

**AMELIORATIVE EFFECT OF VITAMIN C ON ANXIETY AND
MOTOR COORDINATION IN LEAD-INDUCED TOXICITY
IN WISTAR RATS**

By

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**HUMAN PHYSIOLOGY
AHMADU BELLOUNIVERSITY ZARIA,
NIGERIA**

JANUARY, 2013

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AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA**

JANUARY, 2013

DECLARATION

I declare that the work in this thesis entitled Ameliorative effect of Vitamin C on anxiety and motor coordination in sub acute Lead Toxicity in Wistar Rats has been carried out by me in the Department of Human Physiology. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

Suleiman Joseph Bagi

Name of student

Signature

Date

CERTIFICATION

This thesis entitled: Ameliorative effect of Vitamin C on Anxiety and Motor Coordination in Lead-induced Toxicity in Wistar Rats meets the regulation governing the award of the degree of MSc Human Physiology of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Lead (Pb) is a widespread toxic metal found in the environment with potential danger to human health. It is used in the manufacture of batteries, metal products, paints and other domestic substances. A series of lead poisoning in Zamfara State Nigeria has led to the deaths of at least 400 people between March and June 2010. This study investigated the ameliorative effect of vitamin C on anxiety using the open field test and motor coordination using beam walk test and forepaw grip test in lead-induced toxicity in wistar rats. Twenty four male rats divided into four groups of six animals were used for the study. Group I received Distilled water (2ml/kg), group II received diazepam (0.05mg/kg), group III received lead (250mg/kg), group IV received vitamin C (100mg/kg) and 30minutes later was administered lead (250mg/kg). The regimen was administered by oral gavage. The assessment of behaviour was performed on days 0, 14th and 28th in open field (OF) which assessed anxiety function, beam walk (BW) which assessed motor co-ordination and forepaw grip (FG) which assessed motor strength. After the period of 28 days, rats were sacrificed and the brains homogenised, the malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and acetylcholinesterase enzyme (ACHE) were estimated. In the open field There was a significant increase in line crossing 32 ± 5.04 , stretch attend 10.01 ± 2.02 , rearing 11.66 ± 0.17 , a decrease was observed in grooming 1.05 ± 0.43 and freezing 0.83 ± 0.65 while in the beam walk a significant increase and an increase in muscle strength was observed in the group treated with lead and vitamin C. There was a significant decrease in concentration of the enzymes SOD 1.62 ± 0.07 , CAT 40 ± 4.00 and ACHE 20.2 ± 2.49 and a significant increase in MDA concentration 2.08 ± 0.11 in the group treated with lead due to the generation of radical oxygen species ROS as a result of lead toxicity. It can be concluded that the reduced level of anxiety, muscular incoordination, imbalance and muscular weakness in rats following exposure to lead can be ameliorated by the use of pretreatment with vitamin C.

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ABBREVIATIONS, DEFINITIONS, GLOSSARY AND SYMBOLS

| | |
|-------------------------------|--|
| ACHE | Acetylcholinesterase enzyme |
| ADI | Acceptable daily intake |
| APX | Ascorbate peroxidase |
| BAEP | Brainstem auditory evoked potential |
| BLL | Blood lead level |
| Bw | Body weight |
| CAT | Catalase |
| CoA | Coenzyme A |
| DNA | Deoxyribonucleic acid |
| EDTA | Ethylene-diamine-tetracetic acid |
| GABA | γ -aminobutyric acid |
| GGT | γ -glutamyltransferase |
| GLUT1 | Glucose transporters 1 |
| GLUT3 | Glucose transporters 3 |
| GPx | Glutathione peroxidase |
| GR | Glutathione reductase |
| GSH | Glutathione |
| GSSG | Glutathione disulfide (GSSG) |
| H ₂ O ₂ | Hydrogen peroxide |
| MDA | Malondialdehyde |
| metHb | Methylhaemoglobin |
| NOAEL | No observable adverse effect level |
| NHANES | National Health and Nutrition Examination Survey |
| O ₂ U ⁻ | Superoxide ion |

| | |
|------------------|-------------------------------------|
| OHU | Hydroxyl radical |
| oxyHb | Oxyhaemoglobin |
| PBG | Porphobilinogen |
| PbO ₂ | Lead (iv) oxide |
| PTWI | Provisional tolerable weekly intake |
| ROS | Reactive oxygen species |
| SOD | Superoxide dismutase |
| SVCTS | Sodium-Ascorbate Co-Transporters |
| TBA | Thiobarbituric acid |
| Vitc | Vitamin C |
| w/v | Weight per volume |
| WHO | World health organisation |
| ZPP | Zinc protoporphyrin |
| δ-ALAD | δ-aminolevulinic acid dehydrates |
| δ-ALAS | aminolevulinic acid synthetase |

CHAPTER ONE

1.0 INTRODUCTION

Lead is a ubiquitous environmental metal due to its significant role in modern industry (Shalan *et al.*, 2005). Lead is a naturally occurring, bluish-gray metal that is found in small quantities in the earth's crust. The primary use of lead is in the manufacture of batteries. Lead is also used in the production of metal products, such as sheet lead, solder (but no longer in food cans), and pipes, and in ceramic glazes, paint, ammunition, cable covering, and other products. Tetraethyl lead was used in gasoline to increase the octane rating until lead additives were phased out and eventually banned from use in gasoline in the U.S. by the EPA by 1996 (Brady and Holum, 1996)

The codex classification number of lead is ALINORM 01/12A, Appendix XIV. The WHO established a provisional tolerable weekly intake (PTWI) for lead for children of 25 $\mu\text{g}/\text{kg}$ bw, equivalent to an ADI (acceptable daily intake) of approximately 3.5 $\mu\text{g}/\text{kg}$ bw per day (WHO, 1977; Sax, 1989). This PTWI was established on the premise that lead is a cumulative poison and that there should be no increase in the body burden of lead from any source, thus avoiding the possibility of adverse biochemical and neurobehavioural effects in infants and young children (Georges, 2003; Crompton, 2000).

It was based on metabolic studies in infants showing that a mean daily lead intake of 3 to 4 $\mu\text{g}/\text{kg}$ Bw was a NOAEL (no observable adverse effect level) and was not associated with an increase in blood lead levels or in the body burden of lead, whereas a daily intake of 5 $\mu\text{g}/\text{kg}$ Bw or more resulted in lead retention. An unusually small uncertainty factor (less than 2) reflected the conservatism of the end point, the quality of the metabolic data and use of one of the most susceptible groups in the population (Hong *et al.*, 1984; Tucek *et al.*, 2006).

Both occupational and environmental exposures remain a serious problem in many developing and industrializing countries (Yucebilgic *et al.*, 2003). It has many undesired effects, including neurological, behavioural, immunological, renal hepatic and hematological dysfunctions (Loghman-Adham, 1997; Ercal *et al.*, 2000; Moreira *et al.*, 2001; Soltaninejad *et al.*, 2003; Sivaprasad *et al.*, 2003; Patra and Swarup, 2004; De Marco *et al.*, 2005).

Lead has been commonly used in the manufacture of batteries, metal products, paints, and ceramic glazes for thousands of years because it is widespread, easy to extract and easy to work with. It is highly malleable and ductile as well as easy to smelt. Metallic lead beads dating back to 6400 BC have been found in Çatalhöyük in modern-day Turkey. Its density, workability and corrosion resistance were among the metals attractions (Flora *et al.*, 2008).

The biological half-life of lead is extremely difficult to estimate (WHO, 1977). The half-life of lead in erythrocytes is 35 days; in soft tissues (kidney, liver and nervous tissue) the half-life is 40 days; the half-life in bone is 20 to 30 years (Ellenhorn, 1988; Garrettson, 1990).

Lead poisoning (also known as plumbism, colica Pictonum, saturnism, Devon colic, or painter's colic) is a medical condition caused by increased levels of the heavy metal lead in the body. A series of lead poisonings in Zamfara State, Nigeria, led to the deaths of at least 400 people between March and June 2010 (Yahaya, 2010)

Lead is a poisonous metal that can damage nervous connections (especially in young children) and cause blood and brain disorders. Lead poisoning typically results from ingestion of food or water contaminated with lead; but may also occur after accidental ingestion of contaminated soil, dust, or lead based paint. Long-term exposure to lead or

its salts (especially soluble salts or the strong oxidant PbO_2) can cause nephropathy, and colic-like abdominal pains. The effects of lead are the same whether it enters the body through breathing or swallowing (Heskel, 1983; Brown, 2006; Meyer *et al.*, 2008; Rossi, 2008).

Lead can affect almost every organ and system in the body. The main target for lead toxicity is the nervous system, both in adults and children. Long-term exposure of adults can result in decreased performance in some tests that measure functions of the nervous system. It may also cause weakness in fingers, wrists, or ankles (Gallatay, 2005).

Lead exposure also causes small increases in blood pressure, particularly in middle-aged and older people and can cause anaemia. Exposure to high lead levels can severely damage the brain and kidneys in adults or children and ultimately cause death. In pregnant women, high levels of exposure to lead may cause miscarriage (Settle and Patterson, 1980; Squatriti, 2000).

Chronic, high-level exposure has shown to reduce fertility in males. The antidote/treatment for lead poisoning consists of dimercaprol and succimer. Oxidative stress has been implicated in lead poisoning, it represents an imbalance between the production and manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of tissues can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Some reactive oxidative species can even act as messengers through a phenomenon called redox signaling (de Diego-otero *et al.*, 2009).

In humans, oxidative stress is involved in many diseases. Examples include atherosclerosis, Parkinson's disease, heart failure, myocardial infarction, Alzheimer's

disease, schizophrenia. Chemically, oxidative stress is associated with increased production of oxidizing species or a significant decrease in the capability of antioxidant defenses, such as glutathione (Gems *et al.*, 2008).

The effects of oxidative stress depend upon the size of these changes, with a cell being able to overcome small perturbations and regain its original state (Schafer and Buettner, 2001). However, more severe oxidative stress can cause cell death and even moderate oxidation can trigger apoptosis, while more intense stresses may cause necrosis. A particularly destructive aspect of oxidative stress is the production of reactive oxygen species, which include free radicals and peroxides (Lennon *et al.*, 1991; Valko *et al.*, 2005).

Vitamin C or L-ascorbic acid or L-ascorbate is an essential nutrient for humans and certain other animal species, in which it functions as a vitamin (Hemila *et al.*, 2007). In living organisms, ascorbate is an antioxidant, since it protects the body against oxidative stress. It is also a cofactor in at least eight enzymatic reactions, including several collagen synthesis reactions that cause the most severe symptoms of scurvy when they are dysfunctional. In animals, these reactions are especially important in wound-healing and in preventing bleeding from capillaries (Wilson, 1975).

Ascorbate (an ion of ascorbic acid) is required for a range of essential metabolic reactions in all animals and plants (Nualart *et al.*, 2003). It is made internally by almost all organisms, notable mammalian group exceptions are most or all of the order chiroptera (bats), guinea pigs, capybaras, and one of the two major primate suborders, the Anthropoidea (Haplorrhini) (tarsiers, monkeys and apes, including human beings). Ascorbic acid is also not synthesized by some species of birds and fish. All species that do not synthesize ascorbate require it in the diet (Audera, 2001; Meister, 1994).

Deficiency in this vitamin causes the disease scurvy in humans. It is also widely used as a food additive (Gropper, *et al.*, 2004)

1.1 STATEMENT OF RESEARCH PROBLEM

Lead (Pb) is a neurotoxic agent, and even at low doses produce adverse effects on the developing central nervous system (CNS) due to the lack of a functional blood brain barrier (BBB) in children and the intense cellular proliferation, differentiation and synaptogenesis. Studies of developmental lead treatment in rats have demonstrated alteration in complex behaviours such as learning, drug discrimination, spatial-learning and stress behaviours, as well as alterations in motor activity

Lead exposure is associated with decreased intelligence, reduced short-term memory, reading disabilities, and deficits in vocabulary, fine motor skills, reaction time, and hand–eye coordination (Brown, *et al.*, 1999).

1.2 JUSTIFICATION

With the increasing lead mining activities in developing countries like Nigeria, it is important to assess the ameliorative effect of Vitamin C on anxiety and motor coordination, since impairment in anxiety can affect the mental performance and coordination especially in children. The use of vitamin C (a potent antioxidant) in the treatment of lead poisoning is controversial, therefore the need to ascertain this.

1.3 HYPOTHESIS

Ascorbic acid an antioxidant widely used does not have any significant ameliorative effect on anxiety and motor coordination deficit in lead-induced toxicity in rats.

1.4 AIM AND OBJECTIVES

General Aim

The present study is aimed at evaluating the ameliorative effect of ascorbic acid (vitamin C) on anxiety and motor functions in lead-induced toxicity in wistar rats.

Specific objectives

To evaluate anxiety using the open field model.

To evaluate motor coordination using the beam walk model.

To evaluate motor strength using forepaw grip model.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 RELATIONSHIP BETWEEN LEAD TOXICITY, ANXIETY AND MOTOR COORDINATION

Lead toxicity is closely related to its accumulation in certain tissues and its interference with the bio elements, whose role is critical for several physiological processes including the brain. About 99% of the lead present in the blood is bound to erythrocytes. They have a high affinity for lead and contain the majority of the lead found in the blood stream, which makes them more vulnerable to oxidative damage than many other cells especially the brain. This can lead to several imbalance in neurobehaviors like anxiety and motor incoordination. However there is no correlation between motor coordination and anxiety (chiodo, *et al.*, 2004).

Anxiety is a complex, broad concept that can be defined and measured in several ways, and needs to be distinguished from fear. Anxiety and fear differ phenomenologically as well as neurobiologically, although both terms are often used incorrectly interchangeably. Barlow *et al* (2009) defined anxiety as a cognitive-affective structure composed of three key components: a future-oriented negative affective state (sense of uncontrollability focused on possible future negative events), a state of self-focused attention (especially focused on one's (inadequate) capabilities to deal with the threat, and preparedness to attempt to cope with upcoming events. Fear, on the contrary, is more stimulus-bound and limited in time; fear is conceptualized by Barlow *et al* (2009) as a distinct basic emotion that is fundamentally a behavioral act, characterized by the fight or flight response. The neurobiology underlying fear and anxiety is different, and they have divergent effects on somatic pain thresholds especially in muscles leading to motor incoordination.

2.2 ANXIETY

Anxiety is a psychological and physiological state characterized by somatic, emotional, cognitive, and behavioural components. It is the displeasing feeling of fear and concern.

Anxiety is considered to be a normal reaction to a stressor (Davison, 2008).

Anxiety is a generalized mood that can occur without an identifiable triggering stimulus.

As such, it is distinguished from fear, which is an appropriate cognitive and emotional response to a perceived threat. Additionally, fear is related to the specific behaviours of escape and avoidance, whereas anxiety is related to situations perceived as uncontrollable or unavoidable (Bouras and Holt, 2007).

The physical effects of anxiety may include heart palpitations, tachycardia, muscle weakness and tension, fatigue, nausea, chest pain, shortness of breath, stomach aches, or headaches. As the body prepares to deal with a threat, blood pressure, heart rate, perspiration, blood flow to the major muscle groups are increased, while immune and digestive functions are inhibited (the *fight or flight* response). External signs of anxiety may include pallor, sweating, trembling, and pupillary dilation. Someone who has anxiety might also experience it subjectively as a sense of dread or panic (Ohman, 2000).

The emotional effects of anxiety may include "feelings of apprehension or dread, trouble concentrating, feeling tense or jumpy, anticipating the worst, irritability, restlessness, watching (and waiting) for signs (and occurrences) of danger, and, feeling like your mind's gone blank" as well as "nightmares/bad dreams, obsessions about sensations (Barlow, 2002),

The cognitive effects of anxiety may include thoughts about suspected dangers, such as fear of dying. "You may fear that the chest pains are a deadly heart attack or that the shooting pains in your head are the result of a tumour or aneurysm. You feel an intense

fear when you think of dying, or you may think of it more often than normal, or can't get it out of your mind (Sylvers, *et al.*, 2011).

The behavioural effects of anxiety may include withdrawal from situations which have provoked anxiety in the past. Anxiety can also be experienced in ways which include changes in sleeping patterns, nervous habits, and increased motor tension like foot tapping (Neil, *et al.*, 2010; Barker, 2003).

2.3 ANXIOLYTICS

An anxiolytic is a drug used for the treatment of anxiety and its related psychological and physical symptoms. Anxiolytics are also known as minor tranquilizers. It was originally derived from a dichotomy with major tranquilizers, also known as neuroleptics or antipsychotics. There are different types: Benzodiazepines include: Alprazolam (Xanax), Chlordiazepoxide (Librium), Clonazepam (Klonopin, Rivotril), Diazepam (Valium), Etizolam (Etilaam), Lorazepam (Ativan), Oxazepam (Serax) SSRIs (Selective serotonin reuptake inhibitors or serotonin-specific reuptake inhibitor (SSRIs) Nardil and Parnate. Azapirones (buspirone and tandospirone Gepirone), Barbiturates, Hydroxyzine, Pregabalin (Montenegro, *et al.*, 2005; Barlow and Durand, 2009)

2.4 MOTOR COORDINATION

Motor Coordination is the various motor activities caused by sensory stimuli, this is controlled by both the sensory and motor parts of the brain. Anxiety disorders and motor deficits have been implicated in lead toxicity ((De Marco *et al.*, 2005 and Moreira *et al.*, 2001)

Motor cortex is a term that describes regions of the cerebral cortex involved in the planning, control, and execution of voluntary motor functions. The motor cortex can be divided into several main parts:

The primary motor cortex is the main contributor to generating neural impulses that pass down to the spinal cord and control the execution of movement. However, some of the other motor cortical fields also play a role in this function (Rathelot and Strick, 2006).

The premotor cortex is responsible for some aspects of motor control, possibly including the preparation for movement, the sensory guidance of movement, the spatial guidance of reaching, or the direct control of some movements with an emphasis on control of proximal and trunk muscles of the body (Park, *et al.*, 2001).

The supplementary motor area (or SMA), has many proposed functions including the internally generated planning of movement, the planning of sequences of movement, and the coordination of the two sides of the body such as in bi-manual coordination (Sanes, *et al.*, 1995).

The posterior parietal cortex is sometimes also considered to be part of the group of motor cortical areas. It is thought to be responsible for transforming multisensory information into motor commands, and to be responsible for some aspects of motor planning, in addition to many other functions that may not be motor related (Donoghue, *et al.*, 1992).

The primary somatosensory cortex, especially the part called area 3a, which lies directly against the motor cortex, is sometimes considered to be functionally part of the motor control circuitry (Meier, *et al.*, 2008).

Other brain regions outside the cerebral cortex are also of great importance to motor function, most notably the cerebellum, the basal ganglia, and the red nucleus, as well as other sub cortical motor nuclei.

2.5 MOTOR FUNCTIONS AND LEAD POISONING

Lead exposure in young children has been linked to learning disabilities, and children with blood lead concentrations greater than 10 μ g/dL are in danger of developmental disabilities. Increased blood lead level in children has been correlated with decreases in Fine motor skill which is the coordination of small muscle movements which occur in body parts such as the fingers, usually in coordination with the eyes. The effect of lead on children's fine motor skill abilities takes place at very low levels (Moreira *et al.*, 2001).

2.6 BIOLOGICAL SIGNIFICANCE OF VITAMIN C

Vitamin C is purely the L-enantiomer of ascorbate; the opposite D-enantiomer has no physiological significance. Both forms are mirror images of the same molecular structure. When L-ascorbate, which is a strong reducing agent, carries out its reducing function, it is converted to its oxidized form, L-dehydroascorbate. L-dehydroascorbate can then be reduced back to the active L-ascorbate form in the body by enzymes and glutathione (McCluskey, 1985).



Figure 2.1: Oranges as a source of Vitamin C (Padayatty, *et al.*, 2003)

During this process semidehydroascorbic acid radical is formed. Ascorbate free radical reacts poorly with oxygen, and thus, will not create a superoxide. Instead two semidehydroascorbate radicals will react and form one ascorbate and one dehydroascorbate. With the help of glutathione, dehydroxyascorbate is converted back to ascorbate. The presence of glutathione is crucial since it spares ascorbate and improves antioxidant capacity of blood. Without it dehydroxyascorbate could not convert back to ascorbate. L-Ascorbate is a weak sugar acid structurally related to glucose that naturally occurs attached either to a hydrogen ion, forming ascorbic acid, or to a metal ion, forming a mineral ascorbate (Audera, 2001).

2.7 ABSORPTION, TRANSPORT AND DISPOSAL OF VITAMIN C

Ascorbic acid is absorbed in the body by both active transport and simple diffusion. Sodium-Dependent Active Transport Sodium-Ascorbate Co-Transporters (SVCTs) and Hexose transporters (GLUTs)—are the two transporters required for absorption. SVCT1 and SVCT2 import the reduced form of ascorbate across plasma membrane. GLUT1 and GLUT3 are the two glucose transporters, and transfer only dehydroascorbic acid form of Vitamin C (Meister, 1994; Purves, *et al.*, 1998; Hardie, *et al.*, 1991)

SVCT2 is involved in vitamin C transport in almost every tissue, the notable exception being red blood cells, which lose SVCT proteins during maturation. "SVCT2 knockout"

animals genetically engineered to lack this functional gene, die shortly after birth, suggesting that SVCT2-mediated vitamin C transport is necessary for life (Gropper, *et al.*, 2004; Rumsey, *et al.*, 1997).

With regular intake the absorption rate varies between 70 to 95%. However, the degree of absorption decreases as intake increases. Ascorbate concentrations over renal re-absorption threshold pass freely into the urine and are excreted. (Savini, *et al.*, 2007; Nualart, *et al.*, 2003; May, 2003).

Concentrations in the plasma larger than this value (thought to represent body saturation) are rapidly excreted in the urine with a half-life of about 30 minutes. (Banhegyi and Mandl, 2001; Savini, *et al.*, 2007).

Ascorbic acid can be oxidized (broken down) in the human body by the enzyme L-ascorbate oxidase. Ascorbate that is not directly excreted in the urine as a result of body saturation or destroyed in other body metabolism is oxidized by this enzyme and removed (Proctor, *et al.*, 1970; Ohta and Nishikimi, *et al.*, 1999; Nishikimi, *et al.*, 1992; Porter, *et al.*, 1997).

2.8 ANTIOXIDANT ACTIVITIES OF VITAMIN C

Ascorbic acid is well known for its antioxidant activity, acting as a reducing agent to reverse oxidation in liquids. When there are more free radicals (reactive oxygen species, ROS) in the human body than antioxidants, the condition is called oxidative stress, and has an impact on cardiovascular disease, hypertension, chronic inflammatory diseases, diabetes as well as on critically ill patients and individuals with severe burns. Individuals experiencing oxidative stress have ascorbate blood levels lower than 45µmol/L, compared to healthy individual who range between 61.4-80 µmol/L (Goodman, *et al.*, 1998; Milton, 1999; Long, *et al.*, 2003; Montelhagen, *et al.*, 2008).

Ascorbic acid behaves not only as an antioxidant but also as a pro-oxidant. Ascorbic acid has been shown to reduce transition metals, such as cupric ions (Cu^{2+}), to cuprous (Cu^+), and ferric ions (Fe^{3+}) to ferrous (Fe^{2+}) during conversion from ascorbate to dehydroascorbate *in vitro*. This reaction can generate superoxide and other ROS (Hancock, et al., 2000; Pox and Douzery, 2004; Venturi and Venturi, 2007; Venturi, *et al.*, 2000; Haris, 1996; Stone, 1979).

2.9 LEAD INTERACTIONS IN HEME BIOSYNTHETIC PATHWAY

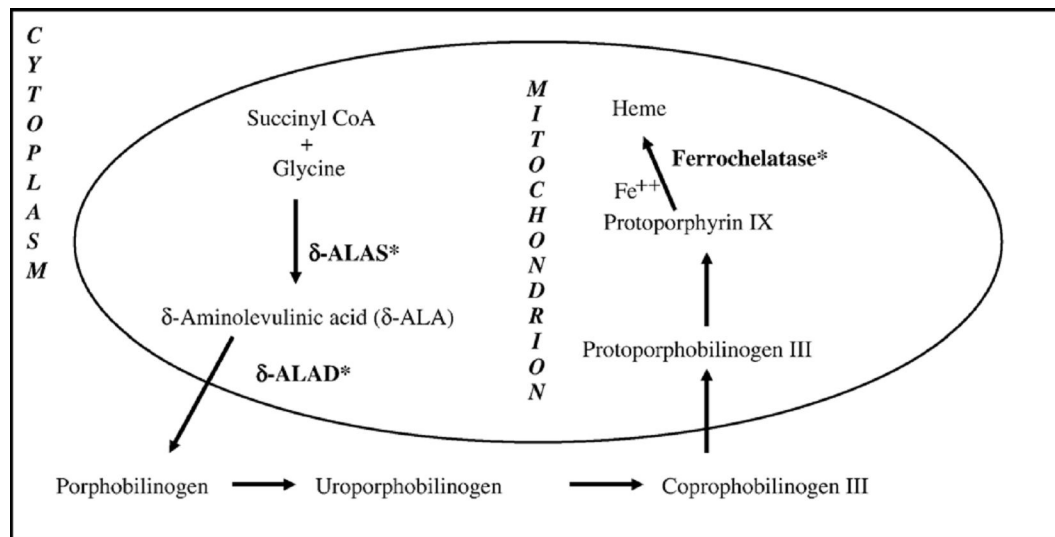
The hematopoietic system is one of the target organs in lead toxicity. The enzymes in the biosynthetic pathway of heme in which the effects of lead are of the clinical interest are δ -aminolevulinic acid synthetase (δ -ALAS), δ -aminolevulinic acid dehydratase (δ -ALAD), and ferrochelatase (Jacob *et al.*, 2000).

The series of reactions leading to heme biosynthesis begins with succinyl coenzyme A (CoA) and glycine and ends with the insertion of an iron (Fe^{++}) into a molecule of protoporphyrin to form heme. In the first step, the enzyme δ -ALAS catalyzes the formation of δ -ALA from glycine and succinyl CoA, whereas in the second step, δ -ALAD catalyzes the formation of porphobilinogen (PBG) from two molecule of δ -ALA. Due to its affinity for $-\text{SH}$ group, lead is known to inhibit δ -ALAD activity that has been used as a laboratory tool for the detection of lead intoxication (Goering, 1993; Bergdahi *et al.*, 1997).

Over 99% of the lead present in the blood accumulates in erythrocytes. Of this, over 80% is bound to δ -ALAD. Austrin et al. found 50% inhibition of δ -ALAD activity at a BLL of $15\mu\text{g/dL}$. In an earlier study, it was found that BLLs $7.1\mu\text{g/dL}$ inhibit the activity of δ -ALAD. Recently, Sakai and Morita found that threshold value of blood lead for δ -ALAD inhibition was extremely low (approximately $5\mu\text{g/dL}$). Inhibition of δ -ALAD by lead

accounts for the accumulation of δ -ALA in blood and urine; urinary δ -ALA has also been used as a biomarker for lead exposure or a marker of early biologic effect of lead. In the last step, ferrochelatase incorporates iron (Fe^{++}) into the protoporphyrin molecule to form heme.

Lead inhibits ferrochelatase activity and therefore, prevents incorporation of iron into protoporphyrin. This reaction leads to binding of zinc, producing zinc protoporphyrin (ZPP). The presence of ZPP has been proposed as an indicator of recent lead intoxication and thus can be used as a biomarker of exposure. However, because of the abundance of hemoglobin (Hb), even in serious cases of lead intoxication, increased ZPP is relatively harmless because it may constitute less than 1% of the total Hb produced.



* Activity of enzymes inhibited by lead

δ -ALAS: Delta-aminolevulinic acid synthetase, δ -ALAD: Delta-aminolevulinic acid dehydratase, Co A: Coenzyme A

Figure 2.2: The inhibition of enzymes by lead in the synthesis of haem (Canfield *et al.*, 2004).

2.10 MECHANISMS FOR LEAD-INDUCED FREE RADICALS GENERATION

Oxidative stress appears to be a possible mode of the molecular mechanism of lead toxicity. Oxidative stress occurs when generation of free radicals (i.e. substances with one or more unpaired electrons) exceed the capacity of antioxidant defense mechanisms (i.e. pathways that provide protection against harmful effect of free radicals) (Thomas *et al.*, 2003).

The depletion of glutathione and protein bound sulfhydryl groups and the changes in the activity of various antioxidant enzymes indicative of lipid peroxidation have been implicated in lead-induced oxidative tissue damage. Lead seems to be quite capable of causing oxidative damage to heart, liver, kidney, reproductive organs, brain, and erythrocytes. The participation of free radicals in lead toxicity may occur at different levels: Inhibition of δ -ALAD by lead accounts for the accumulation of its substrate δ -ALA, that can be rapidly oxidized to generate free radicals as superoxide ion, hydroxyl radical, and hydrogen peroxide, and lead per se has the capacity to stimulate ferrous ion initiated membrane lipid peroxidation (Canfield *et al.*, 2004).

2.11 PRO-OXIDATIVE EFFECT DELTA-AMINOLEVULINIC ACID (δ -ala)

The δ -ALA undergoes enolization and auto-oxidation at pH 7.0–8.0. The conversion of the δ -ALA keto form into the δ -ALA enol form is shown to be necessary for auto-oxidation reactions because levulinic acid, without the amino group ($-\text{NH}_2$) that is thought to facilitate the enolization, has not been found to be active in oxidation reactions. The enolized δ -ALA then autoxidizes and generates O_2^- , as evidenced by the parallel reduction of ferricytochrome c, and also by electron spin resonance spin trapping experiments. Monteiro *et al.*, (1989) reported that δ -ALA/oxyHb coupled oxidation also results in ROS generation (Chiodo *et al.*, 2004).

The steps of the reactions were reported as follows:

δ -ALA enol form is generated following tautomerization and δ -ALA enol acts as an electron donor to molecular oxygen, together with an electron transfer from oxyHb to oxygen resulting in metHb, δ -ALA radical, and H_2O_2 generation. The O_2^- and H_2O_2 , and which are now present as a result of both δ -ALA and δ -ALA/oxyHb coupled auto-oxidation, can interact and generate HOU radicals, which have the highest reactivity among ROS. Inhibition of δ -ALA/oxyHb coupled oxidation by SOD, CAT, and mannitol suggests the involvement of O_2^- , H_2O_2 , and HOU respectively, in the process. Besides oxyHb, metHb and other ferric and ferrous complexes were also shown to trigger δ -ALA oxidation (Chiodo *et al.*, 2004).

This is evidenced by induction of oxygen uptake by δ -ALA in the presence of Fe-ATP and Fe-EDTA complexes as well as oxyHb and metHb. Furthermore, many authors tentatively attribute the neurological symptoms of lead toxicity to the ability of δ -ALA to inhibit either the K^+ -stimulated release of γ -aminobutyric acid (GABA) from preloaded rat brain synaptosomes or the binding of GABA to synaptic membranes (Courtois *et al.*, 2003).

Therefore, it may be concluded that δ -ALA accumulated in lead-intoxication can be suggested as a source of ROS, which is now accepted as being associated with the pathophysiology of lead toxicity. The δ -ALA also has the potential of genotoxicity and demonstrated that the final oxidation product of δ -ALA, 4, 5-dioxovaleric acid, is an effective alkylating agent of the guanine moieties within both nucleoside and isolated DNA. The same group reported increased levels of 8-oxo-7, 8-dihydro-2-deoxyguanosine and 5-hydroxy-2-deoxycytidine in organ DNA of rats chronically treated with δ -ALA, and involvement of HOU in δ -ALA-induced DNA damage. The δ -ALA induces single-strand breaks in plasmid pBR222 DNA.

Hiraku and Kawanishi (1996) reported that free radicals generated by copper-catalyzed oxidation of δ -ALA could cause oxidative damage to DNA fragments obtained from c-Ha-ras proto-oncogene. Taken together, these findings imply agenotoxic potential of δ -ALA. This possible consequence of δ -ALA accumulation deserves further studies of lead toxicity.

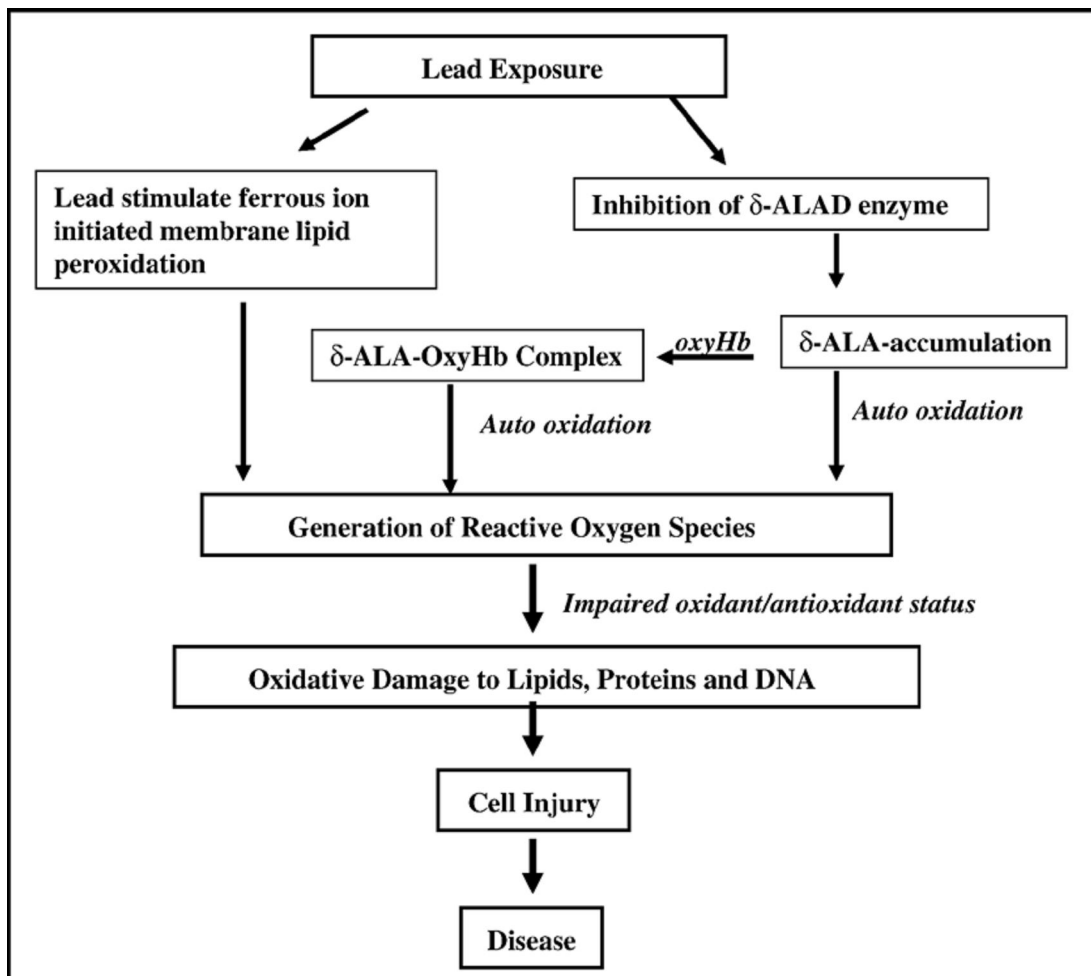


Figure 2.3: Pathophysiology of lead (Banhegyi, *et al.*, 1997)

2.12 LEAD-INDUCED MEMBRANE LIPID PEROXIDATION

Lead is known to have toxic effects on membrane structure and functions. The effects on erythrocyte membranes in particular, have been intensely analyzed because erythrocytes have a high affinity for lead and contain a majority of lead found in the blood stream, and are more vulnerable to oxidative damage than many other cells. On cell membrane, the presence of double bonds in the fatty acid weakens the C–H bonds on the carbon atom adjacent to the double bonds and makes H removal easier (Pollock and Mullin, 1987).

Therefore, fatty acids containing zero to two double bonds are more resistant to oxidative stress than are the polyunsaturated fatty acids with more than two double bonds. After incubation of linoic, linolenic, and arachidonic acid with lead, the concentration of a final product of oxidative stress, malondialdehyde (MDA) was increased with the number of double bonds of fatty acid. Another mechanism for lead-induced membrane oxidative damage is the effect on changes in the fatty acid composition of membrane. Because fatty acid chain length and unsaturation are associated with membrane susceptibility to peroxidation, lead-induced arachidonic acid elongation might be responsible for the enhanced lipid peroxidation in the membrane (Challem and Taylon, 1998; Banhegyi, *et al.*, 1997).

By causing lateral phase separation and/or by increasing lipid peroxidation rates, lead could affect membrane related processes such as the activity of membrane enzymes, endo and exocytosis, the transport of solutes across the bilayer, and signal transduction processes. Taken together, these data suggest that altered lipid composition of membranes due to lead exposure may result in altered membrane integrity, permeability, and function. These would increase the susceptibility to lipid peroxidation (Ariza *et al.*, 1998).

2.13 EFFECTS OF LEAD ON ANTIOXIDANT DEFENSE SYSTEMS OF CELLS

Several antioxidant molecules such as glutathione (GSH) and glutathione disulfide (GSSG) levels and antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) activities are the most commonly used parameters to evaluate lead-induced oxidative damage. GSH is a tripeptide containing cysteine that has a reactive –SH group with reductive potency. Accordingly, GSH plays a vital role in the protection of cells against oxidative stress. Another component of antioxidant defense system, GR, reduces GSSG back to GSH and thereby supports the antioxidant defense system indirectly. GR possesses a disulfide at its active site that was suggested as target for lead, resulting in the inhibition of the enzyme. GPx, CAT, and SOD are metalloproteins and accomplish their antioxidant functions by enzymatically detoxifying the peroxides (–OOH), H₂O₂, and O₂ U– respectively. CAT decomposes H₂O₂ into H₂O and O₂. GPx needs GSH to decompose H₂O₂ or other peroxides with the simultaneous oxidation of GSH into GSSG (Myroie *et al.*, 1986).

CAT has been suggested to provide important pathway for H₂O₂ decomposition at higher steady state H₂O₂ concentration, whereas GPx is believed to play a more important role in H₂O₂ decomposition under lower steady state levels of H₂O₂.

Since these antioxidant enzymes depend on various essential trace elements and prosthetic groups for proper molecular structure and enzymatic activity, they are potential targets for lead toxicity (Batra, *et al.*, 1998).

SOD dismutates the O₂ U– in to H₂O₂ and requires copper and zinc for its activity.

Copper ions appear to have a functional role in the reaction by undergoing alternate oxidation and reduction, where zinc ions seem to stabilize the enzyme instead of having a role in the catalytic cycle. Another type, MnSOD, contains manganese at its active site and is not detected in mammalian erythrocytes, but is present in human liver to some extent observed (Myroie *et al.*, 1986).

Therefore, they have suggested an indirect inhibitory effect on SOD *in vivo* due to the lead-induced copper deficiency. Inhibition of SOD activity by lead was also shown in an *in vitro* study where the authors indicated that this effect of lead can lead to decreased scavenging of ROS and result in oxidative damage.

However, Ariza *et al* (1998) demonstrated rapid induction of cellular H_2O_2 following treatment of AS52 cells with 1M lead, which they suggested to be increased by the stimulatory effect of lead on the activities of CuZn-SOD and xanthine oxidase that produce H_2O_2 . Lead inherence facilitates conversion of Hb into metHb. This reaction is possible not only in pure Hb solution, but also in lysates, wherein antioxidant defense systems are present. It seems that during Hb oxidation in the presence of lead, H_2O_2 is generated, which may induce lipid peroxidation in erythrocyte cell membranes.

Ribarov *et al* (1998). Found that lead significantly enhances the auto-oxidation of Hb in an *in vitro* liposome model. The inhibition of this effect by SOD and CAT suggested that O_2^- and H_2O_2 are somehow involved in the process. As a result, they speculated that lead might induce generation of ROS by interacting with oxyHb, leading to peroxidative damage of erythrocyte membranes.

2.14 OXIDATIVE STRESS IN LEAD-RELATED DISEASES

Investigators have extensively studied that higher levels lead exposure causes oxidative damage to brain, heart, kidneys, and reproductive organs. However, only few studies

demonstrated that low levels lead exposure also induces oxidative stress. We have discussed some studies indicating relevance of oxidative stress to lead-related human diseases with low exposure. Lead may induce oxidative damage to reproductive organs. Sperm ROS generation was significantly higher in lead-exposed rats with BLLs of 33.6 $\mu\text{g}/\text{dL}$ which was associated with the decrease of sperm motility, motile sperm counts, and sperm– oocyte penetration rate (Batra, *et al.*, 1998) .

Furthermore, lead-induced ROS generation was associated with early onset of sperm capacitation and premature acrosome reaction, and reduced zona-intact oocyte penetrating capability. Batra, *et al* (1998) observed a significant decrease in δ -ALAD and SOD activity in rat testis with relatively lower BLL (18.6 $\mu\text{g}/\text{dL}$). Our previous study suggested that moderate BLL 23.4 $\mu\text{g}/\text{dL}$ might be a risk factor the development of prostate cancer in human through generating the ROS. However, low levels lead exposure and oxidative damage to reproductive organs could be the area of worthy investigation.

Exposure to low levels of lead causes hypertension in humans and animals. In a study published in 2006, Menke *et al* (2006) found an increased risk of death from all causes as well as from cardiovascular disease and stroke in association with BLL as low as 2 $\mu\text{g}/\text{dL}$. The study analyzed data from more than 13,946 adult participants in the third National Health and Nutrition Examination Survey (NHANES III) mortality study.

Animal studies show that lead can promote the growth of vascular smooth cells, which play a role in the formation of atherosclerotic plaques. Lead's promotion of oxidative stress is thought to play a role in its cardiovascular effects. In rabbit, after inhalational exposure to lead oxide (PbO) particles between 0.5 and 3.0 μg at 30 $\mu\text{g}/\text{m}^3$ for 4 days

(BLL 1.2–2.0 µg/dL), enhanced H₂O₂, and O₂ U⁻ production was noted, and pulmonary macrophage-mediated functions were disrupted.

Ding *et al* (2001) found that lead-induced hypertension was associated with an increase in ROS, which enhanced vascular reactivity with rather low BLLs (3.2µg/dL) in SD rats. They also showed that lead-induced hypertension might be caused by hydroxyl radical and peroxynitrite generation at average BLL of 12.4µg/dL in lead treated rats. Lead was shown to promote hydroxyl radical generation and lipid peroxidation in cultured aortic endothelial cells. Similar changes were observed in rats given moderate doses of lead (average BLL of 16.8 µg/dL) that caused the increase of serum lipid peroxidation in a dose-response manner. On the basis of these studies, it can be speculated that ROS might be involved in the genesis of lead-related hypertension, through either direct vasoconstrictive effect or by inactivation of endothelium-derived relaxing factor. Recent epidemiological studies of the general population suggest kidney function may be altered at the lowest levels of blood lead studied to date in relation to renal effects.

In a review Ekong *et al* (2006) wrote that lead contributed to kidney damage at concentration below 5µg/dL. Muntner et al. examined the association between low BLLs with chronic kidney diseases among adults of U.S. participating in NHANES III (n=15,211). BLL 4.2µg/dL was strongly associated with chronic kidney disease. In addition, lead's effects on kidney damage are thought to play a major role in its effect on blood pressure. This is because the kidney helps to regulate the blood volume and vascular tone, which are the principle determinants of blood pressure.

The kidney is the organ through which we get rid of the excess salt and fluids. Consequently, impairment of ability of kidney to efficiently excrete salts and fluids can result in the rise in blood volume and, hence blood pressure. Also kidney produces

hormones that regulate the tone of blood vessels. Thus, alterations of kidney function or structure can cause the blood vessels to constrict throughout the body, thereby raising blood pressure. Lead-induced kidney damage also includes the components of ROS.

Farmand *et al* (2005) have shown that rats exposed to lead in drinking water (100 ppm lead acetate) for 12 weeks induce hypertension through the dysregulation of activities of SOD, CAT, GPx, and guanylate cyclase in renal cortex, medulla, and thoracic aorta. Several studies reported that levels of MDA, GSH/GSSG ratio, and activity of antioxidant enzymes were strongly correlated with higher BLL (approximately 30µg/dL to 100µg/dL).

Dursun *et al* (2001) had similar results in a group with a relatively low exposure to lead (BLL 15µg/dL). A study from our group also found that BLL 9.9µg/dL significantly associated with δ -ALAD, MDA and CAT among urban adolescents from general population of Lucknow, India.

Lee *et al* (2006) examined the association of BLL with the oxidative stress markers of γ -glutamyltransferase (GGT), vitamin C, carotenoids, and vitamin E among 10,098 adult participants in the NHNES III. After adjusting for known confounding effects, BLL (2.8µg/dL) showed graded associations, positive with serum GGT and inverse with serum vitamin C, carotenoids, and vitamin E. Authors suggest that strong association of BLL with oxidative stress markers in this population suggests that oxidative stress should be considered in the pathogenesis of lead-related diseases among population with low exposure to lead.

Schafer *et al* (2005) found a significant positive association between BLL (3.5µg/dL) and homocysteine in an older, community-dwelling, adult, population-based sample in a major US urban area. Elevated homocysteine level increases the risk of heart diseases,

strokes, peripheral vascular diseases and cognitive functions. Authors suggest that the mechanisms of this impairment might involve the components of oxidative stress. In pregnant women with low levels of blood lead from 2.7–12.6µg/dL, an inverse relationship was observed between BLL and serum levels of α-tocopherol and ascorbic acid.

The group also examined the association of low levels lead exposure with markers of oxidative stress among children of general population. BLL 7.1µg/dL was significantly associated with δ-ALAD, GSH, MDA, and CAT. Diouf et al (2006) investigated the effects of low levels lead exposure on heme synthesis and some markers of oxidative stress among Senegalese children. They found that BLL 7.3µg/dL was significantly associated with urinary δ-ALA levels, erythrocyte GPx and GR activities, and blood selenium levels. Therefore, low levels lead exposure seems to be capable to induce oxidative stress among human of general population wherein many diseases are thought to be associated with lead-induced generation of free radicals.

2.15 REPRODUCTIVE EFFECTS OF LEAD POISONING

Severe lead toxicity in women has been associated with sterility, miscarriage, stillbirth, and neonatal morbidity and mortality from exposure in utero (Oliver, 1911; Rom, 1976). In men, heavy occupational exposure has been shown to have an adverse effect on semen quality (Lerda, 1992; Hu *et al.*, 1992).

In both men and women, the evidence for low-level exposure effects is weaker. It has been difficult to demonstrate effects on neurodevelopment in infants and children (Assennato *et al.*, 1987; Tuohimaa and Wickmann, 1985; Lerda, 1992; Hu *et al.*, 1992; Assennato *et al.*, 1987, Goyer, 1996;

2.16 EFFECTS OF LEAD POISONING ON THE KIDNEY

The adverse effects of lead overexposure on the kidney have been well documented (Goyer, 1971; Tuohima and Wickmann, 1985; Wildt *et al.*, 1983). Lerda, 1992; Coste *et al.*, 1991; Wildt *et al.*, 1983). These changes may progress to generalized kidney disease, which is characterized by disruption of function of the tubular structures. Chronic and excessive lead exposure may result in end-stage renal disease (Weeden, 1982; Buchet *et al.*, 1980; Goyer *et al.*, 1989).

2.17 EFFECTS OF LEAD POISONING ON HEARING

While less attention has focused on lead and potential effects on sensory functions, there have been some indications of auditory system processing deficits in lead-exposed children (Otto and Fox, 1993).

Otto *et al.* (1985) reported an increased latency of brainstem auditory evoked potential (BAEP—a measure of nerve conduction) in school children with a history of high lead exposure (BLL values ranging from 60 to 90 $\mu\text{g}/\text{dL}$) in early childhood. Other studies have associated deficits in hearing with a relatively low (i.e., 10 $\mu\text{g}/\text{dL}$) BLL, with no apparent lower threshold (Schwarz and Otto, 1987, 1991). Osman *et al.* (1999) reported hearing test results in children indicating that auditory function is impaired at BLL values ranging from 1.9 to 28.1 $\mu\text{g}/\text{dL}$.

2.18 EFFECTS OF LEAD POISONING ON HEMATOLOGICAL PARAMETERS

The effects of lead overexposure on heme (an iron-containing compound involved in oxygen transport by hemoglobin) synthesis have been thoroughly investigated, and there is a consensus that adverse effects on hemoglobin are associated with BLL values of 50 $\mu\text{g}/\text{dL}$ in adults and 80 $\mu\text{g}/\text{dL}$ in children (Goyer, 1996).

2.19 EFFECTS OF LEAD POISONING ON CARDIOVASCULAR SYSTEM

Several reviews have concluded that there is only a weak association between BLL and elevated blood pressure for those with BLL values below 45µg/dL (Hertz-Picciotto and Croft, 1993; Staessen *et al.*, 1995).

This association has been inconsistent across studies because of potential confounders and the inability to establish a clear dose-response relationship. Meta-analyses of the literature on this potential effect of lead indicate that there may be a weak positive association (Nowack *et al.*, 1992; Hertz-Picciotto and Croft, 1993; Rosen *et al.*, 1980).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

Twenty four male rats were used for this study, and were obtained from the Animal House of the Department of Pharmacology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The animals were housed in metal cages in the animal house of the Department of Human Physiology Ahmadu Bello University Zaria and were fed on standard rat feeds and water *ad libitum*. They were allowed to acclimatize for 14 days at room temperature. The experimental animals were divided into four groups of six rats each and the duration of administration was for twenty eight (28) days. Group I received distilled water orally (2ml/kg) and served as negative control. Group II received Diazepam orally 0.05mg/kg and served as positive control. Group III received lead acetate orally 250mg/kg ie 1/20th LD₅₀. Group IV received lead acetate 250mg/kg and vitamin C orally 100mg/kg

3.2 CHEMICALS

Lead acetate (batch no 354781/140797) was obtained from Bijo chemicals store in Kaduna state, Nigeria, and the % purity is 70%. Vitamin C tablets were obtained from Beautiful Gate pharmaceutical store in Zaria, Kaduna state Nigeria, the batch no is 0808381. The Lead acetate and Vitamin C were reconstituted in distilled water prior to daily administration.

3.3 ACUTE TOXICITY STUDY

Acute toxicity studies was carried out using the method described by Lorke (1983) using 12 rats. In the first phase, rats was divided into 3 groups of 3 rats each and was treated with 10mg, 100mg and 1000mg of lead per kg body weight orally. They were observed

for 24 hours for signs of toxicity including death. In the second phase, 3 groups each containing one rat was given orally with three doses 1600mg, 2900mg and 5000mg of lead and was administered according to their body weight based on the result of the first phase. There were no deaths. Therefore $1/20^{\text{th}}$ LD₅₀ (Ambali, *et al.*, 2010) was used for this study.

3.4 NEUROBEHAVIOURAL ASSESSMENTS

The evaluation of neurobehaviour was carried out using the open field model and beam walking test and forepaw grip test.

3.4.1 Test for open field assessment

The open field apparatus was constructed of white plywood and measured 72 x 72 cm with 36 cm walls. One of the walls was clear Plexiglas, so rat could be visible in the apparatus. Blue lines were drawn on the floor with a marker and were visible through the clear Plexiglas floor. The lines divided the floor into sixteen 18 x 18 cm squares. A central square (18 cm x 18 cm) was drawn in the middle of the open field (figure 3.1) (Brown *et al.*, 1999).

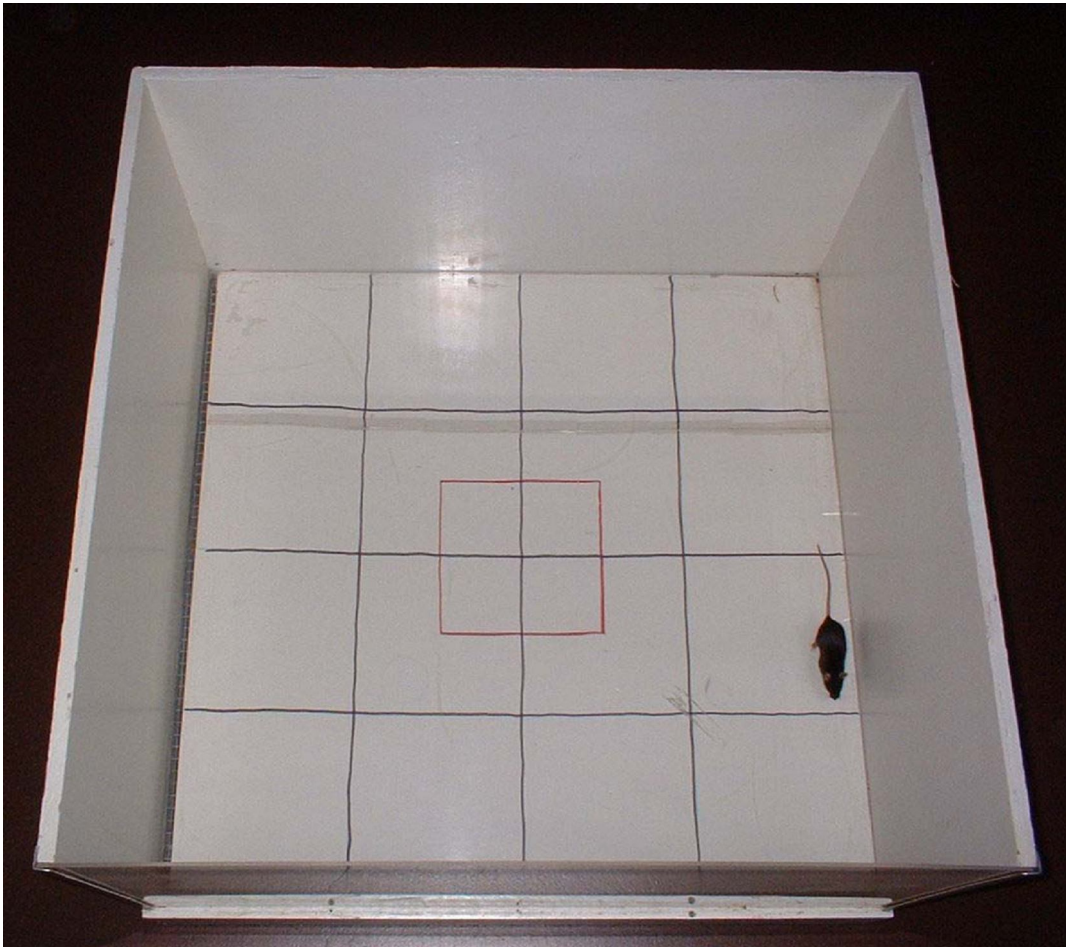


Figure 3.1: Open field

The central square was used because some rats strains have high locomotor activity and cross the lines of the test chamber many times during a test session. Also, the central square has sufficient space surrounding it to give meaning to the central location as being distinct from the outer locations (Carrey *et al.*, 2000). The open field maze was cleaned between each rat using 70 % ethyl alcohol.

Rats were carried to the test room in their home cages and handled by the base of their tails at all times. Rats were placed into the center or one of the four corners of the open field and allowed to explore the apparatus for 5 minutes. After the 5 minute test, rats

were returned in their home cages and the open field was cleaned with 70 % ethyl alcohol and permitted to dry between tests. To assess the process of habituation to the novelty of the arena, rats were exposed to the apparatus for 5 minutes on two consecutive days. This assessment was carried out on day 0, 14 and 28. Rats were trained for 2 consecutive days before the test day.

The following were measured. Line Crossing is Frequency with which the rats crossed one of the grid lines with all four paws. Center Square Entries is frequency with which the rats crossed one of the red lines with all four paws into the central square. Center Square Duration is duration of time the rats spent in the central square. Rearing is frequency with which the rats stood on their hind legs in the maze. Stretch Attend Postures is frequency with which the rat demonstrated forward elongation of the head and shoulders followed by retraction to the original position. Grooming is duration of time the rat spent licking or scratching itself while stationary. Freezing is duration with which the rat was completely stationary. Urination is number of puddles or streaks of urine. Defecation is number of fecal boli produced.

3.4.2 Test for Motor Co-ordination using Beam walk Test (Assay)

The Beam walking test as described by Petrich (2006) was used to assess the motor function. The Beam was made of 1m strips of smooth wood 28 mm in diameter. A narrow support stand was also constructed to hold up the start section of the raised beam (1.5cm cross-section, 50 cm high). Goal box (20 cm on each side, with a 4 × 5cm entrance hole) secured on a narrow support stand (3 cm cross-section, 50 cm high). The middle 80 cm of beam was made by drawing lines 10 cm from the beginning and 10 cm from the end of the beam using a permanent marker (figure 3.2).



Figure 3.2: Beam walk

The rats were made to traverse the beam. Latency (secs) and footslips (number) measurements were taken from this central 80-cm portion of the beam. Thus, any hesitation or pausing, usually associated with either initiation of movement at the start of the beam or entry to the goal box at the end of the beam, did not affect measurements taken.

The time taken to transverse the beam, the number of foot slips (one or both hind limbs slipped from the beam) and the number of falls was measured (Stanley *et al.*, 2005). This assessment was carried out on day 0, 14 and 28. Rats were trained for 3 consecutive days before the test day.

3.4.3 Test for Motor Strength using Forepaw Grip

The forepaw grip time as described by Abou-Donia *et al.* (2001) and modified by Ambali *et al.* (2011) was used to evaluate the effect of treatments on motor strength of the rats. This was assessed by having the rats hung from a 5 mm diameter wooden dowel gripped with both forepaws (figure 3.3).



Figure 3.3: Forepaw grip

The time spent by each rat before releasing their grip was recorded in seconds and this was tried twice during each test session. The results of the two trials were averaged for each testing session. This assessment was carried out on days 0, 14 and 28. Rats were trained for 2 consecutive days before the test day.

3.5 BIOCHEMICAL EVALUATION OF THE BRAIN TISSUE

The biochemical assessments were carried out at the Department of Chemical Pathology, Ahmadu Bello University Teaching Hospital, Shika Zaria Kaduna state. Nigeria. This include: Evaluation of malondialdehyde (MDA) as an index of lipid peroxidation, antioxidant enzymes activity, catalase activity and acetylcholinesterase activity.

3.5.1 Evaluation of Malondialdehyde (MDA) as an Index of Lipid Peroxidation

The level of thiobarbituric acid reactive substance, malondialdehyde (MDA), as an index of lipid peroxidation was evaluated in brain sample using the method of Draper and Hadley (1990) as modified by De Freitas *et al.* (2005). The principle of the method was based on spectrophotometric measurement of the colour developed during the reaction of thiobarbituric acid (TBA) and malondialdehyde (MDA). Brain samples was collected from each animal immediately after decapitation, weighed and then homogenized in a known volume of ice-cold phosphate buffer to obtain a 10% (w/v) homogenate which was then be centrifuged at 600g for 10 min to obtain the supernatant.

The supernatant was mixed with 1 ml 10% trichloroacetic acid and 1 ml 0.67% thiobarbituric acid. The mixture was then heated in a boiling water bath for 15 min and butan-2-ol (2:1, v/v) added to the solution. After centrifugation, the MDA concentration was determined from the absorbance at 532 nm. The MDA concentration in each sample was calculated by the absorbance coefficient of MDA-TBA complex $1.56 \times 10^5/\text{cm/M}$ and expressed as nmol/mg of tissue protein. The concentration of protein in the brain homogenates was evaluated using the Lowry method (Lowry *et al.*, 1951).

3.5.2 Evaluation of Antioxidant Enzymes Activity

The assessment of superoxide dismutase activity was used to determine antioxidant enzyme activity using the method described by De Freitas *et al.* (2005). The 10% (w/v)

homogenate, as obtained above, will be centrifuged at 800g for 20 min and the supernatant obtained was used to assay superoxide dismutase. Superoxide dismutase activity was assayed using xanthine and xanthine oxidase to generate superoxide radicals. They reacted with 2, 4-iodophenyl- 3, 4-nitrophenol-5-phenyltetrazolium chloride to form a red formazan dye. The degree of inhibition of this reaction was used to measure superoxide dismutase activity. The standard assay substrate mixture contained 3 mL xanthine (500 μ m), 7.44 mg cytochrome c, 3.0 mL KCN (200 μ m), and 3.0 mL EDTA (1 mm) in 18.0 mL 0.05 m sodium phosphate buffer, pH 7.0. The sample aliquot (20 μ L) will be added to 975 μ L of the substrate mixture plus 5 μ L xanthine oxidase.

After 1 min, the initial absorbance was recorded and the final absorbance was recorded after 6 min. The reaction was followed at 550 nm. Superoxide dismutase was used to obtain a calibration curve showing the correlation of the inhibition percentage of formazan dye formation and superoxide dismutase activity. Superoxide dismutase activity in the samples was determined from this curve and the results expressed as U/mg of tissue protein.

3.5.3 Evaluation of Catalase Activity

Catalase activity was assessed by the method that uses H₂O₂ to generate H₂O and O₂. The activity was measured by the degree of this reaction. The standard assay substrate mixture contained 0.30 mL H₂O₂ in 50 L 0.05 M sodium phosphate buffer, pH 7.0. The sample aliquot (20 μ L) was added to 980 μ L substrate mixture. The initial absorbance was recorded after 1 min and the final absorbance after 6 min. The reaction was followed at 230 nm.

A standard curve was established using purified catalase under identical conditions. All samples were diluted with 0.1 mmol/L sodium phosphate buffer (pH 7.0) to provoke a

50% inhibition of the diluent rate (i.e. the uninhibited reaction). Results were expressed as mmol¹/min/ μ g of tissue protein. The concentration of protein in the brain homogenates, in both cases, was evaluated using the Lowry method.

3.5.4 Evaluation of Acetylcholinesterase Activity

Each brain was weighed and homogenize with 1% Triton X-100 in 0.1 M sodium phosphate buffer at pH 8 at a ratio of 1/10 (w/v). The homogenate was centrifuge at 1000g for 10 min and the supernatant used for AChE activity. Cholinesterase activity will be determine spectrophotometrically by the Ellman method (Ellman *et al.*, 1961) using acetylthiocholine iodide 30 ml final concentration $\frac{1}{4}$ 0.5 mM) as substrate and 5,50-dithiobis-2- nitrobenzoic acid (DTNB; 200 ml; final concentration $\frac{1}{4}$ 0.33 mM). Assay tubes were completed to 1 ml with Na phosphate buffer, pH 8 as described by Cana[^]das *et al.* (2005). The enzyme activity was calculated relative to protein concentration.

3.6 STATISTICAL ANALYSIS

The statistical analysis was computed using Sigmastat 2.0 for windows. Data collected were analyzed using the one way analysis of variance (ANOVA), followed by post hoc test of scheffe. Results with $p < 0.05$ were considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 ACUTE TOXICITY STUDIES OF LEAD

Table 4.1 Acute Toxicity in wistar rats

Phase I

| DOSE/mg | No of Rats | No of Deaths |
|---------|------------|--------------|
| 10 | 3 | 0 |
| 100 | 3 | 0 |
| 1000 | 3 | 0 |

Phase II

| | | |
|------|---|---|
| 1600 | 3 | 0 |
| 2900 | 3 | 0 |
| 5000 | 3 | 0 |

4.2 EFFECT OF VITAMIN C ON ANXIETY IN LEAD INDUCED TOXICITY IN RATS

The effect of treatments on line square frequency shown on figure II indicates a significant decrease in the total number of lines crossed by rats ($p < 0.05$) in the group treated with lead only 5.83 ± 2.08 . when compared with those treated with normal saline 39.00 ± 4.02 on the 28th day while there was no significant difference in vitamin C and lead + vitamin C groups but an increase of 32.83 ± 5.04 was observed in the group treated with lead + vitamin C after 28 days.

There was a significant decrease in the number of times each rat entered the centre square ($p < 0.05$) in the group treated with lead only, vitamin C and lead + vitamin C when compared with those treated with normal saline (0.5 ± 0.34) on the 14th day (figure II). There was no change in the lead group when compared to the control group. But there was a significant decrease ($p < 0.05$) in the time taken for a rat to spend in the centre square in rats treated with lead only, vitamin C and lead + vitamin C when compared with those treated with normal saline on the 0, 14th and 28th days. There was no change in the lead group when compared to the control (figure 3).

A significant decrease of 2.67 ± 1.50 ($p < 0.05$) in the number of stretches was observed in rats treated with lead only on day 14th and 28th (figure IV) while there was no significant change in groups treated with vitamin C and lead + vitamin C when compared with those treated with normal saline after 28th days. The increase of 10 ± 2.04 in the lead + vitamin C group was also observed (figure 4). A significant decrease 5.83 ± 2.02 ($p < 0.05$) in the number of rears in rats treated with lead only on day 14th and 28th was observed (figure 5) with a considerable increase of 11.67 ± 1.17 while there no

significant change across the groups treated with vitamin C and lead + vitamin C when compared with those treated with normal saline on the 0, 14th and 28th days.

There was a significant increase of 10.17 ± 1.78 ($p < 0.05$) in the number of grooms in rats treated with lead only on day 14th and 28th while there a significant decrease of 9.67 ± 1.02 in the lead + vitamin C group when compared with those treated with normal saline on the 0, 14th and 28th days as shown on figure VI. There was a significant increase of 4.83 ± 1.22 ($p < 0.05$) in the number of times an animal freeze in rats treated with lead only on day 14th and 28th while there was significant decrease of 1.5 ± 0.43 in the group treated with lead + vitamin C when compared with those treated with normal saline on the 0, 14th and 28th days (figure VII).

There was a significant decrease 1.33 ± 0.80 ($p < 0.05$) in the number of boli of faeces in rats treated with lead only on day 14th and 28th (figure VIII) while there no significant change in groups treated with vitamin C and lead + vitamin C when compared with those treated with normal saline on the 0, 14th and 28th days . There was a significant decrease 0.16 ± 0.16 ($p < 0.05$) in the number of streaks of urine in rats treated with lead only on day 14th and 28th (figure 9) while there no significant change in groups treated with vitamin C and lead + vitamin C when compared with those treated with normal saline on the 0,14th and 28th days.

Table 4.2 Parameters of Anxiety in an open field test in wistar rats for day 0.

| GROUPS | LINE SQUARE FREQUENCY | CENTER SQUARE FREQUENCY | CENTER SQUARE DURATION | STRETCH ATTEND | REARING | GROOMING | FREEZING | DEFAECATION | URINATION |
|-------------------------|--------------------------|-------------------------------|------------------------------|-------------------|------------|------------|-----------|-------------|-----------|
| CONTROL | 17.33±6.52 | 0.50±0.34 | 0.83±0.65 | 14.33±4.01 | 8.00±3.56 | 7.50±5.1 | 1.83±0.40 | 3.83±1.30 | 1.16±.83 |
| DIAZEPAM (0.05mg/kg) | 30.83±11.07 | 0.33±0.33 | 1.16±1.16 | 16.83±4.33 | 11.00±4.89 | 12.10±3.90 | 1.83±0.40 | 2.83±0.94 | 0±0 |
| LEAD (250mg/kg) | 35.67±7.29 | 0±0 | 0±0 | 14.16±1.46 | 10.5±3.48 | 14.83±4.57 | 4.00±1.63 | 4.00±0.63 | 3.83±1.07 |
| VITAMIN C (100mg/kg) | 30.00±4.00 | 0±0 | 0±0 | 15.33±2.03 | 7.50±2.43 | 13.5±4.65 | 2.83±0.70 | 1.50±0.71 | 0±0 |

Table 4.3 Parameters of Anxiety in an open field test in wistar rats for day 14.

| GROUPS | LINE SQUARE FREQUENCY | CENTER SQUARE FREQUENCY | CENTER SQUARE DURATION | STRETCH ATTEND | REARING | GROOMING | FREEZING | DEFAECATION | URINATION |
|----------------------|-----------------------|-------------------------|------------------------|----------------|------------|-------------|------------|-------------|-----------|
| CONTROL | 21.50±6.20 | 0.33±0.33 | 0.50±0.50 | 12.00±2.32 | 2.67±1.14 | 5.50±1.52 | 3.03±.74 | 2.67±0.80 | 2.83±1.04 |
| DIAZEPAM (0.05mg/kg) | 24.17±4.44 | 0.33±0.33 | 1.33±1.14 | 8.33±1.40 | 7.00±1.21 | 3.67±2.20 | 3.16±0.60 | 3.50±0.62 | 2.00±0.89 |
| LEAD (250mg/kg) | 8.67±4.09* | 0±0 | 0±0 | 4.00±1.23* | 2.33±0.98* | 15.50±6.25* | 4.83±1.16* | 1.00±0.44* | 1.33±0.80 |
| VITAMIN C (100mg/kg) | 21.00±7.81* | 0±0 | 0±0 | 6.33±1.87* | 7.00±2.46* | 5.67±1.25* | 2.33±0.33* | 1.33±0.50* | 0.83±0.30 |

Table 4.4 Parameters of Anxiety in an open field test in wistar rats for day 28

| GROUPS | LINE SQUARE FREQUENCY | CENTER SQUARE FREQUENCY | CENTER SQUARE DURATION | STRETCH ATTEND | REARING | GROOMING | FREEZING | DEFAECATION | URINATION |
|----------------------|-----------------------|-------------------------|------------------------|----------------|-------------|------------|------------|-------------|------------|
| CONTROL | 39.00±4.02 | 0±0 | 0±0 | 15.50±1.43 | 7.16±1.10 | 1.67±0.33 | 1.67±0.91 | 1.33±0.42 | 5.00±1.63 |
| DIAZEPAM (0.05mg/kg) | 13.17±2.08 | 0±0 | 0±0 | 9.50±0.76 | 2.50±0.56 | 4.00±1.03 | 2.83±0.94 | 1.17±0.60 | 1.67±0.33 |
| LEAD (250mg/kg) | 5.83±2.66* | 0±0 | 0±0 | 2.67±1.50* | 5.83±2.02* | 4.83±1.22* | 1.33±0.80* | 0.16±0.16 | 10.17±1.78 |
| VITAMIN C (100mg/kg) | 32.83±5.02* | 0±0 | 0±0 | 10.01±2.04* | 11.66±1.17* | 1.50±0.43* | 0.83±0.65* | 0.50±0.34 | 9.67±1.02 |

4.3 EFFECT OF VITAMIN C ON MOTOR COORDINATION IN LEAD INDUCED RATS

There was a significant increase 47.25 ± 12.84 ($p < 0.05$) in latency in rats treated with lead only and diazepam on day 28th while there no significant change in groups treated with vitamin C and lead + vitamin C (12.67 ± 4.22) when compared with those treated with normal saline (12.5 ± 1.00) after 28 days of administration as shown on figure 10.

There was a significant increase ($p < 0.05$) in foot slips in rats treated with lead only and diazepam on day 28th (figure XI) while there no significant change in groups treated with vitamin C and an ameliorative effect on lead + vitamin C (10.0 ± 3.2) when compared with those treated with normal saline on the 28th day.

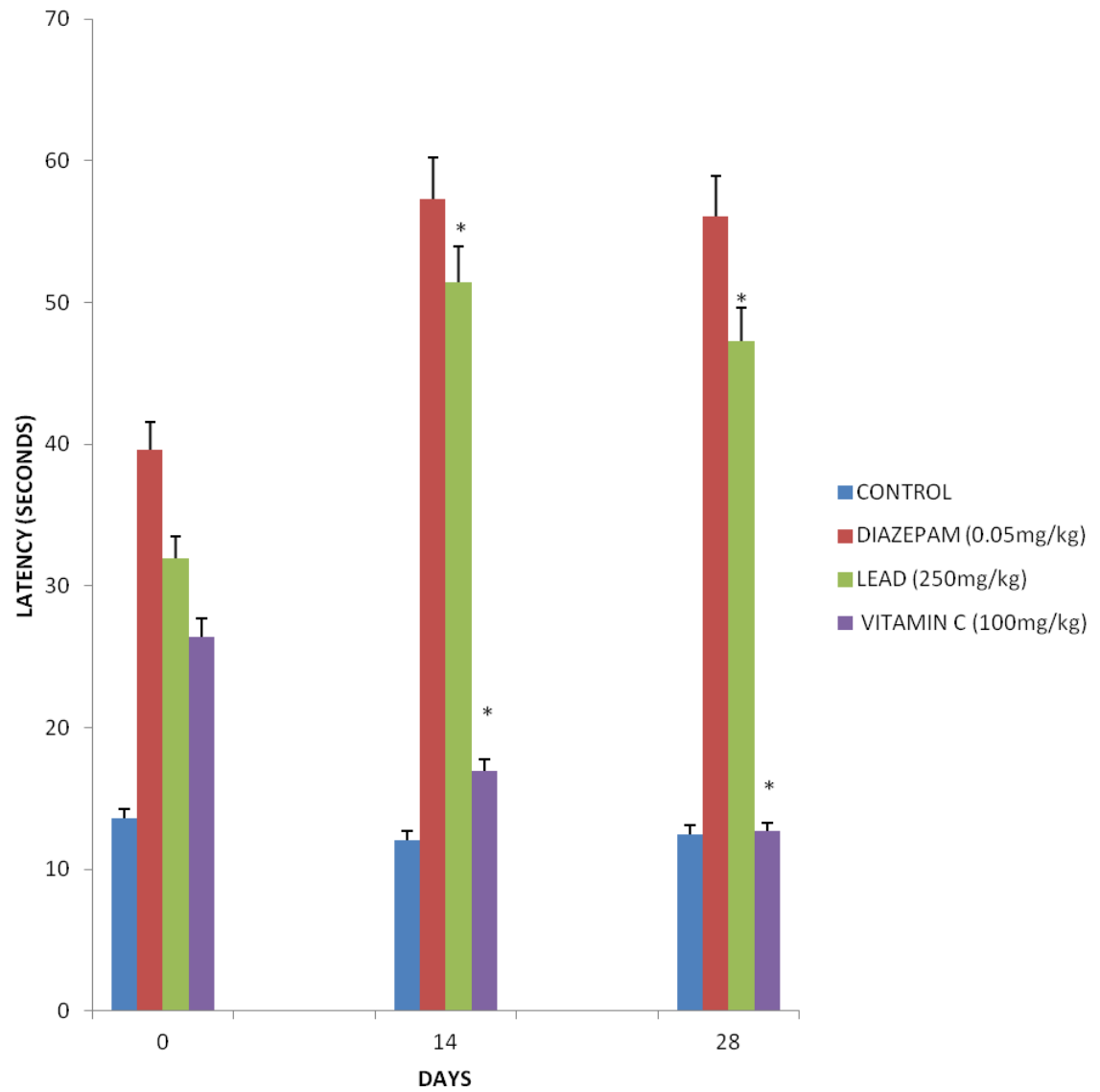


Figure X: Effects of treatment groups on latency in beam walk test. *P<0.05 compared with the control.

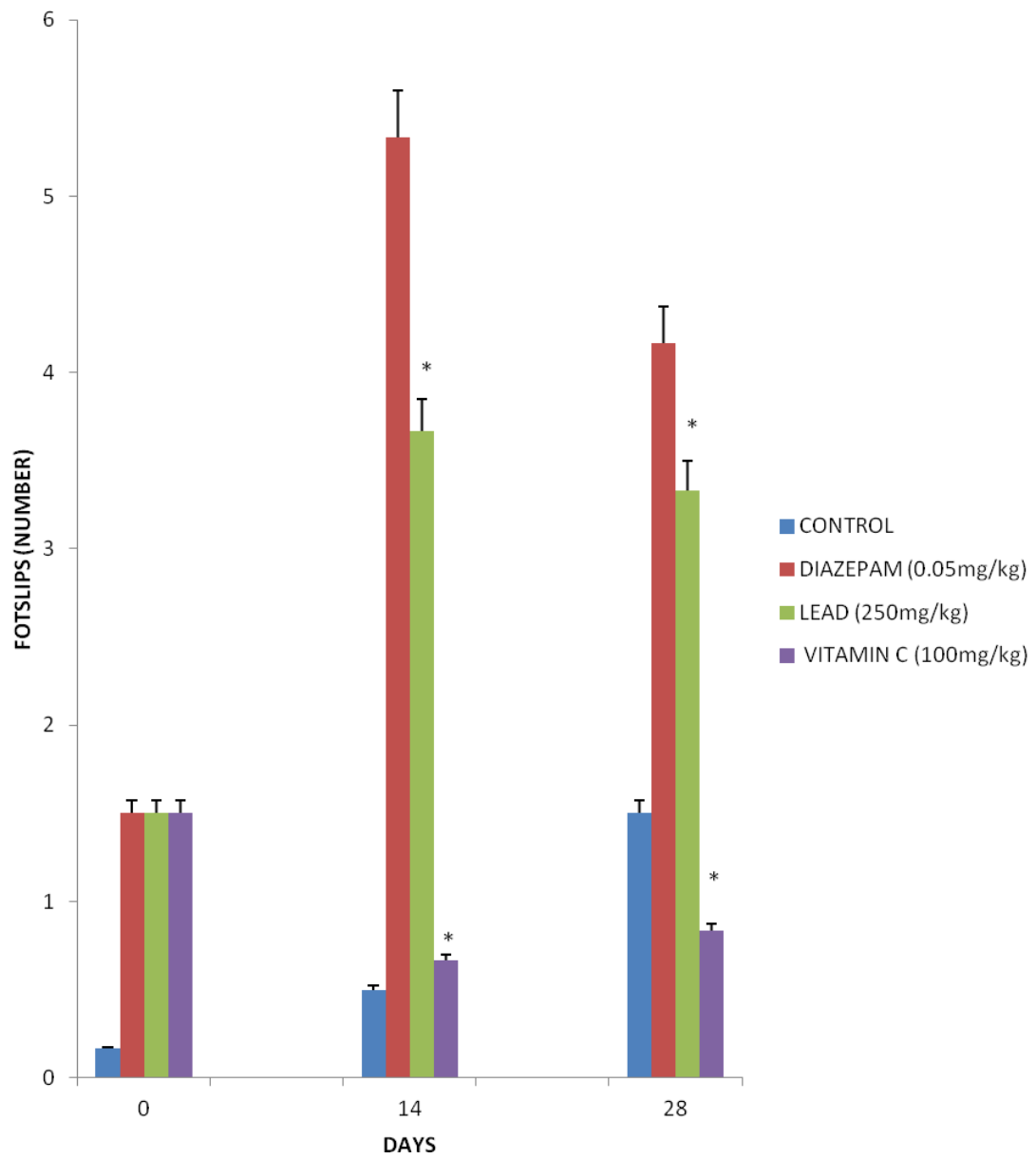


Figure XI Effects of treatment groups on footslips in beam walk test for rats. *P<0.05 compared with the control.

4.4 EFFECT OF VITAMIN C ON MOTOR STRENGTH IN LEAD INDUCED TOXICITY IN RATS

The effect of treatments on forepaw grip is shown on figure XII. There was a significant decrease 10.33 ± 0.89 ($p < 0.05$) in the time taken to hold on to a grip in rats treated with lead only on day 28 while there no significant change in groups treated with vitamin C (24.33 ± 1.34) and lead + vitamin C (19.50 ± 2.48) when compared with those treated with normal saline (25.00 ± 4.33) on the 28 day.

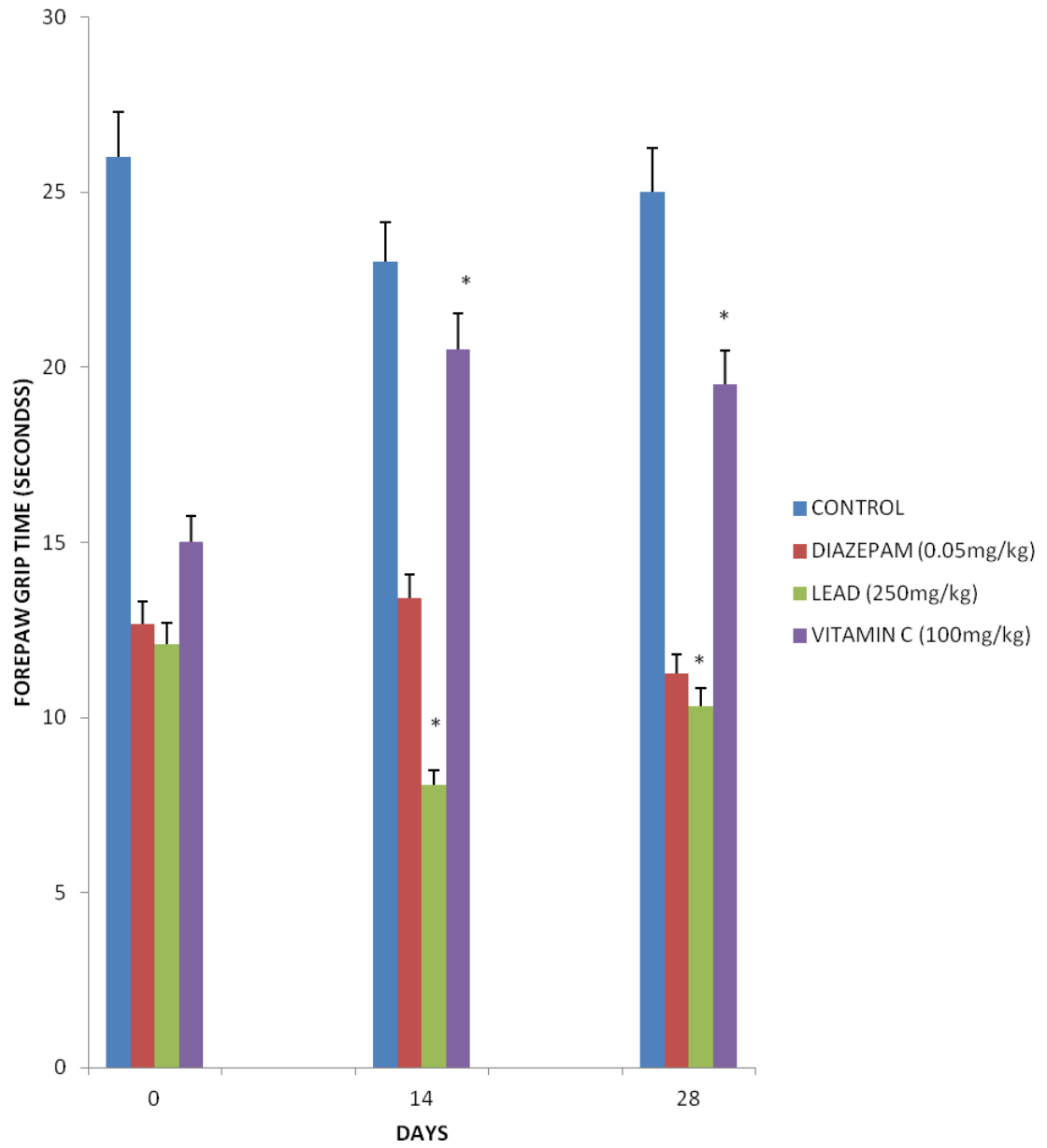


Figure XII Effects of treatment groups on forepaw grip for rats. *P<0.05 compared with the control.

4.5 EFFECT OF VITAMIN C ON OXIDATIVE STRESS IN LEAD INDUCED TOXICITY IN RATS

The effect of treatments on concentration of MDA is shown on figure XIII. There was a significant decrease of 1.8 ± 0.14 ($p < 0.05$) in the MDA concentration in rats treated with lead only while there no significant change amongst groups treated with lead and lead + vitamin C when compared with those treated with distilled water.

The effect of treatments on concentration of superoxide dismutase is shown on figure XIV. There was a significant decrease of 1.86 ± 0.14 ($p < 0.05$) in the superoxide dismutase concentration in rats treated with lead only while there no significant change amongst groups treated with lead and lead + vitamin C when compared with those treated with distilled water .

The effect of treatments on concentration of catalase is shown on figure XV. There was a significant decrease of 40 ± 4.00 ($p < 0.05$) in the catalase concentration in rats treated with lead only while there no significant change amongst groups treated with lead and lead + vitamin C (48.2 ± 3.92) when compared with those treated with distilled water.

The effect of treatments on concentration of Acetylcholinesterase enzyme is shown on figure XVI. There was a significant decrease of 20.2 ± 0.49 ($p < 0.05$) in the Acetylcholinesterase enzyme concentration in rats treated with lead only while there no significant change amongst groups treated with lead and lead + vitamin C (22.0 ± 2.61) when compared with those treated with normal saline.

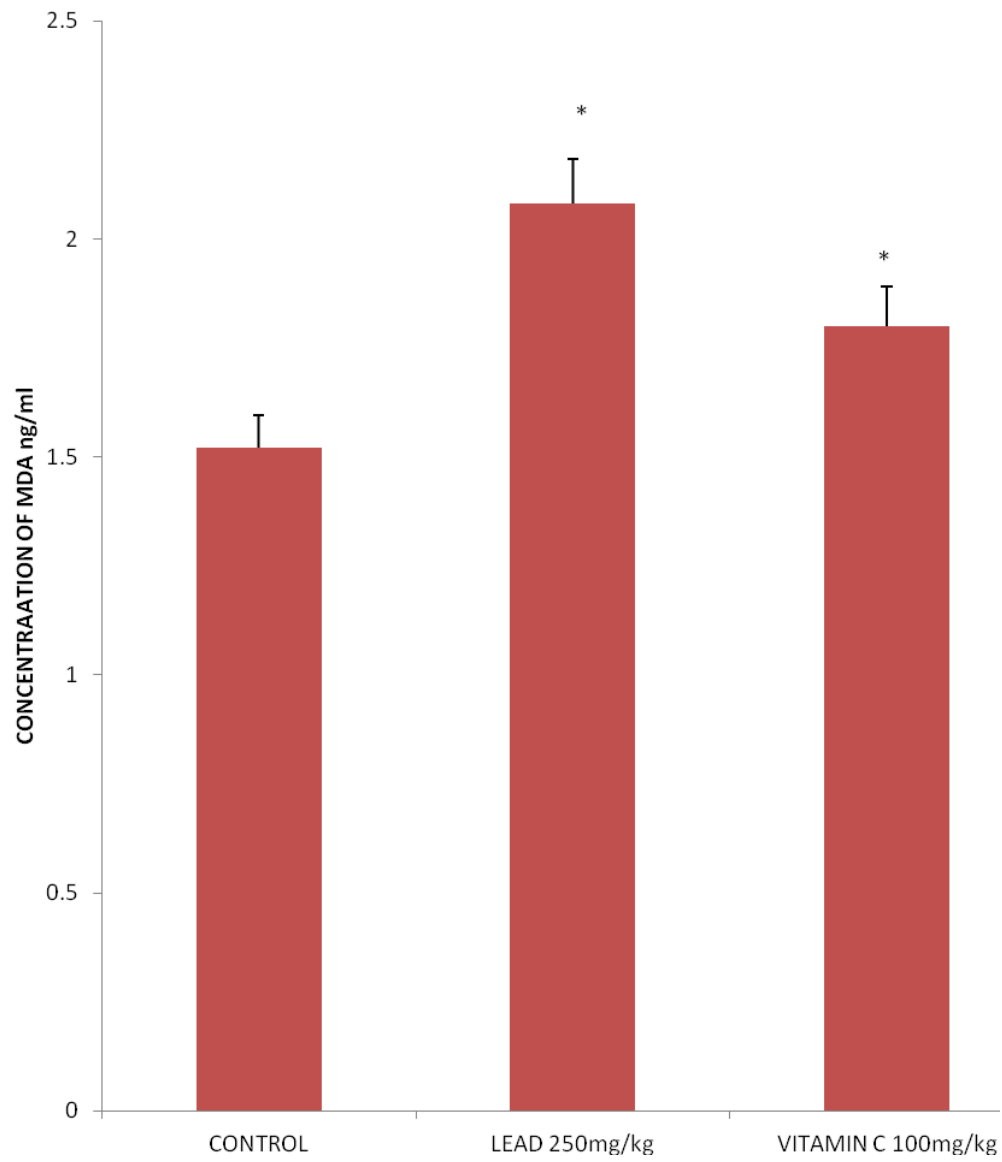


Figure XIII: Effects of Treatment on the concentration of MDA. *P<0.05 compared with the control.

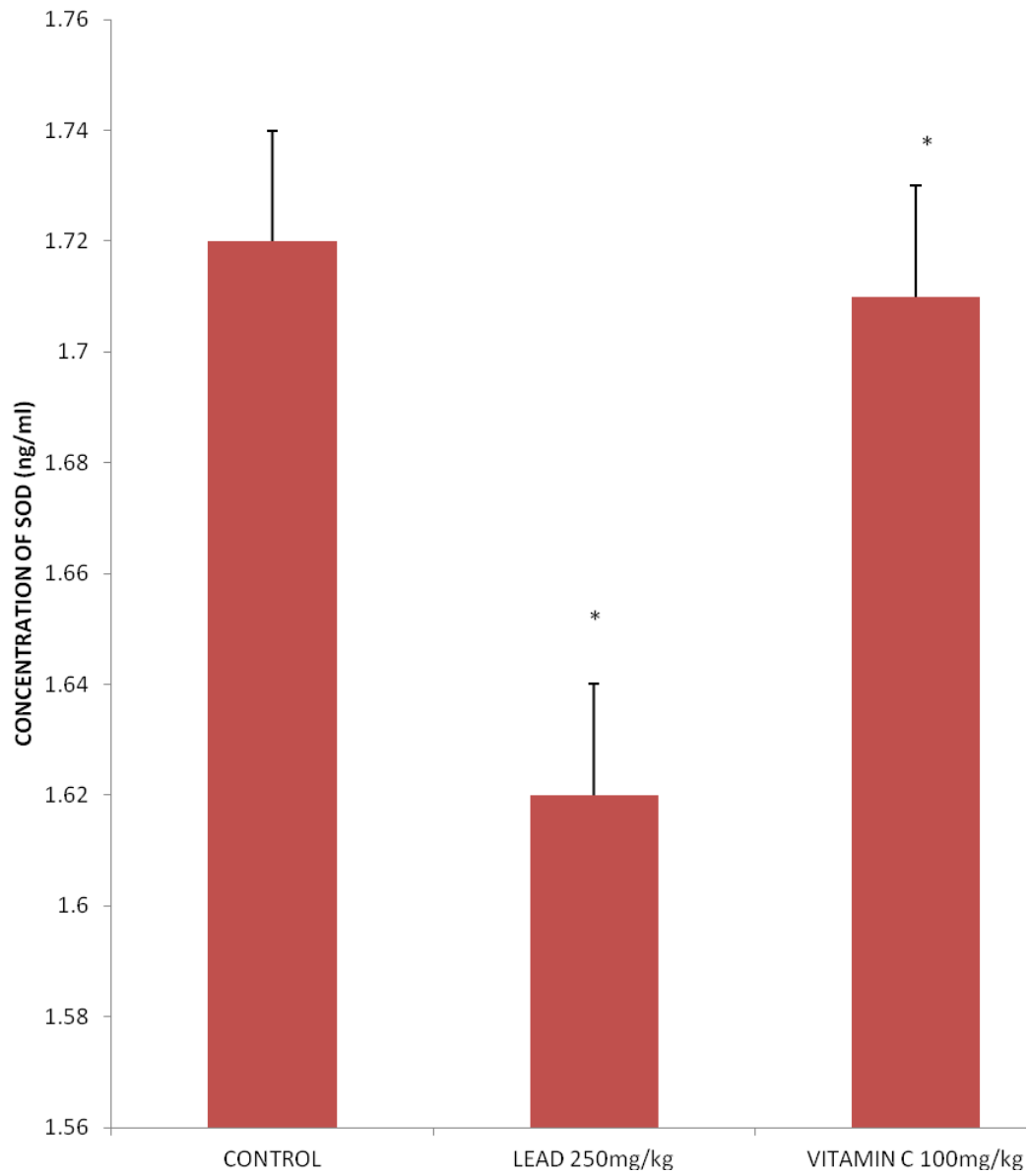


Figure XIV Effects of Treatment on the concentration of SOD. *P<0.05 compared with the control.

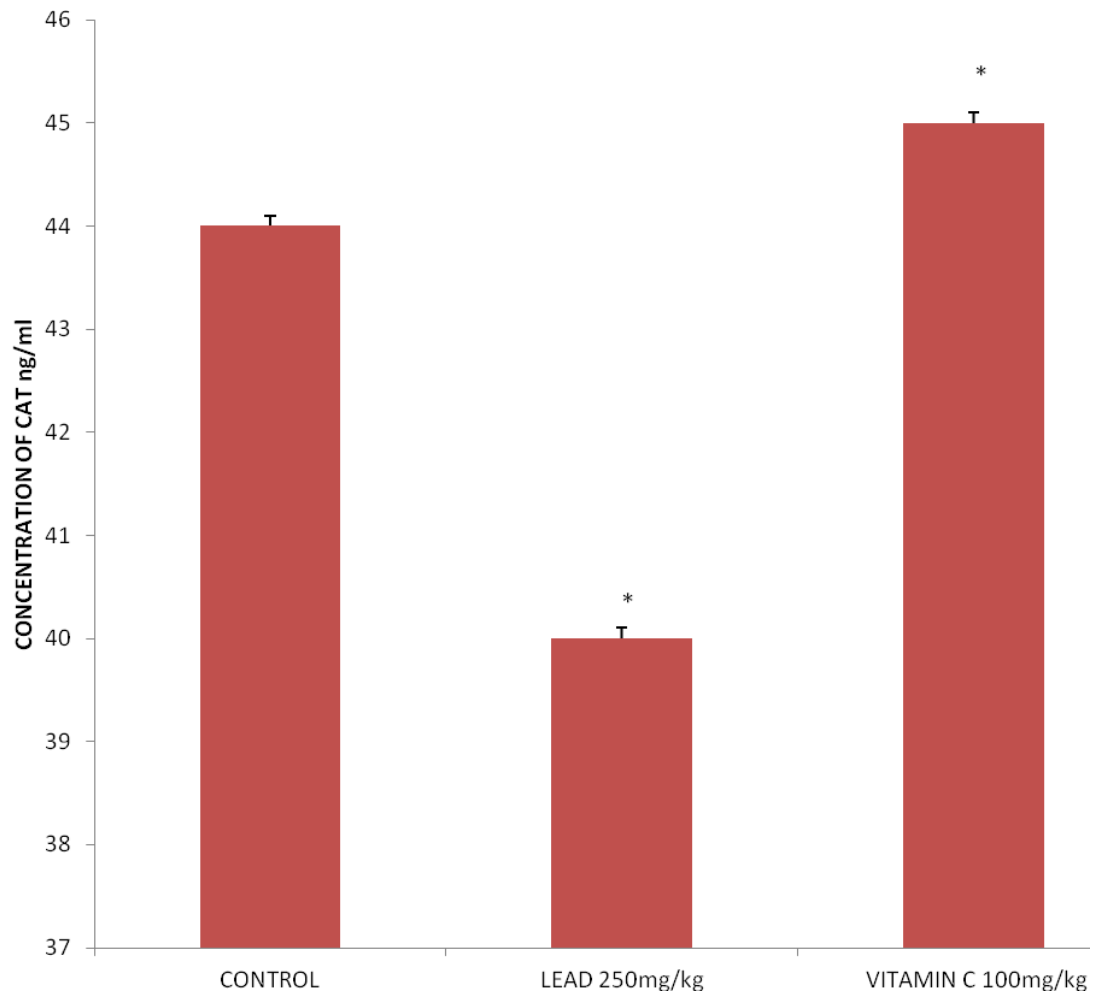


Figure XV: Effects of Treatment on the concentration of CAT. *P<0.05 compared with the control.

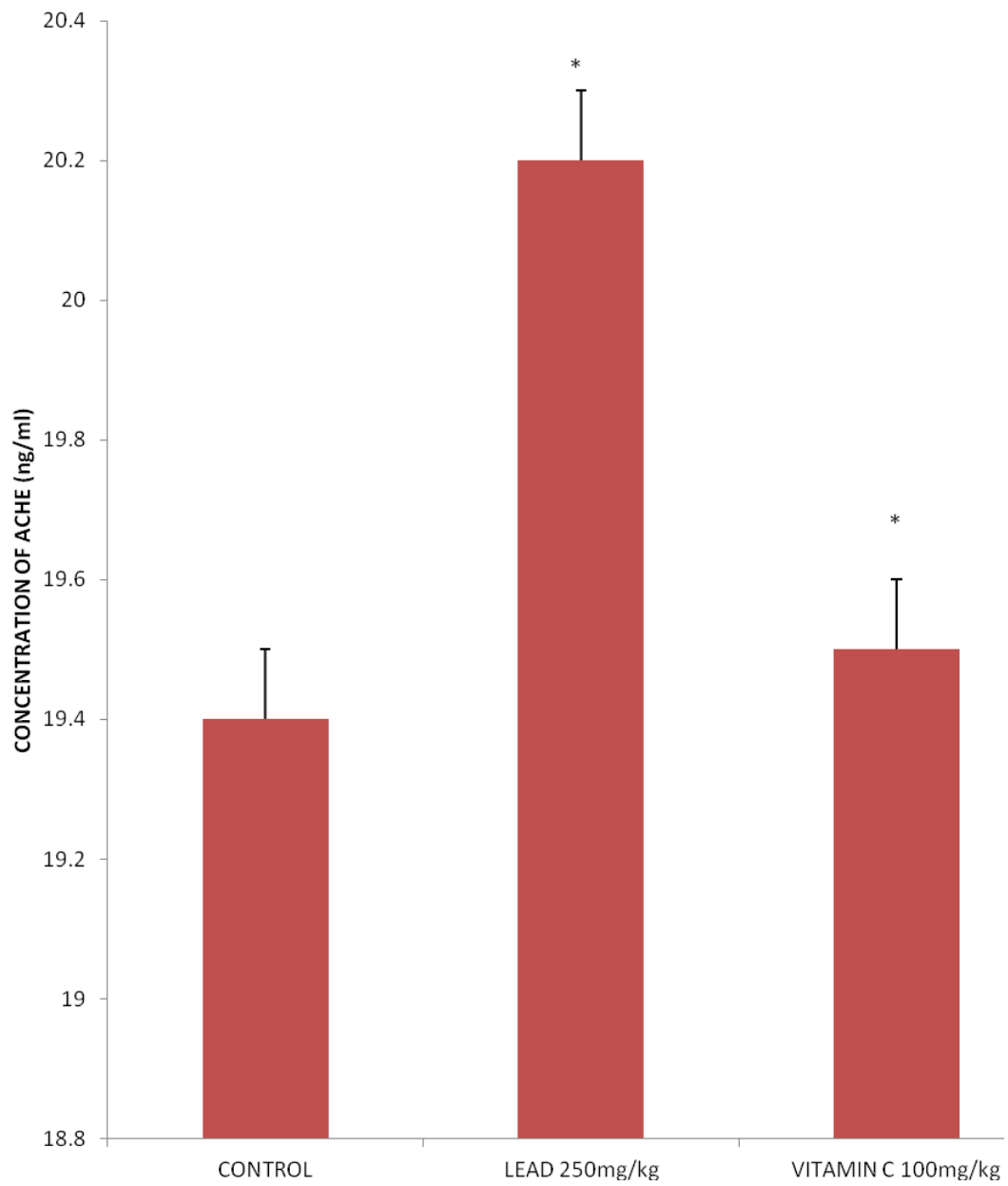


Figure XVI Effects of Treatment on the concentration of ACHE. *P<0.05 compared with the control.

CHAPTER FIVE

5.0 DISCUSSION

There are few studies conducted on anxiety and motor coordination in lead induced rats, therefore in this present study the adverse effect of lead on the brain was established using the open field test, beam walk test and fore paw grip to assess the level of anxiety and motor coordination in rats.

From the results obtained, rats in the group treated with lead were observed to have a decrease in the time taken to cross lines, centre square entries, centre square duration, number of boli, number of streaks of urine, number of grooms and rears on the 14th and 28th day as compared with those treated with normal saline and diazepam on day 0, 14 and 28. The low number of these parameters indicates low amount of anxiety as reported by Walsh and Cummins 1976, but with the administration of lead + vitamin C there was a remarkable increase in crossing of lines, centre squares entries, number of boli, number of grooms and rears.

Hall (1934) describes defecation and urination as indices of anxiety in rodents. He argues that the animal will have reduced locomotion in a novel environment but the autonomic nervous system will be activated which will increase defecation in this noxious arena. However, Bindra and Thompson argue that there is no significant relation between fearfulness, urination and defecation as measured in the Open Field test. Nevertheless, Bindra and Thompson agree that defecation and urination in a novel environment are signs of emotionality, which is not to be equated with fearfulness or timidity.

Therefore this study reveals that there is no relationship between number of boli and streaks of urine with the level of anxiety. It was noted that rats treated with lead did not produce any boli or even streaks of urine as a result of inactivity. They were virtually

immobile throughout the experiment. But those administered with lead + vitamin C showed a lot of mobility.

Anxiety is a motor response to emotion and is controlled by complex mechanisms that involve association areas of the cerebral cortex, the limbic system and the hypothalamus. This study then suggests that lead might have interfered with these areas. The administration of lead in rats showed placidity which is calmness with little or no response to provocation. This might be as a result of the stimulation of the ventromedial nucleus of the hypothalamus. Bilateral amygdaloid lesion has also been seen to inhibit the rage area and facilitate placidity. Lead was observed to have depressed the CNS but with the administration of vitamin C there was a considerable increase in anxiety which probably has a protective effect on the brain and may be due to its antioxidant activity. Apart from its antioxidant properties, some other nonantioxidant activity of vitamin C may have complemented in the brain function.

In the result obtained, there was a statistical significant increase in latency in the group treated with lead when compared to the control group on the 14th and 28th day. But the group treated with lead + vitamin C showed a marked decrease in latency. The lead group showed properties similar to those of the diazepam group. Similarly there was also an increase in the number of footslips in the lead group as compared with the control and vitamin C. The lead + vitamin C group showed a decrease in the number of footslips. However, beam walking across bridges of different cross-sections also provided a well-established method of monitoring motor coordination and balance in rats (Gentile *et al.*, 1978; Feeney *et al.*, 1982; Goldstein and Davis, 1990b; Boyeson *et al.*, 1992; Majchrzak *et al.*, 1992; Soblosky *et al.*, 1996).

The increase in latency and number of foot slips in the lead group indicates a progressive increase in impairment in motor function. This study therefore reveals that lead actually caused muscular in-coordination which was evident in rats treated with lead. The cerebellum plays a minor role in maintaining posture through a servocomparator function might have been affected. Thereby distorting signals from the vestibular apparatus (non auditory labyrinth) and proprioceptive signals from different part of the body and then removes corrective signals that adjusts muscle tone leading to poor posture and balance.

The cerebellum plays a major role in the maintaining equilibrium during rapid motions and motions with rapidly changing direction through a predictive function.

The basal ganglia might also be implicated in lead poisoning. The basal ganglia plays an important role in controlling the muscle tone and voluntary movements the caudate nucleus stimulates muscle tone through stimulation of vestibular nucleus and inferior olive. It receives projection fibers from the motor cortex to corpus striatum, globus pallidus and to several nuclei in the brainstem. The neurons and nerves might also be implicated there by interrupting the nervous pathway.

With the administration of Vitamin C followed by lead there was a considerable amelioration or protection which increased the latency period and footslips. Some level of improvement in the latency and number of footslips in the group pretreated with vitamin C probably underscores the role of oxidative stress in brain dysfunction

The fore grip test is used to assess the motor strength and also the integrity of the muscle. It was observed that the time taken for a rat to fall off a grip decreased in the lead group when compared with the control and vitamin C on day 14 and 28 respectively, the lead + vitamin C showed a significant increase in the time taken to fall off a grip, this may be due inactivity of neuromuscular junction.

With respect to functional strength, grip strength decreased by the end of the repetitive behavioral task which also suggest that reduced grip strength is a measure of inflammation-induced muscle hyperalgesia. However, poor sensorimotor control may also contribute to declines in strength (Ballermann *et al.*, 2001). This study reveals a strong relationship between lead toxicity and motor strength. Increase in lead concentration decrease motor strength. This corroborates with previous studies by Goyer 1990 which suggested that Lead is common environmental toxin that is capable of causing numerous acute and chronic illnesses, that affects each and every organ and system in the body including muscles.

The muscle tone plays a dominant role in the muscle strength. The muscle tone is mild maintained contraction of innervated in muscles, provides a background of facilitation for performing fine movement, it accounts for 20-30% of heat production in the body it helps in venous return by squeezing and preventing dilation of veins between muscles and helps lymphatic drainage by squeezing and preventing dilation of lymphatics vessels between muscles. Neurotoxicity from lead exposure is of concern especially because lead at even very low concentrations can have profoundly detrimental neurological effects (Bellinger, 2004).

Also, lead has been suggested to generate reactive oxygen species (ROS) that result in lipid peroxidation, DNA damage and alteration of antioxidant defense systems of cells represented by superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) (Hsu and Guo, 2002; Ahmed and Siddiqui, 2007). Another role was through the damage of phagocytic activity of Muller cells is by depleting these cells from their GSH content. This consequently affects the harmonical action of the other endogenous antioxidant as SOD and CAT. Several metal chelators have been used to manage lead toxicity in the

event of exposure, but none could be found suitable for reducing lead burden in chronic lead exposure (Osweiler, 1999).

Lead-induced oxidative stress in the brain and other soft tissues has been postulated to be one of the possible mechanisms of lead-induced toxic effects (Pande *et al.*, 2001). Disruption of pro-oxidant/antioxidant balance might lead to the tissue injury. It was reported that lead increased the level of lipid peroxidation (Upasanai and Balaraman, 2001) and altered the antioxidant defense system (Adanaylo, 1999). Several antioxidant enzymes and molecules have been used to evaluate lead-induced oxidative damage in animal and human studies.

In the lead group there was an increase in concentration of MDA as compared with those of the control group. There was no significant difference in the group treated with vitamin C only. The lead + vitamin C showed a significant decrease in MDA. The study revealed a significant increase in the MDA concentration in the brain of lead group as compared to other groups.

This increased MDA concentration is indicative of oxidative damage to the brain tissue this may be attributed to the high metabolic rate in the brain. Oxidative stress induction is one of the molecular mechanisms of lead poisoning. Oxidative stress characterised by elevation in the steady state concentration of reactive oxygen species (ROS), has been implicated in the wide range of biological and pathological conditions. Lead induces lipoperodation by inhibiting the activity of δ -aminolevulinic acid dehydrase leading to accumulation of its substrate δ -aminolevulinic acid, which rapidly oxidize to generate free radicals superoxide ion, hydroxyl radical, and hydrogen peroxide (Oladipo, 2010; Moreira, *et al.*, 2001; Ambali, *et al.*, 2011).

The concentration of catalase in the lead group decreased significantly in the lead group while there was a significant increase in the group treated with lead and an increase in the group treated with lead + vitamin C. Administration of vitamin C significantly inhibited the lipid peroxidation levels of the brain, and increased the catalase (CAT) levels of the brain in lead-exposed rats (Patra *et al.*, 2001).

The concentration of ACHE decreased grossly in the group treated with lead and an increase in the group treated with lead + vitamin C and vitamin C only. This could be as a result of decrease amount of ACHE at the neuromuscular junction and may lead to muscular inco-ordination.

The concentration of superoxide dismutase in the group treated with lead decreased when compared with the group treated normal saline and increase in the lead group. There was no statistical difference in the group treated with vitamin C. Modifications in SOD activity are the most frequently used markers in brain or other tissues. Based on the observation that free radical was generated during the pathogenesis processes induced by lead exposure, it was presumed that supplementation of antioxidants could be an alternative method for chelation therapy (Flora, *et al.*, 2003). Specifically, ascorbic acid, the known chelating agent with antioxidant features, was widely reported with the capability of protecting cells from oxidative stress (Ramanathan, *et al.*, 2002 and Patra, *et al.*, 2001). Besides, additional protective effect of vitamin C on cell apoptosis was revealed in a recent study (Gruss and Fabian, 2002).

This study showed that administration of vitamin C with lead exposed rats exerts an obvious ameliorating as well as treatment effects. This improvement might be related to its antioxidant efficacy that inhibits lipid peroxidation enhanced by lead. Vitamin C (ascorbic acid) is a low molecular mass antioxidant that scavenges the aqueous ROS by

very rapid electron transfer that inhibits lipid peroxidation (Halliwell and Gutteridge, 1985).

5.1 CONCLUSION

It can be concluded that the reduced level of anxiety, muscular incoordination, imbalance and muscular weakness in rats following exposure to lead can be ameliorated by the use of pretreatment with vitamin C. From the data obtained in this study it suggests that that increase in the production of malonadialdehyde, decrease production of catalase, superoxide dismutase and acetylcholinesterase were ameliorated by the antioxidant properties of vitamin C.

5.2 RECOMMENDATIONS

1. A specific part of the brain should be used for the biochemical analysis instead of the whole brain that was used in this study for example the hypothalamus.
2. Some types of maze and models should be used in addition to the ones used in the present study.
3. Histopathology of the brain or a section of brain should be carried out.

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APPENDIX I

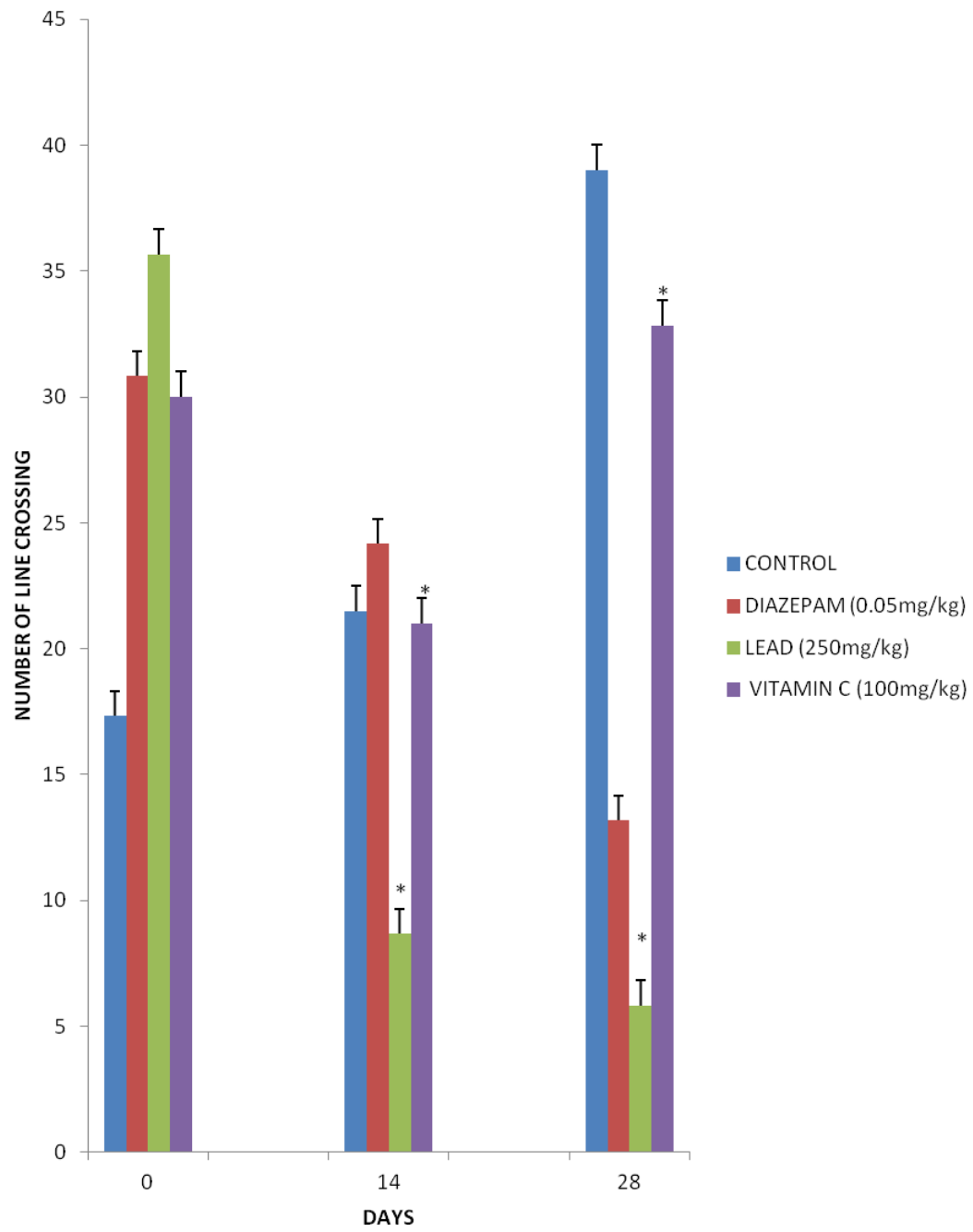


Figure I Effects of treatment groups on Line crossing. Values are expressed as mean \pm SEM (n=6). *P<0.05 compared with the control.

APPENDIX II

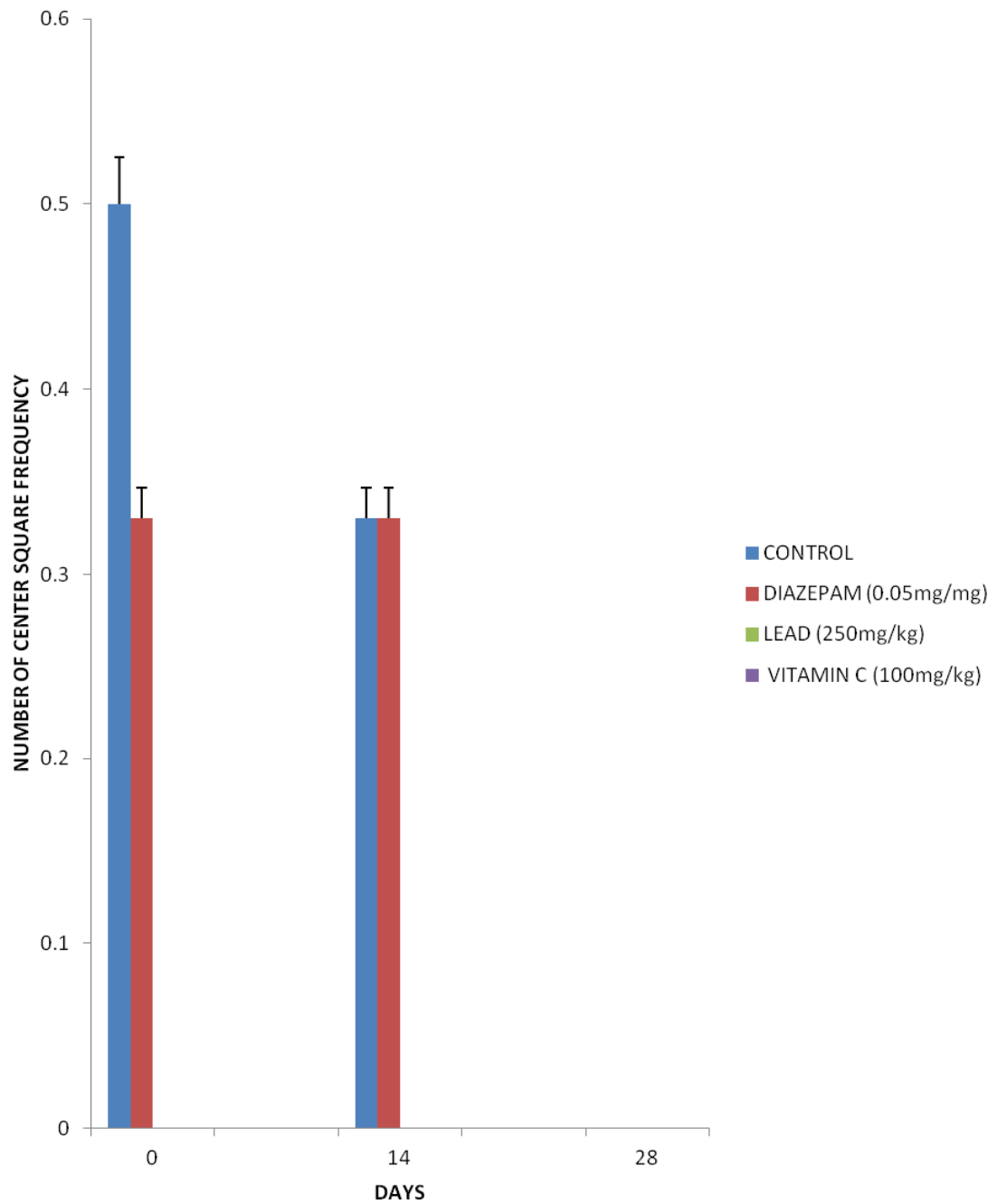


Figure II Effects of treatment groups on center square frequency. Values are expressed as mean \pm SEM (n=6). *P<0.05 compared with the control.

APPENDIX III

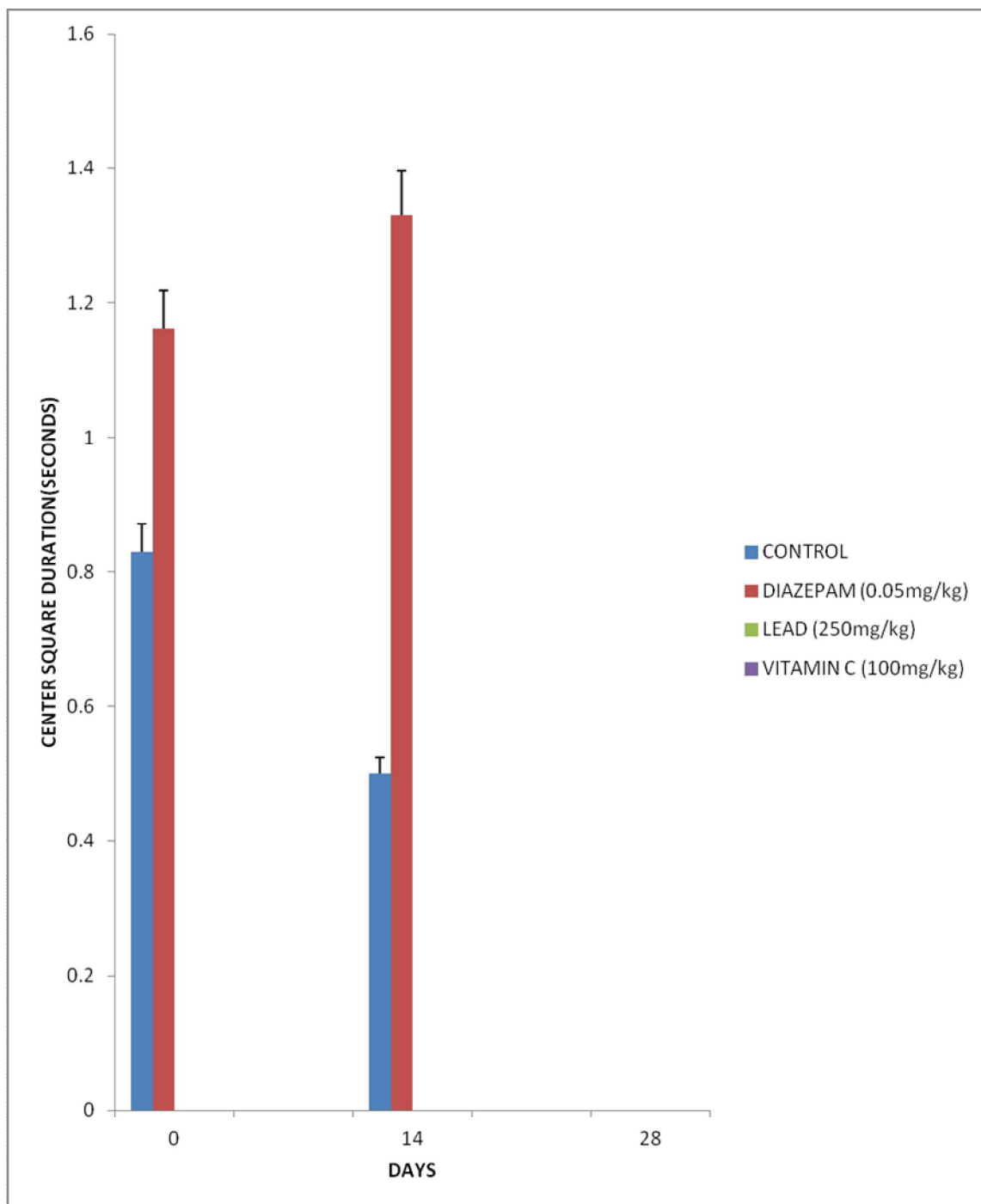


Figure III Effects of treatment groups on center square duration. Values are expressed as mean \pm SEM (n=6). *P<0.05 compared with the control.

APPENDIX IV

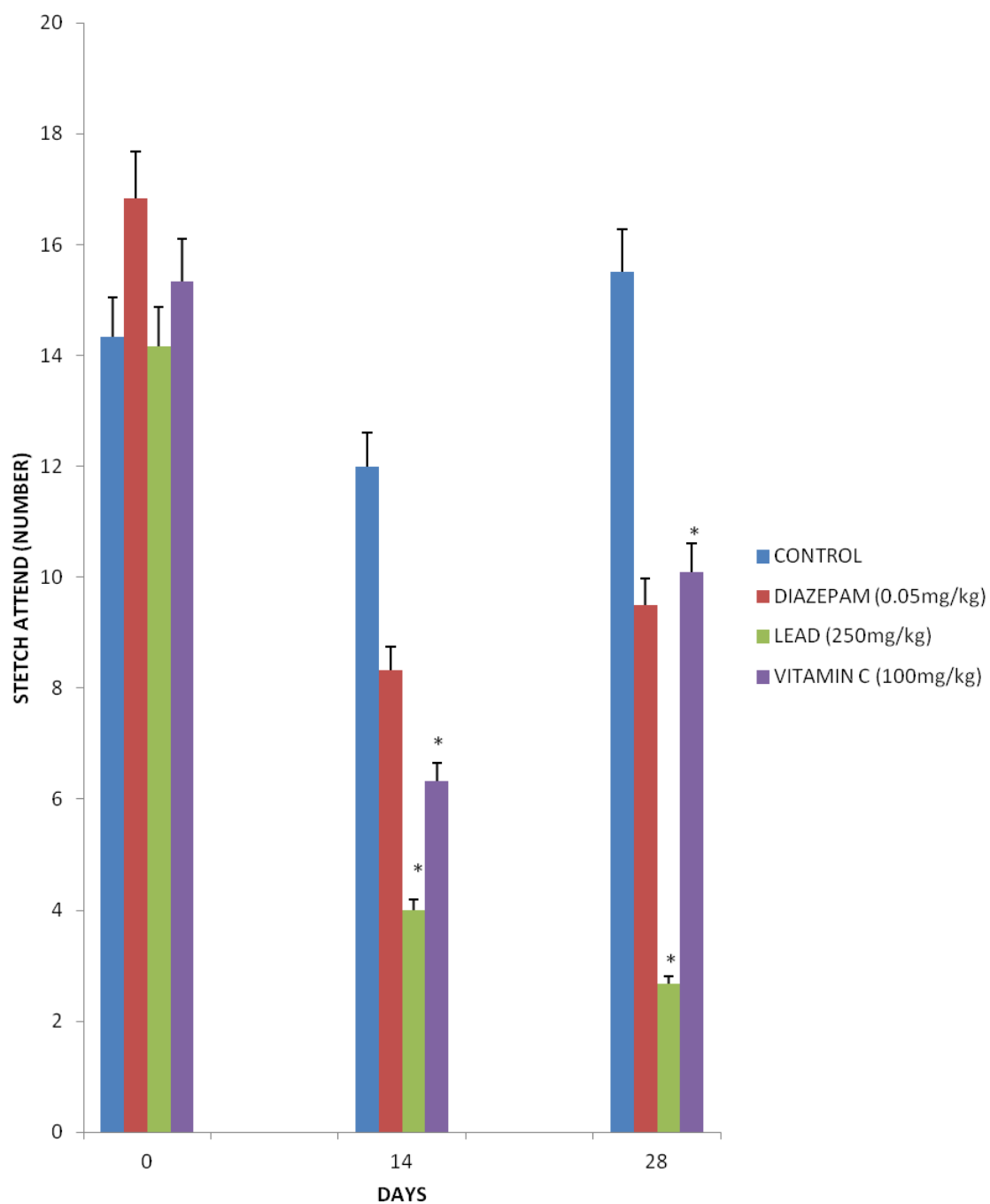


Figure IV Effects of treatment groups on stretch attend. Values are expressed as mean \pm SEM (n=6). *P<0.05 compared with the control.

APPENDIX V

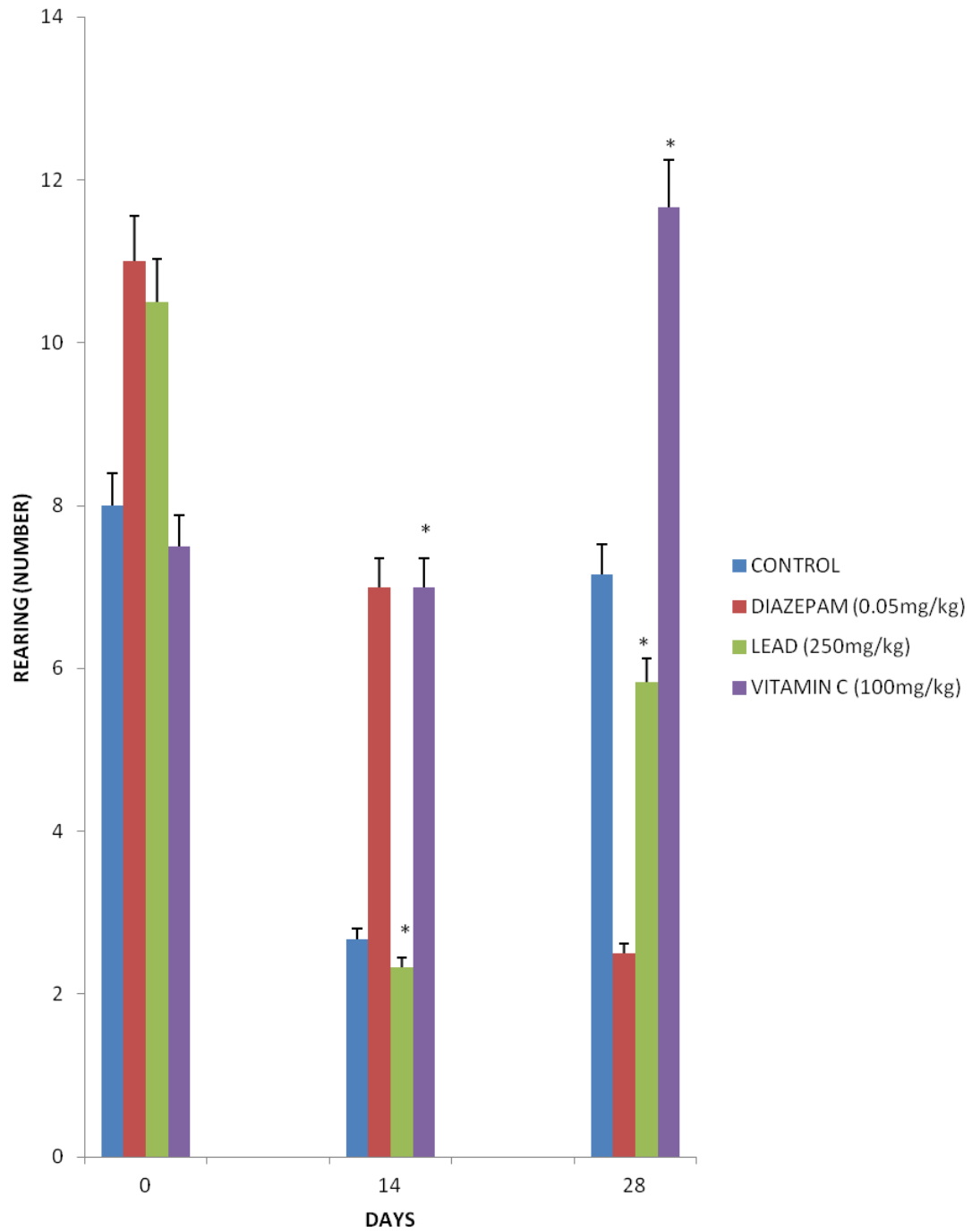


Figure V Effects of treatment groups on rearing. Values are expressed as mean \pm SEM (n=6). *P<0.05 compared with the control.

APPENDIX VI

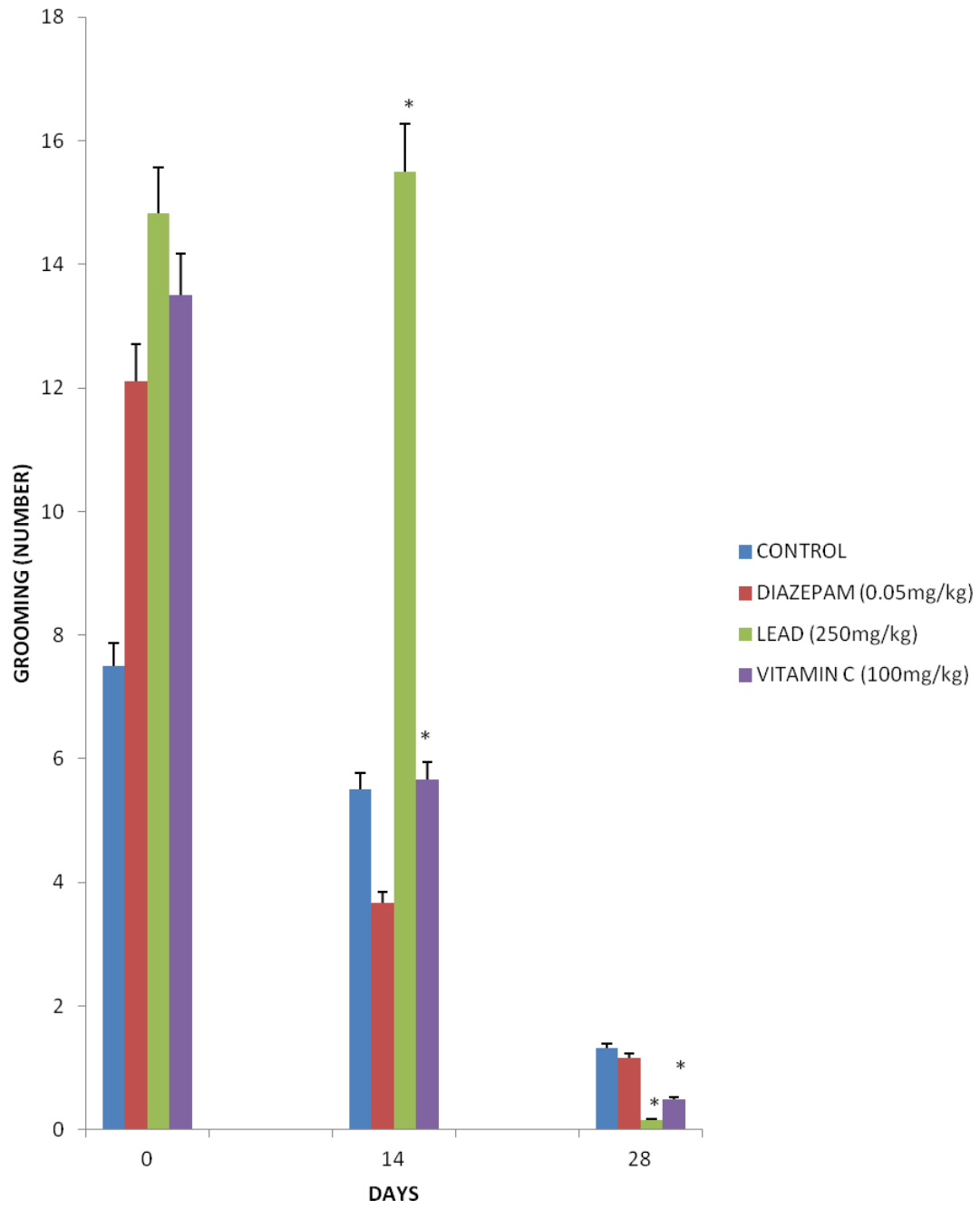


Figure VI Effects of treatment groups on grooming. Values are expressed as mean \pm SEM (n=6). *P<0.05 compared with the control.

APPENDIX VII

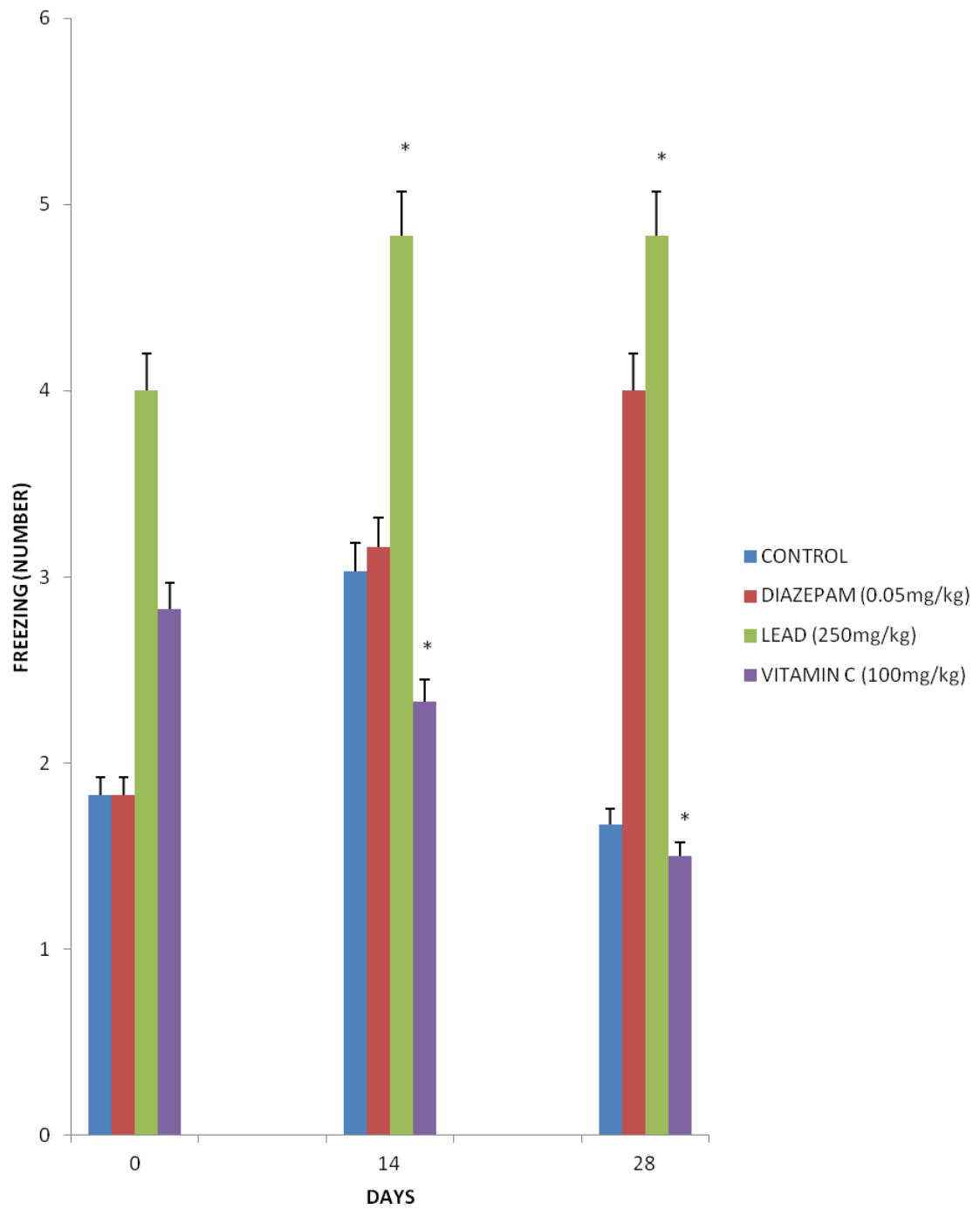


Figure VII Effects of treatment groups on freezing. Values are expressed as mean \pm SEM (n=6). *P<0.05 compared with the control.

APPENDIX VIII

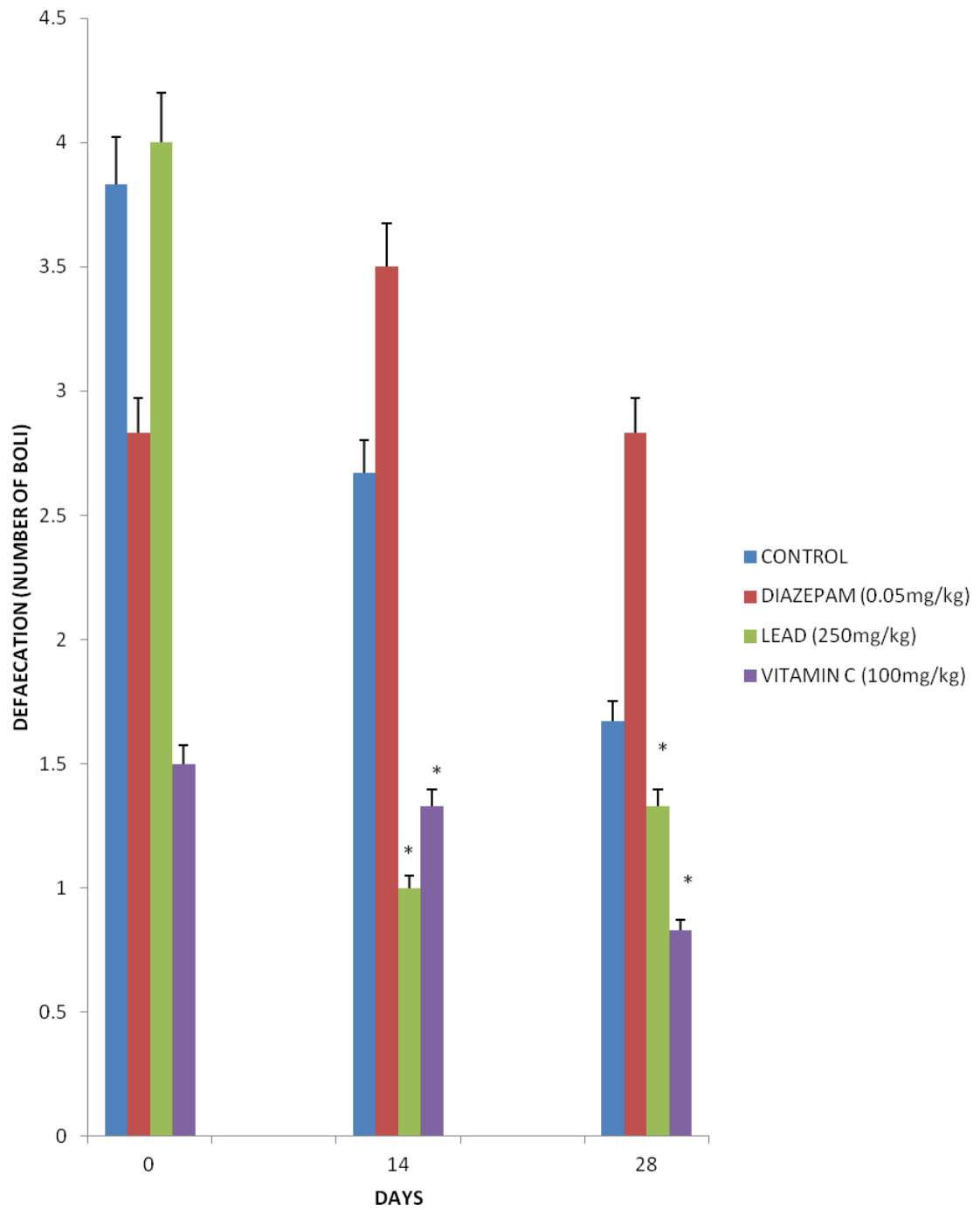


Figure VIII Effects of treatment groups on defaecation. Values are expressed as mean \pm SEM (n=6). *P<0.05 compared with the control.

APPENDIX IX

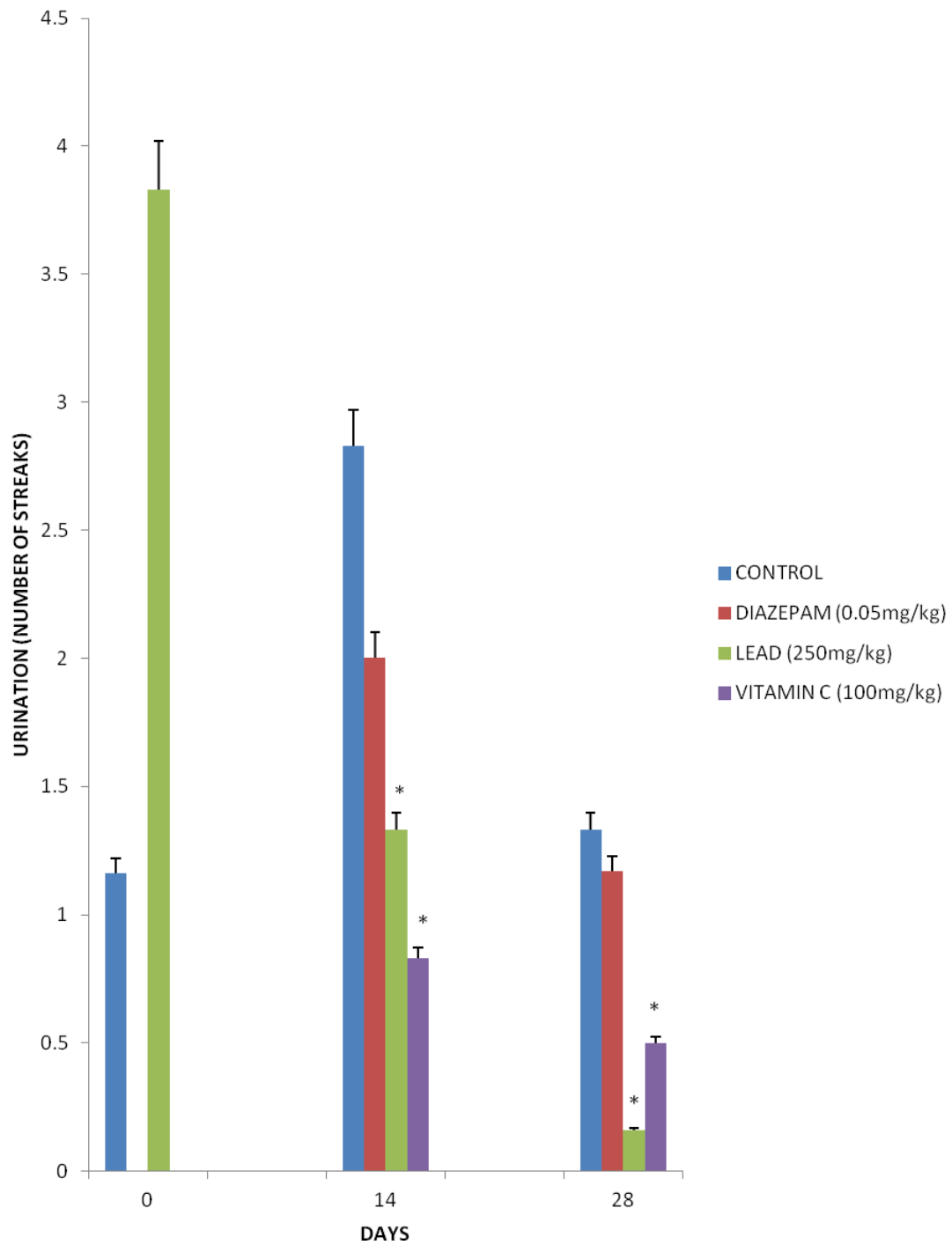


Figure IX Effects of treatment groups on urination. Values are expressed as mean \pm SEM (n=6). *P<0.05 compared with the control.

APPENDIX X

Table 4.5 The mean and standard error of mean of treatment groups on biochemical parameters. * Means $p < 0.05$ was considered significant.

| | CONTROL | LEAD (250mg/kg) | VITAMIN C (100mg/kg) |
|--------------|------------|--------------------|-------------------------|
| MDA (ng/ml) | 1.52±0.07 | 2.08±0.90* | 1.80±0.60* |
| SOD (ng/ml) | 1.72±0.08 | 1.62±0.50* | 1.71±0.50* |
| CAT (ng/ml) | 44.00±3.40 | 40.00±2.8* | 45.00±4.80* |
| ACHE (ng/ml) | 19.40±2.40 | 20.2±2.45* | 19.5±2.41* |

APPENDIX XI

Table 4.6 The parameters of treatment groups on latency in beam walk test. * means $p < 0.05$ was considered significant.

| | CONTROL | DIAZEPAM (0.05mg/kg) | LEAD (250mg/kg) | VITAMIN C (100mg/kg) |
|--------|--------------|-------------------------|--------------------|-------------------------|
| DAY 0 | 13.58± 7.58 | 39.58±12.94 | 31.92±13.85 | 26.42± 8.24 |
| DAY 14 | 12.08 ± 3.06 | 57.33 ± 2.91 | 51.42 ± 6.57 | 16.92 ± 6.76 |
| DAY 28 | 12.5 ± 1.00 | 56.08 ± 5.04 | 47.25 ± 12.84 | 12.67 ± 4.22 |

APPENDIX XII

Table 4.7 The parameters of treatment groups on forepaw grip. *Means $p < 0.05$ was considered significant.

| | CONTROL | DIAZEPAM (0.05mg/kg) | LEAD (250mg/kg) | VITAMIN C (100mg/kg) |
|--------|--------------|-------------------------|--------------------|-------------------------|
| DAY 0 | 26.17 ± 5.08 | 12.67 ± 2.87 | 12.08 ± 1.66 | 15.00 ± 6.08 |
| DAY 14 | 23.00 ± 4.00 | 13.42 ± 4.00 | 8.08 ± 3.14 | 20.50 ± 6.55 |
| DAY 28 | 25.00 ± 4.33 | 11.25 ± 1.93 | 10.33 ± 0.89 | 19.50 ± 2.48 |

APPENDIX XIII

Weights of rats in grams

CONTROL 25/7/11 1/8/11 8/8/11 15/8/11 22/8/11

| RAT/WEEK | 0 | 1 | 2 | 3 | 4 |
|----------|-----|-----|-----|-----|-----|
| 1 | 110 | 122 | 134 | 147 | 155 |
| 2 | 109 | 115 | 124 | 138 | 142 |
| 3 | 115 | 123 | 133 | 156 | 166 |
| 4 | 103 | 113 | 123 | 137 | 141 |
| 5 | 114 | 126 | 140 | 157 | 163 |
| 6 | 99 | 107 | 118 | 125 | 130 |

GROUP II DIAZEPAM

| | | | | | |
|---|-----|-----|-----|-----|-----|
| 1 | 98 | 105 | 115 | 128 | 132 |
| 2 | 105 | 122 | 137 | 154 | 160 |
| 3 | 100 | 113 | 121 | 134 | 142 |
| 4 | 188 | 195 | 204 | 219 | 225 |
| 5 | 114 | 126 | 131 | 147 | 152 |
| 6 | 120 | 131 | 140 | 162 | 165 |

GROUP III VIT C

| | | | | | |
|---|-----|-----|-----|-----|-----|
| 1 | 101 | 105 | 111 | 136 | 143 |
| 2 | 110 | 116 | 120 | 143 | 146 |
| 3 | 120 | 122 | 125 | 147 | 149 |
| 4 | 102 | 108 | 115 | 150 | 155 |
| 5 | 116 | 120 | 122 | 146 | 151 |
| 6 | 136 | 135 | 133 | 157 | 163 |

GROUP IV LEAD

| | | | | | |
|---|-----|-----|-----|-----|-----|
| 1 | 131 | 111 | 91 | 130 | 128 |
| 2 | 140 | 143 | 147 | 177 | 170 |
| 3 | 105 | 110 | 112 | 115 | 116 |
| 4 | 141 | 120 | 95 | 114 | 110 |
| 5 | 112 | 126 | 155 | 169 | 160 |
| 6 | 69 | 101 | 125 | 156 | 154 |

GROUP V LEAD + VIT C

| | | | | | |
|---|-----|-----|-----|-----|-----|
| 1 | 130 | 140 | 147 | 156 | 164 |
| 2 | 122 | 90 | 73 | 120 | 125 |
| 3 | 111 | 107 | 104 | 127 | 130 |
| 4 | 141 | 146 | 151 | 172 | 175 |
| 5 | 135 | 130 | 125 | 150 | 155 |
| 6 | 105 | 99 | 93 | 111 | 119 |

OPEN FIELD ASSESSMENT. (DAY 14)

8/8/11

GROUP I CONTROL

| Rat | Line square freq | Center square freq | Center square duration | Stretch attend freq | Rearing freq | Grooming | Freezing | Defecation | Urination |
|-----|------------------|--------------------|------------------------|---------------------|--------------|----------|----------|------------|-----------|
| 1 | 24 | 0 | 0 | 18 | 2 | 12 | 1 | 2 | 1 |
| 2 | 7 | 0 | 0 | 10 | 0 | 1 | 5 | 4 | 2 |
| 3 | 36 | 0 | 0 | 16 | 2 | 6 | 5 | 5 | 1 |
| 4 | 20 | 0 | 0 | 9 | 5 | 3 | 5 | 0 | 7 |
| 5 | 40 | 2 | 3 | 16 | 7 | 6 | 2 | 1 | 1 |
| 6 | 2 | 0 | 0 | 3 | 0 | 5 | 5 | 4 | 5 |

GROUP II DIAZEPAM

| | | | | | | | | | |
|---|----|---|---|----|----|----|---|---|---|
| 1 | 21 | 0 | 0 | 6 | 6 | 1 | 5 | 5 | 1 |
| 2 | 34 | 1 | 7 | 12 | 11 | 9 | 2 | 5 | 1 |
| 3 | 20 | 1 | 1 | 4 | 6 | 12 | 2 | 3 | 3 |
| 4 | 20 | 0 | 0 | 6 | 3 | 0 | 2 | 3 | 1 |
| 5 | 10 | 0 | 0 | 12 | 10 | 0 | 3 | 1 | 6 |
| 6 | 40 | 0 | 0 | 10 | 6 | 0 | 5 | 4 | 0 |

GROUP III VIT C ONLY

| | | | | | | | | | |
|---|----|---|---|----|---|---|---|---|---|
| 1 | 37 | 0 | 0 | 11 | 3 | 8 | 2 | 7 | 8 |
| 2 | 12 | 0 | 0 | 12 | 3 | 6 | 3 | 1 | 0 |
| 3 | 36 | 0 | 0 | 17 | 4 | 4 | 2 | 8 | 1 |
| 4 | 19 | 0 | 0 | 12 | 3 | 4 | 1 | 0 | 0 |
| 5 | 52 | 0 | 0 | 11 | 7 | 1 | 3 | 5 | 8 |
| 6 | 21 | 0 | 0 | 5 | 1 | 1 | 2 | 3 | 3 |

GROUP IV LEAD ONLY

| | | | | | | | | | |
|---|----|---|---|---|---|----|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 | 11 | 8 | 0 | 0 |
| 2 | 16 | 0 | 0 | 9 | 6 | 45 | 4 | 2 | 0 |
| 3 | 0 | 0 | 0 | 2 | 1 | 6 | 7 | 2 | 1 |
| 4 | 0 | 0 | 0 | 4 | 0 | 6 | 6 | 0 | 0 |
| 5 | 13 | 0 | 0 | 4 | 3 | 6 | 4 | 2 | 5 |
| 6 | 23 | 0 | 0 | 5 | 4 | 19 | 0 | 0 | 2 |

GROUP V LEAD + VIT C

| | | | | | | | | | |
|---|----|---|---|----|----|----|---|---|---|
| 1 | 46 | 0 | 0 | 9 | 12 | 7 | 2 | 0 | 0 |
| 2 | 29 | 0 | 0 | 2 | 16 | 3 | 2 | 0 | 0 |
| 3 | 0 | 0 | 0 | 1 | 0 | 3 | 3 | 1 | 1 |
| 4 | 36 | 0 | 0 | 4 | 6 | 11 | 1 | 2 | 2 |
| 5 | 0 | 0 | 0 | 12 | 2 | 4 | 3 | 5 | 1 |
| 6 | 15 | 0 | 0 | 10 | 6 | 6 | 3 | 0 | 1 |

OPEN FIELD ASSESSMENT. (DAY 28)

21/8/11

GROUP I CONTROL

| Rat | Line square freq | Center square freq | Center square duration | Stretch attend freq | Rearing freq | Grooming | freezing | Defecation | Urination |
|-----|------------------|--------------------|------------------------|---------------------|--------------|----------|----------|------------|-----------|
| 1 | 35 | 0 | 0 | 15 | 6 | 6 | 1 | 1 | 1 |
| 2 | 36 | 0 | 0 | 11 | 11 | 1 | 2 | 0 | 1 |
| 3 | 24 | 0 | 0 | 12 | 6 | 12 | 3 | 2 | 3 |
| 4 | 40 | 0 | 0 | 18 | 10 | 3 | 1 | 1 | 0 |
| 5 | 52 | 0 | 0 | 20 | 6 | 2 | 2 | 0 | 1 |
| 6 | 47 | 0 | 0 | 17 | 4 | 6 | 1 | 6 | 2 |

GROUP II DIAZEPAM

| | | | | | | | | | |
|---|----|---|---|----|---|---|---|---|---|
| 1 | 9 | 0 | 0 | 10 | 1 | 1 | 5 | 6 | 1 |
| 2 | 11 | 0 | 0 | 12 | 2 | 2 | 8 | 5 | 1 |
| 3 | 12 | 0 | 0 | 11 | 3 | 1 | 5 | 2 | 2 |
| 4 | 10 | 0 | 0 | 9 | 2 | 2 | 2 | 3 | 1 |
| 5 | 23 | 0 | 0 | 8 | 5 | 1 | 1 | 1 | 1 |
| 6 | 14 | 0 | 0 | 7 | 2 | 3 | 3 | 0 | 1 |

GROUP III VIT C ONLY

| | | | | | | | | | |
|---|----|---|---|----|----|---|---|---|---|
| 1 | 58 | 0 | 0 | 12 | 4 | 6 | 1 | 6 | 5 |
| 2 | 19 | 0 | 0 | 13 | 10 | 8 | 2 | 2 | 1 |
| 3 | 20 | 0 | 0 | 14 | 9 | 4 | 3 | 2 | 1 |
| 4 | 27 | 0 | 0 | 17 | 5 | 5 | 4 | 2 | 0 |
| 5 | 30 | 0 | 0 | 5 | 11 | 4 | 5 | 1 | 0 |
| 6 | 35 | 0 | 0 | 11 | 12 | 5 | 4 | 1 | 0 |

GROUP IV LEAD ONLY

| | | | | | | | | | |
|---|----|---|---|---|----|----|---|---|---|
| 1 | 11 | 0 | 0 | 5 | 11 | 12 | 5 | 5 | 1 |
| 2 | 10 | 0 | 0 | 2 | 10 | 15 | 2 | 2 | 0 |
| 3 | 0 | 0 | 0 | 0 | 1 | 5 | 9 | 1 | 0 |
| 4 | 14 | 0 | 0 | 9 | 10 | 14 | 1 | 0 | 0 |
| 5 | 0 | 0 | 0 | 0 | 2 | 5 | 7 | 0 | 0 |
| 6 | 0 | 0 | 0 | 0 | 1 | 10 | 5 | 1 | 0 |

GROUP V LEAD + VIT C

| | | | | | | | | | |
|---|----|---|---|----|----|----|---|---|---|
| 1 | 52 | 0 | 0 | 11 | 17 | 10 | 1 | 4 | 2 |
| 2 | 40 | 0 | 0 | 6 | 12 | 11 | 2 | 1 | 0 |
| 3 | 19 | 0 | 0 | 2 | 10 | 9 | 3 | 0 | 1 |
| 4 | 35 | 0 | 0 | 12 | 9 | 11 | 2 | 0 | 0 |
| 5 | 30 | 0 | 0 | 14 | 12 | 12 | 1 | 0 | 0 |
| 6 | 21 | 0 | 0 | 15 | 10 | 5 | 0 | 0 | 0 |

BEAM WALK ASSESSMENT

MOTOR COORDINATION MEASURED IN Seconds while the right and left foot slips are counted

GROUP I CONTROL

25/7/11

8/8/11

21/8/11

| RAT/W k | 0 | | | | | 2 | | | | | 4 | | | | |
|------------|-----------------------------|-----------------------------|-------------|---|---|-----------------------------|-----------------------------|-------------|---|---|-----------------------------|-----------------------------|-------------|---|---|
| | 1 ^S _T | 2 ^N _D | AVERAG E | R | L | 1 ^S _T | 2 ^N _D | AVERAG E | R | L | 1 ^S _T | 2 ^N _D | AVERAG E | R | L |
| 1 | 10 | 15 | 12.5 | 0 | 0 | 8 | 10 | 9.0 | 0 | 0 | 15 | 9 | 12.0 | 1 | 0 |
| 2 | 8 | 5 | 6.5 | 0 | 0 | 12 | 13 | 12.5 | 1 | 0 | 10 | 14 | 12.0 | 1 | 1 |
| 3 | 10 | 12 | 11.0 | 0 | 0 | 15 | 8 | 11.5 | 0 | 0 | 11 | 11 | 11.0 | 1 | 2 |
| 4 | 10 | 13 | 11.5 | 0 | 0 | 17 | 20 | 18.5 | 1 | 0 | 12 | 13 | 12.5 | 3 | 1 |
| 5 | 30 | 30 | 30.0 | 0 | 0 | 10 | 11 | 10.5 | 1 | 1 | 17 | 11 | 14.0 | 2 | 0 |
| 6 | 10 | 10 | 10.0 | 1 | 0 | 14 | 7 | 10.5 | 0 | 0 | 15 | 12 | 13.5 | 1 | 1 |

GROUP II DIAZEPAM

| | | | | | | | | | | | | | | | |
|---|----|----|------|---|---|----|----|------|---|---|----|----|------|---|---|
| 1 | 60 | 60 | 60.0 | 5 | 5 | 60 | 50 | 55.0 | 6 | 6 | 57 | 60 | 58.5 | 5 | 2 |
| 2 | 60 | 40 | 50.0 | 0 | 0 | 55 | 60 | 57.5 | 5 | 4 | 60 | 60 | 60.0 | 5 | 3 |
| 3 | 30 | 45 | 37.5 | 1 | 1 | 60 | 60 | 60.0 | 6 | 6 | 42 | 55 | 48.5 | 1 | 1 |
| 4 | 30 | 30 | 30.0 | 0 | 1 | 60 | 58 | 59.0 | 6 | 6 | 60 | 60 | 60.0 | 6 | 6 |
| 5 | 40 | 40 | 40.0 | 2 | 0 | 45 | 60 | 52.5 | 3 | 2 | 49 | 50 | 49.5 | 2 | 1 |
| 6 | 20 | 20 | 20.0 | 1 | 0 | 60 | 60 | 60.0 | 6 | 6 | 60 | 60 | 60.0 | 6 | 6 |

GROUP III VIT C

| | | | | | | | | | | | | | | | |
|---|----|----|------|---|---|----|----|------|---|---|----|----|------|---|---|
| 1 | 60 | 12 | 36.0 | 0 | 0 | 8 | 7 | 7.5 | 0 | 0 | 12 | 11 | 11.5 | 1 | 1 |
| 2 | 60 | 35 | 47.5 | 0 | 1 | 10 | 11 | 10.5 | 0 | 0 | 8 | 9 | 8.5 | 1 | 0 |
| 3 | 10 | 11 | 10.5 | 0 | 0 | 13 | 15 | 14.0 | 0 | 0 | 13 | 11 | 12.0 | 2 | 1 |
| 4 | 55 | 40 | 47.5 | 1 | 0 | 23 | 20 | 21.5 | 1 | 0 | 15 | 11 | 13.0 | 0 | 3 |
| 5 | 25 | 10 | 17.5 | 0 | 1 | 21 | 19 | 20.0 | 0 | 1 | 12 | 19 | 15.5 | 1 | 1 |
| 6 | 20 | 15 | 17.5 | 0 | 1 | 11 | 11 | 11.0 | 0 | 1 | 20 | 21 | 20.5 | 1 | 1 |

GROUP IV LEAD ONLY

| | | | | | | | | | | | | | | | |
|---|----|----|------|---|---|----|----|------|---|---|----|----|------|---|---|
| 1 | 60 | 45 | 52.5 | 2 | 1 | 60 | 47 | 53.5 | 3 | 3 | 20 | 40 | 30.0 | 2 | 1 |
| 2 | 45 | 40 | 42.5 | 1 | 1 | 48 | 30 | 39.0 | 4 | 1 | 60 | 60 | 60.0 | 3 | 5 |
| 3 | 30 | 35 | 32.5 | 2 | 1 | 60 | 60 | 60.0 | 6 | 6 | 57 | 60 | 58.5 | 4 | 4 |
| 4 | 35 | 30 | 32.5 | 3 | 4 | 52 | 60 | 56.0 | 5 | 4 | 30 | 35 | 32.5 | 4 | 0 |
| 5 | 25 | 20 | 22.5 | 1 | 1 | 60 | 41 | 50.5 | 3 | 2 | 40 | 45 | 42.5 | 1 | 1 |
| 6 | 10 | 8 | 9.0 | 0 | 0 | 49 | 50 | 49.5 | 1 | 2 | 60 | 60 | 60.0 | 6 | 6 |

GROUP IV LEAD + VITC

| | | | | | | | | | | | | | | | |
|---|----|----|------|---|---|----|----|------|---|---|----|----|------|---|---|
| 1 | 25 | 30 | 27.5 | 3 | 3 | 22 | 25 | 23.5 | 1 | 2 | 23 | 20 | 21.5 | 1 | 1 |
| 2 | 20 | 20 | 20.0 | 0 | 0 | 21 | 19 | 20.0 | 0 | 1 | 11 | 12 | 11.5 | 1 | 0 |
| 3 | 60 | 20 | 40.0 | 2 | 1 | 15 | 8 | 11.5 | 2 | 2 | 13 | 11 | 12.0 | 0 | 0 |
| 4 | 20 | 40 | 30.0 | 1 | 0 | 7 | 10 | 8.5 | 0 | 0 | 14 | 12 | 13.0 | 1 | 0 |
| 5 | 20 | 35 | 27.5 | 0 | 0 | 11 | 12 | 11.5 | 0 | 0 | 10 | 9 | 9.5 | 1 | 1 |
| 6 | 15 | 12 | 13.5 | 0 | 2 | 23 | 30 | 26.5 | 1 | 0 | 8 | 9 | 8.5 | 1 | 1 |

FOREPAW GRIP ASSESSMENT

25/7/11

MOTOR STRENGTH MEASURED IN SECS

GROUP I CONTROL

25/7/11

8/8/11

22/8/11

| RAT/WEEK | 0 | | | 2 | | | 4 | | |
|----------|-----|----|-----|----|----|-----|----|----|------|
| | 1 | 2 | AVE | 1 | 2 | AVE | 1 | 2 | AVE |
| 1 | 100 | 62 | 82 | 19 | 21 | 20 | 30 | 15 | 22.5 |
| 2 | 14 | 20 | 17 | 20 | 26 | 23 | 40 | 20 | 30.0 |
| 3 | 13 | 13 | 13 | 11 | 21 | 16 | 17 | 18 | 17.5 |
| 4 | 11 | 13 | 12 | 31 | 25 | 28 | 25 | 35 | 30.0 |
| 5 | 12 | 16 | 14 | 26 | 24 | 25 | 30 | 20 | 25.0 |
| 6 | 18 | 20 | 19 | 26 | 26 | 26 | 25 | 25 | 25.0 |

GROUP II DIAZEPAM

| | | | | | | | | | |
|---|----|----|----|----|----|----|----|----|------|
| 1 | 12 | 18 | 15 | 12 | 18 | 15 | 10 | 11 | 10.5 |
| 2 | 12 | 12 | 12 | 21 | 11 | 16 | 12 | 8 | 10.0 |
| 3 | 11 | 15 | 13 | 10 | 4 | 7 | 13 | 12 | 12.5 |
| 4 | 9 | 5 | 7 | 12 | 10 | 11 | 15 | 11 | 13.0 |
| 5 | 14 | 12 | 13 | 20 | 14 | 17 | 17 | 10 | 13.5 |
| 6 | 16 | 16 | 16 | 19 | 9 | 14 | 9 | 7 | 8.0 |

GROUP III VIT C

| | | | | | | | | | |
|---|----|----|----|----|----|----|----|----|------|
| 1 | 16 | 20 | 18 | 18 | 28 | 23 | 34 | 11 | 22.5 |
| 2 | 10 | 4 | 7 | 52 | 28 | 40 | 33 | 12 | 22.5 |
| 3 | 9 | 11 | 10 | 40 | 20 | 30 | 20 | 30 | 25.0 |
| 4 | 8 | 10 | 9 | 24 | 18 | 21 | 18 | 32 | 25.0 |
| 5 | 21 | 17 | 19 | 11 | 9 | 10 | 15 | 35 | 25.0 |
| 6 | 13 | 11 | 12 | 20 | 18 | 19 | 12 | 40 | 26.0 |

GROUP IV LEAD

| | | | | | | | | | |
|---|----|----|----|----|----|----|----|----|------|
| 1 | 20 | 20 | 40 | 10 | 14 | 12 | 9 | 10 | 9.5 |
| 2 | 13 | 10 | 23 | 7 | 9 | 8 | 11 | 9 | 10.0 |
| 3 | 21 | 11 | 16 | 2 | 10 | 6 | 12 | 10 | 11.0 |
| 4 | 6 | 10 | 8 | 8 | 7 | 15 | 13 | 11 | 12.0 |
| 5 | 6 | 4 | 5 | 6 | 10 | 8 | 10 | 10 | 10.0 |
| 6 | 15 | 9 | 12 | 9 | 5 | 7 | 10 | 9 | 9.5 |

GROUP V LEAD + VIT C

| | | | | | | | | | |
|---|----|----|----|----|----|----|----|----|------|
| 1 | 24 | 26 | 25 | 22 | 18 | 20 | 20 | 16 | 18.0 |
| 2 | 10 | 16 | 13 | 16 | 4 | 10 | 23 | 18 | 20.5 |
| 3 | 8 | 20 | 14 | 28 | 22 | 25 | 24 | 22 | 23.0 |
| 4 | 18 | 10 | 14 | 14 | 16 | 15 | 29 | 12 | 20.5 |
| 5 | 12 | 26 | 19 | 16 | 30 | 23 | 30 | 10 | 20.0 |
| 6 | 4 | 6 | 5 | 39 | 21 | 30 | 15 | 15 | 15.0 |

Weight of brain in grams

| Control | lead + vitc | lead | vitc |
|---------|-------------|---------|---------|
| 1. 1.68 | 1. 1.70 | 1. 1.79 | 1. 1.60 |
| 2. 1.58 | 2. 1.48 | 2. 1.40 | 2. 1.63 |
| 3. 1.83 | 3. 1.59 | 3. 1.55 | 3. 1.68 |
| 4. 1.67 | 4. 1.55 | 4. 1.40 | 4. 1.74 |
| 5. 1.43 | 5. 1.41 | 5. 1.73 | 5. 1.74 |