EFFECT OF AQUEOUS EXTRACT OF COWPEA (*Vigna unguiculata*) ON VISUOSPATIAL LEARNING AND MEMORY IN ACUTE LEAD-INDUCED NEUROTOXICITY IN MICE

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BY

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DEPARTMENT OF HUMAN PHYSIOLOGY, FACULTY OF MEDICINE, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

FEBRUARY, 2016

DECLARATION

I declare that the work in this dissertation entitled "EFFECT OF AQUEOUS EXTRACT OF COWPEA *(Vigna unguiculata)* ON VISUOSPATIAL LEARNING AND MEMORY IN ACUTE LEAD INDUCED NEUROTOXICITY IN MICE" was performed by me in the Department of Human Physiology, Ahmadu Bello University Zaria, under the supervision of Drs. R.A. Magaji and A.U. Zezi. The information derived from the literature has been duly acknowledged in the text and a list of references provided. To the best of my knowledge, no part of this work has been presented for another degree or diploma at any institution.

Yusuf SULAIMAN **Signature** **Date**

CERTIFICATION

This dissertation titled "EFFECT OF AQUEOUS EXTRACT OF COWPEA *(Vigna unguiculata)* ON LEARNING AND MEMORY IN ACUTE LEAD INDUCED NEURO TOXICITY IN MICE" by Yusuf SULAIMAN, meets the regulation governing the award of the degree of M.Sc Human Physiology of the Ahmadu Bello University Zaria, and it is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This dissertation is dedicated to Allah (S.W.T.) for His mercy and support, without which I would have been no one, and also to my dear parents for their kindness and training, and to the parents of all those children, and the children who died as a result of lead poisoning in Zamfara State in 2010.

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LIST OF ABBREVIATIONS

ACE	Angiotesin Convertin Enzyme
AD	Azheirmer's Disease
ATP	Adenosine Triphosphate
B-Clams	(B-Carotene Lindoleic acide Model System
BHA	Butylated Hydroxanisole
BM	Barnes Maze
BPb	Blood lead level
DMSA	Dimercapto succinic acid
DNA	Deoxyribonucleic Acid
DPPH	2, 2- Diphenyl-1- Picrylhydrazyl
FRAP	Ferric Reducing Antioxidant Power
GCP II	Glutamate Carboxy peptidase II
H_2O_2	Hydrogen Perxide
MWM	Morris Water Maze
NADH	Reduced Nicotinamide Adenine Dinucleotide
N-Cams	Neural cell adhesion molecules
NMDA	N – methyl D- aspartate
NOS	Nitric oxide synthase
Pb	Plumbum (Lead)
PKC	Protein kinase C
PM	Pyridoxamine
PPH	Pepsin Pancreation hydrosylate
ROS	Reactive Oxygen Species
TBARS	Thiobarbituric acid reactive substances
TEAC	Trolox Equivalent anti-oxidant Capacity

ABSTRACT

Learning is the act of acquiring new or modifying and re-inforcing existing knowledge, while Memory is relatively the permanent storage of the learned information. Exposure to lead affect brain regions such as hippocampus that are involved in learning and memory. Succimer drug or meso 2,3 – Dimercaptosuccinic acid (DMSA) is a metal chelator which is used as an antidote to lead toxicity. This study aimed at assessing the effect of cowpea (Vigna uinguiculata (L) walp) on learning and memory in acute lead-induced neuro toxicity in mice using Morris water and Barnes mazes. In this study 50 mice (18-22g, aged 6-8 weeks) were used. The animals were divided into two main groups of 25 mice each of the two memory assessment paradigms. Each paradigm has 5 mice allotted to 5 sub-Groups. Distilled water 10 ml/kg, succimmer 20 mg/kg, 250, 500 and 1000 mg/kg Vigna unguiculata aqueous extract were administered orally. Lead acetate solution at 120 mg/kg was also administered orally using canular to induce acute lead toxicity on the first day. The result was not statistically significant in the acquisition sessions and the probe trials for both the Morris water and Barnes mazes when compared to control. At the end of the study, it was concluded that Vigna unguiculata at the doses administered has no effect on learning and memory in acute lead induced neurotoxicity in mice, but that does not mean it lacks total therapeutic benefit. It was recommended that Co-administration of cowpea and succimmer might be of a better therapeutic benefit.

CHAPTER ONE

1.0 Introduction

Lead is a poisonous metal, which exist in both organic (Tetraethyl lead) and inorganic (lead acetate and lead chloride) forms in the environment (Shalan *et al*., 2005). The main sources are medicines, paintings, pipes, ammunition. And more recently, it is found in alloys for welding storage materials for chemical reagents (Garaza *et al*., 2006). Exposure to lead mostly occurs through the respiratory and gastrointestinal systems. Lead is conjugated by the liver and passed to the kidney, where it is excreted out in urine and the rest accumulates in various body organs. This affects many biological activities at the molecular, cellular and intercellular levels, which may result in morphological alterations that can remain even after lead level has fallen (Flora *et al*., 2006; Ibrahim *et al*., 2012).

Lead poisoning or lead intoxication is defined as exposure to high levels of lead typically associated with severe health effects. Poisoning is a pattern of symptoms that occur with toxic effects from mild to high levels of exposure; toxicity is a wider spectrum of effects, including subclinical ones (those that do not cause symptoms) (Guidotfi and Ragain, 2007). The amount of lead in the blood and tissues, as well as the time course of exposure, determines toxicity. Lead poisoning may be acute (from intense exposure of short duration) or chronic (from repeat low-level exposure over a prolonged period), but the chronic is much more common (Rossi, 2008).

Diagnosis and treatment of lead exposure are based on blood lead level measured in micrograms of lead per deciliter of blood (μg/dL). A blood lead level of 10 μg/dL or above is a cause for concern; however, lead may impair development and have harmful health effects even at lower levels, and there is no known safe exposure level (Barbosa, *et al.*, 2005). Authorities such as the American Academy of Paediatrics defined lead

poisoning as blood lead levels higher than 10 μg/dL (Regan and Turne, 2009). Poisoning by organic lead compounds has symptoms predominantly in the central nervous system, such as insomnia, delirium, cognitive deficits, tremor, hallucinations, and convulsions (Karri, *et al*., 2008).

1.1 Acute Lead Toxicity

In acute poisoning, typical neurological signs are pain, muscle weakness, paraesthesia, and symptoms associated with encephalitis such as headache, fever, fatique or weakness, confusion, agitation or hallucinations, seizures, loss of sensation or paralysis in certain areas of the face or body, double vision, perception of foul smells, such as burned meat or rotten eggs, Problems with speech or hearing, loss of consciousness, and so on. Other lead acute symptoms include: Abdominal pain, nausea, vomiting, diarrhoea, and constipation. Lead's effects on the mouth include astringency and a metallic taste. Gastrointestinal problems, such as constipation, diarrhoea, poor appetite, or weight loss, are common in acute poisoning. Absorption of large amounts of lead over a short time can cause shock (insufficient fluid in the circulatory system) due to loss of water from the gastrointestinal tract. Haemolysis (the rupture of red blood cells) due to acute poisoning can cause anaemia and haemoglobin in the urine. Damage to kidneys can cause changes in urination such as decreased urine output. People who survive acute poisoning often go on to display symptoms of chronic poisoning (Pearce, 2007).

1.2 Chronic Lead Toxicity

Chronic poisoning usually presents with symptoms affecting multiple systems, but is associated with three main types of symptoms: gastrointestinal, neuromuscular, and neurological. Central nervous system and neuromuscular symptoms usually result from

intense exposure, while gastrointestinal symptoms usually result from exposure over longer periods. Signs of chronic exposure include loss of short-term memory or concentration, depression, nausea, abdominal pain, loss of coordination, and numbness and tingling in the extremities. Fatigue, problems with sleep, headaches, stupor, slurred speech, and anaemia are also found in chronic lead poisoning (karri *et al,* 2008).

Figure 1.1: Lead Structure (Adopted from Stewart *et al*., 2006).

1.3 Mechanism of Lead Toxicity

Tetraethyl lead, still used as an additive in some fuels, can be absorbed through the skin. Exposure occurs through inhalation, ingestion or occasionally skin contact. Lead may be taken in through direct contact with mouth, nose, and eyes (mucous membranes), and through breaks in the skin. Tetraethyllead, which was a gasoline additive and is still used in fuels such as aviation fuel, passes through the skin; however inorganic lead found in paint, food, and most lead-containing consumer products is only minimally absorbed through the skin (Samarghandian,2013).

The main sources of absorption of inorganic lead are from ingestion and inhalation. In adults, about 35–40% of inhaled lead dust is deposited in the lungs, and about 95% of that

goes into the bloodstream. Of ingested inorganic lead, about 15% is absorbed, but this percentage is higher in children, pregnant women, and people with deficiencies of calcium, zinc, or iron. The main body compartments that store lead are the blood, soft tissues, and bone; the half-life of lead in these tissues is measured in weeks for blood, months for soft tissues, and years for bone (Flora *et al*., 2012).

Lead has no known physiologically relevant role in the body, and its harmful effects are myriad. Lead and other heavy metals create reactive radicals which damage cell structures including DNA and cell membranes. Lead also interferes with DNA transcription, enzymes that help in the synthesis of vitamin D, and enzymes that maintain the integrity of the cell membrane. Anaemia may result when the cell membranes of red blood cells become more fragile as the result of damage to their membranes. Lead interferes with metabolism of bones and teeth and alters the permeability of blood vessels and collagen synthesis. Lead may also be harmful to the developing immune system, causing production of excessive inflammatory proteins; this mechanism may mean that lead exposure is a risk factor for asthma in children. Lead exposure has also been associated with a decrease in activity of immune cells such as polymorphonuclear leukocytes. Lead also interferes with the normal metabolism of calcium in cells and causes it to build up within them (Flora *et al*., 2012).

1.4 Lead Neurotoxicity

Lead Neurotoxicity is a term used to describe neurophysiological changes caused by exposure to toxic agents. Such exposure can result in neurocognitive symptoms and/or psychiatric disturbances. Common toxic `agents include heavy metals, drugs, organophosphates, bacterial, and animal neurotoxins. Among heavy metal exposures, lead

exposure is one of the most common exposures that can lead to significant neuropsychological and functional decline in humans. In the brain exposure of animals to lead caused cerebellar oedema, cerebral satellitosis and encephalomalacia (El-Neweshy and El-Sayed, 2011).

Impairments in cortex, hippocampus and cerebellum were also reported. Lead intoxication in humans can be seen from the recent report of high number of children fatalities in Zamfara Nigeria, an estimated 400 children died. Laboratory testing later confirmed high levels of lead in the blood of the surviving children (MSF, 2012), commonly associated neuropsychological difficulties involve in lead poisoning are: intelligence, learning, memory, executive functioning, attention, processing speed, language, visuospatial skills, motor skills, etc. (Samarghandian, 2013).

1.4.1 Mitigative effects of some chemical agents on lead induced neuro toxicity

In the light of lead associated brain toxicity; researches have also reported cases of lead induced brain toxicities that were mitigated by some chemical agents. One of these researches is the role of exogenous hydrogen peroxide (H_2O_2) in inducing mouse tolerance to lead exposure. Administration of lead was found to significantly ($p<0.05$) inhibit SOD and CAT activities in the brain. Application of 1.2 micro grams H_2O_2 per kg body weight efficiently decrease lead induced injury as revealed by decreased growth suppression, increased antioxidative enzyme activity, reduced lipid peroxidation and protection of nuclear DNA integrity (Li *et al*., 2010). Reckziegel *et al*., (2011) reported the protective effect of garlic in lead induced brain damage.

1.5 Effect of Lead on Different Regions of the Brain

1.5.1 Cerebral cortex

The prefrontal cortex is a likely site of damage responsible for behavioural impairment induced by lead. Indeed, prefrontal cortical damage results clinically in perseveration, inability to inhibit appropriate behavioural response, and increased distractibility. All clinical hallmarks of lead-induced impairment in both monkeys and humans. It has been suggested that basal forebrain and the primary visual cortex may also be damaged by lead. Morphological changes were observed in areas V1 and V2 of the occipital cortex of monkeys following moderate level exposure (Flora *et al*.,2012).

1.5.2 Hippocampus

Morphologic changes were observed in the rat hippocampus following low level exposure to lead during lactation, and at blood lead levels (BPb) of 20 mg/dl. Namely, a significant increase in the size and numerical density of the mossy fibres, the granule cell layer and the commissural–associational area of the dentate molecular layer were reported. This was related to the high zinc content in the hippocampus. The opposite effect i.e. decreased density of cell layers was observed at much higher (BPb) level of 250 mg/dl., which might be irrelevant to our discussion on low-level exposure. However, this latter finding suggests a bimodal effect of lead on the developing hippocampus, and this type of dose–response curve is consistent with those described for some behavioural outcomes in experimental animals (Flora *et al*., 2006).

A recent report has demonstrated long-lasting decrease in the density of cholinergic innervation of the hippocampus as the result of perinatal low-level lead exposure. The

loss of septohippocampal cholinergic projection neurons in neonate animals resulted in a deficit in hippocampal cholinergic innervation that persisted into young adulthood. This may account for persistent cognitive impairments associated with early Pb exposure (Reckziegel *et al*., 2011).

1.5.3 Cerebellum

Lead-induced inhibition of postnatal structuring of the rat cerebellum was indicated by an impaired developmental time course of desialylation of the D2-CAM-N-CAM protein. N-CAM, the neural cell adhesion molecule, regulates neuronal fiber outgrowth and synapse formation. This phenomenon was observed at PbB of 20–30 mg/dl, and may contribute to impairment in fine motor skills. Its possible clinical correlate in human is postural disequilibrium, as was described in a clinical study of 6-year-old children with PbB of 10–14 mg/dl measured during their first 5 years of life (Bhattacharya *et al*., 1993).

1.6 Statement of the Research Problem

Lead poisoning has been a recurrent problem in society for many centuries, and its deleterious effects on central nervous system (CNS) are known as lead encephalopathy or lead neuropathy (Flora *et al*., 2006). Some of the major effects of lead poisoning are neurobehavioral impairments, hyperactivity, alterations in brain structure learning and cognitive deficits in children have been observed even with low blood lead levels (10-20µg/dl) (Needleman, 2004). Although no general hypothesis is known for the mechanism to explain what cellular events underlie the behavioural and cognitive dysfunction of lead, the detrimental effects of lead have warranted interest in this area (karri *et al*, 2008).

One of the reasons for the deleterious effects on lead is its ability to strongly bind to sulfhydryl groups of proteins and to mimic or compete with calcium which is one of the major component of cowpea (*Vigina unguiculata*) (flora *et al*., 2006). Chronic lead toxicity continues to be a leading environmental health issue especially for children (Mushak, 1992). Recent studies have shown that the toxicity of heavy metals such as lead is a problem for ecological, evolutionary and environmental activities in Nigeria (Nagajyoti *et al.,* 2008).

The procedures required in treating lead toxic patients are tedious. The need to seclude patients, use chelating agents or drugs to treat the patients are all not very effective and not easily applicable to the lead patients. Also the problems of affordability, acceptability and compliance to the drugs intake are all other issues to contend with. Therefore, the need to find easier alternative means in alleviating the sufferings of these patients in our environment. Cowpea is found to be rich in protein content, fibres and vitamins, and have many health benefits which led to a number of researches in this area (Kundua *et al.*, 2008). But the question is whether Cowpea (*Vigna unguiculata)* play a role in learning and memory in lead induced neurotoxicity?

1.7 Justification

Chelation therapy is the only available medical counter measure to treat lead or heavy metal toxicity. The thiol and amino carboxylic acid metal chelators have been used for the prevention as well as the raphy for lead toxicity (saxena and flora, 2004). The goal of chelation is to enhance lead elimination before irreversible changes occur, calcium disodium EDTA ($CaNa_2$ EDTA) and 2,3 – dimercaprol have been used conventionally for the treatment of lead intoxication, however, the clinical use of these clelating agents has

been under debate (flora *et al*., 2012). However, the water soluble analog measo - 2, 3 – dimercaptosuccinic acid (DMSA) was found to be an effective chelator without adverse health effects (Jones, 1994). Clinical human and animal studies have shown that succimmer reduces lead levels in blood and other soft tissues (Smith, 2000). However, its hydrophilic properties have hampered its effectiveness in removing lead from brain and skeleton. Also the problems of dosage regimen compliance or treatment protocol and expensive nature of the drugs are other compounding issues (Cremin *et al*., 1999).

Studies have shown that the increasing influx of heavy metals in to water bodies from industrial, agricultural and domestic activities is of global concern because of their well-documented negative effects on human and ecosystem (Nakao *et al.,* 2010). This study intends to determine the role of Cowpea (*Vigna unguiculata)* in cognitive deficits of acute lead induced neuro toxicity, if found to play a role in ameliorating the cognitive deficits of lead toxicity it will reduce the cost of purchasing expensive drugs for treating lead toxicity. This will solve the problems of lack of compliance, acceptability and adverse reactions of the existing drug. *Vigna unguiculata* is bound free and has high bioavailability, and is a common food grown and consumed in Nigeria.

1.8 Hypothesis

Cowpea *(Vigna unguiculata*) has no effect on Visuospatial Learning and Memory in acute lead induced neurotoxicity in mice.

1.9 Aim and Objectives

1.9.1 Aim

The aim of this study is to evaluate the effect of Cowpea (*Vigna unguiculata* (L). Walp). on visuospatial learning and Memory in acute Lead induced neuro cognitive deficits in mice.

1.9.2 Objectives

The specific objectives are:

i. To evaluate the effect of Cowpea (*Vigna unguiculata* (L). Walp) on visuospatial learning and memory in acute lead-induced neurotoxicity in mice using Morris water as a wet maze.

ii. To assess the effect of Cowpea (*Vigna unguiculata* (L).Walp) on visuospatial learning and memory paradigm in acute lead-induced neurotoxicity in mice using Barnes as a dry maze.

CHAPTER TWO

2.0 Literature Review

2.1 Cowpea (*Vigna unguiculata.* (L.) Walp.)

Cowpea or beans also refered to as "Wake" in Hausa, "Ewa" in Yoruba, and "Agwa" in Igbo, is a tropical grain legume which plays an important nutritional role in developing countries of the tropics and subtropics, especially in sub-Saharan Africa, Asia, Central and South America. Because of its high protein content (20-25%), cowpea has been referred to as "poor man's meat". Cowpea young leaves, pods and peas contain vitamins and minerals which have fuelled its usage for human consumption and animal feeding (Aitawade, 2012). It has a long history of cultivation for its edible beans, used both as the dry seed and as unripe fruit, both which are referred to as beans (Triniad *et al*., 2010). White beans are packed with fibres, proteins, carbohydrate, vitamins, minerals and fat. It plays role in preventing Osteoporosis, heart diseases, and certain cancers. The plant sterol esters or phylosterols contained in may help reduce blood cholesterol level (Delgado-Salinas, 2012). White beans or Cowpea is made up of eight vitamins; these vitamins are called B complex. They are: Thiamine (B1), riboflavin (B2), niacin (B3), Panthothenic acid (B5), pyridoxine (B6), cyanocobalamin (B12), folic acid and biotin. These vitaminns are very important for the body cellular activities and metabolism, improves health and function in breakdown of carbohydrates, fats and proteins (Triniad *et al*., 2010).

2.1.1 Biochemical composition of *Vigna unguiculata* grown in Nigeria

Phytochemical studies revealed the presence of bioactive compounds comprising flavonoids (2.36-6.28 mg 100 g^{-1}), alkaloids (1.28-1.64 mg 100 g^{-1}), tannins (0.38-0.77 mg 100 g^{-1}), saponins (0.11-0.23 mg 100 g^{-1}). The protein, carbohydrate, lipids and fiber content were 19.69-39.08, 32.78-67.26, 2.70-21.08 and 1.78-4.68%, respectively. The food energy value ranges from 363.71-477.16 cal g^{-1}. The grains are rich in B-vitamins

such as niacin (1.85-4.01 mg 100 g^{-1}), thiamin (0.46-1.72 mg 100 g^{-1}), riboflavin (0.22-170 mg 100 g^{-1}) and ascorbic acid content ranges from (5.20-55.44 mg 100 g^{-1}) (Obadoni and Ochuko,2002).

The proximate composition of grain, protein, minerals, amino acid and sugar profiles of *Vigna unguiculata* (L.) Walp were analyzed. The crude protein was 23%, fat 3.40% and ash 3.60%. Amino acid analysis indicated the presence of at least 17 amino acids including most of the essential ones. The essential amino acids valine, leucine, phenylalanine and lysine were slightly higher, but sulphur-containing amino acids were lower. Qualitative phytochemical screening of seeds showed fructose, α-glucose, β-glucose, glycerol, manitol, inositol and some oligosaccharides, e.g. raffinose, stachyose, and verbascose. These grains are good sources of minerals comprising calcium, magnesium, phosphorus and potassium while sodium content was low. The legumes can be considered as sources of quality raw materials for food and pharmaceutical industries. Over the years, man has acquired extensive knowledge regarding the utilization of plants around him as food and for maintenance of his health (Okwu and Emenike, 2007).

2.1.2 Phytochemistry of *Vigna unguiculata* extract

Leguminous plants synthesize in their cells a great variety of phytochemicals particularly isoflavones, flavonoids, phenolic compounds**,** lignins, lignans, alkaloids and cyanogenic glycosides (Okwu, 2005). Isoflavones, which are phytoestrogens effectively and efficiently, modulate oestrogen levels in humans. They are of clinical value in low oestrogen states like menopause or imbalanced and toxic oestrogen sensitive conditions such as breast, uterine and prostrate tumour growth (Okwu and Omodamiro, 2005). Phytochemicals regulates, protects and control cancer of prostrate, testicular cancer and

semen quality in men. It prevents breast cancer, cystic ovaries and endometriosis among women (Verger and Leblane, 2003). It is now well recognized that people who consume traditional diets rich in fermented soy foods and beans (mainly the leguminosae) experience less breast, uterine and prostate cancers and increase in semen quality**.** Lignans are weak phytoestrogens that are found in seeds and grains, especially flax seed. They have anti-viral, anti-bacterial, anti-fungal, antioxidant and immune enhancing properties (Okwu, 2005).

Lignins on the other hand are non-carbohydrate dietary fibre that along with polysaccharides occurs in the cell wall of plants. Flavonoids are a group of compounds found in seeds, fruits and vegetables. The family encompasses flavanols, flavanones, flavones and anthocyanidns (Waladkhani and Clemens, 2001). In addition to their free-radical scavenging activity, flavonoids have multiple biological functions, including vasodilator, anti-carcinogenic, anti-inflammatory, anti-bacterial, immune stimulating, anti-allergic, anti-viral and estrogenic effects, as well as being inhibitors of phospholipase A2, cyclo-oxygenase, lipoxygenase, glutathione reductase and xanthine oxidase (Okwu and Omodamiro, 2005). Epidemiological and experimental evidence suggests that consumption of Cowpea is associated with a decreased risk of cancer (Iwe, 2003).

The anti-cancer effects of these crops may be due to the high isoflavone content in Cowpea (Iwe, 2003). The peculiar advantages and health protective properties of cowpea isoflavone prompted its utilization as a nutraceutical and functional foods. Functional foods are those that resemble traditional foods, but render benefits beyond their nutrition and energy value in promoting health and preventing certain chronic diseases especially

cardiovascular disease**,** cancer, diabetes, autoimmune disorders, arthritis and arrhythmia (Shahidi, 2002).

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2.1.3 Roles of Minerals and Vitamins Found in Cowpea (*Vigna unguiculata*)

Calcium is a macro mineral that plays an important role in bone health, muscle contraction, blood clotting, nerve conduction, enzyme regulation, and possibly weight loss (Tremblay and Gilbert, 2011). In humans, intestinal calcium absorption is controlled by complex homeostatic mechanisms involving calcitriol and the parathyroid hormone

(PTH). Calcitriol (1,25(OH2) vitamin D3) increases the synthesis of a cytosolic calcium-binding protein (calbindin) resulting in increased calcium transport in intestinal cells. The PTH indirectly affects intestinal calcium absorption by increasing the formation of calcitriol from its precursor, calcidiol (25(OH) vitamin D3). This internal regulation of intestinal absorption certainly makes it difficult to rely on in vitro availability results as an estimation of calcium bioavailability. The calcium composition in mature cowpea seed per 100g edible portion is 110mg (Watanabe, 2007).

Zinc is a trace mineral with roles in cell growth and replication, bone formation, skin integrity, immune system function, and sexual maturation. Its deficiency is very prevalent in the world, along with deficiencies in iron, vitamin A, iodine, and selenium. Populations with zinc deficiency are more likely to have infants born with neural tube defects have higher incidences of infant and child mortality attributed to respiratory tract pneumonia and diarrhoea and exhibit a high incidence of child stunting. The zinc composition in mature cowpea seed per 100g edible portion is 3.4mg (Dey *et al*., 2010).

Iron deficiency is one of the leading risk factors for death worldwide, affecting an estimated two billion people. The high prevalence of iron deficiency in the developing world has substantial health and economic costs, including poor pregnancy outcome, impaired school performance, and decreased productivity (Zimmermann and Hurrell, 2007).

Copper is a trace mineral that plays an important role in human metabolism, largely because it allows many critical enzymes to function properly. There is, for each crop, a wide variation in copper content. Copper is an essential trace mineral for both physical

and mental health. The highest copper content was found in cowpea, *Vigna unguiculata* (16.95 μg/g of dry weight). Lowest copper content was found in white maize and in yellow maize (Zea mays), with values 1.23 μg/g and 1.38 μg/g of dry weights, respectively. Other foods, such as white sorghum, red sorghum (*Sorghum bicolor*), millet (*Pennisetum glaucum*), and groundnuts (*Arachis hypogea*) had copper contents varying from 2.22 to 11.81 μg/g. These values are well below the supplemental values of 50 mg/day that could interfere with zinc absorption. Thus, among the staple foodstuffs of the areas sampled, cowpea appears to be the richest source of dietary copper followed by groundnut while the two maize varieties are the poorest (USDA, 2011).

Folate is a very important vitamin for pregnant women and those of childbearing age due to its role in the prevention of neural tube defects, which can lead to congenital malformations like spina bifida and/or anencephaly where the brain has not developed. Worldwide, spina bifida and anencephaly are estimated to affect 225,000 children a year (Oakley, 2002). Folate also plays a role in the prevention of certain cancers (Oaks *et al*., 2010; Williams *et al*., 2012), and of neurodegenerative and neuropsychiatric diseases, including Alzheimer's, dementia and depression (Kronenberg *et al*., 2009).

Polyphenols comprise without a doubt the largest group of compounds. Polyphenols consist of several thousand compounds found in fruits, vegetables, and beverages. The polyphenols can be classified as flavonoids and non-flavonoids. Flavonoids consist of the flavonols, flavones, isoflavones, flavanones, anthocyanidins, and flavanols. The non-flavonoids comprise the phenolic acids (hydrobenzoic and hydroxycinnamic acids), lignans, and stilbenes (Fazzari *et al*.,2008).Polyphenols, have so many health benefits, they have been associated with the prevention of cardiovascular heart disease, cancers,

neurodegenerative diseases, and gastrointestinal disorders (González-Gallego *et al*., 2010).

2.1.4 Absorption of cowpea

Proteins consumed in the diet undergo a series of chemical changes in the gastrointestinal tract. The physiology of protein digestion is complicated; pepsin and rennin from the stomach, trypsin from the pancreas and erepsin from the intestines hydrolyse proteins into their component amino acids. Most of the amino acids are absorbed into the bloodstream from the small intestine and thus travel to the liver and from there all over the body. Any surplus amino acids are stripped of the amino (NH2) group, which goes to form urea in the urine, leaving the rest of the molecule to be transformed into glucose. There is now some evidence that a little intact protein is taken up into certain cells lining the intestines. Some of this protein in the infant may have a role in the passive immunity conveyed from the mother to her newborn child (Xiong *et al*., 2013).

A little of the protein and amino acids released in the intestines is not absorbed. The unabsorbed amino acids, plus cells shed from the intestinal villi and acted upon by bacteria, together with gut organisms, contribute to the nitrogen found in faeces. Several components of legume-derived functional foods have recognized benefits on lipid metabolism. Among the necessary characteristics of an efficient lipid lowering functional food exhibited by legumes are Specific Lysine/Arginine ratio of proteins. Binding action of their dietary fibre, which traps fatty acids and bile salts thus inhibiting fat absorption in the small intestine, and the highly fermentable nature of legume fibre and oligosaccharides that significantly increase the production of short-chain fatty acids and exhibit interesting bifidogenic action (Alhaji *et al*., 2010).

2.1.5 Health benefits of *Vigna unguiculata*

According to a work by Philips *et al*., (2003). New functional foods are increasingly sought to improve the treatment of diabetes and cardiovascular diseases. The cowpea (*Vigna unguiculata)* is a widely cultivated legume in Africa, Asia, Central and South America where it is an important constituent of several dishes prepared by soaking, frying, cooking, fermentation, or combinations of them Cowpea has also enormous potential in the development of a variety of new food products such as snacks, weaning foods or fortified traditional foods. Furthermore, cowpea seeds exhibit beneficial health effects related to its antioxidant, hypoglycaemic, hypolipidaemic, and antihypertensive properties (Doblado *et al*., 2005; Frota *et al*., 2008; Guang *et al*., 2012; Xiong *et al*., 2013) which are derived from the specific characteristics of their proteins and from their content of dietary fibre, polyphenols, phytic acid or α-galactoside oligosaccharides (Trinidad *et al*., 2010). The type and content of polyphenols accumulated in the cowpea seed are influenced by phenotype and decrease significantly with the thermal process of cooking (Ojwang *et al*., 2012; Nderitu *et al*., 2013).

Also in a work by Ibrahim *et al*., (2002) The phenolic composition of cowpea seed flour has been shown to include phenolic acids such as p-hydroxybenzoic acid or protocatechic acid, flavonols (e.g. mono and di-glycosides of quercetin), anthocyanins (e.g. delphinidin and cyanidin-3-O-glucosides), and flavan-3-ols (e.g. catechin glucoside). Fermentation is a widely used biotechnological process that improves the sensory characteristics of foods and enhances their nutritive value by increasing the density and availability of nutrients. This process can be spontaneously initiated with the microbiota naturally present in legumes.or controlled by the use of specific cultures or starters from a batch of previously

fermented product. In addition, fermentation has been shown to increase the antioxidant capacity of legume-derived food products, thus reinforcing their beneficial action on human health (Marazza *et al*., 2013).

2.1.6 Metabolic effect of *Vigna unguiculata*

Li *et al* (2014) found that Consumption of raw and fermented *Vigna unguiculata* flours produced clear effects on general metabolism of the experimental animals, affecting their plasma antioxidant capacity, activity of hepatic antioxidant enzymes, liver weight and plasma total-cholesterol and triglycerides. The higher plasma antioxidant capacity found in the groups of animals that consumed fermented Vigna diets is in agreement with the in vitro chemical results and may be derived from the changes in polyphenolic profile to a higher content of flavonoid aglycones at the expense of their corresponding glycosides. Furthermore, such aglycones or flavonoid monoglucosides are absorbed in the small intestine and rapidly metabolised by conjugation with glucuronic acid and/or sulphate or by methylation reactions.

The generated metabolites have been shown to contribute to the beneficial antioxidative effects of flavonoids (Kundua *et al*., 2008). Nevertheless, although the higher antioxidant capacity induced by fermented *Vigna unguiculata* is a relevant feature of this foodstuff, that can make it a valuable nutritional tool in the treatment of diseases with an altered antioxidant status, it should be mentioned that, so far, no beneficial physiological effects as required by regulation have been established in humans exerted by changes in the overall antioxidant capacity of plasma (Li *et al*., 2014). With regard to the biological effects of raw and fermented *Vigna unguiculata* in the liver, the metabolic importance of this organ and its capacity to detoxify xenobiotic and respond to different oxidative

aggressions make it a key organ in the maintenance of body homeostasis. Certain plant foods components, like polyphenols and glucosinolates, are capable of inducing some of the cell detoxification pathways represented by phase I and II detoxifying enzymes (Melegaa *et al*., 2013).

Another work has shown that improving the protection against xenobiotics and their possible deleterious actions. Since the hepatic activity of antioxidant enzymes was induced by fermented Vigna diets, the animals that consumed such diets, and, specially, those that consumed naturally fermented Vigna, were potentially more protected against oxidative stress which may be involved in several inflammatory and metabolic disturbances (Gupte, 2013). Of particular interest is another work by Nishimura *et al*., (2011) which has shown that specific induction of certain antioxidant enzymes like Cu/Zn-SOD and catalase by the Maillard reaction products formed during thermal processing of fermented *Vigna unguiculata* which adds a complementary effect to that previously discussed of its in vitro antioxidant potential. Processes have been shown to significantly modify the content of dietary fibre, phytic acid, free-P and pH of legume flours, and the dialysability of essential nutrients like protein, Phosphorus, Magnesium and Copper. Such changes could in turn affect the metabolic and antioxidant effects of legume (Martin-Cabrejas *et al*., 2004).

The reduction in liver weight caused by raw and fermented Vigna diets that paralleled the improvement in lipid parameters of plasma can be linked to changes in lipid metabolism, since it has been described that increments in liver weight are related to greater fat accumulation in this organ (Merroun *et al*., 2013). In view of its hypolipidaemic action, consumption of *Vigna unguiculata* can be an excellent nutritional strategy for the dietary treatment of diseases in which altered lipid metabolism associated to inflammation brings

about the development of hyper triglyceridaemia, hypercholesterolaemia, hepatomegalia or steatosis. Moreover, in addition to its seeds, other constituents of *Vigna unguiculata* can be used as efficient functional foods with beneficial influence on general metabolism have reported that *Vigna unguiculata* leaves ameliorate cholesterol-induced atherosclerosis and normalise gene expression, cholesterol profile, and antioxidant enzymes (Janeesh and Abraham, 2013).

2.1.7 Anti-oxidant ability of *Vigna unguiculata*

A study of the plants using the vitamin C and the trolox equivalent antioxidant capacity (TEAC) method compared the levels in raw and germinated seeds. Vitamin C was absent in ungerminated seeds, but there was a significant twentyfold increase in levels following germination. Yet, the TEAC values showed that antioxidative effects were present in both ungerminated and germinated seeds and the size of effect was related to the time given allowed in which to germinate. Allowing the seeds to germinate for up to four days allowed for a 58% increase in antioxidant capacity (Oboh, 2006).

Different varieties of cowpea are produced, denoted by colour that varies from white to brown. Vitamin C levels ranged from 0.5 mg/100 g to 0.9 mg/100 g across the different varieties. Although these levels may be considered to be low, it may be the case that the synergism of all the compounds present enables the plant to possess its antioxidant ability. Phytate, a natural antioxidant essential to the development of plants is also present and its levels range from 2.0% to 2.9%. Four main varieties are cultivated – brown, dark brown, brown drum, and light. The brown drum variety of cowpea has both the highest levels of vitamin C and phytate, with the brown variety showing the highest concentration of total phenols at 1 mg/g, indicating that this variety may serve as a potential candidate for future testing. The levels of phenols have been shown to be an important in determination of

antioxidative ability However, this has correlated with a poor free radical scavenging ability. The light and dark brown varieties have showed the greatest ability with values of 705 mg and 618 mg needed to quench 50% of the free radicals present in DPPH, respectively, and this can be compared with a twenty-fold more potent activity for the standard, butylated hydroxanisole (BHA) (Siddhuraju and Becker, 2007).

Similar findings were observed using the ferric reducing antioxidant power (FRAP) assay with raw cowpeas exhibiting the greatest antioxidant ability. In addition, the β-carotene linoleic acid model system (β-CLAMS) assay also showed that raw, brown variety had significantly higher antioxidant ability than heated or soaked cowpeas, although BHA exhibited a far superior inhibition of β-carotene Coupled with the fact that a dose dependent inhibition of superoxide was observed, and that good levels of hydroxyl scavenging were observed, it may be in fact a summation of all the different antioxidant abilities presented that allow *Vigna unguiculata* to effectively combat oxidative damage(Contour-Ansel and Torres-Franklin, 2006).

The polyphenol composition of *Vigna unguiculata* has been related to the antioxidant potential of this legume (Nderitu *et al*., 2013). Studies on polyphenol distribution within the different anatomical parts of cowpea seed point out to a noticeable content of these compounds in the seed coat in which they are evenly present as free and bound phenolics, among which the main antioxidant activity corresponds to the free fraction (Gutierrez-Uribe, 2011).

Lactic fermentation process using the microbiota naturally present in the seed or adding a starter culture increases the titratable capacity and decreases the pH of the food to levels

below (Porres *et al*., 2003; Li *et al*., 2014) thus improving its reducing capacity. Furthermore, fermentation significantly modifies the polyphenolic profile and antioxidant capacity of legumes, giving rise to the appearance of phenolic compounds with proved biological activity, such as tyrosol and quercetin aglycone, derived from acid hydrolysis of glycosides caused by low pH and the action of microorganisms, which were not present in the raw seed flour (Duenas *et al*., 2005; Cheng *et al*., 2013).

In another study by a group of researchers, they reported that: natural or controlled fermentation of *Vigna unguiculata* seed flour significantly increased its antioxidant capacity as measured by assays based on electron transfer like the Folin–Ciocalteu, Prussian blue or reducing capacity. Those improvements were in turn related to a significant increase in hydroxyl radical scavenging capacity that prevented lipid peroxidation of brain homogenates. Moreover, the higher antioxidant capacity of fermented *Vigna unguiculata* extracts was not related to a higher Fe^{2+}- complexing capacity, thus supporting its hydroxyl radical scavenging potential rather than the inhibition of free radical formation due to chelation of Fe that could interfere in the correct development of the antioxidant assay. Nevertheless, in order to further confirm the free radical scavenging action of our samples, we decided to test their potential using the Cu^{2+}- induced oxidation of Low density Lipo-protein and subsequent changes in electrophoretic mobility. The antioxidant capacity of raw and fermented *Vigna unguiculata* extracts was also evident using this methodology, and a higher antioxidant potential was observed in the extract of heated naturally fermented seed flour (Setchell *et al*., 2002).

The effects of drought stress in *Vigna unguiculata* , suggestive of its antioxidant capacity and using levels of glutathione reductase (involved in the regeneration of reduced glutathione) as a marked showed relatively robust protection Similar examination of the plants the defensive capabilities assessed through the measurement of antioxidant activity in the periphery of the plant also revealed moderate levels of vitamin C but also high levels of important free radical scavenging catalase and glutathione reductase activity in the periphery (Dalton *et al*.,1998).

2.1.8 Anti hypertensive effect of *Vigna unguiculata*

Segura-Campos and Chel-Guerrero, (2011) work on anti-hypertensive effect has shown that Interesting antihypertensive effects have been suggested for *Vigna unguiculata* via methods involving Flavourzyme protease hydrolysis. Unrefined hydrosylated products yielded by this method caused small levels (IC50 value of 2634.4 μg/ml) of inhibition of Angiotensin Converting Enzyme (ACE), a primary pharmacological target for the treatment of hypertension. However, subsequent ultrafiltration of the hydrosylated products showed a more enhanced effect, with the largest effect produced by lower molecular weight peptides. The largest effect was from the smallest peptide derivative with a weight of less than 1 kDa that produced an IC50 of 0.04 μg/ml for the inhibition of ACE. The lowest levels of inhibition resulted from a peptide of over 10 kDa in weight with an IC50 of 170.6 μg/ml reported.

The potent ACE inhibitory activity observed in the lowest molecular weight peptide is suggestive of a potential treatment for AD, since reduction of blood pressure is largely considered to be beneficial in reducing risk of AD. Indeed, the fact that the inhibition is of ACE, is also important however since some current pharmacological inhibitors of Acetyl

Cholinesterase Enzyme have been reported to have varying levels of beneficial effect on rates of cognitive decline and risk of Alzheimer's Disease in various studies (Davies *et al*., 2011). An alternative approach to explore the properties of *Vigna unguiculata* using different enzymatic hydrosylation, with pepsin pancreatin hydrosylate (PPH), showed an enhanced IC50 prior to ultrafiltration of 1397.9 μg/ml. Yet this is significantly more potent than the equivalent value produced by unrefined products yielded by flavourzyme, which may be due to the production of different or more bioactive peptides when PPH was used (McGuinness *et al*., 2009).

2.2 Learning

Learning is the process by which we acquire knowledge about the world, while memory involves encoding, storage and retrieval of such information for future use (Sharma *et al*., 2010). Memory can be classified according to its duration, into short term memory and long term memory. Short term memory is a type of memory that lasts for about 20-40 seconds or at most minutes, it involves processing of information in the hippocampus and the medial temporal lobe, to create memory traces that later can be converted to the long term memory. Long term memory can store larger quantities of information that last for years and sometimes for a lifetime (Sharma *et al.*, 2010).

Memory is broadly divided into three viz: sensory, short-term and long-term memories. Sensory memory holds sensory information for a few seconds (10 to 20) or less after an item is perceived. The ability to look at an item, and remember what it looked like with just a second of observation, or memorization, is an example of sensory memory. The brain stores sensory information for very short periods of time in a working memory, to be able to use it later (Arash *et al.*, 2014).

Working memory is a form of short term memory that allows one to temporarily store and process information. This temporary store enables one to complete or work on a complex tasks while being able to keep information in mind. For instance, the ability to work on a complicated mathematical problem utilize one's working memory (Chein and Feiz, 2010). Brain areas involved in the neuroanatomy of memory include the hippocampus, the amygdala, the striatum, or the mammillary bodies which are thought to be involved in specific types of memory. For example, the hippocampus is believed to be involved in spatial learning and declarative learning, while the amygdala is the brain center involved in emotion (Labark and Cabeza, 2006).

2.2.1 Memory encoding

The term memory encoding defines the neural processes that change an experience into the memory of that experience. In other words, the physiological events that lead to memory formation. This section addresses the questions: Are there different kinds of memories, where do they occur in the brain, and what happens physiologically to make them occur? New scientific facts about memory are being generated at a tremendous pace, and there is as yet no unifying theory as to how memory is encoded, stored, and retrieved (Florian and Anders, 2014). Encoding allows information that is from the outside world to reach senses in the forms of chemical and physical stimuli. Memories for facts and events are not acquired in their definite form. Rather, some post-learning processes are known to take place that gradually stabilize new memories (Florian and Anders, 2014).

2.2.2 Consolidation

The process by which short-term memories become long-term memories is called consolidation. Long-term potentiation is a process in which synapses become stronger the more frequently signals are passed between two neurons. This mechanism is believed to play a major role in the learning and memory processes. When two neurons fire at the same time repeatedly, they become more likely to fire together in the future. Eventually, these two neurons will become sensitized to one another (Gerrow, 2010).

As we acquire new experiences, information, and memories, our brains create more and more of these connections. Essentially, the brain is able to rearrange itself, establishing new connections while weeding out old ones. By rehearsing or recalling information over and over again, these neural networks become strengthened. For example, if you study the same material regularly over a long period of time, the pathways involved in remembering that information become stronger and more familiar. The repeated firing of the same neurons over and over again makes it more likely that those same neurons will be able to repeat that firing again in the future. As a result, you will be able to remember the information later with greater ease and accuracy (Gerrow, 2010).

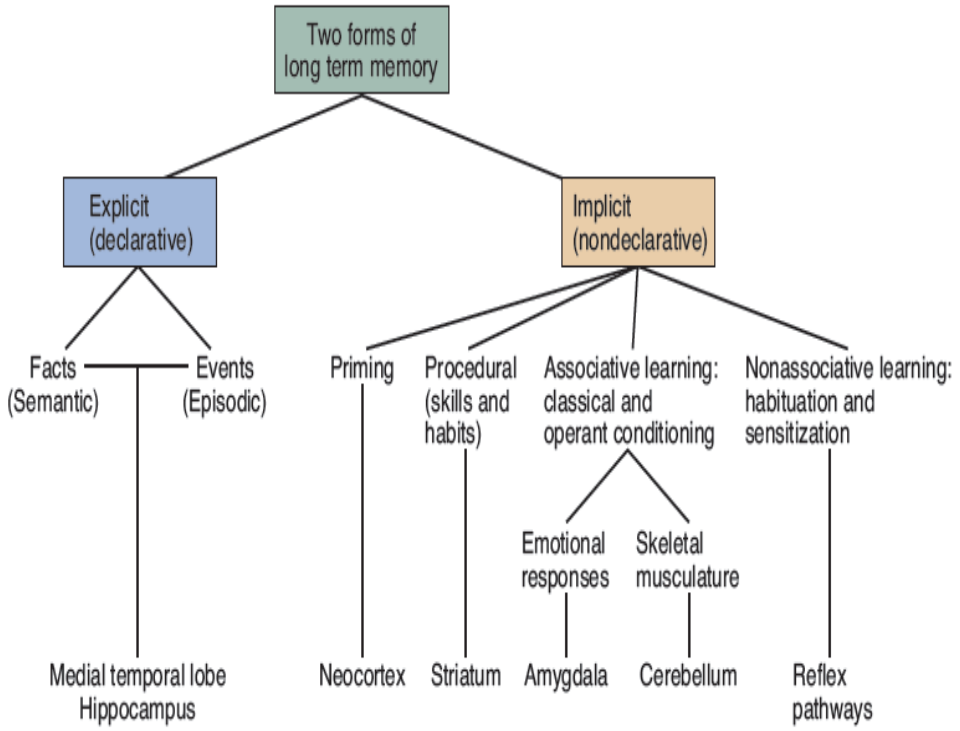

Figure 2.1: Types of Memory (Adopted from Flora *et al*., 2012).

2.3 Areas of the Brain Involved in Learning and Memory

2.3.1 Amygdala

The amygdala receives inputs from all senses as well as visceral inputs. Since the amygdala is very important in emotional learning it is not surprising that visceral inputs are a major input source. Visceral inputs come from the hypothalamus, septal area, orbital cortex, and parabrachial nucleus. Olfactory sensory information comes from the olfactory bulb. Auditory, visual and somatosensory information comes from the temporal and anterior cingulate cortices. The ventral striatum includes part of the caudate, putamen, and the nucleus accumbens septi (nucleus that reclines on the septum) (Anthony, 2009).

2.3.2 Hippocampus

Is a major component of the brains of humans and other vertebrates. Humans and other mammals have two hippocampi, one in each side of the brain. It belongs to the limbic

system and plays important roles in the consolidation of information from short-term memory to long-term memory and spatial navigation. The hippocampus is located under the cerebral cortex; and in primates it is located in the medial temporal lobe, in rodents, the hippocampus has been studied extensively as part of a brain system responsible for spatial memory and navigation (Conrad, 2008).

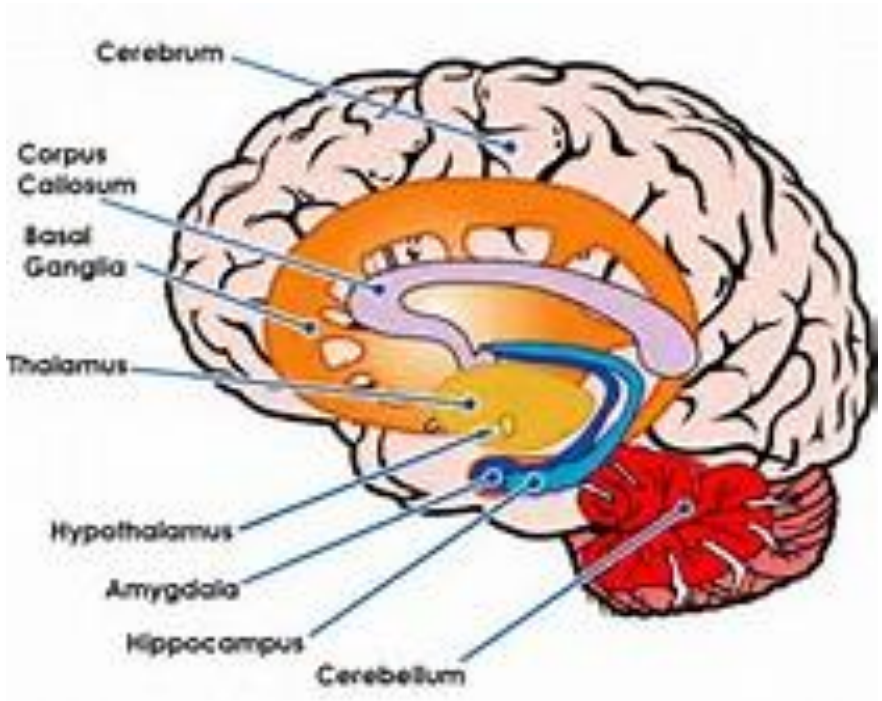

Figure 2.2: Hippocampus and Amygdala Structure of the Brain **(**Adopted from Nakazawa *et al*., 2004).

2.3.3 The anterior cingulate cortex (ACC)

Is the frontal part of the cingulate cortex that resembles a "collar" surrounding the frontal part of the corpus callosum. It consists of Brodmann areas 24, 32, and 33. It appears to play a role in a wide variety of autonomic functions, such as regulating blood pressure and heart rate. It is also involved in rational cognitive functions, such as reward anticipation, decision-making, empathy, impulse control, and emotion. The ACC seems to be especially involved when effort is needed to carry out a task such as in early learning and problem-solving (Taylor *et al*., 2006).

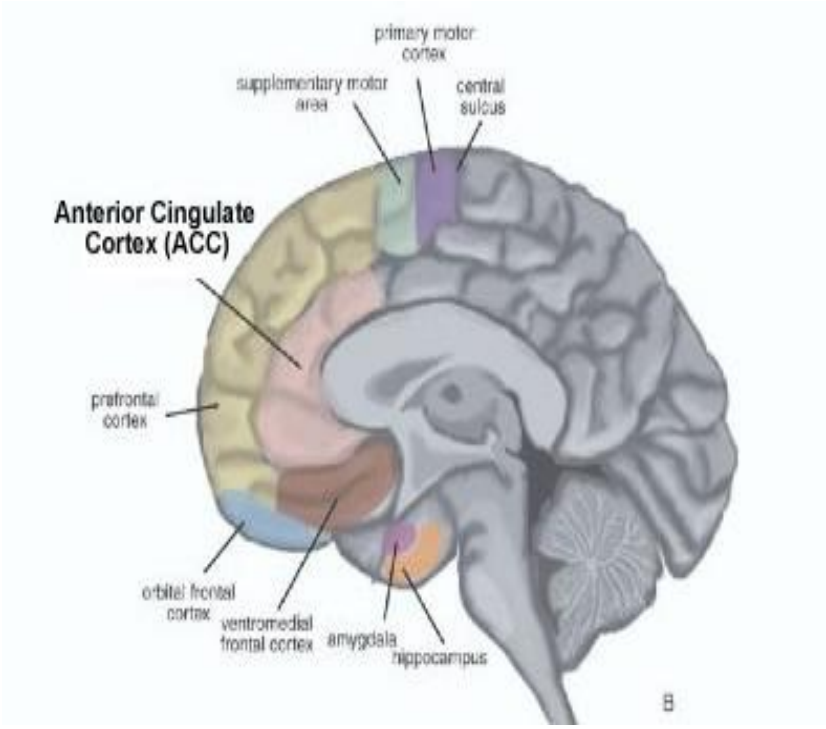

Figure 2.3: Anterior Cingulate Cortex diagram (Adopted from Polli *et al*., 2005).

2.4 Amnesia

Amnesia refers to an inability to recall information that is stored in memory. In simple terms, amnesia is the loss of memory. Organic causes of amnesia may include brain damage through injury, or the use of specific drugs - usually sedative drugs. Amnesia may be one of the symptoms of some degenerative brain diseases, such as Alzheimer's disease. People with amnesia also find it hard to imagine the future, because our constructions of future scenarios are closely linked to our recollections of past experiences. Researchers used advanced brain imaging techniques to show that remembering the past and envisioning the future may go hand-in-hand, with each process sparking strikingly similar patterns of activity within precisely the same broad network of brain regions (Kopelman, 2002).

Being a little forgetful is completely different to having amnesia. Amnesia refers to a large-scale loss of memories that should not have been forgotten. These may include important milestones in life, memorable events, key people in our lives, and vital facts we have been told or taught. Amnesia is different from dementia. Although dementia includes memory loss, it also involves other important cognitive problems which may affect the patient's ability to carry out daily activities. Amnesia can present itself in two fundamentally different ways (Squire *et al*., 2004).

2.4.1 Forms of amnesia

2.4.1.1 Neurological amnesia

There is bilateral damage to structures of the medial temporal lobe or midline diencephalon and a characteristic pattern of impairment. The hallmark of the condition is an impaired capacity for new learning (anterograde amnesia), which occurs together with variable loss of information acquired before the onset of impairment (retrograde amnesia). The impairment affects declarative memory, that is, conscious memory for facts and events regardless of sensory modality or type of material (for example, names, places, faces, objects, odors, and sounds). Other forms of memory that are not accessible to conscious recollection remain intact (e.g., skills, habits, and simple forms of conditioning), and these nondeclarative forms of memory depend on brain systems outside the medial temporal lobe and midline diencephalon (McHugh, 2008).

2.4.1.2 Functional amnesia

This condition has been termed psychogenic amnesia, hysterical amnesia, or dissociative amnesia. In this kind of amnesia, memory is impaired in the absence of structural damage due to brain injury or disease. Here, we use the term functional amnesia. Although

functional amnesia is not associated with structural brain damage, there is evidence of hypometabolism, especially in the frontal lobe as measured by neuroimaging. Similar findings have also been observed in other psychiatric and neurological conditions (i.e., transient global amnesia) (Brand *et al*., 2009).

2.4.2 Types of amnesia

There are many different types of amnesia. Below is a list of the most common ones:

2.4.2.1 Anterograde amnesia

This is a type of amnesia in which the patient cannot remember new information. Things that happened recently, information that should be stored into short-term memory disappear. This is usually caused by brain trauma (brain damage from a blow to the head, for example). However, a patient with anterograde amnesia can remember data and events which happened before the injury.

2.4.2.2 Retrograde amnesia

This type of amnesia is often thought of as the opposite of anterograde amnesia. In this type of amnesia the patient cannot remember events that occurred before his/her trauma, but remembers things that happened after it normally.

2.4.2.3 Transient global amnesia

This is the temporary loss of all memory. The patient with transient global amnesia also finds it very hard to form new memories - he/she has severe anterograde amnesia. The loss of past memories is milder.

2.4.2.4 Traumatic amnesia

This is memory loss caused by a hard blow to the head. People who lose their memory as the result of a car accident may have traumatic amnesia. People with traumatic amnesia may experience a brief loss of consciousness, or even go into a coma. In the majority of cases the amnesia is temporary - how long it lasts usually depends on how severe the injury is.

2.4.2.5 Wernike-Korsakoff's psychosis

This type of memory loss is caused by extended alcohol abuse. The disorder tends to be progressive - it gradually gets worse and worse over time. Patients with Wernike-Korsak off's psychosis also tend to have neurological problems, such as poor coordination, and the loss of feelings in the toes and fingers. It can also be caused by malnutrition. It is linked to thiamin deficiency (Reinhold *et al*., 2006).

2.5 Succimmer

Succimer or Meso-2, 3-dimercaptosuccinic acid (DMSA) is a sulfhydryl-containing, water-soluble, non-toxic, orally administered, metal chelator which has been in use as an antidote to heavy metal toxicity since the 1950s. DMSA's water solubility, oral dosing, large therapeutic window, and low toxicity make it superior to other chelating agents available (Gracia and Snodgrass, 2007). Dimercaptosuccinic acid is a dithiol (containing two sulfhydryl, or S-H, groups) and an analogue of dimercaprol, a lipid-soluble compound also used for metal chelation. Approximately 20 percent of an oral dose of DMSA is absorbed from the gastrointestinal tract of healthy individuals. DMSA has been shown to be successful in lowering blood lead levels in children and adults with lead toxicity, and is approved for use in chelation of lead in children (Bradberry and Vale, 2009).

Dosing protocols for heavy metal toxicity treatment using DMSA vary depending on physician preference and individual patient need, but currently two protocols are most often used. In one protocol, 10-30 mg/kg is given per day in three divided doses, using a three-days-on, 11-days-off cycle, with a minimum of eight cycles. A second protocol involves giving 500 mg/kg (in two or three divided doses) every other day for a minimum of five weeks. Dimercaptosuccinic acid appears to be absorbed best when taken between meals (Bradvery and Vale, 2009).

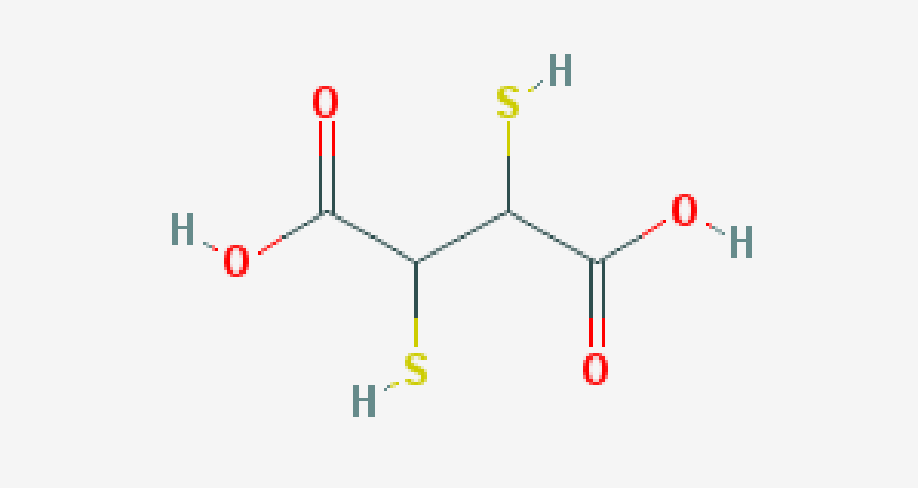

Figure 2.4: Structure of Succimmer (Adopted from Dart *et al*., 1994).

2.5.1 Absorption, distribution and excretion

In a study performed in healthy adult volunteers, after a single dose of Succimer at 16, 32, or 48 mg/kg, absorption was rapid but variable with peak blood radioactivity levels between one and two hours. On average, 49% of the radiolabeled dose was excreted: 39% in the faeces, 9% in the urine and 1% as carbon dioxide from the lungs. Since faecal excretion probably represented non absorbed drug, most of the absorbed drug was excreted by the kidneys. The apparent elimination half-life of the radiolabeled material in

the blood was about two days. In other studies of healthy adult volunteers receiving a single oral dose of 10 mg/kg, the chemical analysis of succimer and its metabolites in the urine showed that succimer was rapidly and extensively metabolized. Approximately 25% of the administered dose was excreted in the urine with the peak blood level and urinary excretion occurring between two and four hours. Of the total amount of drug eliminated in the urine, approximately 90% was eliminated in altered form as mixed succimer-cysteine disulphides; the remaining 10% was eliminated unchanged (Miller, 1998).

The excretion of altered succimer reached a peak between 2 and 4 hr after administration. There were small but significant increases in the excretion of zinc, copper, and lead after succimer. The chelating agent did not influence the urinary excretion of 27 other metals and elements (Walker *et al*., 1992). Dimercapto succinic acid was administered to mice intravenously; the mice were frozen by immersion in dry ice/hexane at 6 and 20 min and 1, 3, 9, and 24 hr after injection. The frozen mice were sectioned and processed for whole-body autoradiography for soluble substances.

The radioactivity was highly localized in extracellular fluids such as the sc, intrapleural, ip, and periosteal spaces. There was a pronounced accumulation in the periosteal fluid above that in other fluids during the first hour after injection. Most of the radioactivity was eliminated by the kidney and liver. Pretreatment of a mouse with $HgCl_2$ subcutaneously an hour before. DMSA produced an increase in radioactivity in the liver and decrease in lung. A high concentration of radioactivity was seen at the subcutaneous site of injection of the $HgCl_2$. The results are interpreted to indicate that most of the DMSA is in the extracellular space but that it can cross cellular membranes to some extent. The pronounced accumulation in periosteal fluid may be an interaction of DMSA

with Ca^{2+} in this space. No tissue had a pronounced retention of the compound, but lung retained more than most other tissues (Kreppel *et al*., 1995).

2.5.2 Mechanism of Action

Succimer is a lead chelator, it forms water soluble chelates and, consequently, increases the urinary excretion of lead. DMSA chelates by coordination of one sulphur and one oxygen atom with Pb. Solubility of the lead chelates depends on the ionization of the non-coordinated thiol and carboxylic acid groups. Bimane derivatization, HPLC, and fluorescence, as well as gas chromatography can be used for analysis of DMSA in biological fluids. The acid dissociation curve. Succimer is a heavy metal chelator. It binds with high specificity to ions of lead in the blood to form a water-soluble complex that is subsequently excreted by the kidneys. Succimer can also chelate mercury, cadmium, and arsenic in this manner (Balnusa *et al*., 2005).

CHAPTER THREE

3.0 Materials and Methods

3.1 Plant Collection, Identification and Preparation of the Extract

Fresh Seed of *Vigna unguiculata* was purchased from Samaru Market Zaria. It was identified in the Department of Biological Sciences A. B.U. Zaria by U.S. Gallah, (herbarium officer and a taxonomist) with a Voucher No. 463 on 10/7/2013. And it was prepared into aqueous extract in the Department of Pharmacognosy, A.B.U. Zaria, Nigeria, using cold maceration method. Five hundred grammes (500g) of the seed was grinded, soaked in water (solvent) for 24 hours, the solution was then filtered using muslin cloth, and the filtrate was placed under hot water bath set at 60^0c to evaporate the water and form the extract. Five hundred (500) mg of the extract was dissolved in 5ml of distilled water to obtain 100 mg/ml stock concentration corresponding to 1000 mg/kg dose. Serial dilution was made from the stock solution to obtain 500 and 250 mg/kg doses of the extract respectively.

3.2 Animals and their Management

Fifty (50) apparently healthy mice (18-22g) aged 6-8 weeks were purchased from the animal house of the Faculty of Pharmaceutical Sciences Ahmad Bello University (A.B.U.) Zaria. The mice were housed in cages containing dust-free sawdust bedding. They were fed with pellets made from grower's mash and water *ad libitum*.

The animals were divided into two main groups of 25 mice each of the two memory assessment paradigms; each paradigm had 5 mice allotted to 5 subgroups respectively as follows:

Group I: Administered distilled water (10ml/kg) orally

Group II: Administered Succimmer 20 mg/kg orally

Group III: Administered *Vigna unguiculata* extract at 250mg/kg orally

Group VI: Administered *Vigna unguiculata* extract at 500mg/kg orally

Group V: Administered *Vigna unguiculata* extract at 1000mg/kg orally

All treatments were administered to the animals one hour before inducing the acute lead toxicity by administering 120 mg/kg of lead acetate orally using canular in the first day (Manal *et al.*,2013; Magaji *et al*., 2014a).

3.3 Drugs

Succimer (200mg) was obtained from the office of Zamfara Division of Medicins Sans Frontieres (MSF) Zamfara, Nigeria. Lead Acetate of analytical grade product number: 27929, BDH Laboratory Chemicals Limited, Poole, England was used at the dose of 120 mg/kg body weight (Manal *et al.*, 2013; Magaji *et al*., 2014b).

3.4 Acute Toxicity Study

3.4.1 Median lethal dose (LD_{50}) of *Vigna unguiculata*

The LD_{50} of the aqueous extract of *Vigna unguiculata* was determining using the method described by Lorke (1983) in mice. This test was carried out in two phases in the first phase, three are (3) groups each containing 3 animals, the animals were treated with *Vigna unguiculata* extract at the doses 10, 100 and 1000 mg/kg body weight per oral and observed for signs of toxicity and death for 48 hours. In the second phase, four (4) groups each containing one mouse were administered with four more specific doses of the extract

based on the result of the first phase (1200, 1600, 2900 and 5000 mg/kg respectively). The LD_{50} value was determined by calculating the geometric mean of the lowest dose that cause death and the highest dose at which all the animals survived.

$$LD_{50} = \sqrt{MLD \times MTD}$$

Where LD_{50} = median lethal dose

MLD = minimum lethal dose

MTD = maximum tolerated dose

3.5 Neurobehavioural Assays

3.5.1 Assessment of spatial memory using Morris water maze (Wet-Maze)

The Morris water maze was invented by Richard G.M. Morris in 1981 (Morris, 1981). This device is one of the widely used models in the study of spatial learning and memory in rodents (Morris *et al*., 1981; Sharma *et al* 2010). Morris water maze consists of a circular pool. The pool was about 75-150 cm in diameter and 20 cm in depth filled with water. A circular platform (10-12 cm in diameter) made from Polyvinylchloride was submerged below the surface of the water 0.5 cm depth from the surface. The water in the pool was filled and drained daily. The experimenter and cues in the test room remained in the same place throughout the duration of the experiment. They served as visual cues to the mice and helped them to navigate themselves to the hidden platform (Gary, 2013).

Testing in the Morris water maze lasted for three days, with two days of acquisition training, and one day of probe trial. The pool was divided into four quadrants (1, 2, 3, and 4) in a clockwise fashion, which served as a starting point for the trials per day. In addition, the platform was fixed in the fourth quadrant. The acquisition training began by

placing the mouse into the water at quadrant 1, facing the side of the pool, and a stopwatch was switched on. The mouse was permitted to explore the pool and search for the hidden platform for two minutes. When the animal locates the platform, the stopwatch was switched off. When it failed to find the platform during the two minutes, it was gently guided to the platform and allowed to stay on the platform for ten seconds to explore its surrounding. The mouse was removed and placed in its holding cage with tissue paper bedding that was replaced when it became completely wet (The Morris water maze, 2006). It allows the mice to get dry quickly. The second mouse was then placed in the pool and follow the same procedure as that of the first mouse.

Each mouse completed four trials per day (each quadrant being the starting point for each trial) with an inter trial interval of 8 min giving a total of eight trials during the acquisition training (2 days). In the probe trial, the platform was removed and each mouse was allowed to explore the platform for 60s (Morris, 1981). Each mouse was placed in quadrant one to avoid cue bias and allowed to explore the pool. During the acquisition phase; swim latency (the time it took the mouse to swim and mount on the hidden platform), frequencies of thigmotaxis behaviour were recorded. During the probe trial, the time spent in each quadrant and number of times the mouse crosses the former platform location in the fourth quadrant was also recorded which served as a measure of memory (Gary, 2013).

Figure 3.1: Morris water maze (Snapped by the candidate 09/02/2015)

3.5.2 Assessment of spatial memory using barnes maze (Dry-Maze)

The Barnes maze was developed by Carol Barnes to avoid the stress, induced by swimming in Morris water maze (Barnes, 1979). It was later adopted for mice (Pompl *et al*, 1999). The Barnes maze consists of a circular platform (69 cm in diameter) painted with white non-toxic oil based paint. Forty circular holes measuring (4.45 cm) in diameter were evenly spaced around the perimeter of the circular platform. The maze was elevated 48.4 to 50 cm above the floor by a stand. A target box (13 cm x 29 cm x 14 cm) made of plywood containing a small ramp was placed under a selected escape hole. An electric

bulb of 200 watts was hung 76 cm above the maze platform to provide aversive bright light. Three visual cues of various geometric shapes were pasted in semi random locations on the inside wall of the test room. These served as extra mazes (Walker, 2010).

Behavioural testing consists of acquisition training for two days, which was made up of one trial per day. In this acquisition session mice were placed on the centre of the platform and the light bulb was switched on. This triggers the escape behaviour and the mouse was allowed to explore the maze for five minutes to locate the escape hole and escape from the maze. Once the mouse was inside the escape hole. The light was switched off (Ingram *et al*., 1994; Moscovitch *etal*., 2005). When the mouse failed to locate and enter the escape hole within 5 minutes it was gently guided into the escape hole.

Immediately after the mouse enters the target box, the mouse was allowed to stay in the target box for 10 seconds. On day three, probe trial (no escape box present) during which the maze was divided into four quadrants which was used to evaluate memory within a duration of two minutes. During the acquisition session latency (time it took for the mouse to find and enter the escape platform) and total errors (head dips into incorrect holes) were recorded. During the probe trial, entries into the correct quadrants, number of errors (head dips into incorrect hole) and number of targets (head dips into correct hole) were recorded to access memory (Brown *et al*., 2013).

Figure 3.2: Barnes Maze (Snapped by the candidate 09/02/2015)

3.6 Statistical Analyses

Data obtained from the study was expressed as mean ± standard error of the mean. The parameters observed were measured using factorial-repeated measure ANOVA with bonferroni post hoc test. Also parameters for the prove trials of both Morris water and Barnes mazes were measured using one-way ANOVA and Kruskall Wallis H-test respectively. Values of $p \leq 0.05$ were taken to imply statistical significance.

CHAPTER FOUR

4.0 Results

4.1 The Extract Yield and LD_{50}

4.1.1 Yield of the extract

The yield of the aquous extract of cowpea (*Vigna unguiculata*) was formed from the 500g seed. it was 175 grammes (g) after weighing, and the percentage yield was calculated as follows:

$$\frac{175}{500} \times \frac{n}{100} = \frac{17500}{500n}$$

$$n = \frac{175}{5} = 35\%$$

4.1.2 Acute Toxicity Study of Aqueous Extract of Cowpea (*Vigna unguiculata*) seed in mice

No sign of toxicity was observed in all the animals in the groups during the two phases of the toxicity study. The animals were active and alive during the study. The oral median lethal dose (LD_{50}) value in the mice was estimated to be greater than 5000mg/kg (table 4.1).

4.2 Assessment of Spatial Memory Using Barnes Maze (Dry-Maze)

4.2.1 Time taken (latency) to find escape hole

During the two day training session, there was no difference in performance (latency to find the escape hole) on the *Vigna unguiculata* treated groups when compared to control in both day one and two, $F(4,20) = 0.857$, $P = 0.506$. the latency (time taken to find the escape hole in seconds) for day one was, 144.40 ± 45.1 for the control group and 247.40 ± 50.6, 202.80 ± 44.3 and 221.80 ± 48.3 for 250, 500, and 1000 mg/kg *Vigna unguiculata* treated groups respectively. The latency (time taken to find escape hole in seconds, for

day two was 121.60 ± 49.6 for control group and 208.80 ± 57.5, 192.80 ± 50.6 and 250.00 ± 32.6 for 250, 500, 1000 mg/kg *Vigna unguiculata* treated groups respectively (Table 4.2).

Table 4.1: Results of acute toxicity study showing the different doses of aqueous extract of cowpea (*Vigna unguiculata)* seed administered orally to mice, number of death and the percentage mortality

Phase I

Group (n=3)	Dose (mg/kg)	Number of Death	% Mortality
1	10	0/1	0
2	100	0/1	0
3	1000	0/1	0

Phase II

Group (n=1)	Dose (mg/kg)	Number of Death	% Mortality
1	1200	0/1	0
2	1600	0/1	0
3	2900	0/1	0
4	5000	0/1	0

Table 4.2: Effect of cowpea (*Vigna unguiculata*) on learning in mice during 2 – day training session in Barnes maze

Groups	**Latency (s) Mean ± SEM**	
	Day 1	**Day 2**
Control distilled water 10ml/kg	144.40 ± 45.1	121.60 ± 49.6
Succimer 20mg/kg	190.40 ± 48.3	200.20±43.4
Vigna unguiculata 250mg/kg	247.40 ± 50.6	208.80 ± 57.5
Vigna Unigiculata 500mg/kg	202.80 ± 44.3	192.80 ±50.6
Vigna unguiculata 1000mg/kg	221.80 ± 48.3	250.00±32.6

Mean in the various groups are not significantly different (P>0.05)

4.2.2 Number of incorrect (error) head dips

During the two day training session there was no difference in errors (incorrect head dips) on *Vigna unguiculata* treated groups when compared to control in both day one and two, F (4. 20) = 1.599, P = 0.214, the number of incorrect (error) head dips for day one was 12.00 ± 3.0 for the control group and 08.20 ± 4.2, 0.8.40 ± 4.2 and 15.80 ± 6.1 for 250, 500, 1000 mg/kg *Vigna unguiculata* treated groups respectively. The number of incorrect (error) head dips for day two was 18.80 ± 6.2 for control group and 06.60 ± 4.3, 17.40 ± 5.7 and 19.60 ± 4.8 for 250. 500 and 1000 mg/kg *Vigna unguiculata* treated groups respectively (Table 4.3).

4.2.3 Number of correct head dips

On day three which was the probe trial session, there was no significant difference in performance (as a frequency of head dips into the correct hole) in the *Vigna unguiculata* treated groups when compared to control f = (4,20) = 1. 130, p = 0.371. The frequency of correct head dip was 3.40 ± 1.4 for the control group and 2.00 ± 0.8, 0.00 ± 0.0 and 2.80 ± 1.8 for 250, 500 and 1000 mg/kg *Vigna unguiculata* treated groups respectively.

4.2.4 Time spent in each quadrant

During the probe trial session, both groups spent more time in Quadrant three (3) which was adjacent to the quadrant that contained the escape hole f = (4, 20) = 0.391 p = 0.812, but there was no significant difference in time spent in all the Quadrants between the *Vigna unguiculata* treated groups when compared to that of the control group (p > 0.05) .

Table 4.3: Effect of cowpea (*Vigna unguiculata)* on learning in mice during 2 – day training session in Barnes maze

Groups	**Number of incorrect head dips Mean ± SEM**	
	Day 1	**Day 2**
Control distilled water 10ml/kg	12.00 ± 3.0	18.80 ± 6.2
Succimer 20mg mg/kg	22.60±6.9	27.00±10.2
Vigna unguiculata 250mg/kg	08.20±4.2	06.60±4.3
Vigna unguiculata 500mg/kg	08.40±4.2	17.40±5.7
Vigna unguiculata 1000mg/kg	15.80±6.1	19.60±4.8

Mean in the various groups are not significantly different (P>0.05)

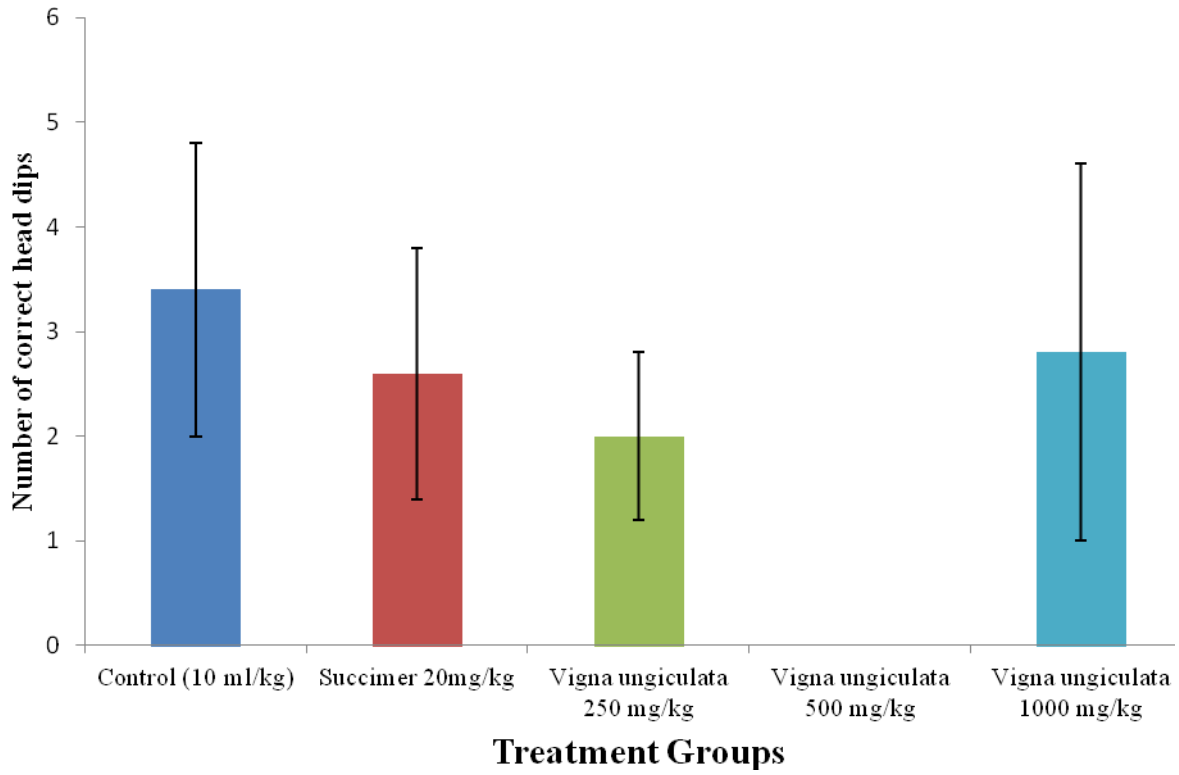

Figure 4.1: Effect of *Vigna unguiculata* on Visuospatial Memory During 1-day Probe-Trial Session in Barnes Maze

Mean in the various groups are not significantly different (P>0.05)

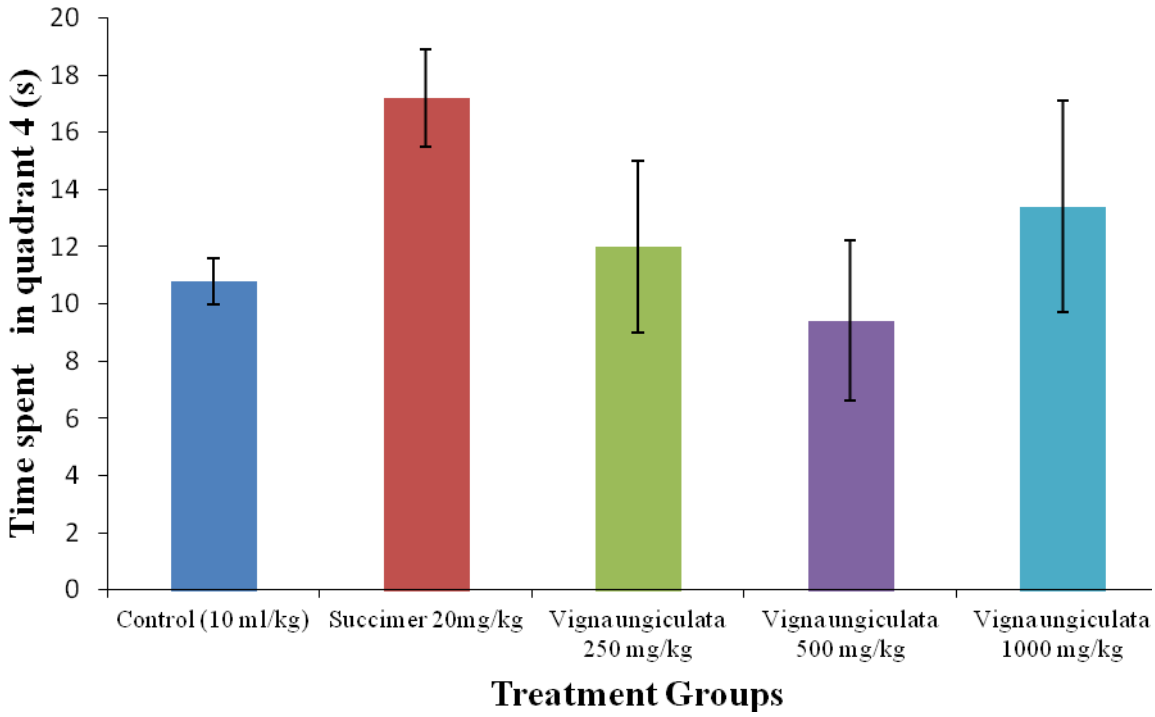

Figure 4.2: Effect of *Vigna unguiculata* on Visuospatial Memory During 1-day Probe-Trial Session in Morris Water Maze

Mean in the various groups are not significantly different (P>0.05)

4.3 Assessment of Spatial Memory Using Morris Water Maze (Wet- Maze)

4.3.1 Time taken (latency) to find escape platform

During the two day training session, there was no significant difference in performance hr(latency) to find escape platform) on the *Vigna unguiculata* treated groups when compared to the control f = (4.20) = 3.451, p = 0.0 27. The latency, time taken to find escape platform in seconds, for day one was 320.60 ± 51.7 for the control group and 343.80 ± 44.5, 285.60 +38.7, and 433.00+17.8 for 250, 500 and 1000mg/kg. *Vigna unguiculata* treated groups respectively. However, during the second day of the training session, performance improved across the groups when compared to day one ($p < 0.05$), the values being 433.00 ± 17.85 and 387.20 ± 37.4 for day one and two respectively. But there was no difference in performance between the groups. ($P > 0.05$) the latency (time taken to find the escape platform in seconds) for day two was 236.40 ± 58.7 for the control group and 313.20 ± 32.4, 278.00 ± 39.2 and 387.20 ± 37.4 for 250, 500 and 1000 mg/kg *Vigna unguiculata* treated groups respectively.

4.3.2 Frequency of platform crossing

In day three, which was the probe trial session, there was no significant difference in performance (as a frequency of former platform location crossing) when compared to control. F = (4,20) = 0.976, p = 0.442 the frequency of platform crossing was 2.20 ± 0.8 for the control group and 3.00 ± 0.3, 2.00 ± 0.4 and 1.40 ± 0.5 for 250, 500 and 1000 mg/kg *Vigna unguiculata* treated groups respectively.

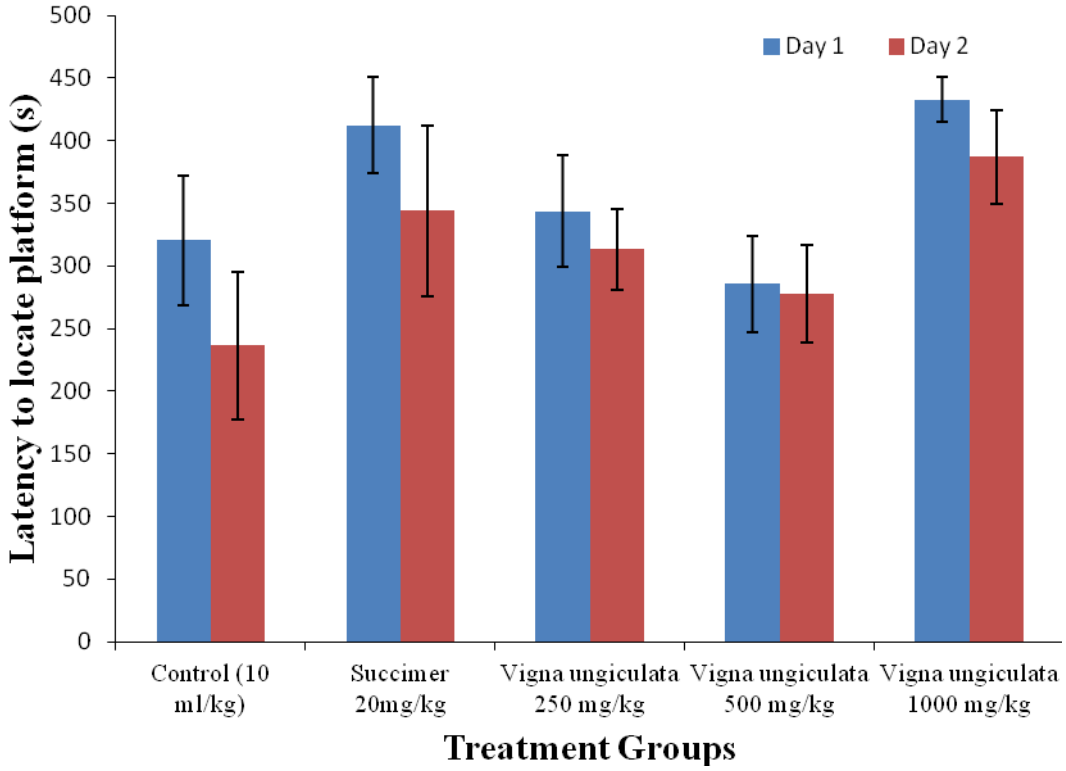

Figure 4.3: Effect of *Vigna unguiculata* on Visuospatial Learning During 2-day Acquisition Session in Morris Water Maze

Mean in the various groups are not significantly different (P>0.05)

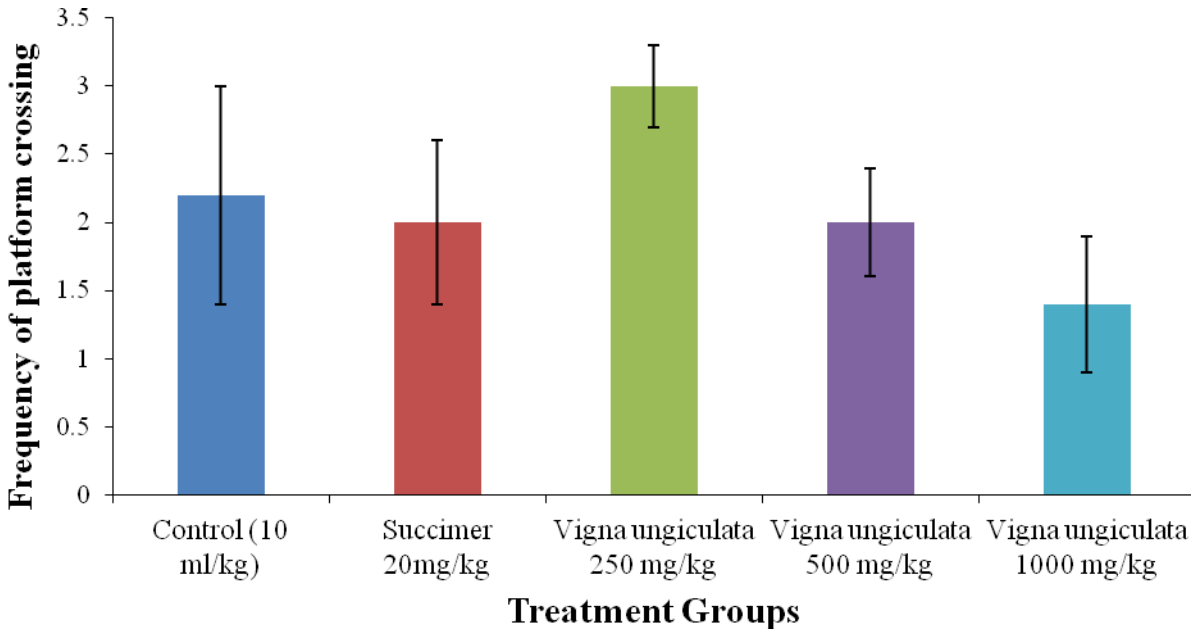

Figure 4.4: Effect of *Vigna unguiculata* on Visuospatial Memory during 1-day Probe-Trial Session in Morris Water Maze

Mean in the various groups are not significantly different (P>0.05)

4.3.3 Number of quadrant crossing

During the probe trial session, both groups crossed the quadrant where the escape platform was located during the training session, but there was no significant difference during the training between the Vigna Unciculata treated groups when compared to the control group. F = (4,20) = 0.739 P = 0.576 (Table 4.4).

Table 4.4: Effect of cowpea (*Vigna unguiculata)* on memory in mice during probe trial in Morris water maze

	Mean ± SEM			
Groups	**Quad 1**	**Quad 2**	**Quad 3**	**Quad 4**
Control distilled water 10mg/kg	19.80±2.6	9.60±1.5	9.60±0.9	10.80±0.8
Succimer 20mg/kg	12.20± 3.7	11.60±1.7	17.40±5.3	17.20±1.7
Vigna unguiculata 250mg/kg	21.00± 4.1	11.60±1.5	12.00±1.5	12.00±3.0
Vigna unguiculata 500mg/kg	18.60±1.8	13.60±3.4	17.00±3.7	9.40±2.8
Vigna unguiculata 1000mg/kg	15.20±1.9	15.80±1.8	12.60±1.2	13.40±3.7

Mean in the various groups are not significantly different (P>0.05)

CHAPTER FIVE

5.0 Discussion

This study was designed to study the effect of aqueous extract of cowpea *(Vigna unguiculata.* (L.) Walp*)* on visuospatial learning and memory in acute lead induced neurotoxicity in mice in Morris water and Barnes mazes paradigms. Using three different dosages of *Vigna unguiculata* (250,500 and 1000 mg/kg) respectively. Similar to the Morris Water Maze, the Barnes Maze allows for evaluation of spatial reference memory and learning but without inducing despair and anxiety that commonly are seen in the water maze in the form of floating and thigmotaxis (Roariguez *et al.*, 2013). At the same time, compared to the Morris water maze, learning in the Barnes maze was slow, that may be due to explorative behaviour of mice and the modest nature of the motivating stimuli. Notwithstanding these differences between the two sets, many studies using mice have utilized the Barnes maze successfully to assess spatial memory (Sun and Alkon, 2004). The main advantage of the Barnes maze is that being a dry-land maze, it does not induce stress as in the Morris water maze that involves swimming. In addition, like the Morris water maze, it allows evaluation of learning working memory and spatial reference memory (Sunyer *et al*., 2007).

It is particularly suitable for mice since these animals exhibit a lower performance on the Morris water maze. Various studies in mice have successfully utilized the Barnes maze to assess spatial memory (Barrett *et al*., 2010). One disadvantage of the Barnes maze is that learning can be very slow or even absent in some cases. This can be explained by the lack of stressful stimuli, thereby producing more exploratory behavior than escape responses in animals that are not sufficiently motivated to escape (Sunyer *et al.*, 2007). This can result in an increase in the number of errors due to further exploration of the maze although the mouse learned the association between the spatial cues and the escape

location previously. Harrison *et al.* (2006) proposed a solution to this extra-exploratory behavior by calculating latency, path length and number of errors to the first encounter of the escape hole, called primary latency, primary path length and primary errors, respectively. Another disadvantage of the Barnes maze is that it can also stimulate non-spatial strategies like a serial strategy that can then affect performance. If the maze is not cleaned appropriately, the animals can use "aromatic cues" to solve the maze. One of the studies using multiple mazes to study spatial memory demonstrated that mice committed considerably more errors than expected by random and this could be due exploratory behavior of mice and may not indicate impairment in spatial memory (Lewejohann *et al.*, 2004).

The work of Rojana Thammanee *et al.*, (2013) which corroborate with the findings of this work has shown that Acquisition in the training phase typically is assessed as a decrease in latency and in the number of erroneous holes searched (HS) before finding the target hole, though not necessarily going into the escape cage. Entering the escape cage through the target hole often is not used as an end – point. Because, unlike the water maze the environment is not aversive enough to require immediate escape and mice may continue to explore after having identified the target hole. Other measures such as pat length or speed, also may be used. Long term memory was evaluated in the probe phase, which occurs following training and a delay by removing the escape cage and observing search behaviour for a set amount of time. Practically, this was measured as the time spent and holes searched (HS) in the target quadrant. A mouse with intact memory is expected to spend more than 25% (Chance level of their time in the target quadrant (Rojana Thammanee *et al.*, 2013).

The study demonstrated that after inducing acute lead toxicity, the administration of *Vigna unguiculata* extract did not cause any significant change in learning and memory performance when compared to control in Barnes maze. This might be attributed to the aversive nature, less stressful and high exploratory behaviour of mice which make them commit more errors in target hole search as it conforms to the findings of Harrisson *et al., (*2009) who showed that Barnes maze is less stressful when compared to Morris water maze.

Also Rojanthamanee *et al., (*2013) showed that rodents tend to commit more errors as training increases due to less aversive nature of Barnes maze not merely an indication of spatial memory impairment. This also conforms with the work of Aida *et al*., (2013). Who showed that much of the training after trial form was redundant and leading to elimination of cognitive difference between groups. Furthermore, based on the findings of this result on Morris Water Maze, it has shown that *Vigna unguiculata* administration in mice did not significantly affect or improve learning during the acquisition session when compared with the control. This corroborate with the work of Moscovitch, *et al*., (2005). Which showed that the central nervour System damage caused by heavy metals such as lead cannot be reversed by succimer leading to impairment in neuro psychological function.

However, this finding is contrary to the work of Hosseini *et al.,* (2011) who showed that rodents progressively learn to find the Hidden platform in the Morris water paradigm assessment. This was observed by significant reduction of escape latency in the four day trained animals compared with one day trained animals. The animal learns to locate the platform hidden under the water from four different starting points. Over a number of trials animals learn the location of the hidden platform based on distal cues and with time

the latency to locate the platform decreases. The strength of learning was tested afterwards by a probe trial in which the hidden platform is removed and the amount of time spent in the former region of platform is measured. In this maze, mice can use three different strategies. to locate the escape platform: a praxic strategy, when the animal learns the sequence of movements needed to reach the platform, a taxic strategy, when the animal uses cues or visual proximal guides to reach the platform, or a spatial strategy, when the animal reaches the target using information about the spatial location of the platform (Dudchenko, 2004).

The Morris water maze permits the accurate and reproducible study of reference memory, spatial working memory and learning. The Morris water maze has been shown to be highly sensitive in the assessment of damage to the hippocampus. Furthermore, aged animals exhibit consistent impairment in learning the location of the escape platform (Pan *et al*., 2008). Recently, the assessment of age-related decline in memory in mice was conducted using the Morris water maze to better elucidate the mechanisms involved (Zhao *et al.*, 2009).

Exposure to lead is unavoidable as it occurs through many routes including contaminated air, water, soil, food, and consumer products. The safe threshold for lead exposure has not been identified, as there is no accurate amount specified for lead toxicity (Rossi, 2008). Lead exists in different forms and is a constituent of a variety of organic compounds, which have the ability of direct penetration of the skin, respiratory tract, and it has a predominant effect on central nervous system (Kosnett *et al*., 2007). Consequently, it constitutes a significant public health problem, despite efforts to reduce its level in the ecosystem (Rojas-castaneda *et al.*, 2011).

Neurological damage induced by lead toxicity is a well-known condition that has been reported to be a base for several disorders like mental retardation; behavioural problems; nerve damage; Alzheimer's disease; Parkinson's disease; and possibly schizophrenia (Liu *et al.,* 2010). The ability of lead to pass through the blood-brain barrier is due in large part to its ability to substitute for calcium ions. At the molecular level, lead interferes with the regulatory action of calcium on cell functions and disrupts many intracellular biological activities (Sanders *et al.,* 2009).

Acute high-dose exposure to lead is not the only source of lead-based neurotoxicity. Acute low-dose exposure also appears to produce measurable, if less dramatic, effects on nervous system function. Epidemiological studies have failed to find evidence of a threshold for neurological effects; recent large-scale, prospective studies suggest that blood lead levels below 10 µg/dl significantly worsen intellectual functioning in children and that the strength of association is stronger at the low range of exposure. The presence of lead in the human body causes damage to the nervous system through several mechanisms. Direct effects on the nervous system may be classified as either morphological or pharmacological. Morphological effects alter the development of the nervous system, particularly from the prenatal period through childhood. Such effects include disruption of key molecules during neuronal migration and differentiation interference with synapse formation, mediated by a reduction in neuronal sialic acid production and premature differentiation of glial cells (Surkan *et al*.,2007).

Pharmacological effects result from the action of lead as a pharmacological agent in the CNS. Lead substitutes for calcium and, to a lesser extent, zinc and inappropriately triggers processes reliant on calmodulin. Lead also interferes with neurotransmitter release,

disrupting the function of gabaergic, dopaminergic, and cholinergic systems as well as inhibiting NMDA-ion channels during the neonatal period. *In vitro* studies have shown that lead activates protein kinase C in capillary cells and inhibits Na+/K+-ATPase in the cell membrane, interfering with energy metabolism. Within the cell, lead appears to interfere with calcium release from the mitochondria resulting in formation of reactive oxygen species, speeding mitochondrial self-destruction through formation of the permeability transition pore, and priming activation of programmed cell death processes. Indirect effects on the nervous system result from interference with other body systems that support nervous system function. Lead exposure has been found to increase risk of numerous conditions that may have adverse effects on nervous system function, including hypertension, impaired renal function, impaired thyroid function, vitamin D deficiency, and preterm birth There has been some debate as to whether lead exposure affects peripheral nerve conduction velocity (Lanphear *et al*., 2005).

The most severe neurological effect of lead exposure is lead encephalopathy a response to very high doses of lead that results in development of irritability, headache, mental dullness and attention difficulty, memory loss, tremor, and hallucinations within weeks of exposure. Symptoms abruptly worsen to paralysis, convulsions, delirium, coma, or death. Children may develop lead encephalopathy at lower doses of lead than adults. Post-mortem pathological findings include oedema, capillary disruption, proliferation of glia, and diffuse anoxic injury (Bellinger *et al*., 2003).

The succimmer group also which was administered as a standard drug serving as a therapy for lead toxicity presented with a result which was not statistically significant, but this might be as a result of the damage caused by lead in the brain areas involved in

learning and memory and inability of the drug to reverse the brain damage and improve neuro psychological functions (Dietrich *et al*, 2004; Muran, 2006). Similarly, this corroborate with the works of Bridges *et al.,* (2009) and Ruha *et al.,* (2009) whom has shown that succimmer does increase the urinary excretion of heavy metals in rodents and in Humans. They further explained that Both cessation of lead exposure and chelation therapy effectively lower blood lead levels, reducing pharmacological effects of lead, but they show no therapeutic benefit against neuro morphological changes.

However, the work of Lindgren *et al*., (2003) is contrary to these findings which have shown that effects of acute lead exposure on neurocognitive performance do show some evidence of reversibility. Where removal of exposure and subsequent reduction of blood lead levels have been associated with improvements in verbal memory performance, visual memory performance, gross motor speed and visual discrimination speed. Moreover, in line with this result on the performance of mice in both Morris water and Barnes mazes. The work of Rogan *et al*., (2001) and Chuang *et al*., (2005) has shown that there is no compelling evidence for effective neuro rehabilitation deficits due to lead exposure.

Chelation therapy is the only available medical counter-measure to treat lead/metal toxicity. The thiol and amino carboxylic acid metal chelators have been used for the prevention as well as therapy. The goal of chelation is to enhance lead elimination before irreversible changes occur. Calcium disodium EDTA ($CaNa_2EDTA$) and 2,3-dimercaprol have been used conventionally for the treatment of lead intoxication; however, the clinical use of these chelating agents has been under debate (Swaran *et al*., 2007).

However, the water-soluble analogue of meso-2,3-dimercaptosuccinic acid (DMSA), was found to be an effective chelator without adverse health effects. Clinical human and animal studies have shown that Succimer (trade name of DMSA) reduces lead levels in blood and other soft tissues. However, its hydrophilic properties have hampered its effectiveness in removing lead from brain and skeleton. Recently, a number of mono and diesters of DMSA were synthesized and tested for metal intoxication, especially against lead cadmium mercury and gallium arsenide. Among these monoesters, Mono iso Amyl Dimercapto Succinic acid (MiADMSA). has been proved to be effective in gaining intracellular access through various endogenous ligands, thereby having an added advantage over DMSA. It can be inferred from the result of this work that combined administration of two structurally different chelators might be a better treatment protocol than monotherapy. However, it is not known whether this treatment protocol (i.e., combined administration of two chelating agents) is equally effective in the recovery of altered neurological disorders (Swaran *et al*., 2007).

So Vigna unguiculata might have a significant effect on learning and memory due to its nutritious content, and other studied health benefits. But there is need to ascertain its chelating property, and ability to reverse brain damage so as to improve neuropsychological functions. But there have been a limited number of published studies in animal models that have addressed this issue (Ware *et al.*, 2004) hoping that this work will serve as a stepping stone.

CHAPTER SIX

6.0 Summary, Conclusion and Recommendations

6.1 Summary

Learning is the acquisition and storage of information as a consequence of experience. It is the act of acquiring new, or modifying and reinforcing, existing knowledge, behaviours, skills, values, or preferences and may involve synthesizing different types of information (Daniel *et al*., 2011). Memory is relatively permanent storage form of the learned information. It is the ability of an individual to record sensory stimuli, events, and other information. It is also the ability to retain them over short or long periods of time and recall the same at a later date when needed (Jarrad and Gina, 2013). The cowpea *(Vigna unguiculata)* is a widely cultivated legume in Africa, Asia, Central and South America where it is an important constituent of several dishes prepared by soaking, frying, cooking, fermentation, or combinations of them (Philips et al., 2003). Cowpea has also enormous potential in the development of a variety of new food products such as snacks, weaning foods or fortified traditional foods. Furthermore, cowpea seeds exhibit beneficial health effects related to its antioxidant, hypoglycaemic, hypolipidaemic, and antihypertensive properties (Doblado *et al*., 2005; Frota *et al*., 2008; Guang *et al*., 2012; Xiong *et al*., 2013) which are derived from the specific characteristics of their proteins and from their content of dietary fibre, polyphenols, phytic acid or α-galactoside oligosaccharides (Trinidad *et al*.,2010).

6.2 Recommendations

Based on the result of this study, the following recommendations were made;

i. Further neurobehavioral tests should be carried out using other models to evaluate the effect of cowpea on learning and memory.

ii. Co-administration of cowpea and succimmer might give a better result due to synergistic effect of drugs.

iii. A research with neuro-enhancers to test the effect on lead neuro-impairment.

iv. Clinical studies are also needed to determine if chelation theraphy lowers or improve blood lead level using blood samples, M.R.I., histological slides, and so on in both human subjects and animal studies.

6.3 Conclusion

Vigna unguiculata does not statistically improve learning and memory in acute lead induced neurotoxicity in mice using Morris water and Barnes Mazes.

6.4 Contribution to Knowledge

i. Administration of aqueous extract of Cowpea at the dosages of 250, 500 and 1000 mg/kg does not statistically improve cognition in acute lead induced neurotoxicity in mice in Morris water Maze at ($F(4,20) = 0.976$, $P = 0.442$

ii. Administration of aqueous extract of Cowpea at the dosages of 250, 500 and 1000 mg/kg does not statistically improve cognition in acute lead induced neurotoxicity in mice in Barnes Maze at ($F(4,20) = 0.857$, $P = 0.506$

iii. Chelation therapy may not improve neuronal damage in acute lead induced neurotoxicity in mice.

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APPENDIX I

Effect of beans (*Vigna unguiculata)* on memory in mice during probe trial in Barnes maze

Groups	**Number of correct head dips** **Mean ± SEM**
Control distilled water 10ml/kg	3.40 ± 1.4
Succimer 20mg/kg	2.60± 1.2
Vigna unguiculata 250mg/kg	2.00 ± 0.8
Vigna unguiculata 500mg/kg	0.00
Vigna unguiculata 1000mg/kg	2.80± 1.8

Mean in the various groups are not significantly different (P>0.05)

Effect of beans (*Vigna unguiculata)* on memory in mice during probe trial in Barnes maze

Groups	**Time spent in each Quadrant** **Mean ± SEM**			
	Quad 1	**Quad 2**	**Quad 3**	**Quad 4**
Control distilled water 10ml/kg	20.60±6.7	16.00±3.5	29.80±6.1	25.40±4.6
Succimer 20mg/kg	25.60±7.1	19.60±1.7	25.00±8.1	25.40±5.5
Vigna unguiculata 250mg/kg	25.60±11.3	9.20±5.1	14.20±6.1	33.80±9.9
Vigna unguiculata 500mg/kg	5.00±2.09	26.20±8.5	26.20±5.4	35.00±15.3
Vigna unguiculata 1000mg/kg	35.40± 15.9	22.00±8.4	28.60±11.1	07.00±2.0

Mean in the various groups are not significantly different (P>0.05)

Effect of beans (*Vigna unguiculata*) on learning in mice during 2 – day training session in Morris water maze

Groups	**Latency to locate plat form (s)** **Mean ± SEM**	
	Day 1	**Day 2**
Control distilled water 10ml/kg	320.60±51.7	236.40±58.7
Succimer 20 mg/kg	412.20±38.3	344.00±67.9
Vigna unguiculata 250mg/kg	343.80±44.5	313.20±32.4
Vigna unguiculata 500mg/kg	285.60±38.7	278.00±39.2
Vigna unguiculata 1000mg/kg	433.00±17.8	387.20±37.4

Mean in the various groups are not significantly different (P>0.05)

Effect of beans (*Vigna unguiculata*) on memory in mice during probe trial in Morris Water maze

Groups	**Number of plat form crossing** **Mean ± SEM**
Control distilled water 10mg/kg	2.20 ± 0.8
Succimer 20mg/kg	2.00 ± 0.6
Vigna unguiculata 250mg/kg	3.00 ± 0.3
Vigna unguiculata 500mg/kg	2.00 ± 0.4
Vigna unguiculata 1000mg/kg	1.40 ± 0.5

Mean in the various groups are not significantly different (P>0.05)

APPENDIX II

Phase IA

Weight (kg)	Dose (mg/kg)	Number of Death	Mortality
0.18	100	0/3	0
0.22	100	0/3	0
0.19	100	0/3	0

Total: 0/3 mortality

No death and sign of toxicity observed in the 2nd phase of the toxicity study

Phase IB

Weight (kg)	Dose (mg/kg)	Number of Death	Mortality
0.20	100	0/3	0
0.18	100	0/3	0
0.18	100	0/3	0

Total: 0/3 mortality

No death and sign of toxicity observed in the 2nd phase of the toxicity study

Phase IC

Weight (kg)	Dose (mg/kg)	Number of Death	Mortality
0.20	1000	0/3	0
0.19	1000	0/3	0
0.18	1000	0/3	0

Total: 0/3 mortality

No death and sign of toxicity observed in the 2nd phase of the toxicity study

Phase II

Weight (kg)	Dose (mg/kg)	Number of Death	% Mortality
0.20	1200	0/1	0
0.18	1600	0/1	0
0.19	2900	0/1	0
0.18	5000	0/1	0

Total= 0/4 mortality

No death and sign of toxicity observed in the 2nd phase of the toxicity study