three minutes (sections would be examined microscopically at this stage to confirm sufficient degree of staining);
- Differentiated in acid alcohol (0.5% HCL in 70% ethanol for two to three minutes;
- Rinsed well in running water for 10-15 minutes;
- Counterstained in 1% aqueous eosin for two to four minutes;
- Excess stain would be washed off in running water and examined under microscope;
- Dehydrated rapidly in ascending grades of ethanol (50% through absolute ethanol), cleared in xylene and mounted in a synthetic resin medium (DPX).

3.13 PHOTOMICROGRAPHY

The processed slides was captured with a LABO® trinocular microscope (Labo Microsystems GmbH, Germany) on which would be mounted an Omax 9.0MP USB Digital Microscope Camera (made in Korea). The camera features 9 megapixels (3488 × 2616 pixel) high-resolution colour digital camera and 0.5X reduction lens. It was connected to a laptop on which would be installed ToUpView software (version ×64, 3.7.71.49; built-in 2016). A panoramic view of the slides was captured using ×4 and ×10 objective lenses. The images would be merged and processed automatically with Adobe Photoshop CS6 (version 13.0, ×64) for final output.

3.14 STATISTICAL ANALYSIS

Data was analyzed using Graphpad Prism Statistical package, version 7 (developed by Graph pad software Inc.; released in 2016). Statistical significance would be determined by means of Analysis of Variance (ANOVA), followed by turkey's multiple comparison post-hoe test.

CHAPTER FOUR

4.0 RESULTS

4.1 PHYTOCHEMISTRY

Table 4.1 Phytochemical components of *curcuma longa* root.

Carbohydrates	+
Saponins	+
Phenolics	+
Flavonoid	+
Steroids	+
Reducing sugar	+
Alkaloids	+
Tannins	+

+ signs indicate the presence of the compounds listed.

4.2 EFFECTS OF TREATMENTS ON BRAIN AND BODY WEIGHT

Table 4.2 shows the body and organ weight of animals in the experimental groups A-F. In group B, results show a significant decrease (p<0.05) in the final body weight compared to control. However, an increase (p>0.05) was observed in the final body weight of groups C-D when compared to group B.

For group B, the absolute whole brain weight, a decrease (p>0.05) was observed in group B when compared to the control group. In groups C and D, there was a decrease (p>0.05) and an increase (p>0.05) respectively in the absolute whole brain weight when compared to control.

Table 4.2: Body weight, absolute whole brain and relative brain weights of control and treatment groups after 21 days.

Groups	Initial BW (g)	Final BW (g)	Absolute whole brain weight (g)	Relative brain weight (%)
Control	163.6 ± 3.919	177.9 ± 7.417	1.700 ± 0.058	0.960 ± 0.015
Cd	164.3 ± 6.692	151.2 ± 2.194	1.367 ± 0.067[#]	0.900 ± 0.031
Cd + CL1	165.7 ± 9.135	164.2 ± 7.541	1.600 ± 0.100	0.980 ± 0.042
Cd + CL2	164.0 ± 6.372	166.1 ± 4.727	1.567 ± 0.033	0.947 ± 0.022
CL1	167.6 ± 4.675	177.2 ± 7.686	1.667 ± 0.033	0.943 ± 0.032
CL2	166.0 ± 3.795	177.0 ± 4.930	1.633 ± 0.033	0.923 ± 0.015

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

4.3 EFFECTS OF TREATMENT ON NEUROBEHAVIORAL ACTIVITY (Y-MAZE)

Figure 4.1 shows the spontaneous alternation (%) during Y-maze test in experimental groups A-F. Here, a significant decrease (p<0.05) was observed in group B when compared to control. On the contrary, a significant increase (p<0.05) in group C and D when compared to the Cadmium only group.

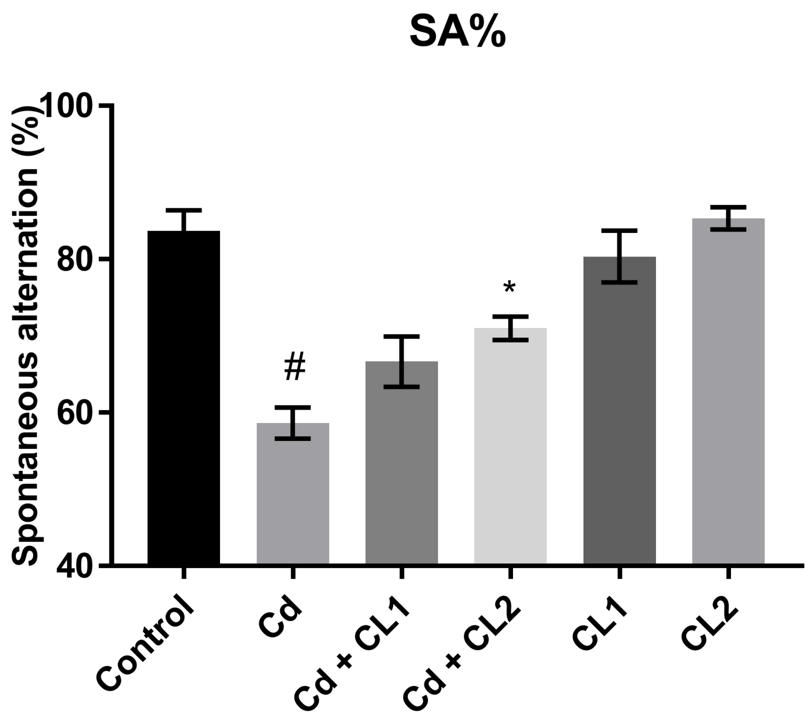

Figure 4.1: Spontaneous alternation (Y-Maze test) of control and treatment groups after 28 days. Bars represent the mean ± SEM. # *p*< 0.05 compared with the control group; * *p*< 0.05 compared with the Pb-alone group.

4.4 EFFECTS OF TREATMENT ON ANTIOXIDANT ACTIVITY

Figure 4.2 illustrates the activity of antioxidant enzymes in experimental groups A-F. Here, a significant decrease in SOD, CAT and GPx ($p<0.05$) was observed in group B when compared to control. However, an increase in SOD, CAT and GPx ($p>0.05$) was observed in groups C and D when compared to cadmium treated only group. Furthermore, a significant increase in MDA ($p<0.05$) was observed in group B when compared to the cadmium treated only group.

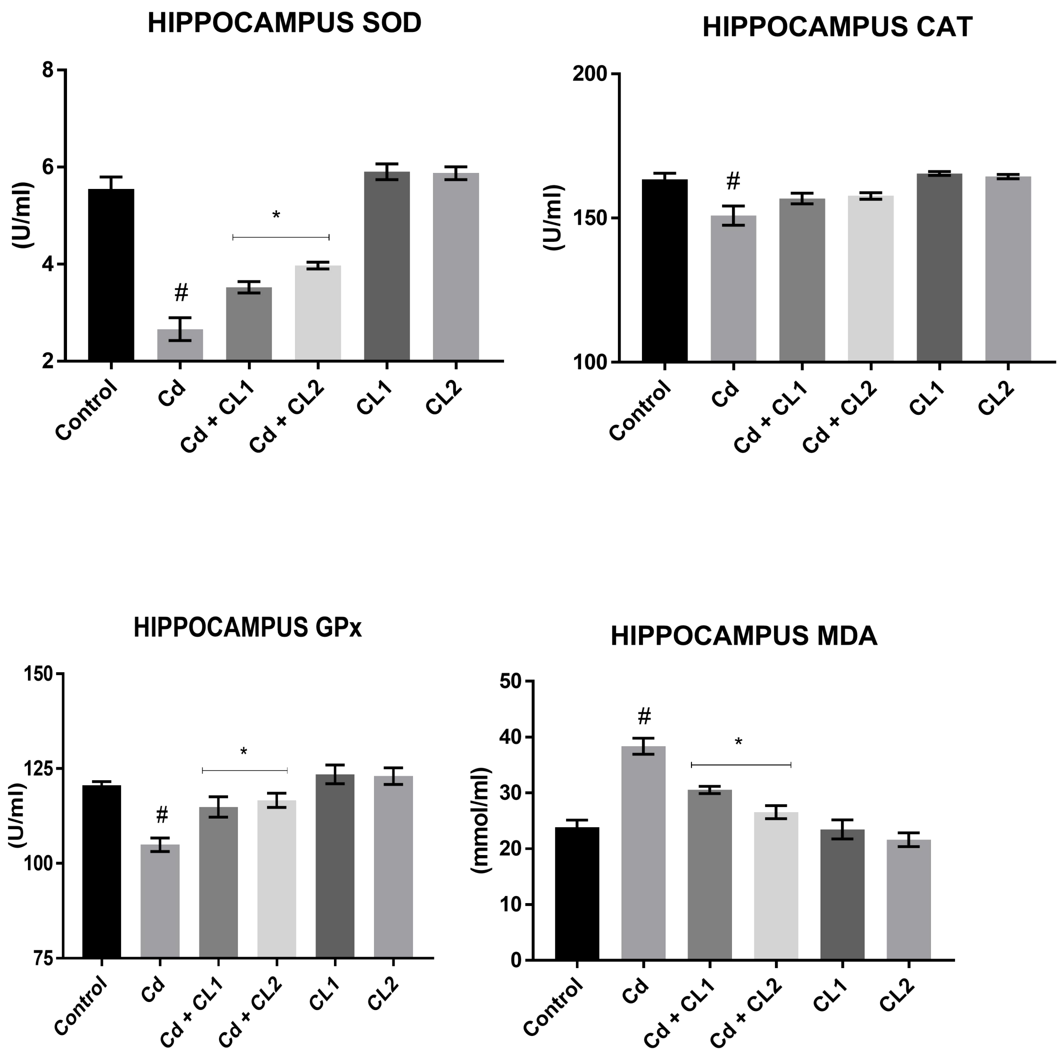

Figure 4.2: Activity of antioxidants in the hippocampus of control and treatment groups after 21 days. Bars represent the mean ± SEM. # *p*< 0.05 compared with the control group; * *p*< 0.05 compared with the Cd-alone group.

4.5 EFFECTS OF TREATMENT ON THE HISTOLOGY

Figure 4.5 shows the histology of the hippocampus. Plate A shows the normal hippocampal histology. Plate B shows altered morphology with vacuolated cellular structure, pyknotic nuclei and few cells due to the administration of cadmium. Plate C and D shows a better architecture of tissue compared to Cadmium treated only group(B). Plate E and F shows normal hippocampal histology, having similar tissue morphology when compared to control (Plate A).

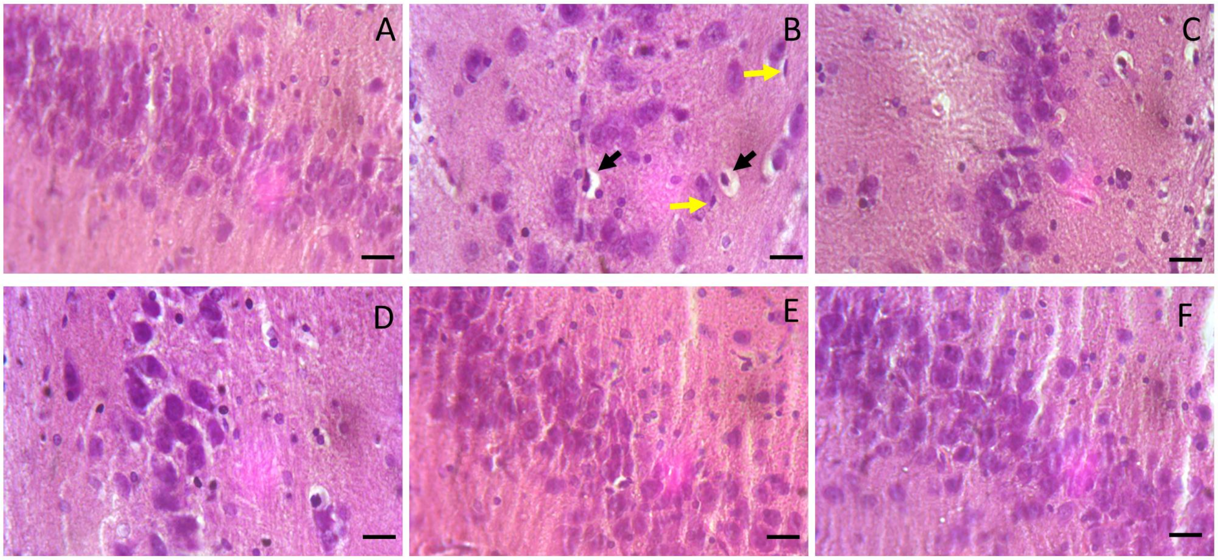

Figure 4.3 Effect of CL on Cd-induced changes in the hippocampus of control and experimental rats. (A) Control. (B) Cd-induced group 5mg/kg. Sections showing altered morphology with vacuolated tissue architecture (black arrows) and pyknotic nuclei (yellow arrows) and fewer cells observed in the CA1 region (C) Cd + 100mg/kg CL (D) Cd + 200mg/kg CL (E) 100mg/kg CL (F) 400mg/kg CL (H&E x400). Scale bar: 25µm

45 2

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

Cadmium, a heavy metal posing severe risks to human health, has been reported to have numerous adverse effects on the body. This study aims to evaluate the protective activity of ethanolic *curcuma longa* root extract on cadmium-induced hippocampal damage in Wistar rats.

Phytochemical analysis *curcuma longa* rhizomes showed the presence of carbohydrates, saponins, phenolics, flavonoid, steroids, reducing sugar, alkaloids and tannins. Carbohydrates, made up of carbon, hydrogen, and oxygen, are organic compounds that serve as a source of energy for animals and humans (Bach et al., 2013). They may be classified according to their degree of polymerization: monosaccharides, disaccharides, oligosaccharides, and polysaccharides. The main monosaccharide is glucose, which is utilized as an energy source by animals. Glucose can be derived from starch and sugars in the diet, from glycogen that is stored in the body (Salvin 2013). Saponins are naturally occurring glycosides described by the soap-like foaming, and consequently, they produce foams when shaken in aqueous solutions. Saponins exhibit a biological role and medicinal properties such as anti-inflammatory, anti-bacterial (Sparg et al., 2004), anti-fungal, and anti-cancer properties (Cheng et al., 2011). Phenolics are compounds that are found in all food groups. They exert antioxidant activity by scavenging hydroxyl radical, and superoxide radical anion (Chirinos et al., 2009). Steroids are anti-inflammatory medicines used to treat a range of conditions. They are also known as corticosteoroids. They also exhibits anti-fungal activity (Sautaur et al., 2005). Alkaloids are a class of naturally occurring organic nitrogen-containing bases. They have important physiological effects on humans and animals. Well-known alkaloids include morphine, strychnine, quinine, ephedrine, and nicotine. Tannins (commonly referred to as tannic acid) are water-soluble polyphenols that are present in many plant foods. Tannins possesses anti-carcinogenic and anti-mutagenic properties which can be related to their antioxidative property that is important in protecting cellular oxidative damage, including lipid peroxidation (Chung et al., 1998).

A decrease in final body weight observed in rats administered with cadmium indicates the toxicity of cadmium. This agrees with the findings by Ercal and colleagues reporting that harmful chemical reduces the body weight of Wistar Albino rats (Ercal et al., 2001).

The hippocampus is associated primarily with spatial memory and it is very prone to oxidative stress (Bliss, et al., 1993; Brunson et al., 2001). Cognitive dysfunction can be prevented by the maintenance of a normal redox state in the hippocampus. The mechanism of cadmium toxicity can be by an oxidative stress pathway, which modifies neuronal morphology and causes the death of neurons and consequently affecting cognitive tasks (Rinaldi et al., 2017). Y-maze test is a spatial working memory test, used to measure cognitive dysfunction (Ben-Azu et al., 2016). There was a significant decrease in the spontaneous alternation of cadmium treated only group when compared to control which indicates cognitive dysfunction (Ben -Azu et al., 2016). Contrarily, there was a significant increase in the spontaneous alternation of *Curcuma longa* pretreated extract groups when compared to cadmium only group. This means that *Curcuma longa* was able to produce a cognition-enhancing effects. These findings demonstrate the ability of this extract to protect against cognitive dysfunction related to cadmium toxicity. This is in correlation with the study by Shrikant and Kalpana where they reported that treatment with *Curcuma longa* improves memory (Shrikant and Kalpana, 2008).

Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPx) are a collection of antioxidants that act to suppress or prevent the formation of free radicals. These enzymes breakdown hydrogen peroxides and hydroperoxides to harmless molecules (H_2O_2/alcohol and O_2) (Akinloye, 2019). Results from this study showed increase in oxidative stress demonstrated by the depletion of hippocampal SOD, CAT and GPx in the group treated with cadmium only when compared to control. This is in agreement with a previous study by Karul and colleagues, where they reported a decrease in SOD, CAT and GPx (Karul et al., 2001).

Malondialdehyde (MDA), is an end product of lipid peroxidation. It is used indirectly to measure lipid peroxidation. A significant increase in MDA observed in the hippocampus of rats treated with cadmium only indicates an increase in oxidative stress. However, a significant decrease was observed in the MDA level in groups pre-treated *Curcuma longa*, when compared to the Cadmium only group. The findings shows that *Curcuma longa* was able to protect against Cadmium inducted oxidative stress; This is in line with previous studies of Mohammed Alizadeh and Sorayya Kheirouri, that supplementation with curcumin was effective in decreasing MDA and improving levels of antioxidants in diseased individuals under conditions of oxidative stress (Mohammed and Sorayya, 2019).

Histological findings of control group showed normal histology of the CA1 region in the hippocampus. However, Cadmium treated group showed altered morphology with vacuolated tissue architecture, pyknotic nuclei and fewer cells in the CA1 region of the hippocampus. This agrees with the histological data from previous reports that cadmium exposure damage the neuron of the hippocampus (Faten et al., 2019). In *Curcuma longa* pretreated group; the severity of the hippocampal damage is decreased when compared with that of cadmium only treated group.

Administration of the extract was able to reduce pyknotic nuclei formation and atrophy of pyramidal cells in the CA1 region of the hippocampus. These indicate the neuroprotective effect of the extract. Our findings shows that animals treated with cadmium extract had similar histological appearance when compared to that of control. This, therefore signifies that *Curcuma longa* had no toxic effects on the hippocampus of the rats.

5.2 CONCLUSION AND RECOMMENDATION

The findings from this study demonstrate that *Curcuma longa* extract was able to protect against cadmium-induced hippocampal damage. The antioxidant activity of *Curcuma longa* may be due to the presence of curcumin which is a potential substance that inhibits oxidative stress-induced hippocampal damage owing to its radical scavenging activity.

In consideration to numerous established findings on the therapeutic applications and effects of curcumin based on its antioxidant mechanism of actions, *Curcuma longa* could be used both as a protective and therapeutic agent against cadmium-induced toxicity. It is recommended that further studies be done to validate the protective activity of ethanolic *Curcuma longa* root extract in other animal models of Cadmium toxicity.

REFERENCES

Addis, DR., Schacter, DL. (2011). The hippocampus and imagining the future.5:173.

Amaral, D., Lavenex, P. (2007). Hippocampal neuroanatomy; the hippocampus book. P.37.

Ammon, HP., Anazodo, MI., Safayhi, H., Dhawan, BN., Srimal RC.(2011). Curcumin: A potent inhibitor of leukotriene B4 formation in rat peritoneal polymorphonuclear neutrophils (PMNL).58:226.

Barbier, O., Jacquillet, G., Tauc, M., Cougnon, M., Poujeol, P. (2005). Effect of heavy metals on, and handling by the Kidney.99 (4):105-110.

Barclay, LR., Vinqvist, MR., Mukai, K., Goto, H., Hashimoto, Y., Tokunaga, A., Uno, H. (2000). On the antioxidant mechanism 0f Curcumin classical methods are needed to determine antioxidant mechanism and activity.2 (18):2841-2843.

Ben-Azu, Bulus Adzu, Olusegun G Ademowo.(2016). Drug development research.3(78):135-145.

Bliss, TV., Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature. 1993;361:31–39.

Brunson, KL., Eghbal-Ahmadi M., Bender R., Chen Y., Baram TZ. Long-term, progressive hippocampal cell loss and dysfunction induced by early-life administration of corticotropin-releasing hormone reproduce the effects of early-life stress. Proc Natl Acad Sci U S A. 2001b;98:8856–8861.

Chattopadhyay, L., Biswas, K., Bandyopadhyay, U., Banerjee, RK. (2004). Turmeric and Curcumin. Biological actions and medicinal applications.87:44-53.

Cheah, YH., Nordin, FJ., Sarip, R., Combined xanthorrhizol-curcumin exhibits synergistic growth inhibitory activity via apoptosis induction in human breast cancer cells MDA-MB-231. Cancer Cell Int. (2009)., 9:1. doi: 10.1186/1475-2867-2869.

Chirinos, I.R., Betalleluz-Pallardel, A. Huamán, C. Arbizu, R. Pedreschi, D.Campos, HPLC-DAD characterisation of phenolic compounds from Andeanoca (Oxalis tuberosa Mol.) tubers and their contribution to the antioxidant capacity, Food Chem. 113 (2009) 1243–1251.

Dall'igna OP, Fett P, Gomes MW. 2007. Caffeine and adenosine A(2a) receptor antagonists prevent beta-amyloid (25-35)-induced cognitive deficits in Mice. Exp Neurol. 203:241-245.

Ercal N, Gurer-Orhan H, Aykin-Burns N (2001) Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. Curr Top Med Chem 1:529-539.

Faten,Y. Mahmoud., Ashraf, H. Abd, El-Hakim., Ayman, S. El Deen Amer., Mariam, W. Fidal. (2019). Effect of exposure to cadmium on the hippocampus in adult albino rat and the possible role of L-carnitine. 3(4): 240-250.

Fresquez, M.R., Pappas, R.S., Watson, C.H. (2013).Establishment of toxic metal reference range in tobacco from US Cigarettes.37:298-304.

Genchi, G., Caroci, A., Lauria., Sinciropi., M.S., Catalano, A., Nickel. (2020). Human health and environmental toxicology. Internation.Environ.Res.Public Health.17

Halliwell, B., Gutterridge, JMC. (2003). Free radicals in biology and medicine.247-251.

Hogervost, J., Plusquin, M., Vangronsvel, J., Nawrot, T., Cuypers, A., Van Hecke, E., Roels, H. A.., Carleer, R., Staessen, J.A.(2007). House dust as possible route of environmental exposure to cadmium and lead in adult general population Environment Res.103: 30-37.

https://images.app.goo.gl/oW427MvFRcNczES49

https://images.app.goo.gl/pWMH6gxe92ZWvJ2aA

Jarup L. (2003).Hazards of heavy metal contamination. 68(10):167-182.

Jin, T., Nordberg, M., French, W., Dumont, X., Bernard, A., Ye, TT.Kong Q Wang Z., li P, Lundstrom NG., Li, Y., Nordberg, GF. (2002). Cadmium biomonitoring and renal dysfunction among a population environmentally exposed to Cadmium from smelting in China.15(4):397-410.

Jomova, K., Valko,M. (2011). Advances in metal induced oxidative stress and human disease. Toxicology 283:65-87.

Joseph, P., Muchnok, T. K., Klishis, M.L. (2001).Cadmium- induced Cell transformation and tumorigenesis are associated with transcriptional activation.

Jurenka, JS. (2009). Anti-inflammatory properties of curcumin, a major constituent of Curcuma longa. A review of preclinical and clinical research.14(2):141-53.

Karlowicz Katarzyna., Bodalska. (2017). Curcuma longa as a medicinal herb in the treatment of diabetic complications.

Kikusawa., Ritsuko., Reid., Lawrence, A. (2007). "Proto who utilized turmeric, and how?" (PDF). In Siegel, Jeff., Lynch, John., Eades., Diana (eds.). Language Description, History and Development: Linguistic indulgence in memory of Terry Crowley. John Benjamins Publishing Company. pp. 339–352.

Kim,W., Kim,D.W., Yoo, D.Y., Jung, H.Y., Nam, S.M., Kim. J.W. (2014). DendropanaxmorbiferaLéveille extract facilitates Cadmium extraction and prevents oxidative damage in the hippocampus by increasing antioxidant levels in cadmium-exposed rats.14:428.

Leong-Škornickova, Jana., Šida, Otakar., Wijesundara, Sirtl., Marhold, Karol. (May 2008). "On the identity of turmeric: the typification of curcuma longa L. (Zingiberaceae)". Botanical Journal of the Linnean Society. 157 (1): 37–46.

Li, GP., Chu, T.,Yang, F., Beech, W.,Frautschy, S.A., Cole G.M. (2001). The curry spice curcumin reduces oxidative damage and amyloid pathogenesis on Alzheimer’s transgenic mouse.21:8370.

Meepun, N., Saguansakbaramee, N., Wongchuphan, R.(2014). Analysis of lead and cadmium contents in local Vegetables in Surat Thani,Thailand.6:455-461

Menon, V. P., Sudheer, A. R. (2007). Antioxidant and anti-inflammatory properties of curcumin. Adv. Exp. Med. Biol.595:105–125.

Mezynska, M., Brzóska, M.M. (2018). Environmental exposure to Cadmium. A risk for health of the general population in industrialized countries and preventive strategies.25:3211-3232.

Mittler R. (2002).Oxidative Stress, Antioxidants and Stress tolerance; Trends Plant Science.7:405-410.

Mohammad, A., Sorayya, K Curcumin reduces malondialdehyde and improves antioxidants in humans with diseased conditions: a comprehensive meta-analysis of randomized controlled trials(2019). 9(4): 23.

Monte AS, Greicy CS, Roger SM, Joanna KS, Junia VS, Rafaela CC. 2013. Prevention and reversal of ketamine-induced schizophrenia related behavior by minocycline in mice: possible involvement of antioxidant and nitrergic pathway. J Psychopharmacol. 11:1032–1043.

Nair, K.P., Prabhakaran (2013). The Agronomy and Economy of Turmeric and Ginger. The Invaluable Medicinal Spice Crops. Newnes. pp. 7–10.

Nelson, K.M., Dahlin, JL., Bisson, J. (2017). "The Essential Medicinal Chemistry of Curcumin. Journal of Medicinal Chemistry. 60 (5): 1620–1637.

Nordberg, GF. (2004). Cadmium and health in the twenty-first century historical remarks and trends for the future.Biometals. 17(5):485–489.

Pickersgill., Barbara (2005). Prance., Ghillean., Nesbitt., Mark (eds.). The cultural history of plants. Routledge. p.170.

Pickersgill., Barbara (2005). The cultural history of plants.175.

Pinot, F., Kreps, S.F., Bachelet, M., Hainaut, P., Bakonyi, M., Polla, B.S.(2000).Cadmium in the environment: sources, mechanisms of biotoxicity and biomarkers. Reviews on Environmental health,15(3): 299-323.

Priya., Darsimi, K.I. (2014). The Chemistry of Curcumin. From extraction to therapeutic Agent. Journal of molecules 19(12):100-112.

Rahimzadeh , M.R.(2017). Cadmium toxicity and treatment: An update.Caspian J.Intern.Med.8:135-145.

Rahmani S., Asgary S., Askari G., Keshvari M., Hatamipour M., Feizi A. (2016). Treatment of Non-alcoholic Fatty Liver Disease with Curcumin: A Randomized Placebo-controlled Trial. Phytother. Res.30:1540–1548

Ravindran, P.N., Nivmal Babu, K., Sivaraman, K.(2007).Turmeric, the golden spice of life. The Genus Curcuma.1-14.

Renugadevi, J., and Prabu, S.M.(2010).Cadmium-induced hepatotoxicity in rats and the protective effect of naringenin:Experimental and Toxicologic pathology.62(2):171-181.

Rinaldi M., Micali A., Marini H., Adamo EB., Puzzolo D., Pisani A., Trichilo V., Altavilla D., Squadrito F., Minutoli L. (2017) Cadmium, organ toxicity and therapeutic approaches: a review on brain, kidney and testis damage. Curr Med Chem 24(35):3879–3893.

Robin, A., Beinhoft.(2013).Cadmium Detoxification. Elimination of persistent toxicants from the human body,7.

Romeo-Puertas, M.C., Corpas,F.J., Rodriguez-Serrano, M., Gómez, M., Del Rio, L.A., Sandalio LM(2007).Differential expression and regulation of anti-oxidative enzymes by cadmium in pea plants. Plants physiology. (164):1346-1357.

Rusconi, M., Pollesello, S.(2010).Water and Soil monitoring for the protection of environment and human health.

Sarraf, P., Parohan, M., Javanbakht, M.H., Ranji-Burachaloo, S., Djalali M.(2019).Short-term curcumin supplementation enhances serum brain-derived neurotrophic factor in adult men and women: a systematic review and dose-response meta-analysis of randomized controlled trials.69:(1-8).

Sautaur, M., Miyamotu, T., Lacaille-Dubois, M.A (2005). Steroidal saponins from smilax medica and their antifungal activity. *Journal of natural products.* 68(10): 1489-1493.

Scott., Ashley., Power., Robert, C., Altmann-Wendling., Victoria., Artzy., Michal., Martin., Mario, A. S., Eisenmann, Stefanie., Hagan, Richard., Salazar-García., Domingo, C., Salmon, Yossi., Yegorov, Dmitry., Milevski, Iani. (2020).

Shrikant Mishran and Kalpana Palaniveru. Ann Indian Acad Neurol. 2008 Jan-Mar; 11(1): 13–19

Singh, V. (2014). Textbook of Anatomy: Head, Neck and Brain, Volume III, 2nd Edition. Reed Elsevier India Private Limited. 402-403.

Sirirugsa, P., Larsen, K. & Maknoi, C. (2007). The genus Curcuma L. (Zingiberaceae): distribution and classification with reference to species diversity in Thailand Gardens' Bulletin Singapore 59: 203-220.

Slavin, J.L., Lloyd, B. (2012).Health benefits of fruits and vegetables. Advances in Nutrition. 3(4):506-516.

Soisungwa, S.(2019).Dietary Cadmium intake and its effects on kidneys. Toxics.6:15.

Tang, H., Lu, D., Pan, R., Qin, X., Xiong, H., Dong, J., Curcumin improves spatial memory impairment induced by human immunodeficiency virus type 1 glycoprotein 120 V3 loop peptide in rats. Life Sci. 2009;85(1–2):1–10.

Tinkov, A.A., Filippini, T., Ajsuvakovae, O.P., Skalnaya, M.G., Aasethf,J., Bjorklundh, G., Gatia, Hailinai, E.R., Popova E.V., Nemereshinai, O.N., Huangk, P.T., (2018).Cadmium and atherosclerosis. A review of toxicological mechanisms and a meta-analysis of epidemiologic studies.162:240-260.

Turmeric". Unabridged Random House Dictionary. Dictionary.com. 2013.

Wang,X. C.(2016).Novel insights into redox system and the mechanism of redox regulation.43:607-628.

Wei li., Miaoran, Ning., KwiHyc,Koh. (2014). Drug metabolism and disposition.42(4):796-802.

Yamada K, Noda Y, Hasegawa T, Komori Y, Nikai T, Sugihara H,Nabeshima T. 1996. The role of nitric oxide in dizocilpine-induced impairment of spontaneous alternation behavior in mice. J Pharm Exp Ther. 276:460–466.

Zalups, RK AS(2003):Molecular handling of Cadmium in transporting epithelia.186(3):163-188.