CO-ADIMINISTRATION OF LEAD ACETATE AND CADMIUM CHLORIDE ON ERYTHROCYTE MORPHOLOGY AND BONE MARROW CYTOLOGY IN MALE WISTAR RATS

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JANUARY, 2023.

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A PROJECT WRITTEN IN THE DEPARTMENT OF PHYSIOLOGY AND SUBMITTED TO THE SCHOOL OF BASIC MEDICAL SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) DEGREE OF THE UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA.

JANUARY, 2023.

DEDICATION

I dedicate this work to God Almighty my creator my strong pillar my source of inspiration wisdom knowledge and understanding.

ACKNOWLEDGE

I would like to express my special thanks gratitude to God Almighty for making this a success.

My gratitude to my awesome supervisor **Mrs. Peter U.A.** for her patience, guidance, correction and her time during this Project

I want to sincerely appreciate my parent Mr. and Mrs. Linus Umezulike for their kindness financial support and encouragement so far in my education.

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ABSTRACT

Heavy metals are metallic elements that have a relatively high density compared to water. Some examples are lead and cadmium. These metals distributed into the body through ingestion or through inhalation of air. Fifteen (15) Adult male Wistar rats weighing between 100-130g were used for this study. They were assigned into three (3) groups of (n=5) in each group. Group 1 served as (control Group) while Group 2 and 3 serve as experimental group. Group 1: (control group) were give pellet and distilled water. While the group 2 and 3 were administered $CdCl_2$ and $pb(C_2H_3O_2)_2$ 100ppm for 14 days and 28 days respectively. After four weeks of administration, the blood collection was through orbital sinus using heparinized capillary tube into EDTA bottles. Thin blood smear from the EDTA bottles was placed on microscope slide. The slide was allowed to air dry after that it was subsequently fixed with absolute methanol for about 15 mins staining for 20 mins each and were viewed understand microscope. The bone marrow was experimented using flushing techniques. The result actualized from this study shows in the erythrocyte morphology, lead acetate and cadmium chloride affect the shape (slightly rounded or blunted) and color (faded) of the cells and there are microcytes which are unusual red blood cells which are seen scattered in the entire field.the bone marrow cytology shows abundant erythroid series in the treatment groups, also lymphoid cellular series recruitment interspersed by the other reticulocyte of the bone marrow when compared with the control. In conclusion, it was observed from this study that acute co-exposure to lead acetate and cadmium chloride affect the erythrocyte morphology of Wistar rats, this effects may result in a condition called poikilocytosis . The resulting effects on the bone marrow may eventually lead to anemia.

CHAPTER ONE
INTRODUCTION

1.1 Background of study

Heavy metals are though metallic elements that have a relatively high density compared to water (Fergusson, 1990). They include metalloids such as lead, cadmium, that are able to induce toxicity at low level of exposure (Duffus, 2002). Cadmium (Cd) and lead (Pb) are appearing and are those that cannot be decomposed or dissolved pollutants representing a great concern to human health. These metals are toxic and are distributed into the environment, but industrial development (Satarug *et al*., 2010) associated with smelting and mining, manufacturing of batteries, pigments, and ceramic are well-known emitters of Cd and Pb. Lead and cadmium can enter the soil through the disposal of sewage sludge, or the application of pesticides or phosphate fertilizers (cadmium) (ATSDR, 2012). Increased release of both metals in the environment and their non-biodegradability has increased the risk of human exposure. The main routes of Cd and Pb exposure are ingestion and inhalation due to their presence in food stuffs as well as contaminated water and inhalation of air. (Tchounwou *et al*., 2012). The World Health Organization (WHO) has published a list of 10 chemicals or groups of chemicals of concern for human health, which includes Cd and Pb. Additionally, the US Agency for Toxic Substances and Disease Registry (ATSDR) ranked Cd in seventh and Pb in

second place on the priority list of dangerous substances (Matovic *et al*., 2015). Many in vivo and in vitro studies have been conducted to determine the exact mechanisms of toxicity of Cd and Pb. The present body of comprehension suggests oxidative stress as one of the critical mechanisms of toxicity of both metals, even though neither of these metals is a Fenton's metal (Flora *et al.,* 2012). Other possible mechanisms of toxicity are bin ding to oxygen, nitrogen, and sulphur ligands, which may affect numerous enzymes and proteins (Matovic *et al*., 2015), interaction with bio elements (Bulat *et al.,* 2017); inhibition of apoptosis (Rani *et al.,* 2014); and changes in DNA structure and the inhibition of damaged DNA repair, which may lead to aberrant gene expression (Waisberg *et al.,* 2003; Joseph 2009; Ahmed *et al*., 2010). Lead acause in bone marrow segment neutrophils and myeloid series cells, and increased myeloid:erthroid ratios. Blood lead concentration and myeloid: erythriod ratios decreased after cessation of lead administration. pb and Cd has been recognized as a biological toxicant. They are widely dispersed in the environment and at excessive levels; they are toxic to humans (Jarup, 2003). Absorbed cadmium and lead following oral ingestion is carried via blood to soft tissues. In this respect, this study was designed to evaluate the toxic effects of co-administration of lead acetate and cadmium chloride on erythrocyte morphology and bone marrow cytology on male Wistar rats.

1.2 Justification of study

Humans are mostly exposed to heavy metals in combined form. Several investigations have been carried out on these metals individually. Lead and cadmium has been widely recognized as the single most significant health threat in human and all living things at large. Lead and cadmium can cause toxicity in the bone marrow with short red cell survival and the development of microcytic hypochromic anemia. In the past, many research works have been conducted to discover the extent of effect of some of these heavy metals (pb and Cd on human. For that cause, it is necessary to check the effect of co- exposure of lead acetate and cadmium chloride on bone marrow cytology and erythrocyte morphology as humans are exposed to these metals in combined form.

1.3 Aim and objective

The aim of this research is to evaluate the effects of co- administration of lead acetate and cadmium chloride on erythrocyte morphology and bone marrow cytology on male Wistar rats.

1.3.1 The objective of the study is to

1. To determine the effect of co- administration of lead acetate and cadmium chloride on bone marrow cytology of male Wistar rats.
2. To determine the effect of co- administration of lead acetate and cadmium chloride on erythrocyte morphology of male Wistar rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Lead

Lead (Pb) and cadmium (Cd) are common environmental occupational toxic metal, Cd and Pb poisoning remains a health threat (Zbakh *et al.*, 2012). Inhalation and ingestion are the two main routes of exposure to Pb and Cd (John *et al*., 2010), of which ingestion is the primary route of environmentally exposed people. After absorption, most Pb binds to proteins in erythrocytes and is distributed to soft tissues and home; the latter is the main depot for this metal (Hambach *et al*., 2013). Pb can cause mitochondrial damage, reactive oxygen species (ROS) production, glutathione depletion and apoptosis (Sabath and Robies-Osorio, 2012). Cd is transported in blood by albumin to liver, where it bounds to metallothionenin (MT). The Cd-MT complex is then released back into circulation (Hambach *et al*., 2013). Found that Pb and Cd can interact with each other in a complex way (John *et al.,* 2010). The co-exposure to Pb and Cd may induce addictive or synergistic interactions or new effects that are not observed for single element exposure (Wang and Fowler, 2008).

2.2 Lead is a chemical element with the symbol pb and atomic number of 82. Lead is soft and malleable. It is a slightly bluish, bright silvery metal in a dry atmosphere. The main sources of lead exposure include drinking water, food,

cigarette, industrial processes and domestic sources (Lockitch *et al 1991*). Pb exposure mainly arises from contact with Pb based paints in home, fertilizers, cosmetics, automobiles, disposable batteries, etc., especially in developing countries (Nevin, 2007). Several lines of evidence implicate that Pb exposure causes many pathological incidences including cardiac (Voors *et al.,* 1982), hepatic (Patra *et al.,* 2001), behavioral (De Marco *et al*., 2005), immunological (Rosenberg *et al*., 2007), neurological (Bellinger, 2008), renal (Rastogi, 2008) and hematological (Khalid and Fartosi, 2008) dysfunctions. Although the precise mechanism of Pb toxicity is not clear, there is evidence that Pb can cause generation of reactive oxygen metabolites and inhibits the activity of antioxidant enzymes in tissues (Pulido and Parrish, 2003; Jurczuk *et al.,* 2007; Franco *et al.,* 2009).high lead level can affect multiple organs such as its long – term accumulation in the bone while causing toxicity in the bone marrow with shortened red call survival and the development of the microcytic hypochromic anemia with basophilic stippling(Silbergeld *et al.,* 2000; Patrick, 2006).lead is known to delay fracture healing and may contribute to osteoporosis. Yet the exact mechanism by which lead affect normal cellular function in bone and cartilage is poorly understood. Considering the relationship between Pb exposure and oxidative stress, attention has been focused on compounds having antioxidant properties in order to combat against Pb induced toxicity.

2.3 Mechanism of toxicity

Lead toxicity is due to increase generation of reactive oxygen species (ROS) and interference with generation of antioxidants. Lead causes the generation of ROS like hydroperoxide, hydrogen peroxide, and singlet oxygen. ROS are stabilized by glutathione in the body. Ninety percent of glutathione in the cell exists in reduced form and 10%in oxidative forms, and it typically acts as an antioxidant defense mechanism. Glutathione stabilizes ROS, and after being converted (oxidizing) to glutathione disulfide, it is reduced back to GSHby glutathione reductase. Lead inactivates glutathione by binding to GSH's sulfhydryl group, which causes GSH replenishment to become increasing oxidative stress. Lead also interferes with the activity of other antioxidant enzymes including superoxide dismutase and catalase. The increase in oxidative stress leads to cell membrane damage due to lipid per oxidation. Higher lead level can affect multiple organs such as its long –term accumulation and retention (over years) in bone and teeth while causing toxicity in the bone marrow with shortened red cells survival and the development of microcytic hyochromic anemia with basophilic stippling.

2.4 Cadmium

This metal is mostly used in industries for the production of paints, pigments alloys, coatings, batteries as well as plastics. Majority of cadmium, about three-fourths is used as electrode component in producing alkaline batteries. Cadmium is emitted through industrial processes and from cadmium smelters into sewage sludge, fertilizers, and groundwater which can remain in soils and sediments for several decades and taken up by plants. Therefore, significant human exposure to cadmium can be by the ingestion of contaminated foodstuffs especially cereals, grains, fruits and leafy vegetables as well as contaminated beverages Also, humans may get exposed to cadmium by inhalation through incineration of municipal waste.

2.5 Mechanisms of cadmium toxicity

These effects are the result of more than one mechanisms of toxicity, all interrelated in their complexity. Thus it is difficult to identify a fine line between these mechanisms of Cd toxicity, making their understanding highly complicated. The most important mechanism by which cd manifests its toxic effect include change in gene expression and inhibition of damaged DNA repair, interference of apoptosis and autophagy, oxidative stress, and interaction with bioelements. In this review we will give a brief overview of the recent development and findings on the most relevant general and specific mechanisms and molecular pathways of Cd toxicity. Cd inhibits the differentiation of bone marrow mesenchymal stem cells (BMSCs) into osteoporosis, Cd mainly affect the activation of osteoclasts and promotes bone desorption.

2.6 Cadmium exposure

Following acute oral exposure to cadmium, an asymptomatic period of up to 60 minutes may precede clinical symptoms. Exposure to lower doses of cadmium results in gastrointestinal irritation, vomiting, abdominal pain and diarrhoea according to Risk Assessment Information System (RAIS) (1991). Higher doses may affect the nervous system, liver, cardiovascular system and may lead to renal failure and death [International Programme on Chemical Safety (IPCS)].

Chronic oral exposure to cadmium leads to renal failure, characterised by proteinuria due to renal tubular dysfunction. The accumulation of cadmium in the kidney affects renal vitamin D metabolism, which subsequently disturbs calcium balance that may lead to osteomalacia and osteoporosis [Environment Agency (EA) (2009).]. This, as well as the increased excretion of calcium may result in bone disease (Agency for Toxic Substances and Disease Registry (ATSDR) (2008).

Acute inhalation of cadmium may initially cause irritation of the upper respiratory tract, although symptoms may be delayed for 4-8 hours. Dyspnoea, chest pain and muscle weakness may also occur. Pulmonary oedema, bronchitis, chemical pneumonitis, respiratory failure and death may occur within days of exposure. In the long-term following exposure, progressive pulmonary fibrosis and impaired lung function may occur. Chronic inhalation of cadmium causes loss of renal tubular function, leading to proteinuria and impairs lung function by causing bronchitis, obstructive lung disease and in some cases interstitial fibrosis [International Programme on Chemical Safety (IPCS)].

2.7 Bone marrow

The bone marrow is a specialized connective tissue that has blood vessels and is found in the center of the bones .is a loose, spongy network of reticular fibers and associated cells fills. the medullary cavities of bone and provides a supporting framework (stroma) for the hemopoietic cells (monga *et al.,* 2022). The network of fibers and cells is continuous with the endosteum of the bone and is intimately associated with blood vessels that pervade the marrow. Within the meshes of the reticular fiber network are all the cell types normally found in blood, their precursors, fat cells, plasma cells, and mast cells. These constitute the free cells of the marrow (Rubin, 2007). The reticular cells are fixed cells that have no special phagocytic powers and do not give rise to precursors of hemopoietic cells. They are modified fibroblasts responsible for the formation and maintenance of reticular fibers. There are two types of bone marrow: red and yellow. Red marrow contains blood stem cells that can In toxicological research, bone marrow cytologic evaluation is invaluable in determining potential hematotoxicity or prognosis of xenobiotics-induced pathologies (Bolliger, 2004).Investigated the effect of Cd and Pd on the bone lesion in growing swine (Pond *et al.,* 1982).

Reticular cells have large, palely stained nuclei and irregularly branched cytoplasm that extend long slender processes along the reticular fibers Bone marrow comprises approximately 5% of total body mass in healthy adult humans, such that a man weighing 73 kg (161 lbs) will have around 3.7 kg (8 lbs) of bone marrow (Hindorf *et al.,* 2010) There are two type of bone marrow, red and yellow.

2.8 Red marrow is actively engaged in the production of blood cells and represents the active or hemopoietic marrow. The red color is due to the content of red cells and their pigmented precursors. Red bone marrow contains blood stem cells that can become red blood cell (Chan *at al.,* 2016)

2.9 Yellow (fatty) marrow is inactive, and its principal cellular components are fat cells. Fat cells also are scattered sparingly throughout the red marrow. The amount and distribution of fatty marrow vary with age and the need for blood cells. Fatty marrow is very labile and easily replaced by active marrow. Yellow marrow serves as a reserve space for the expansion of active marrow to meet increased demands for blood. Active marrow first replaces the fat cells scattered within the red marrow itself, but if demands for blood remain high or are increased, red marrow gradually encroaches into the areas of fatty marrow (Poulton *et al.*, 1993).

2.10 Hematopoietic stem cells in the bone marrow give rise to two main types of cells: myeloid and lymphoid lineages. These include monocytes, macrophages, neutrophils, basophils, eosinophils, erythrocytes, dendritic cells, and megakaryocytes, or platelets, as well as t cells, b cells, and natural killer (nk) cells. The different types of hematopoietic stem cells vary in their regenerative capacity and potency. They can be multipotent, oligopotent or unipotent, depending on how many types of cells they can create (Arikan *et al., 2014*).

Pluripotent hematopoietic stem cells (Reya, 2003) have renewal and differentiation properties. They can reproduce another cell identical to themselves, and they can generate one or more subsets of more mature cells. It is involved in a complex array of functions ranging from hematopoiesis, locomotion, protection of delicate and vital organs, regulation of calcium and phosphate homeostasis, and the development and maintenance of the immune system (Walsh *et al.,* 2018; Ponzetti and Rucci 2019).The development of mature erythrocytes stems from the bone marrow and terminates in the circulatory system. The biochemical and physiological activities such as DNA synthesis, erythropoietin production, iron (Fe) availability and metabolism, stem cell factors production, response to erythropoietin, Heinz body formation, clotting factor production, and stromal reaction, which are extremely sensitive to xenobiotics interference thereby making a wide room for the possibility of production of abnormal red cells.

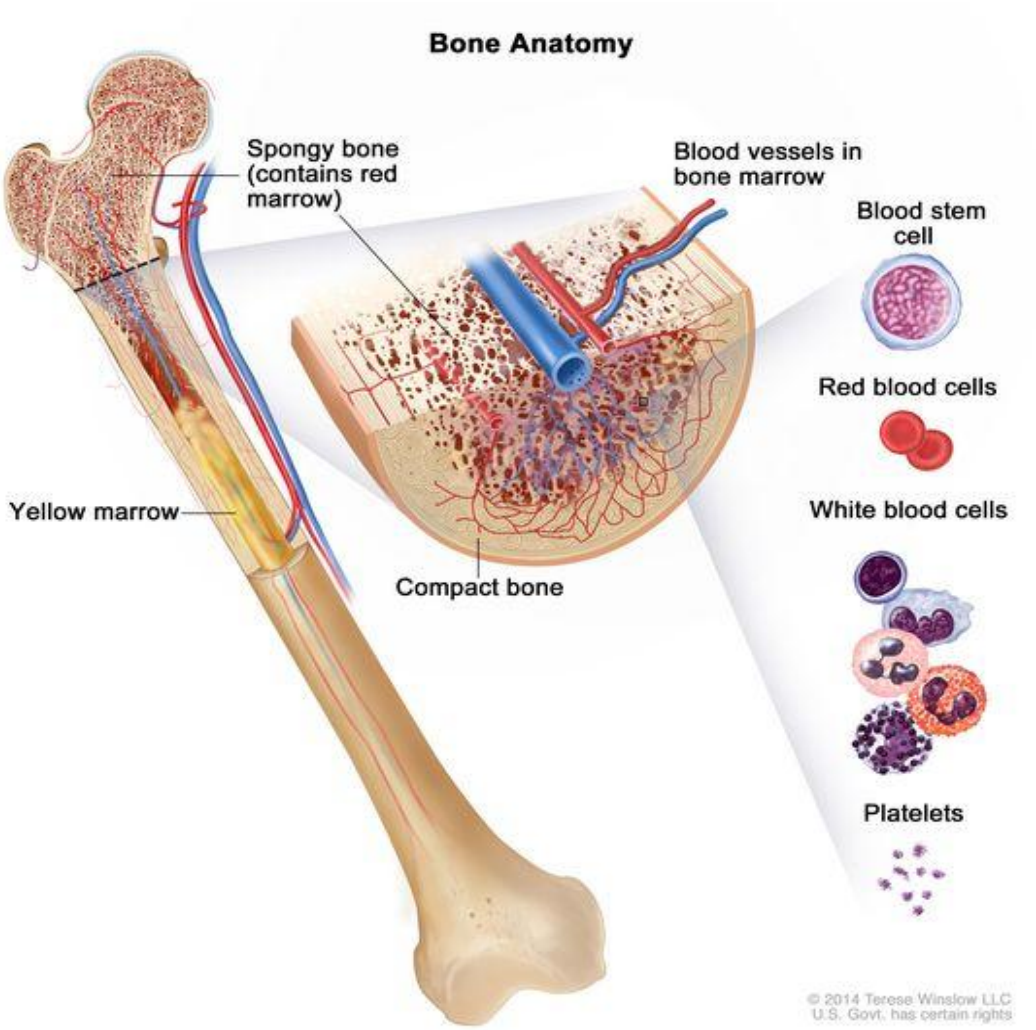

Fig 1: Bone anatomy (Rubin, 2007)

2.11 Erythrocyte Morphology

Erythrocytes are the major cellular component of the circulating blood. Erythrocytes have a consistent diameter of 7-8 μm, making them the perfect 'histologic rulers' during routine examinations. Roughly, erythrocytes in circulation average is about 5 million cells per cubic millimeters of blood. Erythrocytes have a biconcave discs, with very high plasma membrane integrity and cytoskeleton stability. This means that their periphery is thicker than their central part. Erythrocytes are sensitive cells and can serve as cellular indicators (Pretorius, and Kell, 2014) to indicate onset of any physiological change occurring in the body. This feature maximizes the total surface of the cell membrane facilitating gas exchange and transport. In addition erythrocytes do not have a nucleus (anuclear) or any other intracellular organelles, as they are all lost during erythropoiesis. The average life span of about 100–120 days, erythrocyte production and senescence is maintained in constant equilibrium. Any imbalances affecting production or destruction of red cells result in red cell disorder. In essence, red cells are maintained at a constant volume in the body, depending on several factors. Physiologic factors such as age, sex, altitude, smoking status or pregnancy account for slight inter-individual and intra-individual variations. Typically, there are different measures of red cell counts and they include red cell mass, red cell volume, red cell count, haematocrit and haemoglobin concentration.

Normally, a red cell has a round form, shaped like a disc, well-haemoglobinised cytoplasmic rim with a central pallor covering inner third of the red cell. Deviations in morphology size, shape, color, contents/inclusion or distribution (Hoffman, 1987) may be associated or perhaps diagnostic of disease entities. Circulating red cells are formed from bone marrow stem cells. Stem cells are pluripotent; they self-replicate and differentiate to specialized cells in circulation through different lineages. Red cells are formed from the myeloid stem cell lineage (colony forming unit—granulocytes, erythroid, myeloid and megakaryocytes). The earliest recognizable red cell precursor in the bone marrow is the pronormoblast. The pronormoblast undergoes series of maturation to become the orthochromatic normoblast. Upon extrusion of its nucleus, the late normoblast becomes the shift reticulocytes, which is released into the circulation. Finally, DNA remnants and other chromatin materials in the reticulocytes is removed by the pitting action of the spleen, hence the mature red cells. Erythrocytes cannot be seen with the naked eyes. Typically, morphology of red cells is performed on peripheral blood smears, once there is an indication. Erythrocyte morphology is either indicated by a clinical request or laboratory flags. Erythrocyte morphology may also be indicated when significant deviations from the normal are seen in the laboratory during blood work (full blood count) irrespective of a clinical request. For instance, a significantly reduced hemoglobin level with low MCV and raised RDW may suggest iron deficiency anemia. This is an indication for red cell morphology

and other ancillary investigation for iron deficiency. The morphological shape of erythrocytes in a blood smear is dominated by target cells (Landis-Piwowar, *et al., 2015*). Target cells appear in the peripheral blood smear shaped like a bull's eye cells. The target cell appears as hypochromic with a volume and a thin layer of hemoglobin located at the center (Ciesla, 2007).

2.12 Red cell morphologic disorders

The red cell morphology is reviewed by a haemato-morphologist under the compound microscope and any significant abnormalities are noted for reporting/diagnosis. Red cell morphology is evaluated in terms of size, shape, color, distribution and intra cytoplasmic inclusions. In general, red cells have a fairly uniform variation in size, with a red cell distribution width of 11–15% in normal individuals. Abnormal variations in sizes and shape are termed anisocytosis and poikilocytosis, respectively (Jones, 2009).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Equipment and reagent

- EDTA bottle
- Universal bottle
- Dissecting set
- Capillary tube
- Slide rack
- Spectrophotometer (Model: 7219 search tech)
- Automated pipette
- Weighing balance (Model: S. Mettler)
- Mayrunwald and giemsa stains
- Microscope
- Cadmium chloride ($CdCl_2$) (product of Carmel chemicals china)
- LEAD ACETATE $PB(C_2H_3O_2)_2$ =379.33 Isohem Laboratory Angamaly Kochi-683573. India
- Distilled water

Pelleted manufactured by top feed and flour mill limited were obtained from anatomy animal home. University of Benin, Edo state.

3.2 Preparation Dosage of administration

Cadmium chloride(0.1g) and lead acetate(0.1g), were measured with a sensitive scale .cadmium chloride(0.1g) was mixed with one liter of distilled water and 100ppm of lead acetate was mixed with one liter of distilled water (Ding *et al.,*

1998). Both concentration was properly mixed together, which was given to group 2 as drinking water for 14day and group 3 for 28days (Carmingnani *et al*., 2000).

Experimental animals

Fifteen (15) male rats of the Wistar stain were used for study. All fifteen Wistar rats were purchased from the Department of Anatomy, School of Basic Medical Science Animal house. They were housed in breeding cage in photo-period controlled environment (12hours light, 12 hours dark cycle).

The rats were allowed acclimatization for one week. Body weight range of these rats used was 100g-130g just before acclimatization.

3.3 Experimental designs

The Wistar rats were selected randomly and divided into three groups which are control, 14days exposure and 28days exposure. Acclimatization to new environment under normal care was for one (1) week with all fifteen (15) rats on distilled water.

The animals were cared for according to the guideline of the National Institute of Health (NIH) U.S.A for the care and use of laboratory animals.

Group 1 (control group) were given distilled water and pellet feed throughout the administration.

Group 2 (28days exposure) Pb $(C_2H_3O_2)_2$ and Cd Cl 100ppm was administered to the animal for 28days.

Group 3(14days exposure) were administered Pb $(C_2H_3O_2)_2$ and CdCl 100ppm for 14days.

3.4 Blood collection /sacrifice

After four weeks of administration of distilled water and $Pb(C_2H_3O_2)_2$ and $CdCl_2$ to the respective group. All animal in group 1,2and 3 were sacrificed through cervical dislocation.

Blood sample were collected from retro- orbital sinus of each animal using heparinzed capillary tube into EDTA bottles. Thin blood smears on microscope slides were made from the blood in the EDTA bottle. The slides were air-dried in clean, dry environment and subsequently fixed with absolute methanol for 15 min before staining with Maygrunwald and Giemsa stains respectively for about 20 min each. They were viewed under light microscope (PEC MEDICAL USA).

3.5 Erythrocyte Morphology

Erythrocyte morphology and quantification was carried using smear techniques. A drop of blood was placed on the slide for the preparation of thick and thin smear. The prepared smears were then dried in air for a small time. After this ethanol were used for the fixation of blood smears and allowed it in air for few minutes to dry. When it became dry then deep it in water for removing of ethanol from slide. Staining of Slides: The fixed smears are then kept in gimesa

stain approximately for 15 minutes. After 15 minutes the stain slides are removed from the gimesa stain and allowed in air to become dry.

Microscopy of Slides: When slides were fully dried then present it for microscopy. The microscopy of slides was performed on 100X of a microscope of a microscope. When the slide was observed under 100X of microscope, an oil emersion was used on the site of slide. For better detection oil emersion is used. During the microscopy of slides, different morphological changes were identified (Muzafar *et Al.,2022)*.

3.6 Procedure for bone marrow cytology

Twenty four hours after last administration, animal were sacrifice through cervical dislocation. Using flush technique, cytologic evaluation of bone marrow was according to the method described by (Bolliger, 2004). They were placed on the center of the dissection tray. Forceps and scissors were used to shave out flesh from the rat bones. Each femur from the animals in the test and control groups were identified and surgically removed. Each bone head and epiphysis was transversely sectioned in order to access the femoral internal matrix (medulla). The content was flushed into Eppendorf tube using 2.2ml sodium citrate. The mixture was carefully vortexed to make a homogenous mixture, cytocentrifuged at –5 °C (Centurion Scientific Model K241R) with the supernatant removed; thin “drawback and push-away” smear readily made on the microscope slide from the sediment using a pasture pipette. The slide was allowed to air dry, fixed in absolute methanol for 15 min, stained with May-Grunwald and counter-stained with Giemsa before it was viewed under a light microscope.

3.7 Statistical Analysis

All data were subjected to statistical analysis using graph pad prism software (Version 8.0). One way analysis of variance (ANOVA) was carried out and data were presented as mean ± standard error of mean (SEM). Values of $P \leq 0.05$ were considered statistically significant in all cases while n-values denote number of animals in each experimental group.

CHAPTER FOUR

RESULTS

4.1 Body weight

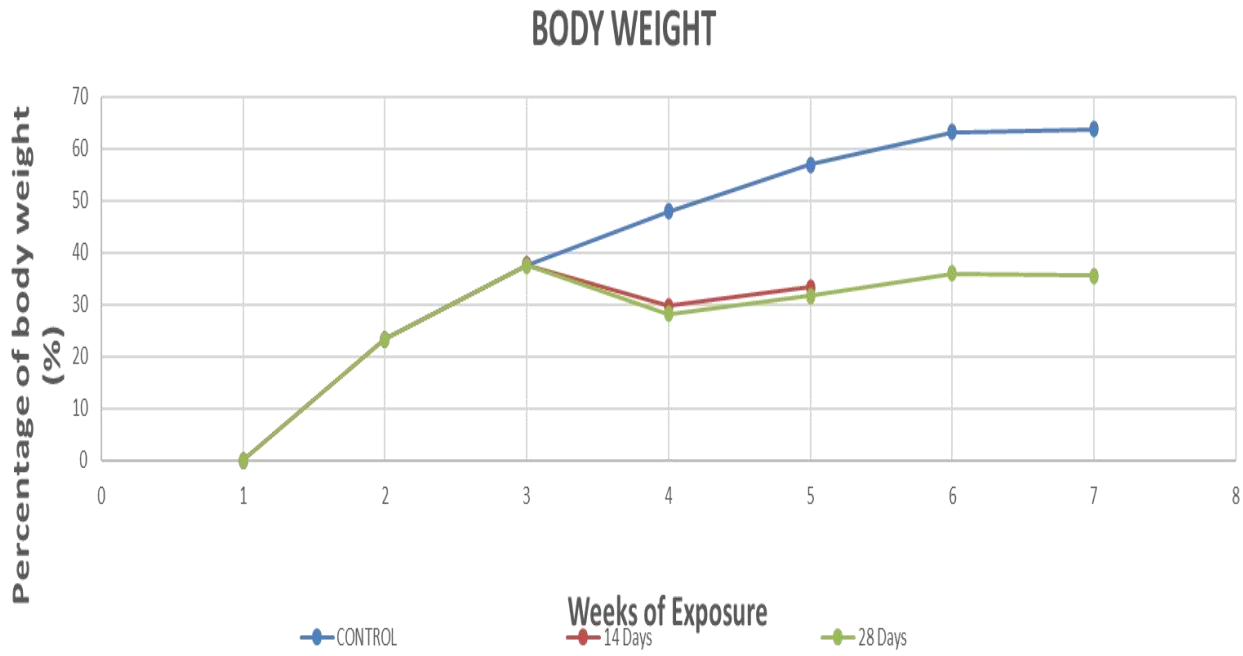

Fig.1: Effects of co-administration of lead acetate and cadmium chloride on the body weight of Wistar rats for 14 days and 28 days.

The body weight of the rat significantly decrease following co-exposure to lead acetate and cadmium chloride for 14-day and 28-day groups when compared with the control. P≤.0.05.N=5;±SEM.

4.2 Erythrocyte Morphology

Control group

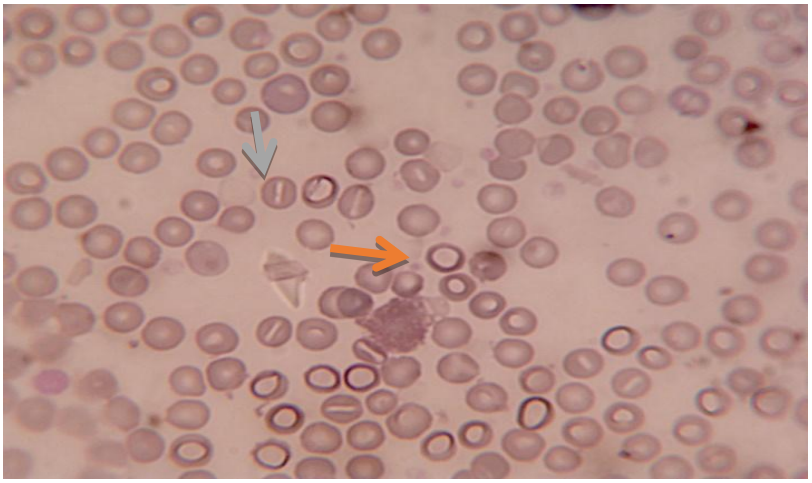

Plate1a: Acanthocyte (black arrow), Stomatocytes (ash arrow), hypochromasia (orange arrow).

14days group

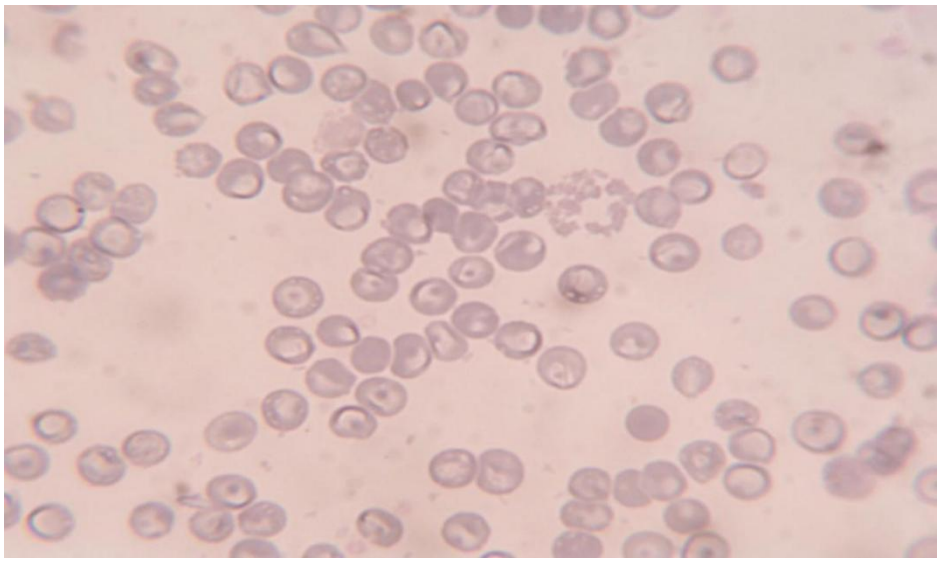

Plate 2a:Grossly stippled erythrocytes, acanthocyte, target cells, fragmenting erythrocytes and hypochromasia

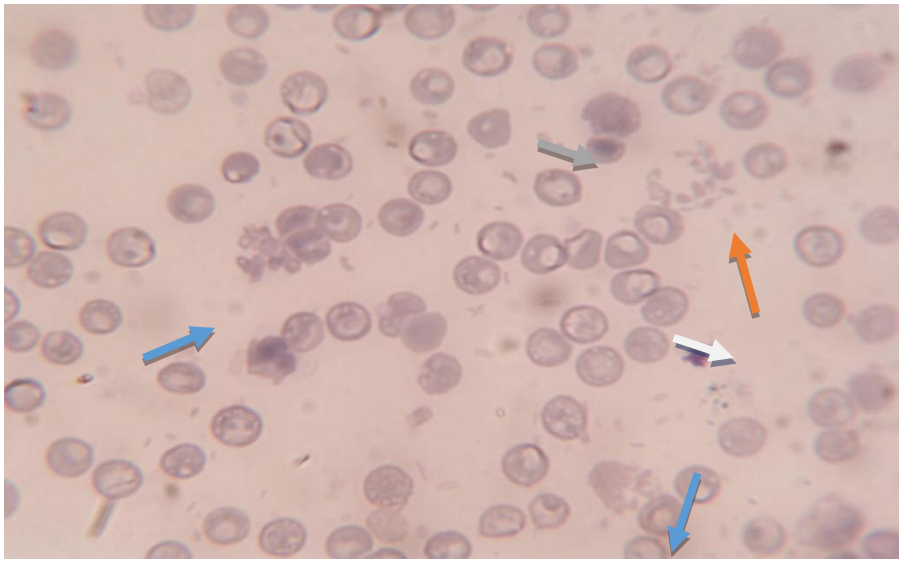

Plate2b: Grossly stippled erythrocytes, acanthocyte, target cells, fragmented erythrocyte (blue arrow) fragmenting erythrocytes (red arrow), bite cell (ash arrow) and hypochromasia.

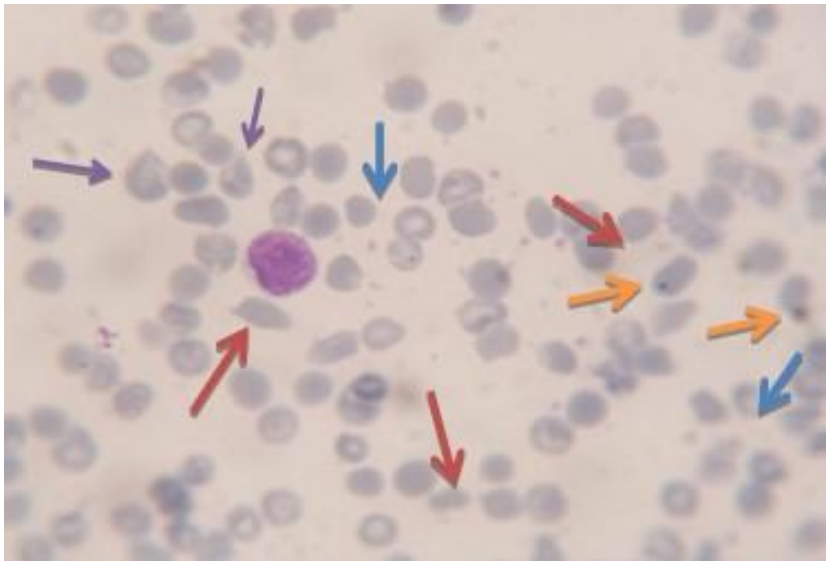

Plate3a: Howell-Jolly body (orange arrow), fragmented erythrocyte (brown arrow), tear drop cell (purple arrow) Microcytes are seen dispersed in the entire field.

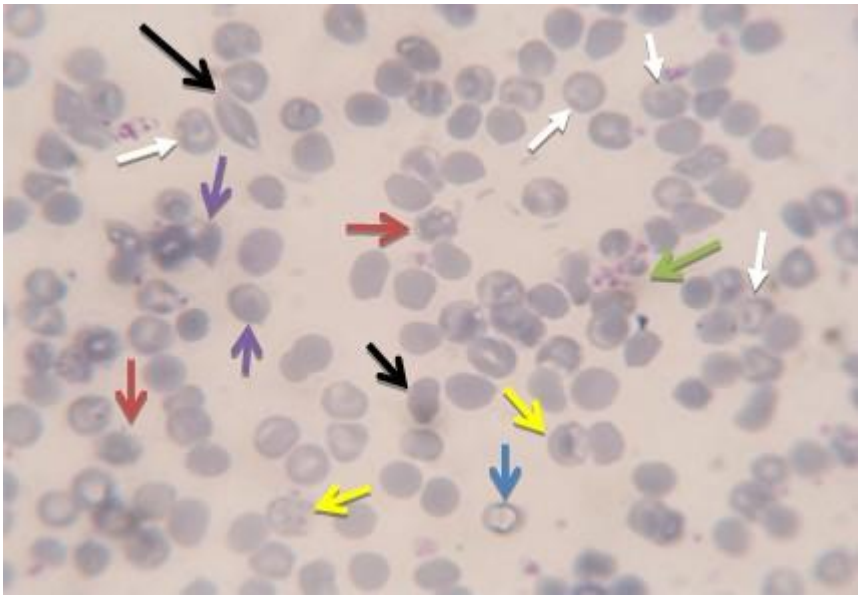

Plate3b: Tear drop cell (purple arrow) with evidence of traumatic erythrolysis (green arrow) , stippled erythrocyte (yellow arrow), elliptosis (black arrow), acanthocyte (red arrow), codocyte/target cells (white arrow)

4.3 Bone_marrow

Control group

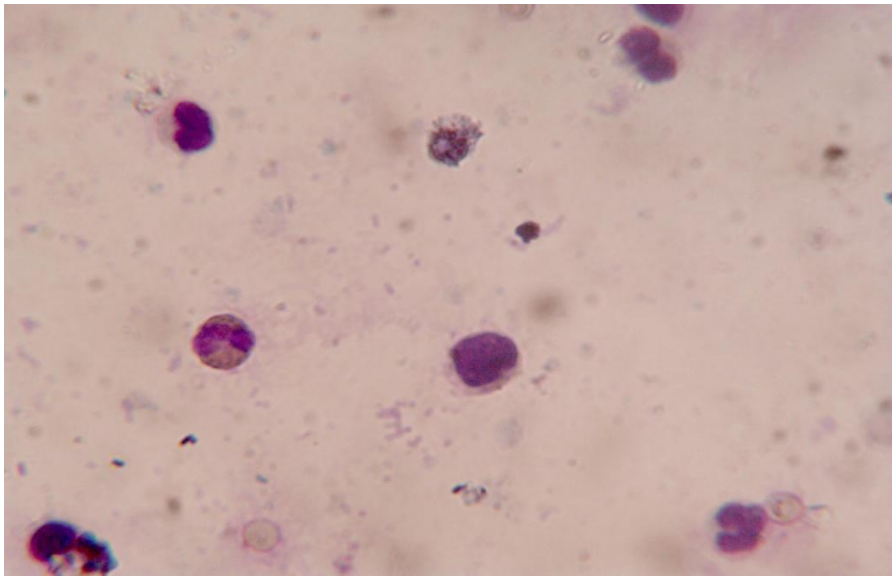

Plate4.

14days group

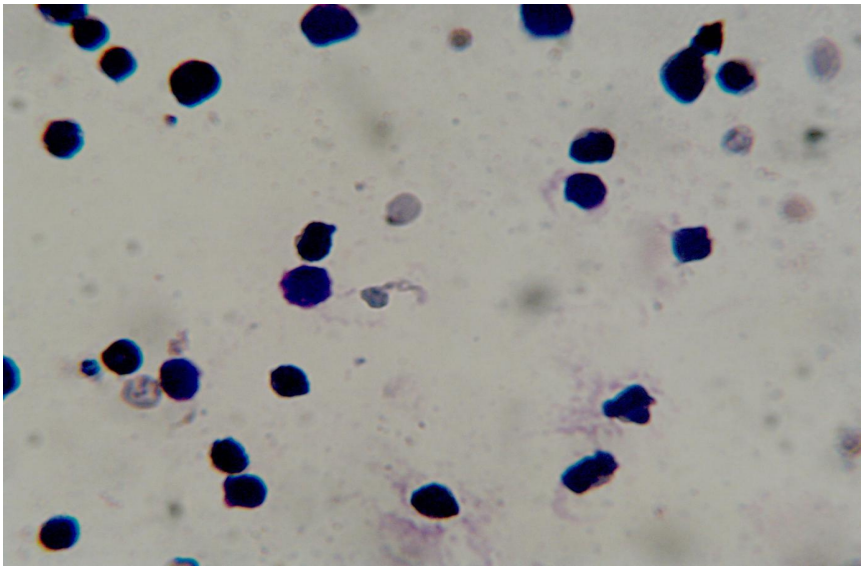

Plate5.

28days group

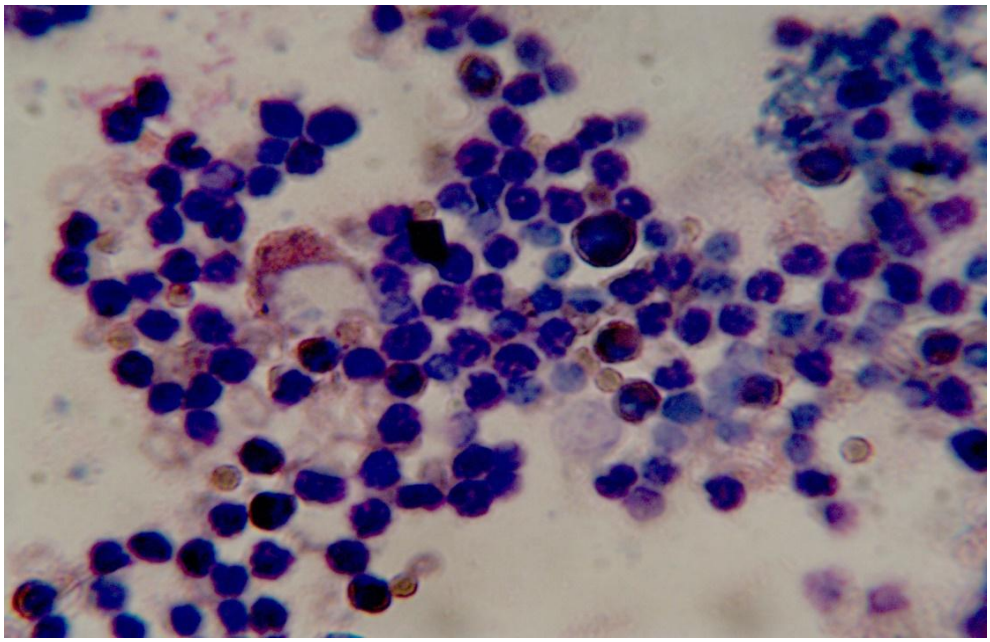

Plate6a:Large aggregate of erythroid, lymphoid cellular series recruitment. Reticulocytes are also seen distributed within the field.

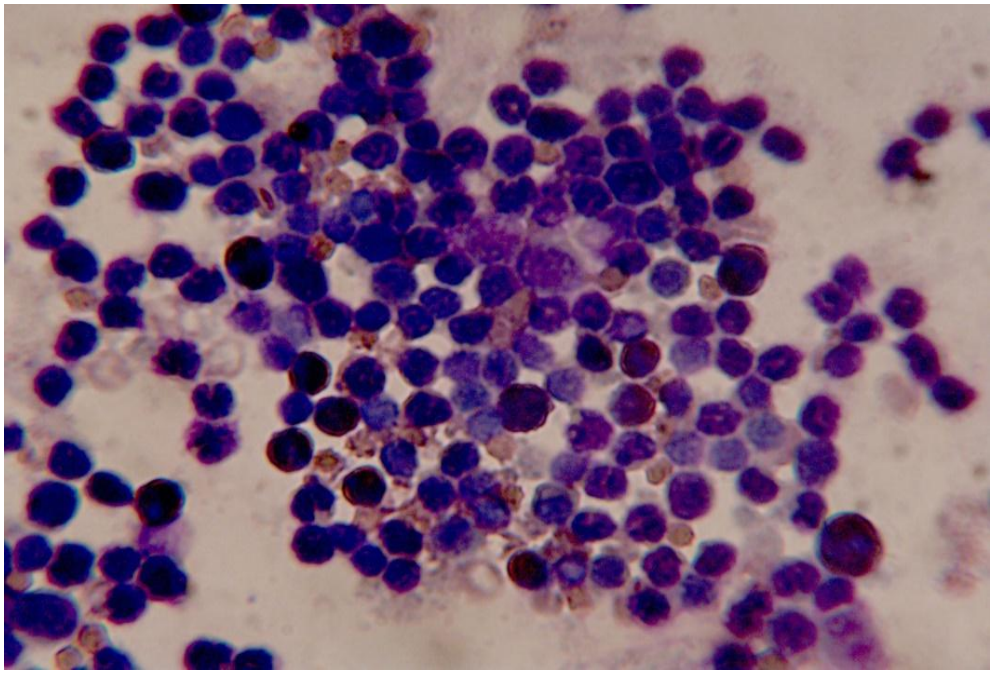

Plate6b: Massive erythroid and lymphoid cellular series recruitment interspersed by the presence of reticulocyte in the bone marrow.

CHAPTER FIVE

DISCUSSION

The bone marrow is a soft, specialized connective tissue that has blood vessels and is found in the center of the bones. Is a loose, spongy network of reticular fibers and associated cells fills the medullary cavities of bone and provides a supporting framework (stroma) for the hemopoietic cells (monga *et al.,* 2022). Erythrocytes are the major cellular component of the circulating blood. Erythrocytes have biconcave discs, with very high plasma membrane integrity and cytoskeleton stability. This means that their periphery is thicker than their central part. Erythrocytes are sensitive cells and can serve as cellular indicators (Pretorius, and Kell, 2014).

In this study male Wistar rats were administered lead acetate and cadmium chloride orally for14 days for groups 2 and 28days for group 3 to observe the effect erythrocyte morphology and bone marrow cytology. The result for the bone marrow cytology shows abundant erthroid series in the 28days co exposure group, also lymphoid cellular series recruitment interspersed by the other reticulocyte of the bone marrow when compared with the control. Reticulocytes are also seen distributed with in the field. The 14days treatment also have a massive erthroid and lymphoid cellular series recruitment interspersed by the other reticulocyte of the bone marrow when compared with control.

This indicates that sub-acute co-exposure to lead acetate and cadmium chloride affects the bone marrow cytology in which they increase the erythroid series

and lymphoid series and also there is presence of undeveloped red blood cells also called reticulocyte scattered in the cell of the bone marrow. Then in the erythrocyte morphometry, lead acetate and cadmium chloride affects the shape (slightly rounded or blunted) and color (faded) of the cells and there are microcytes which are unusual red blood cells which are seen scattered in the entire field. The microcytes appear when the cell is damaged or when there is a disease found in the cell. e.g Anaemia. Acanthocyte and Tear drop significantly increased in the 28days co-exposure group when compared with 14days and control group. There was a significant increase in Bite cell on the 28th days exposure group when compared with the control. Stippled erythrocyte significantly decreased in 28th days exposure group but increased in 14days administered group. Hypochromasia and Stomatocyte increased significantly in the treatment groups when compared with the control. Increase in stomatoyctes are associated with very rare hereditary disorders of red cell cation permeability leading to increased or decreased red cell water content, and this could lead to cardiovascular disease (Gallagher, 2018). The overall observation from this result was that lead acetate and cadmium chloride affect the erythrocyte morphology of adult Wistar rats (Acanthocyte, tear drop, bite cell, stippled erythrocyte, hypochromana, stomatocyte, and howell cell/codocyte). This result agrees with a study by (Saini, 2021) in their study on erythrocyte morphology, osmotic fragility and hematological studies after short term dietary copper deficiency in male Wistar rats. Their study observed there was occurrence of

stomatocyte, acanthocytes, howell-jelly in the rats after 2 and 4weeks of copper deficiency.

CONCLUSION

In conclusion, it was observed from this result that sub-acute co exposure to lead acetate and cadmium chloride affect the erythrocyte morphology in terms of the biconcavity of the RBCs of adult Wistar rats.

This exposure also causes erythrocyte morphology disorder called poikilocytosis which affects bone marrow by causing anemia.

REFERENCES

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.Nordberg, G., Nogawa, K., Nordberg, M., and Friberg, I. (2007).Cadmium In:*Handbookontoxicology of metals*.Pp.65-78.

Agency for Toxic Substances and Disease Registry (ATSDR)(2012). Toxicological profilefor cadmium. Atlanta, GA: US Department of Health and Human Services, Public Health Service.

Ahmed, I., Nawaz, M.,an Ahmad, Z (2010). Does Service Quality Affect Students' Performance? Evidence from Institutes of Higher Learning. African Journal of Business Management, 4, 2527-2533.

Arikan, Hüseyin; Çiçek, and Kerim (2014). "Haematology of amphibians and reptiles: a review" (PDF). North-Western Journal of Zoology. 10: 190–209.

Bolliger, A.P., (2004). Cytologic evaluation of bone marrow in rats: indications methods and normal morphology. Vet ClinPathol 33(2):58–67.

Bressler J.P., Olivia, L., Cheong, J.H., Kim, Y., and Bannona, D., (2004). Divalent metal transporter in lead and cadmium transport. Ann N Y Acad Sci.; 1012:142–52.

Brzó;ska, M. M., Moniuszko, B. and Sawicki, J. (2002).Liver and kidney function in rats exposed to cadmium *Lab. Anim. Sci*.**56(11)**:549-554.

Bulat, J., Hayes, A., Macon, W., Richa, R., And Abery, B.(2015).School and classroom disabilities inclusion guide for low- and middle-income countries Research triangle Park, NC: RI Press

Bulmer, F.M.R., Rothwel, H.E. and Frankish, E.R. (1938).industrial cadmium poisoning. *Can.Public.Hlth J.* **29**:19-26.

Byorklund, G. (1995). "Mercury and acrodynia" *Journal of Orthomolecular Medicine.***10 (3 and 4)**:145-146.

Carafoli, E., (1987). Intracellular calcium homeostasis. *Annu Rev Bioche* **56**:395–433.

Chan, Brian Y.; Gill, Kara G.; Rebsamen, Susan L.; Nguyen, and Jie C. (2016). "MR Imaging of Pediatric Bone Marrow". RadioGraphics. 36 (6): 1911–1930.

Ciesla, B.(2007).Hematology in Practice. Philadelhia: F. A.Davis Company.

Duffus, J.H. (2002). Heavy metals—A meaningless term? Pure and Applied Chemistry. 74(5): 793 – 807.

Fergusson, J.E. (1990.) The Heavy Elements: Chemistry, Environmental Impact and Health Effects. Pergamon Press, Oxford, 85-547.

Flora S.J.S., Pachauri, V., and Saxena, G.,(2010). Academic Press; Arsenic, cadmium and lead. Reproductive and Developmental Toxicology; pp. 415–438.

Fowler, F., H. Wang, and E. Lightbourn,(2008). Optimizing Operations for MATS Compliance and Improved Unit Economics. 15th Annual Electric Power Conference & Exhibition. Rosemont, IL. May 14-16.

Gallagher, P.G., (2018).Update on the clinical spectrum and genetics of red blood cell membrane disorders. Curr Hematol Rep. ;3:85–91.

Genchi, G., Sinicropi, M.S., Lauria, G., Carocci, A., and Catalano, (2020). The effects of cadmium toxicity. Int J Environ Res Public Health.17(11).

George, J.W., and Duncan, J.R., (1979) . The hematology of lead poisoning in man and animals. Vet ClinPathol; 8(1):23–30.

Green, R., and Widder, K. J., (1987). Methods in Enzymology, vol. 149.San : Academic Press. vational study. Cephalalgia, 0(0), 1-6.

Guyton, A. C., and Hall, J. E., (1996) .Red blood cells, anemia and polycytemia.In Textbook of Medical Physiology, 425–433.

Hambach, A., Evers, S., Summ, O., Husstedt, I.W., and Frese, A. (2013). The impact of sexual activity on idiopathic headaches: An observational study. Cephalalgia, 0(0),

Herron, N. (2001). Cadmium compounds i: kirk-othmer Encyclopedia of chemical Technology, 5th ed. Vol. 4, New York: John Wiley and Sons.Pp.507-523.

Hindorf, C.; Glatting, G.; Chiesa, C.; Lindén, O.; Flux, G. (2010). "EANM Dosimetry Committee guidelines for bone marrow and whole-body dosimetry". Eur J Nucl Med Mol Imaging. **37** (6): 1238–1250.

Hoffman, J.F. (1987).On the mechanism and measurement of shape transformations of constant volume of human red blood cells Blood Cells, 12,pp. 565-588.

Horiguchi, H., Oguma, E., and Kayama, F. (2011). Cadmium induces anemia through interdependent progress of hemolysis, body iron accumulation, and insufficient erythropoietin production in rats. ToxicolSci; 122(1):198–210.

humanredblood cells and intracellular distribution. Kitasato Arch ExpMed63(4):15–23.

International Programme on Chemical Safety (IPCS).Cadmium. Poisons Information Monograph. PIM 089.

Jarup, L., Berglund, M., Elinder, C.G., Nordberg, G., and Vahter, M. (2003). Health effects of cadmium exposure—a review of the literature and a risk estimate. Scand J Work Environ Health; 24 (Suppl 1): 1–51.ularis

John, P., Jonesa, Juan, P., Carricart-Ganivetb, Roberto, I. P., Susana, E., Michael, A., Rinat, I., And Gabito (2010)Microstructural variation in oxygen isotopes and elemental calcium ratios in the coral skeleton of Orbicella annularis. chemical geology 419.

Lacombe, C., Dasilva, J.L., Bruneval, P., Casadevall, N., Camilleri, J.P., and Bariety, J., (1991) Erythropoietin—sites of synthesis and regulation of secretion. Am J Kidney Dis; 18(4):14–9.

Landis-Piwowar, K, Landis J, and Keila P, (2015).The complete blood count and peripheral blood smear evaluation. In: Clinical laboratory hematology. 3rd ed. New Jersey: Pearson; p. 154-77.

Lewis, D. A., andAlpar, H. O. 1984. Therapeutic possibilities of drugsencapsulated in erythrocytes. Int. J. Pharm. 22:137–146. 4.

Lockitch G, Berry B, Roland E, Wadsworth L, Kaikov Y, and Mirhady F. (1991) Seizures in a 10-week-old infant: lead poisoning from an unexpected source. Can Med Assoc J;145:1465–8.

Lund JE (2000) Toxicologic effects on blood and bone marrow. In: Feldman BF, Zinkl JG, Jain NC (eds) Schalm's Veterinary Hematology, 5th edn. Lippincott Williams and Wilkins, Philadelphia PA, pp 44–50.

Matovic′, V.; Buha, A.; Bulat, Z.; Dukic′-′Cosic′, D.; Đukic′-′Cosic′, D., Cadmium toxicity revisited: Focus on oxidative stress induction and interactions with zinc and magnesium. Arh. Hig .RadaToksikol. 2011,62,65–76.

Mojiminiyi FBO, Merenu IA, Ibrahim MTO, Njoku CH (2008).The effect of cement dust exposure on haematological and liver function parameters of cement factory workers in Sokoto Nigeria. Niger J PhysiolSci 23(1–2):111–114.

Monga I, Kaur K, Dhanda S (2022). "Revisiting hematopoiesis: applications of the bulk and single-cell transcriptomics dissecting transcriptional heterogeneity in hematopoietic stem cells". Briefings in Functional Genomics. **21** (3): 159–176..

Muzafar S., Haseena, K., Noor S., Sania, S., Akhtar, R., Fazal, A., and Muhammad I,(2022).Morphological Study of Human Blood for Different Diseases, Biomedical Journal of Scientific & Technical Research,Vol 30,(1), pp 23047-23057.

Partick, Lyn. (2006). Lead toxicity, a review of the literature. Part 1: Exposure evaluation, and treatment. Alternative Medicine rewiew11(1): 2-22.

Pond, WG, Yen, JT., Hill, DA., Ferrell, CL., Krook L. (1982). Bone lesions in growing swine fed 3% cement kiln dust as a source of calcium. J AnimSci 54(1):82–88.

Pond., W.G., Yen JT, Hill, D.A., Ferrell, C.L., and Krook ,L., (1982).Bone lesions in growing swine fed 3% cement kiln dust as a source of calcium. J AnimSci 54(1):82–88.

Ponzetti M, Rucci N (2019) Updates on Osteoimmunology: What's New on the Cross-Talk between Bone and Immune System. Front Endocrinol 10.

Poulton, T B., Murphy, W., Duerk, J L.,Chapek, C C., and Feiglin, D H., (1993). "Bone marrow reconversion in adults who are smokers: MR Imaging findings". American Journal of Roentgenology. 161 (6): 1217–1221.

Pretorius, E., and Kell, D.B., (2014).Diagnostic morphology: Biophysical indicators for iron-driven inflammatory diseases. Integr Biol .6(5):486-510.

Reya, T (2003). Regulation of hematopoietic stem cell self-renewal. Recent Prog Horm Res 58:283-95.

Risk Assessment Information System (RAIS) (1991). Toxicity summary for cadmium.Chemical Hazard Evaluation and Communication Group, Biomedical and Environmental Information Analysis Section, Health and Safety Research Division.

Ropars, C.,Chassaigne, M.,and Nicoulau, C.,(1987).AdvancesintheBloscicences, vol. 67. Oxford: Pergamon Press.

Rubin R,david S and Strayer.(2007). Ruubin's pathology:clinicopathological foundation of medical.lippincott William &wilkins;90

Saini, A. R., Kurup and Neena N.,(2021).Erythrocyte morphology, osmotic fragility, hematological parameters-hemoglobin, Indian J Pharm Sci;84(1):27-34

Sanchez, JR. and Lynch DT. (2020). Histology, basophilic stippling.StatPearls. Treasure Island (FL). PMID: 31424843.

Satarug. S., Baker, JR., Urbenjapol, S., Haswell-Elkins, M., Reilly PE., and Williams, DJ., (2020). A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. Toxicol Lett;137(1–2):65–83.

Sethi, p. k.,and Khandelwal., D.J. (2006). Cadmium exposure: health hazards of silver cottage industry in developing countries. *Med. Toxicol*. **2**:14-15.

Sugawaram ,E., Nakamura, K., Fukumura, A.,and Seki Y. (1990).Uptake of lead by

Tchounwou, P.B.; Yedjou, C.G.; Patlolla, A.K.; and Sutton, D.J. (2012) Heavy metal toxicity and the environment, 101, 133–164.

Telen, M. J. 1993. The mature erythrocytes. In Winthrob'sHematology, 9th ed., eds. R. Lee, T. C. Bithel, J. Foerster, J. W. Athens, J. N. Lukens, 101–133.

Thompson, W.G., Meola, T., Lipkin, M., and Freedman M.L. (1988). Red cell distributionwidth, mean corpuscular volume, and transferrin saturation in the diagnosis of iron deficiency. Arch Intern Med; 148.

Walsh, MC., Takegahara, N., Kim, H., Choi, Y. (2018).Updating osteoimmunology: regulation of bone cells by innate and adaptive immunity. Nat Rev Rheumatol 14(3):146–156.

Ward, JM., Cherian, S., Linden, MA. (2018).Hematopoietic and lymphoid tissues. In: Treuting PM, Dintzis SM, Montine KS (eds) Comparative Anatomy and Histology a mouse rat and human atlas, 2nd edn. Academic Press, Elsevier, pp 365–401.

Zbakh, H., Salhi, G., Bochkov, V., Ciudad, C.J., Noé, V., Hassoun, M.,and Riadi, H(2012).Potential use of olive mill wastewater in the preparation of functional beverages.vol(4):p234

Zimmermann, U. (1983). Cellular drug-carrier systems and their possible targeting.InTargetedDrugs,ed.E.P.Goldberg,153–200.

APPENDIX

Red Cell Morphology

Red Cell Morphology	Control	High Dose	Low Dose
Acanthocyte	0.0±0.0	10.0±1.5	6.0±0.2
Tear drop	0.0±0.0	9.0±1.2	3.0±0.0
Bite cell	0.0±0.0	6.0±0.4	5.0±0.1
Stippled erythrocyte	0.0±0.0	2.0±0.6	35.3±2.9
Hypochromasia	2.0±0.0	11.0±2.0	26±3.1
Stomatocyte	1.2±0.0	6.0±0.2	14.2±2.2
Target cell/Codocyte	-	12.0±1.0	-
Howell-Jolly body	0.0±0.0	7.0±0.2	5.0±0.5
MIN erythrocyte	-	4.0±0.9	-

*The difference between the values of red cell morphology of the experiment groups (High dose and Low dose) and the control group at 0.05.

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Stomatocyte	1.2±0.0	6.0±0.2	14.2±2.2
Target cell/Codocyte	-	12.0±1.0	-
Howell-Jolly body	0.0±0.0	7.0±0.2	5.0±0.5
MIN erythrocyte	-	4.0±0.9	-
Fragmenting erythrocyte	1.0±0.0	-	6.0±0.9

Bone marrow

Groups	Erythroid series (%)	Lymphoid series (%)	Lymphocyte (%)
control	21	11	8
14days exposure	73	16	11
28days exposure	49.5	42.5	14