COMPARATIVE HISTOMORPHOLOGICAL AND HISTOSTEREOLOGICAL TERATOGENIC EFFECTS OF PRENATAL EXPOSURE TO PHENOBARBITAL AND PHENYTOIN ON THE DIFFERENTIATION OF EPIPHYSEAL GROWTH PLATE OF THE LONG BONE IN ALBINO RATS (*Rattus norvegicus*)

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Comparative Histomorphological and Histostereological Teratogenic Effects of Prenatal Exposure to Phenytoin and Phenobarbital on the Differentiation of Epiphyseal Growth Plate in Albino Rats (*Rattus norvegicus*)

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DECLARATION

Thesis is my original work and has not been presented for a degree in any other university

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DEDICATION

I dedicate this research to my lovely wife Catherine Mwihaki, and My daughters Joy, Precious and Ivanna for their support, prayers and encouragement.

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ACRONYMS AND ABBREVIATION

AEDS	Antiepileptic drugs
ANOVA	Analysis of variamce
FDA	Food,Drug and Substance Organisation
JKUAT	Jomo Kenyatta University of Agriculture and Technology
MANOVA	Multivariate Analysis of Variance
PT	Phenytoin
PB	Phenobarbital
RLS	Resource-Limited Setting
SAFARI	Small Animal Facility For Research and Innovation
SUDEP	Sudden Unexpected Death in Epilepsy
TM 1	Trimester One
TM 2	Trimester Two
TM 3	Trimester Three
UON	University of Narobi
WHO	World Health Organization
WWE	Women With Epilepsy

DEFINITION OF OPERATIONAL TERMS

Histomorphological refers to the study of the structure of tissues, specifically in this context, the examination of changes in the structure of the epiphyseal growth plate due to prenatal exposure to phenytoin and phenobarbital by use of microscopy analysis.

Histostereological is a histo-quantitative approach used to analyze three-dimensional structures within tissues. In this case, it involves the quantitative assessment of changes in the epiphyseal growth plate caused by exposure to phenytoin and phenobarbital.

Teratogenic Effects refers to the adverse effects on the developing embryo or fetus when exposed to substances, such as drugs or chemicals, during pregnancy. In this context, it pertains to the impact of phenytoin and phenobarbital on the development of the epiphyseal growth plate, which is a critical region for bone growth during the prenatal period.

Prenatal Exposure This term signifies that the exposure to phenytoin and phenobarbital occurs during the period of pregnancy.

Phenytoin and Phenobarbital These are medications that are often used to treat epilepsy and they have potential teratogenic effects when used during pregnancy, which can impact bone growth.

Epiphyseal Growth Plate is a cartilaginous region located at the proximal and distal ends of long bones in the body, where bone growth occurs during childhood and adolescence. It's crucial for proper bone development and lengthening.

ABSTRACT

The prenatal exposure to phenytoin and phenobarbital in the management of maternal neurological disorders like epilepsy has been associated with a wide range of fetal neurological and musculoskeletal malformations. What is yet to be established is their comparative histostereological and histomorphological teratogenic effects on the fetal skeletogenesis of the long bones, hence the basis of this study. In carrying out this study, a post-test-only experimental study design was adopted. All the animal experimentation was carried out in the animal research facility situated at the University of Nairobi (UON), while the bone tissue processing for histology and histostereological was carried out in the Department of Human Anatomy of JKUAT. A Sample size of 30 albino rat dams weighing between 220±30 grams were used, for each of the study medicine. This sample size was determined by the use of the resource equation for the one-way Analysis of Variance method (ANOVA). The 30 Albino rats in each of these two study categories of phenobarbital and phenytoin were first broadly divided into two study groups of 3 rats for the control group and 27 rats for the experimental group. To evaluate whether the teratogenic effects of both medicines are dose-dependent, the 27 rats in their experimental groups were further subdivided into three study subgroups of 9 rats as follows; (i) 9 rats for low doses of phenobarbital and phenytoin group (31mg/kg/3.1mg/kg, respectively), (ii) 9 rats for medium doses of phenobarbital and phenytoin group (medium 62mg/kg and 19mg/kg, respectively), (iii) 9 rats for the high phenobarbital and phenytoin group (124mg/kg and 41.35mg/kg, respectively). To further evaluate whether the observed teratogenic effects are time-dependent, the 9 rats in each of the three-dose categories were further sub-divided into three subgroups of 3 rats each according to the trimesters of exposure as follows; (i) 3 rats for trimester one (TM_1); (ii) 3rats for trimester two (TM_2) and (iii) 3 rats for trimester three (TM_3). All rats in both control and experimental groups were fed on rodent pellets and water *ad-libitum* and sacrificed on the 20^{th} gestational day. The fetal bones were harvested for both histomorphological and stereological analysis. Histomorphological data information of photomicrographs was taken using a Swift 3.0 microscope digital camera (20mega pixels), and uploaded to Swift 3.0 software for labelling. The maternal and fetal pregnancy outcomes and stereological data (parametric) were collected using structured checklists, stored in Excel spreadsheets Windows 10, version 2016, and then exported for analysis into the SPSS program for Windows version 25 for analysis (Chicago Illinois). Data was expressed as mean ± SD for all values. The analysis applied both one-way ANOVA for intragroup comparisons, MANOVA for interaction effects and pairwise comparisons. All results whose $P<0.05$ were considered significant. The findings of this study established that both phenobarbital and phenytoin had teratogenic inhibitory effects on the developing epiphyseal growth plates of the long bones in a dose and time-dependent manner particularly at TM_1 and TM_2, with phenobarbital having more detrimental effects than phenytoin. It was therefore recommended that high dosages of the two medicines where possible should be avoided at TM_1 and TM_2. Further studies with non-human primates were recommended to help come up with findings that will apply to humans.

CHAPTER ONE

INTRODUCTION

This chapter starts by giving a brief introduction to phenytoin and phenobarbital chemical constituents, their mode of teratogenicity, followed by the problem statement, justification and significance of the study, research questions and the study objectives, the hypothesis of the study, the aim of the study, assumptions of the study, the limitation, the delimitations of the study and the conceptual framework

1.1 Background Information

Phenytoin is sold under the brand name dilatine and has a chemical formula of $C_{15}H_{12}N_2O_2$ and a molecular weight of 252.3 g/mol, on the other hand, phenobarbital is a barbotine derivative with a chemical formula of $C_{12}H_{12}N_2O_3$ and a molecular weight of 232.235 g/mol. Phenytoin and phenobarbitone are anticonvulsant medicines that are among the most prescribed medicines in the management of maternal neurological conditions like epilepsy, seizures, and bipolar diseases among others (Nevitt *et al*., 2019). Studies have shown that all anticonvulsant medicines have some degree of teratogenicity to the fetal organogenesis including the teratogenic perturbations to the fetal musculoskeletal development. As such, the American Food, Drug and Substance Organization (FDA) has classified these medicines under class C medicines where they have to always be used with caution during pregnancy (Gedzelman & Meador, (2012); Etemad *et al., (*2012). Although the teratogenic perturbations of the two medicines on the developing fetal skeletogenesis have been reported, it is still not clear on their comparative histomorphological and histostereological teratogenic effects of the two medicines on the epiphyseal growth plates of the developing long bones. At the same time, whether or not their teratogenic effects on the developing epiphyseal growth plates of the long bones are dose and time-dependent is yet to be elucidated.

Existing literature has shown that many anticonvulsant drugs have well-established safety profiles in adults, but less is known about their teratogenic outcomes in the

developing foetuses when prenatally exposed at varying doses and at different gestational periods (Al Watatr *et al.,* 2015), similarly, the comparative histomorphological and histostereological teratogenic effects of phenobarbital and phenytoin when exposed prenatally on fetal skeletal growth and development *in-utero* is not well elucidated (Taylor *et al.,* 2003). Further-more whether or not the observed effects on the fetal skeleton are dose and time-dependent is also not very clear.

Phenobarbital retails by among other names as Nembutal, and Luminal, among others while phenytoin retails by the name Dilantin. Phenobarbital is a substituted pyrimidine derivative while phenytoin is a hydantoin derivative with molecular formulas of C12H12N2O3 and C15H12N2O2 and molecular weights of 232.235 g/mol 252. 268 g/mol respectively. Phenytoin and phenobarbital are both first-generation anticonvulsant medicines with similar effectiveness (Dizon *et al.,* 2019). Both medicines are currently being used as a first-line treatment in several developing countries including Kenya as anti-convulsants acting in the central nervous system, or as sedatives in the management of maternal epilepsy, anxiety, and anxiety-related disorders among other neurological conditions (Lutes, 2020; Kwan & Brodie, 2004) They exert their anticonvulsive effects by inhibiting serotonin (5-HT) and γ-amino butyric acid (GABA) neurotransmitters in the brain (M. Keppel Hesselink, 2017).

Their prenatal teratogenicity is thought to be caused by the ability of their metabolites namely 4′-hydroxylated DP and 3′,4′-Dihydroxylated product (3′,4′-diHPPH) to cross the blood-placenta barrier between the mother's blood to the developing fetal tissues hence these metabolites accumulate in the fetal tissues interfering with the process of embryogenesis, organogenesis and morphogenesis. Past literature is not clear on their effects on developing fetuses based on the duration of exposure (Czeizel et al., 2011).

Past literature has associated the use of phenobarbital, phenytoin and other related medicines with detrimental effects on the developing fetus that include developmental delay, and cognitive impairment among other major congenital malformations (Birnbaum *et al*., (2020; Weston *et al*., (2016); Gedzelman & Meador,

(2012), their use cannot be discontinued in pregnancy because of the risk of uncontrolled seizures that can be harmful to both the mother and the fetuses (Galappatthy *et al*., 2018; Weston *et al*., 2016). This is despite the fact that the comparative histomorphological and histo-steological data on fetal skeletal of developing fetuses upon prenatal exposure to phenobarbital and phenytoin medicines is scarce in sub-Saharan Africa and especially in Kenya, hence the basis of this study.

1.3 Statement of the Problem

Skeletal disorders are common conditions that are associated with children born of women taking antiepileptic drugs thus interfering with quality of life later in life (Pack, 2003). It's estimated that about 6.7% of these children will develop growth retardations (Giménez *et al*., 2019). The best care for women with epilepsy during pregnancy aims at achieving complete seizure control while decreasing fetal exposure to the potentially harmful effects of anti-seizure medications (McAuley *et al*., 2012). Antiepileptic drugs are crucial to control seizures and other epileptic symptoms and untreated epilepsy can cause harm to both the mother and the unborn baby. Phenobarbital and phenytoin are used in Kenya to manage epilepsy among pregnant women. Phenobarbital and phenytoin have good seizure control effects, but their effect on fetal skeletogenesis is not well elucidated (Abou-Khalil, 2016). This raises a dilemma faced by healthcare workers and expectant mothers on what is the least teratogenic drug among the two drugs and at what dosages. This dilemma is further exacerbated by the scarcity of data concerning the histomorphological and histostereological alterations in fetal growth plates that occur following exposure to phenytoin and phenobarbitone. Therefore this study aims at addressing this knowledge gap on the histomorphological and histostereological effect of the two drugs on prenatal skeletogenesis, hence guiding healthcare decisions to determine which of these medications might have lesser teratogenic effects and therefore can be safer for use during pregnancy.

1.4 Justification of the Study

Skeletal disorders that are on the increase will continue to rise if proper intervention will not be established. Antiepileptic medications are among the most common teratogens prescribed to women of reproductive age (Meardor *et al.* 2018). Many studies have linked exposure to antiepileptic drugs (AEDs) to negative child outcomes that include major congenital malformations, developmental delay, and cognitive impairment. In addition, the occurrence of maternal seizures during pregnancy has also been linked to poor neonatal outcomes and cognitive impairment in children (Weston *et al.*, 2016). The balance between maintaining maternal seizure control during pregnancy and not over-exposing the developing fetus to AEDs remains a challenge for clinicians (Lb *et al*., 2001). Concerns regarding the effects of AEDs on the fetus often result in discontinuation or reduction in the dose of the AEDs, thereby increasing the woman's risk of convulsions (Meardor *et al.* 2018). Due to an increase in the use of phenobarbital and phenytoin in many clinical settings and the limited information on its safety in pregnancy, there is a critical need for evidence to help pregnant women or women of childbearing age and their healthcare providers to balance the risks and benefits of the drugs with regard to pregnancy-related outcomes.

1.5 Significance of the Study

The availability of teratogenic histomorphological and histostereological information will guide the clinical decision and helps in policy-making guided by scientific finding. Any service that cares for women with epilepsy will need to provide evidence-based information on the risks to the mother and baby and the benefits of appropriate treatment. Through the findings of this study, pregnant women with epilepsy can be made aware of their condition and the possible effects of the drugs. Through this, the women can take deliberate steps to manage both their condition and their pregnancy. Understanding the teratogenic effects of phenobarbital and phenytoin will be substantially valuable because it will help to potentially guide clinical practice and inform studies aimed at understanding the expected pregnancy outcomes. Clinicians can therefore take appropriate actions to mitigate the side

effects of the two medicines. Health workers can tailor interventions to the high-risk nature of pregnant women with epilepsy and to help prevent unnecessary instances of morbidity in this patient group. The findings of this study can act as a baseline for future intervention studies.

Although use of these antiepileptic drugs during pregnancy is associated with major congenital malformations in the fetus, they cannot be discontinued in many women planning pregnancy because of the risk of uncontrolled seizures that can be harmful to the mother as well as to the child (Galappatthy *et al*., 2018). Unfortunately, histomorphological and histosteological data on pregnancy outcomes of women on antiepileptic drugs is scarce in sub-Saharan Africa, especially in Kenya. This study therefore seeks to comparatively evaluate histostereological and histomorphological teratogenic effects of exposure to phenobarbital and phenytoin on developing foetal skeleton in albino rats.

1.6 The Broad objectives, Research question and Specific Objectives of the Study

1.6.1 The Broad Objective

To comparatively evaluate histomorphological and histostereological teratogenic effects of prenatal exposure to phenobarbital and phenytoin on the differentiation of epiphyseal growth plates in albino rats.

1.6.2 Specific Objectives

i) To comparatively compare how maternal and fetal pregnancy outcomes between phenobarbital and phenytoin compare when prenatally administered at varied doses in albino rats.
ii) To establish the comparative histomorphological teratogenic effects of phenobarbital and phenytoin on the differentiation of epiphyseal growth plate in albino rats
iii) To compare histoquatitative teratogenic effects of phenobarbital and phenytoin on the differentiation of epiphyseal growth plate in albino rats

iv) To establish whether histomorphological and stereological teratogenic effects of phenobarbital and phenytoin are both time and dose-dependent.

1.6.3 The Research Questions

i) What are the maternal and fetal pregnancy outcomes between phenobarbital and phenytoin when prenatally administered at varied doses in albino rats?
ii) What are the histomorphological teratogenic effects of phenobarbital and phenytoin on the on the differentiation of epiphyseal growth plate in albino rats?
iii) What are the histostereological teratogenic effects of phenobarbital and phenytoin on the differentiation of epiphyseal growth plate in albino rats ?
iv) What are the histomorphological and histostereological teratogenic effects of phenobarbital and phenytoin both time and dose-dependent?

1.7 Null Hypothesis

There are no significant comparative differences in the histomorphological and the histo-stereological teratogenic effects of phenobarbital and phenytoin on the development of the fetal bones when exposed in varied doses and at different gestation periods in albino rats (*Rattus norvegicus*).

1.8 Study Assumptions

i) The current study assumes that the structure of the skeleton of the albino rats (*Rattus norvegicus)* resembles those of humans
ii) The current study assumes that the albino rat model is a suitable surrogate for studying the teratogenic effects of phenobarbital and phenytoin in humans during the prenatal period. This assumption implies that the biological responses and mechanisms in albino rats are reasonably representative of those in humans.
iii) The current study assumes that the administration of phenobarbital and phenytoin to pregnant albino rats accurately reflects the drug exposure scenarios encountered by pregnant women.

iv) The current study also assumes that there are no uncontrolled variables or confounding factors that significantly influence the observed teratogenic effects. This assumption is essential for attributing changes in fetal bone development specifically to the administered drugs.

v) The study also assumes that the gestational periods during which albino rats are exposed to phenobarbital and phenytoin are comparable to critical periods in human fetal bone development. This assumption is important for drawing meaningful conclusions about potential human implications.

1.9 Study Limitation and Delimitation

1.9.1 Study Limitation

i) One of the primary limitations of this study was the lack of an electron microscope, which could have significantly improved the quality and details of histomorphological results. Without this advanced imaging tool, the study might miss out on finer structural information within the fetal bones, potentially limiting the depth of analysis.

ii) The Albino rats of the species *Rattus norvegicus* used in this study may not precisely mirror the response to phenobarbital and phenytoin seen in other species, including humans. This species-specific variability could limit the direct applicability of the study's findings to clinical situations involving pregnant women.

1.9.2 Study Delimitation

i) This study was delimited to investigate the teratogenic effects of phenobarbital and phenytoin on fetal bone development. It did not explore other potential impacts of these drugs on the developing fetus, such as neurodevelopmental outcomes or organ development.

ii) The study is delimited to using an albino rat model as a surrogate for human pregnancies. While animal models provide valuable insights, they do not fully replicate the complexities of human gestation.

iii) The study concentrates on histomorphological and histo-stereological methods to assess epiphyseal growth plate changes. While these methods are informative, the study does not incorporate other diagnostic techniques, such as molecular or genetic analyses, which might provide additional insights.

1.10 Conceptual Framework

The independent variable **Dependent Variable** **Study Outcome**

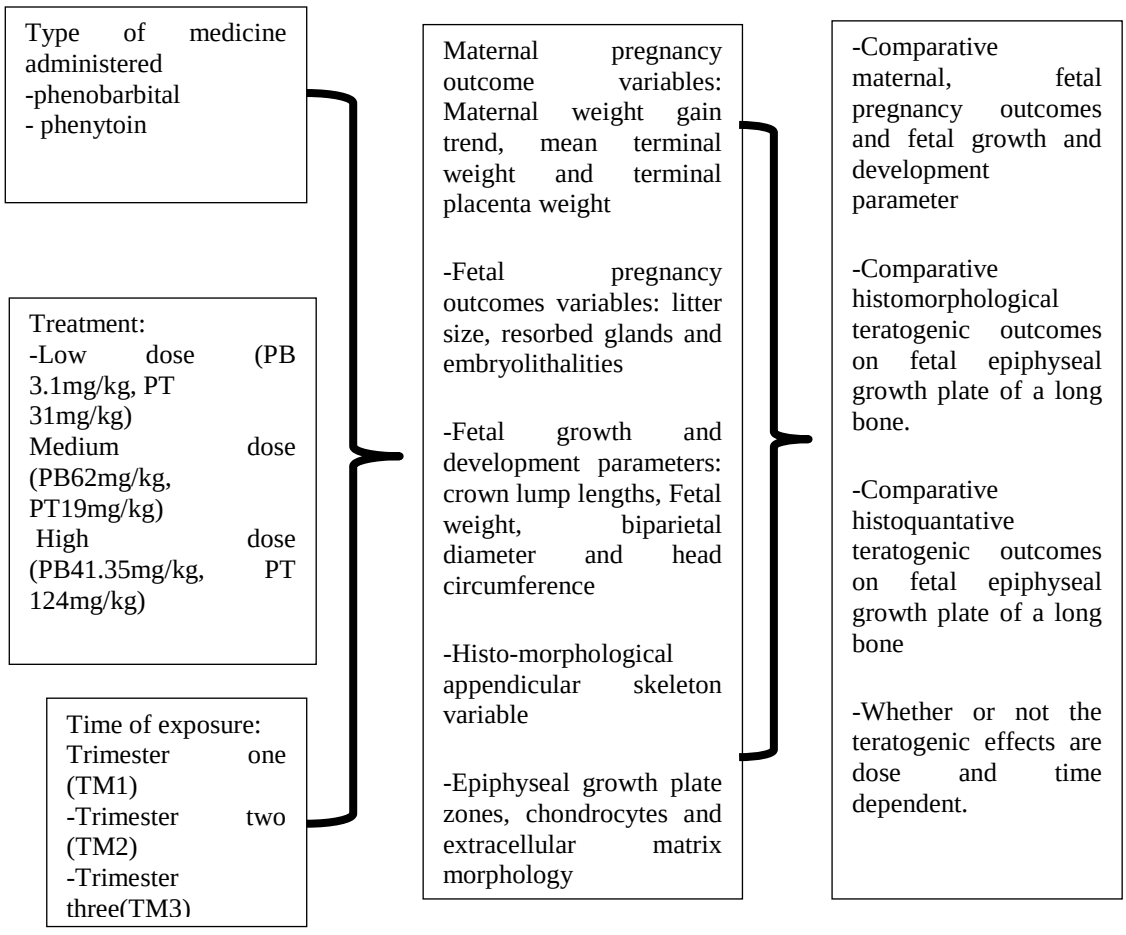

Figure 1.1: Conceptual Framework

CHAPTER TWO

LITERATURE REVIEW

2.1 Chapter Introduction

This chapter begins by describing the pharmacology of phenobarbital and phenytoin in terms of their brand names, chemical formula, molecular weight classes, solubility, mode of action, and mode of excretion. This is followed by their mode of teratogenicity on the fetal bones, both intramembranous and endochondral ossification under bone embryogenesis, morphology of growth plate, mechanism of phenobarbital and phenytoin teratogenicity on fetal bones, maternal and fetal pregnancy outcomes and lastly histomorphology and histostereology

2.2 Pharmacology of Phenobarbitone and Phenytoin

Phenobarbital which retails by among other names as Nembutal, Luminal, and others, is a barbiturate drug (Bankstahl *et al*., 2013). It has been used in clinical practice as an anticonvulsant and sedative among pregnant women. Its effects on the fetus are influenced by the crossing of the placenta blood barrier which is dependent on the duration of treatment and pregnancy duration (Czeizel *et al*., 2011). Barbiturates are depressants acting on the central nervous system that were originally designed to treat anxiety, anxiety disorders, and seizures. Although its use has declined in many developed nations, Phenobarbital is still a first-line treatment in several developing countries across the globe (Lutes, 2020).

Phenytoin, a hydantoin derivative is a common first-line AEDs with similar effectiveness as phenobarbital (Dizon *et al*., 2019). It has similar effects to barbiturates, but with minimal sedative effects and has been used primarily to treat epilepsy. Phenytoin inhibits the firing of action potentials, by slowing the rate of recovery of the sodium channels. It selectively inhibits the Na+ channel to prolong the neuronal refractory period (Patocka et al., 2020). It's also believed that phenytoin exerts its antiepileptic effect by inhibiting serotonin (5-HT) and γ-aminobutyric acid

(GABA) neurotransmitters in the brain (M. Keppel Hesselink, 2017). Phenytoin is taken orally or parenterally and its soluble in water (Patocka *et al.,* 2020).

2.3 Comparative Similarities and Differences in the Skeletogenesis between Humans and Rats

In the process of skeletogenesis between rats and humans, bones are formed through two mechanisms embryologically; endochondral or intramembranous ossification. Intramembranous ossification forms the flat bones of the clavicle, skull, and most of the cranial bones. Endochondral ossification mesenchymal tissue transforms into cartilage and later ossifies to bone and forms an axial skeleton and long bones.

The process of intramembranous ossification involves the direct transformation of mesenchymal cells into osteoblasts, which are responsible for bone formation(Hara et al., 2022). This intramembranous ossification in both rats and humans begins with the differentiation of mesenchymal cells into osteoblasts (Giffin *et al.,* 2019). These osteoblasts play a critical role in synthesizing the bone matrix, a fundamental step in the formation of flat bones. However, significant differences emerge in the scale and timing of these flat bone development due to shorter gestation periods and life cycles, exhibiting a more rapid intramembranous ossification process compared to humans.

In both rats and humans, bone tissue develops from intra-embryonic mesoderm where the ones of the axial skeleton are sourced from the sclerotome of paraxial mesoderm, limbs from the somatic lateral plate mesoderm and cranial bones from the bronchial arches as well as the neural crest cells (Wu *et al.,* 2016**).** In both rats and humans, the bone tissue is impregnated with minerals, especially calcium and phosphorous. During bone osteogenesis there are several cells involved mainly; osteoblasts, osteoclasts, and osteocytes(Diomede *et al*., 2020). Further, in both rats and humans, the endocrine signalling mechanisms make bone into dynamic tissue that entails remodelling as well as modelling (Florencio-silva *et al., 2*015). Bones are formed through two mechanisms embryologically; endochondral or intramembranous ossification. Intramembranous ossification forms the flat bones of the clavicle, skull, and most of the cranial bones. Endochondral ossification

mesenchymal tissue transforms into cartilage and later ossifies to bone and forms an axial skeleton and long bones.

2.3.1 Intramembranous Ossification

This entails the formation of bone by direct transformation of mesenchymal cells into osteoblasts which are the bone-forming cells. Through intramembranous ossification, flat bones, some bones of the viscerocranium and cranial suture lines are formed (Berendsen & Olsen, 2015). Mesenchymal to osteoblast transformation is under great influence of epithelia via several growth factors, transcription factors and receptor interactions at different times (Giffin *et al.,* 2019).

2.3.2 Endochondral Ossification

This entails an initial cartilaginous model that is later ossified transforming it into bone as exampled in the appendicular skeleton and some parts of the axial skeleton (Berendsen & Olsen, 2015). The cartilaginous model has a cartilaginous shaft which is surrounded by the perichondrium, an epiphysis at the proximal and distal ends as well as the epiphyseal growth plate juxtaposed between the epiphysis and metaphysis (Berendsen & Olsen, 2015). The epiphyseal growth plate contributes to appositional growth due to its active production of chondroblasts from the groove of Ranvier (Burdan *et al.,* 2009). The chondroblasts from the perichondrium, later on, differentiate into chondrocytes that secrete type II collagen and components of the extracellular matrix. At the same time, the perichondrium gets invaded by capillaries and at this point, it differentiates into the periosteum whereas osteoblasts mature to secrete type I collagen which is the major type of collagen found in bone (Berendsen & Olsen, 2015). Longitudinal bone growth is primarily in epiphyseal growth plates that provide the cartilage source (Berendsen & Olsen, 2015). The process of calcification will continue with the cartilaginous model being replaced with bone that contains calcium, zinc and magnesium as well as the anion phosphate that strengthens the bone.

2.4 The Morphology of Epiphyseal Growth Plate

Longitudinal bone growth from cartilaginous mesenchymal cell model of the hyaline type of the epiphyseal growth plate. Its located between metaphysis and epiphysis of all long bones. It is a multilayer structure that is formed from a proliferation of cells that synthesise an extracellular matrix. The reserve zone is responsible for germinal structure and protein synthesis. There is an increased multiplication of cells at the zone of proliferation where cells duplicate rapidly(Abubakar *et al*., 2019). Subsequently, at the zone of transformation, there are morphological changes that consist of hypertrophic layers (lower and upper layers). Mineralization of the cartilage increases rapidly where calcium and alkaline phosphatase are deposited. At this level, primary zone and secondary zones as well as chondrocytes undergo apoptosis and are eliminated (Fernández-Iglesias *et al*., 2021). Differentiation and proliferation of chondrocytes are regulated by endocrine agents including thyroid, sex hormone, growth hormones and vitamin metabolites(Burdan *et al*., 2009)

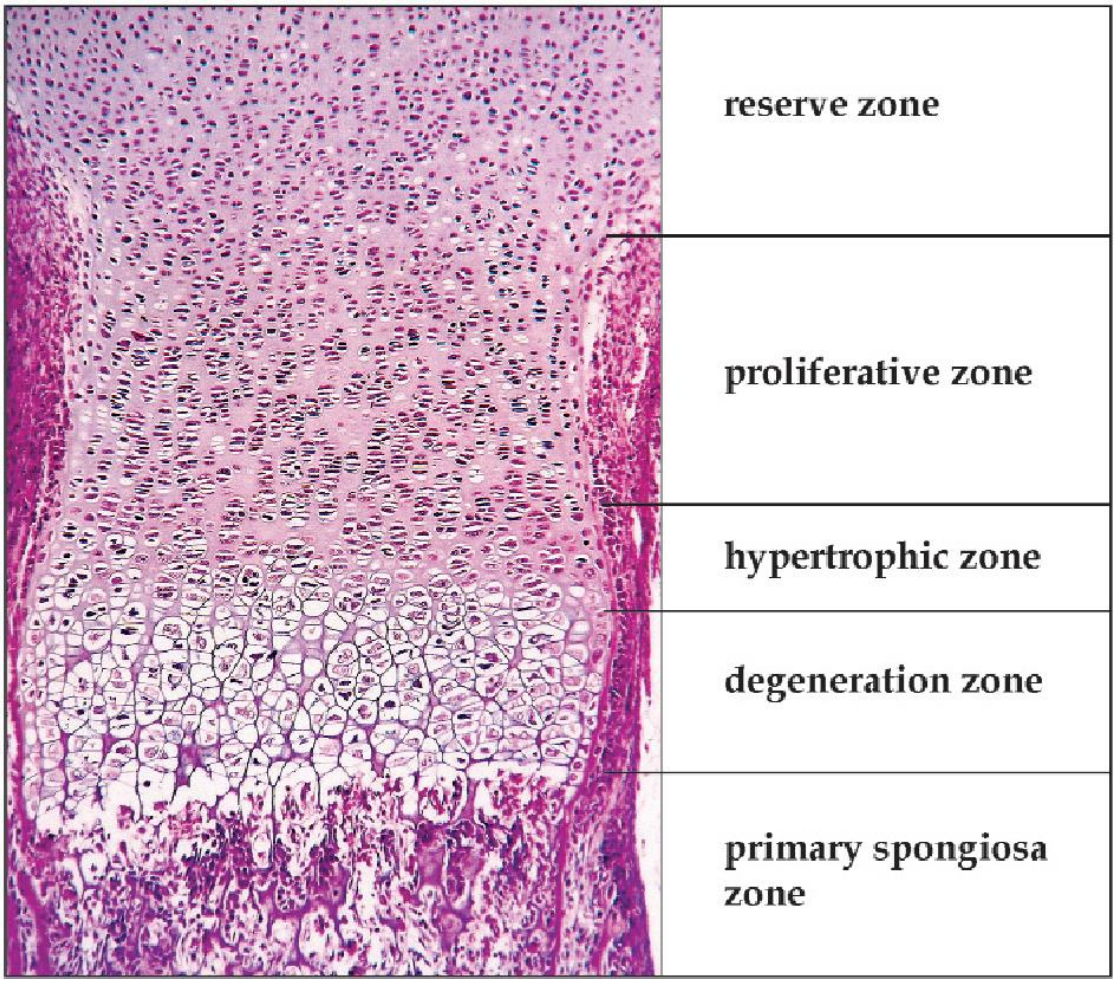

Figure 2.1: Histological Zones of an Epiphyseal Growth Plate

Adopted from (Burdan et al., 2009)]

2.5 The Mechanism of Phenobarbital and Phenytoin Teratogenicity on Fetal Bones

Teratogenic agents are compounds and environmental conditions which interfere with normal *in-utero* development (Martinez et *al.,* 2018). Teratogenesis is a process that causes birth defects or malformations in an embryo or fetus. The frequency of major growth retardation, malformations, and hypoplasia of the midface and fingers as a result of antiepileptic drugs collectively known as embryopathy is increased in infants exposed to anticonvulsant drugs *in-utero* (Verrotti *et al*., 2014). Both phenobarbital and phenytoin cross the placental barrier freely hence accumulating in the fetus resulting in an associated variety of effects on somatic development and risk of other birth defects in the offspring and possibly also other adverse effects(Bath & Scharfman, 2013). The prolonged use of phenobarbital decreases chondrogenesis and inhibits chondrocyte proliferation during embryogenesis(Pack, 2003)

2.6 The Maternal and Fetal Pregnancy Outcomes Following Prenatal Exposure to Varied Doses of Phenobarbital and Phenytoin

Women with epilepsy receiving antiepileptic drugs are known to be at a greater risk of having seizures during pregnancy, as well as other complications (miscarriage, preterm labour, low birth weight, and maternal or fetal death); their offspring are more likely to have congenital malformations or developmental delays and increased risk of teratogenicity (Berendsen & Olsen, 2015). Combined, studies conducted in rodent models suggest that phenobarbital exposure during periods of early bone development disrupts key developmental processes, including neurogenesis and apoptosis, and results in widespread structural changes (Lutes, 2020). Antiepileptic drugs like carbamazepine are significantly more harmful to the treatment group than the control, but lamotrigine and levetiracetam are not (Veroniki *et al*. 2017). Most antiepileptic drug administration has been shown to cause bone teratogenicity, especially diaphysis of long bones more so in fetal radius and the ulna (Pack, 2003). It also causes reduced ossification of the femur, tibia and fibula as well as metatarsal bones (Bath & Scharfman, 2013).

Phenobarbital exposure has been shown to cause a significant reduction in fetal outcomes more so causing birth defects that include reduced fetal growth parameters including reduced head circumference among other defects (Pack, 2003). Both phenobarbital and phenytoin are known to cause fetal hydantoin syndrome which includes developmental fetal malformation (Pack, 2003).Outcomes from the International Registry of Antiepileptic Drugs in Pregnancy (EURAP) prospective pregnancy register recently demonstrated a dose-dependent risk of malformations with a range of AEDs, including Phenobarbital and Phenytoin (Brodie & Kwan, 2012)**.** Further, another study showed higher doses of carbamazepine, valproic acid and phenobarbital have fewer means of crown-rump length, fetal weight, bi-parietal diameter and head circumference (Tomson *et al.*, 2011). Additionally, carbamazepine has the same mode of action as phenobarbital and phenytoin established that as the dose of medicine increases there is a reduction in biparietal diameter and crown-rump length (Dennis *et al*., 2021)

2.7 The Histomorphological and Histostereological Effects Following Prenatal Exposure to Varied Doses of Phenobarbital and Phenytoin

Bone histomorphometry allows quantitative evaluation of bone micro-architecture, bone formation, and bone remodelling by providing insight into cellular changes. It plays an important role in monitoring changes in bone properties because of systemic skeletal disorders(Rauch *et al*., 2000). This quantitative evaluation plays an important role in studies that involve the bones. It helps to quantify different parameters to explore the fetal bone effects of phenobarbital and phenytoin (Rauch *et al*., 2000). It's usually based on the shape of cells or the morphology of tissue. Histomorphometry describes the quantitative aspect of analysis, such as the number of cells per area, surface area and size (Eriksen *et al*. 1994, Ott 2002; Dempster *et al*. 2009). The histomorphometric data of cells and other structures are often used to describe the histomorphology of a specific tissue by using variables such as minimum and maximum length to calculate a circularity index(Bagheri *et al*., 2015).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Chapter Introduction

The chapter describes the study setting, followed by the study design, the description of the study subjects, the sample size determination, the grouping of the animals, inclusion and exclusion criteria, the feeding of albino rats, breeding and confirmation of pregnancy, determination, calculation and administration of phenobarbital and phenytoin histomorphological and stereological procedures, data analysis, ethical considerations and approvals.

3.2 Study Setting

This study was carried out at the animal research house. The animal experimental procedures, including breeding, randomization, feeding, weighing, administration of drugs, and harvesting of fetal skeletal structures, were carried out at the animal research house at the University of Nairobi (UON), Department of Biomedical Science Chiromo campus. While tissue processing, histomorphological and histostereological analysis were done in the histology laboratory, department of Human Anatomy, School of Medicine-Jomo Kenyatta University of Agriculture and Technology (JKUAT).

3.3 Study Design

A post-test only with control experimental study design was adopted since the fetal bones were harvested and processed for both the histomorphological and histostereological analysis after prenatal exposure to the two medicines [i.e. phenobarbital and phenytoin] this study design was considered the most appropriate.

3.4 Study Sample/Subject

The study subjects used were female albino rats of species *Rattus norvegicus* from the 3rd series breed of pure colony. The reason for selecting this species followed

known scientific facts that include; (i) their litter size is large with an average of 6-12 fetuses, (ii) they have low chances of developing spontaneous congenital malformation, (iii) it is easy to get the study subjects since they have short gestational period, (iv) the cost of maintaining albino rats is lower, (v) there is readily available reproductive information vi) they are small in size and this makes it easy to handle and care for them during an experiment (vii) they are more tolerant in withstanding many experimental medicines (Hard, T., Barnes, H., Larsson, C., Gustafsson., Lund, 1995; Modlinska & Pisula, 2020)

Sexually mature albino rats of pure bleed aged between 8-9 weeks and of the 3rd series weighing between 200-250g were used as the study animals. They were sourced from the lower Kabete veterinary animal house at the UON. The Albino rats were chosen because they are cheap to maintain, have a short gestational period of 21 days, deliver a large litter of 11-16 fetuses and have a low incidence of spontaneous congenital anomalies(Anatomy, 2020; Mathematics, 2016)

3.5 Description of this Species of Albino Rats Used in the Study

In this particular species of albino rats used in this study, both the male and the female albino rats resemble **'**Japanese hooded rats', hence identical in genetic composition from a common ancestor (Pritchett & Corning, 2016). Female rats acquire reproductive maturity at 3 and 4 months of age, with a gestation period of 21 days(Clark & Price, 1981). Each trimester takes 7 days after conception, with the first trimester being between day one to day seven, trimester two from day eight to day fourteen while third trimester is from day fifteen to day twenty first. Pregnancy is detectable two weeks post-conception. Baby rats are deaf and blind at birth. Weaning takes place on the 21st day after birth. The weight of adult females is 220 to 250 grams while that of male rats weighs 230 to 280 grams (Bailey *et al.*, 2014; Pallav Sengupta, 2013**)**.

3.6 The Acquisition of the Albino Rats Used in the Study

The 30 albino rats weighing 220±30g used in this study were procured from the Institute of Primates and Research situated in Nairobi county and were ferried to the Chiromo animal research facility at the University of Nairobi.

3.7 Sampling Method

In determining the sample size of albino rats used in this study, a resource equation for One Way Analysis of Variance (ANOVA) was used (Arifin & Zahiruddin, 2017). In this equation, E which is the acceptable range of degrees of freedom (DF) in the analysis of variance (ANOVA) range between 10 and 20. A value lower than 10 as per the formula requires adding more animals, and the highest value of 20 subsequently increases the power of the study.

The formula is **n = DF/k + 1,** where DF = total number of subjects, k = number of groups, and n = number of subjects per group(Charan & Kantharia, 2013). **Therefore, n=20/10+1 =3.** To eliminate bias and to ensure objectivity, a systematic uniform random sampling method was applied to select the fetuses to use in this study. Three fetuses from each rat were chosen making a total of 90 fetuses. The rest of the foetuses were preserved in a 10% formaldehyde solution for future use in case of any problem arising from the experiment.

3.8 Breeding of Rats

Sexually mature albino male rats from a pure colony of the 3rd series were introduced overnight in standard cages measuring 143 square inches of floor space each assigned to two female rats from 2100HRS (+/- 30 minutes) to 2100 HRS (+/- 30 minutes) the following day, after which they were taken back to their separate cages.

3.9 Pregnancy Determination

The determination of pregnancy was done in 2 steps as follows: -

Step 1: Was to determine whether mating took place.

Spermatozoa on the vaginal smear if observed under the microscope was an indication that coitus had taken place.

Step 2: Determination of Whether Fertilization Had Taken Place

a) Materials to be Used in the Determination of Pregnancy

i) Cotton tipped swab
ii) 0.85% phosphate-buffered saline
iii) Microscope slides
iv) Ethanol (95%)
v) Absolute alcohol
vi) 10mls blunt-tipped disposable pipettes
vii) Giemsa stain

b) The Procedure that was Followed in the Determination of Pregnancy

1. The rats were restrained with a gauze holder against the body
2. 1ml of saline was introduced into the vaginal cavity using a blunt-tipped disposable pipette (vaginal wash)
3. Cotton tipped swab moistened with phosphate-buffered saline was then gently inserted into the vaginal cavity
4. The swab was slightly rolled before withdrawing
5. The moist swab was then withdrawn and rolled onto a clean glass microscope
6. The specimen was spray-fixed using 95% ethanol
7. The slides were subsequently air dried and others by dipping in 100% alcohol
8. The slides were stained with Giemsa stain
9. The slides were observed under the BP Olympus microscope

c) Observations to Confirm Fertilization

To determine whether fertilization took place, the presence of large, polyhedral epithelial cells, many neutrophils on the smear and scattered epithelial cells served as an indicator that fertilization took place and that was counted as the first day of pregnancy (gestation day one). Those that had not conceived, were allowed for

another 24 hours with the males after which the test was repeated to confirm their pregnancy

3.10 Selection Criteria

3.10.1 Inclusion Criteria

i) Rats that conceived
ii) All healthy rat dams
iii) All alive fetuses at the time of sacrificing the rat dams

3.10.2 Exclusion Criteria

i) Rats that had tested negative for pregnancy test.
ii) All rats that developed signs of a disease
iii) Any dead fetuses found in the uterine horn at the time of sacrificing the rats.

3.11 The Grouping of Dams

The 30 dams used in the study were randomly assigned to either 3 rats as the control and 27 rats in the experimental category per group. To determine whether the effects of phenobarbital and phenytoin are dose-dependent, the 27 rats in each experimental category were further divided into three broad study sub-groups of 9 rats each based on doses applied as follows: 9 rats for the low phenobarbital and phenytoin group; 9 rats for the medium phenobarbital and phenytoin group; and 9 dams for the high dose phenobarbital and phenytoin group. To determine whether the effects of phenobarbital and phenytoin are time-dependent, the 9 rats in each of the three study categories of the low, medium and high phenobarbital and phenytoin groups were further subdivided into three subgroups of three rats each based on the trimester of exposure as follows three (3) rats for trimester one (TM_1), 3 rats for trimester two (TM_2) and 3 rats for trimester three TM_3 **(*Figure 3.10*)**

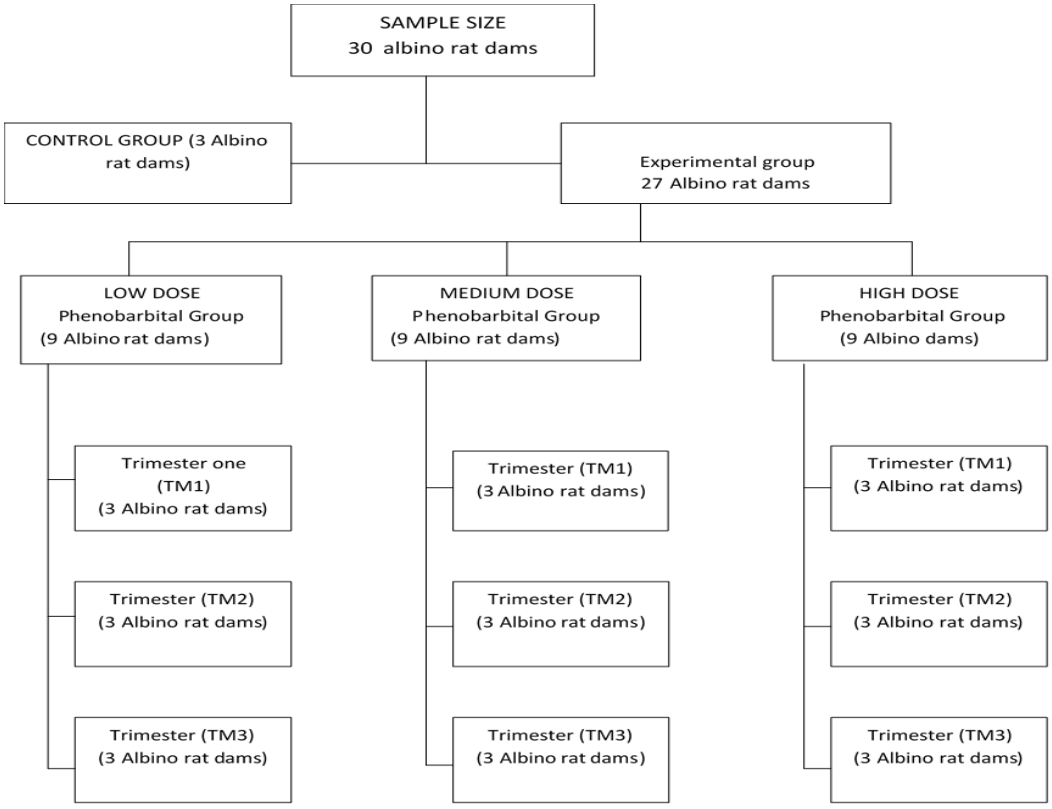

Figure 3.1: Flow Chart Showing the Grouping of Albino Rats Dam

3.12 The Feeding Process of the Rat

These dams were fed at 0900hrs on rodent pellets and water *adlibitum* procured from Unga Feeds Limited in Thika town. Feeds were done in spacious standard cages (Allen *et al.*, 2016). All animals were allowed to stay in their cages for seven days to adjust to the new environment before the experimentation began. The animals in the control and the experimental categories were fed as follows: -

1. **The control group;** Received a standard diet as determined by the Academy of Nutrition and Dietetics containing by weight (100g): 68% starch,4% cellulose, 5% lipid (corn oil) and 20% protein) and by calories: 20% proteins, 72% carbohydrates, 12% lipids, and 54mg/kg zinc and water *ad libitum* for the whole of the gestation period day 1-20. The mothers were then sacrificed on the 20th day of gestation.
2. **The experimental groups:** The animals in the experimental groups were similarly fed on standard rodent pallets as above in the control and water *ad-*

libitum but in addition received either phenobarbital or phenytoin treatment based on their doses of low, medium and High as well as according to the trimester of exposure (TM_1, TM_2 and TM_3) as follows:

(a) The Low-Dose Phenobarbital/Phenytoin Groups

The rats receiving low dosage received a standard diet, water *ad-libitum* and a constant daily dose of phenobarbital 3.1 mg/kg, phenytoin 31mg/kg administered as a single bolus through gastric gavage gauge 16 at 0900hrs. The 3 rats in trimester one (TM_1) received phenobarbital or phenytoin treatment daily from day one (GD_1) to gestation day twenty (GD_{20}); those in trimester two (TM_2) received the treatment daily starting from gestational day seven (GD_7) all through to gestation day twenty (GD_{20}), while those in trimester three (TM_3) received daily phenobarbital/phenytoin treatment daily from gestational day fourteen (GD_{14}) all through to gestational day twenty (GD_{20}), the last day of gestation.

(b) Medium Dose Phenobarbital/Phenytoin Groups

Rats in this group received a standard diet, water *ad-libitum* and a constant daily dose of phenobarbital 19.1mg/kg/ or phenytoin 62mg/kg administered as a single bolus through gastric gavage gauge 16 at 0900hrs. The 3 rats in trimester one (TM_1) received phenobarbital or phenytoin treatment daily from day one (GD_1) to gestation day twenty (GD_{20}); those in trimester two (TM_2) received the treatment starting daily from gestational day seven (GD_7) all through to gestation day twenty (GD_{20}), while those in trimester three (TM_3) received daily phenobarbital/phenytoin treatment daily from gestational day fourteen (GD_{14}) all through to gestational day twenty (GD_{20}), the last day of gestation.

(C) High-Dose Phenobarbital/Phenytoin Groups

Rats in this group received a standard diet, water *ad-libitum* and a constant daily dose of phenobarbital, 41.4mg/kg/ phenytoin 124mg/kg administered as a single bolus through gastric gavage gauge 16 at 0900hrs. The 3 rats in trimester one (TM_1) received phenobarbital or phenytoin treatment daily from day one (GD_1) to gestation

day twenty (GD_{20}); those in trimester two (TM_2) received the treatment starting daily from gestational day seven (GD_7) all through to gestation day twenty (GD_{20}), while those in trimester three (TM_3) received daily treatment phenobarbital or phenytoin daily from fourteenth-day gestation (GD_{14}) all through to gestational day twenty (GD_{20}), the last day of gestation.

3.13 Handling of Rats

In handling the rats, the process began by acclimating the rats to the new environment where the experiments were being carried out at the animal house in Chiromo Campus University of Nairobi. This entailed putting them in their respective cages for 7 days before the start of the experiment to enable them to acclimatise to the new environment. To ensure the humane and consistent handling of the rats, the rats were handled by the investigator and his trained assistant as per the recommendations of the animal ethics committee. They were weighed every morning between 0830 hrs and 0900 hrs. All procedures performed were as per the stipulated guidelines for the care of laboratory animals by The Norwegian National Research Ethics Committees, (2018).

3.14 Determination, Calculation and Administration of the Doses

3.14.1 The Human Dose Equivalent

In determining the highest, medium and the lowest dose to be administered, the adult dose was determined first. The Phenobarbital dose in humans ranges between 30mg-400mg per day while the Phenytoin range is between 300-1200mg in divided dosages (Azar & Abou-Khalil, 2008). Both drugs were obtained through Kobian ltd company, an Indian supply firm.

3.14.2 The Procedure for Determination of Phenobarbital and Phenytoin Doses

To determine the dosages of Phenobarbital and Phenytoin to be used, a simple guide for conversion of animal dosages from human dosages was applied (Nair & Jacob, 2016), which states that, Human Equivalent Dose mg/kg = Animal dose mg/kg multiplied by a constant ratio (Km) 6.2.

3.14.3 Phenobarbital and Phenytoin Dosages Calculation

The highest therapeutic dose of Phenobarbital dose in humans is 400mg, the medium dose is 185mg and the minimum dose is 30mg. The average weight of an adult human is 60kg (Nair & Jacob, 2016). On the other hand, the highest dose of phenytoin in human beings is 1200mg; the medium dose is 600mg while the lowest dose is 300mg.

I) The Determination of phenobarbital dosages

a) Determination of high-dose phenobarbital group

Highest dose of phenobarbital 400mg

The average weight of a man's kg

400mg = 60kg

X=1kg

X=1x400/60 =6.67mg/kg

AED = HED X Km factor

Therefore, 6.67mg/kg x 6.2 =41.4mg/kg

b) Determination of Medium Dose Phenobarbital Group

Medium dose phenobarbital -185mg

The average weight of a man-60kg

185mg = 60kg

X=1kg

X=1x185/60 =1mg/kg

AED = HED X Km factor

Therefore, 3.08mg/kg x 6.2 =19.10mg/kg

c) Determination of low-dose phenobarbital group

Lowest dose phenobarbital -30mg

The average weight of a man-60kg

30mg = 60kg

X=1kg

X=1x30/60 =0.5mg/kg

AED = HED X Km factor

Therefore, 0.5mg/kg x 6.2 =3.1mg/kg

ii) Determination of Phenytoin Dosages

a) Determination of High-Dose Phenytoin Group

Highest dose of Phenytoin-1200mg

The average weight of a man-60kg

1200mg = 60kg

X=1kg

X=1x1200/60 =20mg/kg

AED = HED X Km factor

Therefore, 20mg/kg x 6.2 =124mg/kg

b. Determination of Medium Dose Phenytoin Group

Medium dose phenytoin-600mg

The average weight of a man-60kg

600mg = 60kg

X=1kg

X=1x600/60 =10mg/kg

AED = HED X Km factor

Therefore, 10/kg x 6.2 =62mg/kg

c. Determination of Low-Dose Phenytoin Group

Lowest dose phenytoin-300mg

The Average weight of a man-60kg

300mg = 60kg

X=1kg

X=1x300/60 =5mg/kg

AED = LED X Km factor

Therefore, 5mg/kg x 6.2 =31mg/kg

Since the weight of rats to be used in the study ranges between 200-250g, the dosage needs to be converted into mg/kg to mg/g as follows;

iii) Calculation of Specific Rat Dosages

If for example, the weight of the rat is **250 g** and the Low phenobarbital dose is **3.1mg/kg,** then the calculation is done as follows;

(3.1mg/kg/1000) =0.031mg/g

0.031mg/g x250g=7.75mg

If the phenobarbital tablet is 30mg, and reconstitution is done in 10ml of distilled water, then

30mg=10ml

7.75mg=$\frac{7.75\ mg \times 10ml}{100mg}$=0.775ml

3.15 Administration of Phenobarbital and Phenytoin

Both phenobarbital and phenytoin were administered by the researcher daily at 0900hrs.

a) Materials required for administration

i) Pregnant dams (30)
ii) Tablets of phenobarbital and phenytoin
iii) Gavages' needle gauge 16
iv) 20 ml beaker for dilution
v) Syringes (2ml and 5ml)
vi) Deionized water (500mls)
vii) A table cloth

b) The Procedure for Administering Various Doses of Phenobarbital and Phenytoin Using Gastric Gavage

1) The rat was held carefully from the neck region using the left hand
2) The rat was then wrapped with the tablecloth to prevent the animal from soiling the investigator's clothing.

3) It was then rested against the body with the animal's mouth facing the investigator
4) The gastric gavage needle (gauge 16) was gently inserted into the mouth of the rat turning it gently to pass the oesophageal constrictions and the cardiac sphincter
5) The treatment bolus was then put in the stomach of the rat
6) The gavage needle was then gently removed

3.16 Sacrificing of the pregnant albino rats

The Female dams were humanely sacrificed through inhalation of concentrated carbon dioxide between 0900HRS and 1100HRS on the 20th day of gestation to avoid devouring dead fetuses or the congenitally deformed foetus.

a. Materials for Humane Sacrificing the Rats

i.) The pregnant rat GD_{20}
ii.) Carbon dioxide
iii.) Cotton gauze or cotton wool
iv.) Bell or dissector jar
v.) Physiological saline 0.85% concentration
vi.) Mounting board
vii.) Mounting pin
viii.) A pair of scissors
ix.) A pair of forceps (toothed)
x.) Scalpel blade
xi.) Scalpel blade handle
xii.) Drip set 2 in number
xiii.) Fixatives- 10% Formaldehyde
xiv.) Hypodermic needle gauge 20
xv.) Surgical gloves
xvi.) Magnifying glass for the light microscope
xvii.) Ruler
xviii.) Electronic weighing machine
xix.) Specimen collection bottle

b. Procedure for Humane Sacrificing the Rats

i.) Concentrated carbon dioxide was introduced into a bell jar
ii.) A tight-fitting lid was then put into the bell jar
iii.) The pregnant rat was then put into the bell jar
iv.) The rat then waited for 15 minutes to be anaesthetized
v.) The rat was then removed from the bell jar and mounted onto the board using mounting pins with the dorsal side on the board
vi.) Using a pair of scissors and forceps the rat was cut through the ventral medial side from the xiphisternal joint to the symphysis pubis

3.17 Harvesting of Fetuses

i) Twenty minutes after anaesthetizing the rats with concentrated carbon dioxide, a longitudinal incision along the abdominal of the mother was done from the xiphisternal joint to the symphysis pubis along the linear alba and the full extent of both uterine horns was exposed.
ii) Before opening either of the placental horns, fetal positions within the horns as well as the number of live and dead fetuses indicated by their movement following a gentle prodding with a probe will be determined and recorded as litter size.
iii) The number of the "devoured endometrial glands", characterized by yellowish nodules found along the anti-myometrial margin of the uterine horns that mark any original implantation site was counted and recorded. Thus, the endometrial glands unoccupied by living or recently dead fetuses represented the number of prior resorptions.
iv) The uterine horns were excised along the anti-myometrial border to expose the fetuses, embryonic membranes and placentas using a pair of scissors.
v) They were gently removed in totality from the uterus, utilizing the blunt end of a pair of forceps.
vi) An incision along the dorsal surface of the membranes revealed the fetuses,

3.7.1 Each Fetus and Its Placenta were Removed and Weighed and the General Fetal Morphology was Examined and Recorded Immediately

i) A general examination was done to check for any abnormalities
ii) The initial Crown-Lump length and Bi-Parietal diameter for each fetus were taken and recorded.
iii) The fetuses were inserted in 10% formalin to continue with fixation fixative used during perfusion fixation

3.18 Procedure for Harvesting Fetal Bones

Three fetuses were chosen objectively guided by their weights (lowest, median and highest weights) and their bones were harvested for both histological and morphometric analysis according to the following procedure;

a) Fetuses were mounted onto the dissection board using mounting pins ventral side facing the board.
b) Using a pair of scissors and forceps the tibia and humerus bones were removed
c) To avoid damaging the fetal bone, the skin was removed between the knee and ankle joints and between the elbow and wrist joints
d) The entire tibia and humerus were removed
e) Each bone was examined for general external features and obvious congenital malformations
f) The bone lengths were assessed using a string and a ruler
g) The bones were immersed in the formaldehyde, to proceed with processing either for light microscopy or stereology for 24 hours

3.19 Processing for light microscopy

a. Materials used for Staining

a) The specimen's fetal bones
b) Zenker's solution (1 litre)
c) Dibutyl phthalate Polystyrene Xylene (DPX) mountant

d) Glass slides and cover slips
e) Hematoxylin and eosin
f) Glass staining square jars
g) Paraffin wax
h) Microtome knives
i) Rotary microtome
j) Heater and water bath container
k) Specimen bottles
l) Slide holders
m) Distilled water
n) Formaldehyd40%concentration
o) Xylene
p) Isopropyl alcohol
q) Van Grisons stain
r) Wood blocs
s) Glassware for preparation of dilutions
t) Beakers
u) Egg albumin
v) Dropper
w) Cedarwood oil

b.) The Procedure for Processing the Fetal Bone for Light Microscopy and Stereology

a) The bones were fixed in the Boiun's (Zenkers' solution) for 24 hours
b) They were then dehydrated in an ascending concentration of alcohol (50%, 60%, 70%, 80%, 90%, 95% and 100% (absolute) each for one hour.
c) They were then cleared by immersion with cedarwood oil for 12 hours.
d) They were then infiltrated with paraplast© wax for 12 hours at 56^0c
e) The bone tissues were then orientated in the longitudinal axis
f) They were then embedded in paraffin wax on the wooden blocs
g) Excess wax was trimmed off from the bone tissue

h) 5µm thick longitudinal sections were cut from head to tail regions with Leitz sledge rotary
i) microtome
j) The cut sections were floated in water at 37^0c to spread the tissue
k) The sections were then stuck onto glass slides using egg albumin, and applied as thin film with a micro-dropper.
l) The slides were then dried in an oven at 37^0c for 24 hours
m) Blinding was done by coding all the slides by the researcher or the assistant
n) They were then stained with different stains including: -Hematoxylin and Eosin (H&E), based on the cellular structures that needed to be studied.

3.20 Histostereological Analysis

3.20.1 The Process of obtaining a Specimen for Histoqualitative and Histoquantative Analysis

i) The bones were obtained from the appendicular skeleton for histostereological studies
ii) Tibia and humerus were chosen after simple random sampling.
iii) The soft tissue from the tibia and humerus bones of different fetal rat groups was removed by placing them in 2% KOH for eight days to achieve complete chemical maceration of the soft tissue leaving the bone intact.
iv) The Hercules© digital veneer callipers were used to take the length of the tibia and humerus upon calibration to the 0.00 mark each time a measurement was made.
v) The measurement was made from the medial malleolus to the tibia to inter condyle eminence and from the trochlear to the head of the humerus in humeral bones.
vi) Bones were then infiltrated and embedded in paraffin wax and later placed on an electric cold plate for cooling for 24 hours.
vii) The tissue blocks were oriented along their long axis and microtome at 5-micrometre thickness for histomorphological and histoquantative analysis by Leitz sledge rotatory microtome.

viii) Slides were selected using a systemic uniform random sampling technique, and longitudinal sections were obtained.

ix) The tissue sections to be stained were picked based on the K^{th} value (skip) of 10 calculated.

x) The sections obtained were placed in a water bath at 37^0 degrees and fishing was done on glass slides and then placed on a slide holder for staining.

xi) The dehydrated and deparaffinized sections of the tibia and humerus were dipped three times for 2 minutes sequentially in xylene, 100% ethanol, 80% ethanol, and in de-ionized water for 5 seconds.

xii) Then they were dipped in haematoxylin for 2 minutes, rinsed with de-ionized water for 5 seconds, stained with acid ethanol, and later rinsed with de-ionized water.

xiii) Then, they were stained with Eosin for 2 minutes and rehydrated sequentially with 95% and 100% ethanol (3 dips each).

xiv) Three dips in xylene for 15 seconds and the coverslips were applied and left to dry overnight in the hood.

3.21 Histoquantative Methods of Obtaining the Surface Area of the Epiphyseal Growth Plate

i) To establish the beginning and terminal end of the proximal and distal epiphyseal growth plates of the tibia several criteria were used based on cell size and organization(Craig et al., 2004)

ii) To establish the length of epiphyseal growth plate zones, an established procedure by Jillian P was used (Rapid, 2011).

iii) The junction between the zones was outlined based on the chondrocytes' morphological characteristics and changes in histological matrix staining.

iv) The vertical height of the total epiphyseal growth plates of both proximal and distal tibia and humerus was measured.

v) The surface area of the growth plate was determined using Stepanizer version beta 2.28. Proximal and distal tibia and humerus epiphyseal growth plates were used for the study after tissue processing and staining with haematoxylin and eosin.

3.22 Histo-Photomicrography (Materials and Procedure)

a. Materials

i.) Digital camera (32 megapixels)
ii.) BP Olympus microscope
iii.) Memory card
iv.) Histological glass slide

b. Procedure Followed in Taking Histo-Photomicrographs

i.) Histological slides were mounted on the stage of the microscope
ii.) The focus was adjusted until the image to be photographed was in focus
iii.) The field was magnified appropriately
iv.) Photographs of the regions were taken as viewed best under the focus of the microscope
v.) Photographs were transferred to the computer by use of a memory card

3.23 Statistical Data Management and Analysis

- Histomorphological qualitative data was collected using photomicrographs at different magnifications using a swift 3.0 (20 megapixel) digital camera and then exported to Adobe Fireworks for qualitative analysis.
- Data on pregnancy and histostereological outcomes that form the parametric data (inferential data) was collected using structured checklists and stereological data sheets respectively, stored and coded in Excel spreadsheets Windows 10, version 2013. It was then exported for analysis to the SPSS program for Windows version 25 for analysis (Chicago Illinois).
- Comparative descriptive analysis of parametric data was computed by use of ANOVA followed by Tukey's post hoc multiple comparison t-tests, while MANOVA was done to obtain main and interaction effects as well as mean difference results between phenobarbital and phenytoin. Data was expressed

as mean± standard deviation (SD) for all values, and results whose $P<0.05$ were considered to be statistically significant.

- Parametric data was presented in the form of tables, while discrete data was presented in the form of graphs

3.24 Ethical Consideration

All procedures for animal handling, feeding, human sacrificing and harvesting of organs were performed as per laid down protocols, with approval from the Animal Ethics Committee Jomo Kenyatta University of Science and Technology. All procedures were carried out as per laid down protocols and regulations by the International Animal Research Institute (IARI) of the USA as outlined by (Gomez et al., 2010) and the care of laboratory animals' guidelines (Bayne, 1986). Ethical approval was sought and approved by the Animal Care and Use Committee based in the University of Nairobi (UON), Faculty of Veterinary Medicine, Department of Veterinary Anatomy and Physiology, before initiation of the study (FVM BAUEC/2021/332).

CHAPTER FOUR

RESULTS

The findings of this study are presented in line with the study objectives: however, the findings of the 4th objective on whether or not the teratogenic effects of the two medicines are dose and time-dependent are integrated within the findings of the 1st, 2nd and the 3rd objectives. [NB> Some tables and figures are huge enough and go beyond the margins while some even spill over from one page to the next]

4.1 Objective 1: The Comparative Evaluation of How Prenatal Exposure to the Varied Doses of Phenobarbital and Phenytoin Influence and Maternal Pregnancy Outcomes

In carrying out the comparative evaluation of how the two medicines influenced the maternal and fetal pregnancy outcomes, the findings are presented in three stages as follows: -

Stage 1: The comparative effects of the two medicines on maternal pregnancy outcomes including; **(i)** the daily maternal weight gain treads; (**ii**) the mean terminal weights, (**iii**) the mean total weight gain, and **(iv)** the terminal placental weights.

Stage 2: The comparative effects of the two medicines on the fetal pregnancy outcomes including; **(i**) the litter sizes, (**ii**) the number of dead fetuses, and **(iii**) the number of resorbed endometrial glands.

Stage 3: The comparative effects of the two medicines on the fetal growth and developmental parameters including; (**i**) the fetal weights, (**ii**) crown-rump length (**iii**) the bi-parietal diameters (**iv**)the head circumference.

Stage 1: The comparative findings on how the two medicines influenced the maternal pregnancy outcomes

In evaluating how the two medicines influenced the maternal pregnancy outcomes, the following parameters were evaluated; **(i**) the means of the total maternal weight, (**ii**) the Means of the maternal weight gain, and (**iii**) the means of the terminal placental weights. This study established that there was a statistical significance reduction ($p<0.005$) in all three maternal pregnancy outcome parameters evaluated in both treatment groups of the phenytoin and the phenobarbital treated groups as compared with the control. On further carrying out the ANOVA analysis to find out how the two treatment groups differed from the controls, the findings were as follows;-**(a**) terminal placental weights, ($F(18,38) = 156.082$ $P = 0.001$), (**b**) Mean terminal weight ($F(18,38) = 13.639$ $P = 0.042$), (**c**) Mean maternal weight gain ($F(18,38) = 33.963$ $P = 0.049$) as shown in the table below (***Table 4.1***)

Upon evaluating the effects of time of exposure of phenobarbital and phenytoin on the three maternal pregnancy outcome parameters, it was noted that the most deleterious effects on the maternal pregnancy outcomes were when the treatments were instituted in TM1 and TM2 with the least effects seen at TM3. (***Table 4.1***). It can further be deduced from Table **4.1.1** below that phenytoin had more deleterious effects in influencing the maternal pregnancy outcomes parameters as compared with the phenobarbital-treated groups.

Table 4.1: The ANOVA Table Showing the Comparative Findings on How The Two Medicines Influenced the Three Maternal Pregnancy Outcome Parameters when Administered at Varied Doses of Low, Medium and High and at TM1, TM2 and TM3 Compared with the Control

The study groups	Study groups and dosage levels.	The time of exposure	The comparative mean terminal weight, maternal weight gain and placental weight for various study groups		
			Mean terminal weight (g)+ SD)	Mean maternal weight gain (g) + SD)	Mean placental weight (g)(mm) + SD)
Control.	Control (C) No treatment	None.	292.6923±. .0287	98.000 ±.0007	0.4378±.0003
The Phenobarbital treatment groups	Low-dose treatment group (LDPB)- [3.1 mg/kg/b.w)	TM1	247.1205± .0215	51.2717±.0033	0.2906±0.0028
		TM2	264.2559± .0938	57.2645±.0048	0.3410 ±0.0036
		TM3	292.0472± .0033	87.2643±.0019	0.3760 ±0.0135
	Medium dose treatment group(MDPB)- [19.2mg/kg/bw)	TM1	243.2458±.0868*	45.3050±.00326*	0.2959 ±0.0032*
		TM2	249.2110± .0646	55.2890±.0012	0.3239 ±0.0046
		TM3	260.1454± .0312	69.2835±.00165	0.3691 ±0.0007
	High-dose treatment group (HPB)(41.5 mg/kg/bw)	TM1	243.1873± .0513*	37.3350±.0011*	0.2318±0.0012*
		TM2	244.1703± .7589*	50.3262±.0005*	0.2777 ±0.0034*
		TM3	267.1454± .0312	59.3126±.0008	0.3165 ±0.0012
The phenytoin treatment groups	Low dose treatment group (LPT)-(31 mg/kg/bw).	TM1	257.2136± .9245	56.00 ±.0001	0.3111 ±0.0011
		TM2	277.3776± .0744	62.2795±.0018	0.3627 ±0.0031
		TM3	292.4914± .0315	92.2672±.0033	0.3949 ±0.0007
	Medium dose treatment group (MPT)-[62 mg/kg/b .w).	TM1	255.0318± .0979	50.3179±.0068	0.2906 ±.0003*
		TM2	259.0939± .0131	60.2925±.0017	0.3239±.0003
		TM3	271.2283± .0753	74.2882±.0024	0.3691 ±.0032
	High dose treatment group (HPT) (124 mg/kg/bw).	TM1	253.3111± .0135*	42.3666±.0025*	0.2747 ±.0034*
		TM2	254.5431± .0223*	55.3553±.0033*	0.3020 ±.0040*
		TM3	278.0696± .0072	64.3446±.0038	0.3392 ±.0012
ANOVA Statistics			F(18,38)= 13.639 P= 0.042	F(18,38)= 33.963 P= 0.049	F (18,38) =156.082 P= 0.001

Key: *indicates that the differences are statistically significant with the control.

Upon carrying out the MANOVA level one analysis to assess how the three independent variables (drugs, dosage and trimesters) globally influenced the three maternal pregnancy outcomes, when each of the three independent variables of the drug, dose and time either acting alone, or when combined in two ways, or when combined in three ways, it was noted that there were statistically significant individual main effects, two-way interaction effect and three-way interaction effects at varying proportions as shown by the values of the Partial Eta squared as follows:

At the individual level, global effects of how the three independent variables including drug, dose or trimester, individually influenced the three dependent maternal pregnancy outcomes:(**a**) Drugs, Wilk's lambda a=.001, F (6, 72) = 2237.227b, p=0.001, Partial Eta Squared = .798, **(b**) Doses (TM1, TM2, TM3) wilk's lambda=.000, f (6, 72) = 5237.227b, p<0.001, Partial Eta Squared = .998 and (**c**) Trimester, wilk's lambda= .003, f (6, 72) =759.846b, p<0.001, partial Eta Squared = .596 (***Table 4.1.2***)

At the two-way interaction effects level, where either of the two independent variables of drug, dosage or trimester when combined, there was statistical significant in their effects as follows:

a)Drugs*doses Wilk's Lambda a=.011, F (6, 72) = 430.541b, p=0.001, Partial Eta Squared = .473, (**b**) doses * trimester (TM1, TM2, TM3) Wilk's Lambda =.011 , f (6, 72) = 323.450b, p<0.001, Partial Eta Squared = .323 and **(c**) drugs * trimester, Wilk's Lambda= .220, f (6, 72) = 108.624b, p<0.001, partial Eta Squared = .240 was observed (***Table 4.2***)

At the three-way interaction effects level, where either of the three independent variables of drug, dosage and trimester were combined, there was statistical significant in their effects as follows Wilk's lambda=.296, f (12, 95.539) = 96.840, p<0.001, partial Eta Squared = .228 (***Table 4.2***)

Overall it can deduced that the three independent variables of drugs, dosage, and trimesters individually contributed significantly to maternal pregnancy outcomes, with partial percentage contributions of 79.8%, 99.8%, and 59.6%, respectively. Additionally, two-way interactions between these variables showed moderate contributions, ranging from 24% to 47.3%. At the highest level, the three-way interaction had a substantial but smaller contribution of 22.8%, revealing the intricate interplay among these factors in influencing maternal pregnancy outcomes.

Table 4.2: The Manova Level 1 Table Findings on How the Two Medicines (Phenobarbital and Phenytoin), Their Dosages and Trimesters Plus Their Interactions Globally Influenced the Three Maternal Outcome Parameters

Types of MANOVA evaluation level 1	The comparative effects global were assessed.	The parameters used	The multivariate statistical test parameters were applied. MANOVA test statistics (Wilks' Lambda)	Statistics (F)	Hypothesis degree of freedom	Error degree of freedom	Sig.<0.05	Proportion of variance (Partial Eta Squared)
(**i**) Test on whether the observed results were due to chance.	To find out whether the observed effects were due to treatment.	The Intercept parameter	<0.001	2805517.894b	3.000	36.000	<.001	1.000
(**ii**) The Individual Main effects of the drug, time of exposure and dosages on the maternal dependent variables	To find out whether or not the observed overall effects were due to varied doses in terminal weight, Maternal Weight gain, and placental weight.	Doses (Low, Medium, High Dose)	<0.001	5237.227b	6.000	72.000	<.001	.998
	whether or not the observed overall effects were due to phenobarbital or phenytoin on terminal weight, Maternal Weight gain, and placental weight.	Drugs (Pb, PT)	.001	2237.227b	6.000	72.000	<.001	.798
	To find out whether or not the observed overall effects were due to differing trimesters in terminal weight, Maternal Weight gain, and placental weight.	Trimester (TM1,TM2,TM3)	.003	759.846b	6.000	72.000	<.001	.596
(**iii**) Two-way interaction effects On the maternal dependent variables	To find out whether or not the observed overall effects were due to interaction between varied doses and the drugs in terminal weight, Maternal Weight gain, and placental weight.	Doses (Low, Medium, High Dose)* Drugs (PB, PT)	.011	430.541b	6.000	72.000	.001	.473
	whether or not the observed overall effects were due to interaction between and differing trimesters in terminal weight, Maternal Weight gain, and placental weight.	Doses (Low, Medium, High Dose) * Trimester (Tm1, Tm2, Tm3)	.103	323.450 b	12.000	95.539	.002	.323
	whether or not the observed overall effects were due to interaction between phenobarbital or phenytoin and differing trimesters in terminal weight, Maternal Weight gain, and placental weight.	Drugs (Pb,Pt) * Trimester (Tm1,Tm2,Tm3)	.220	108.624b	6.000	72.000	.004	.240
(**iv**) The three-way interaction effects	whether or not the observed overall effects were due to an interaction between terminal weight, Maternal Weight gain, and placental weight.	Doses (Low, Medium, High Dose)* Drugs (Pb,Pt) * (Trimester (Tm1,Tm2,Tm3)	.296	96.840	12.000	95.539	.006	.228

Key: - *Indicates interaction effects, b: -Exact statistic using MANOVA.

Upon carrying out MANOVA **level 2** the comparative multiple analysis results on how the individual drug, dose and the time of exposure plus their interactions influenced each of the three maternal outcome parameters this study established the following: -

The individual effects of how the three dependent variables of maternal weight, terminal maternal weight, or placenta weight were influenced by either of the following dependent variables of trimester, dosage and drugs.

a. At the individual level, the highest contribution of the observed effects was trimester with partial eta ranging from 96.8% t o 99.1%, followed by doses at93.9% to 96% and lastly drug at 38.2% to 81.6% (***Table 4.3***)
b. At two-way level interaction, when two variables were combined the observed effects were highest between doses * trimesters with partial eta ranging from 30.3% to 82.8% followed by dosages and drugs ranging from 11.9% to 23.6% and lastly drug and trimester ranging from 11% to 17.6% on the following variables: maternal weight gain, placental weights and terminal maternal weight as shown in (***Table 4.3***)
c. at three-way interaction, when the three independent variables (drug, dosage and trimester) were combined they were noted to have the highest effects on terminal maternal weight with partial eta at 42.4%, followed by maternal weight gain at 21.1% and finally placenta weight at 10.1%. (***Table 4.3***)

overall it can be deduced that time of exposure that is the trimester had the highest individual contribution, ranging from 96.8% to 99.1%, followed by dosage at 93.9% to 96%, and drug at 38.2% to 81.6%. Two-way interactions were most prominent in doses * trimesters (30.3% to 82.8%), followed by dosages and drugs (11.9% to 23.6%), and drug and trimester (11% to 17.6%). In three-way interactions, the combined influence of drug, dosage, and trimester had the highest impact on terminal maternal weight (42.4%), followed by maternal weight gain (21.1%), and placenta weight (10.1%). These findings underscore the varying degrees of influence of these factors and their interactions on maternal outcomes.

Table 4.3: The MANOVA Level 2 Table Findings on How The Individual Medicine (Phenobarbital and Phenytoin), Their Dosages and Trimesters Plus Heir Interactions Influenced Each of the Three Maternal Outcome Parameters

Types of MANOVA evaluation At level 2	The group being tested (independent varaibles-intervention and dosage being compared)	The three dependent variables.	Type IIIDf Sum of Squares (measure of the amount of variability in the dependent variable after the controlling for the effectsin the model)	Mean Square	F statistics	Sig.	Partial Eta Squared
(i)The evaluation of the correctness of the model used for the study	Corrected Model: - TheWilks Labda model)	Maternal Weight gain	46.067a	18	2.559	332.303	<.001	.994
		Placental weights.	.134b	18	.007	156.082	<.001	.987
		Terminal maternal weight	23645.509c	18	1313.639	219.582	<.001	.990
(ii) Test on whether the observed results were due to chance	Intercept (total)	Maternal weight gain	1162.152	1	1162.152	150897.516	<.001	.773
		placental weight	4.772	1	4.772	100378.178	<.001	.664
		Terminal maternal weight	2875148.613	1	2875148.613	480596.689	<.001	.337
	Doses (Low, medium, high	Maternal weight gain	.028	2	.014	294.149	<.001	.939
		Placental weights.	7.113	2	3.556	461.786	<.001	.960
(iii) The Individual independent variable and its effects on each of the three maternal Dependent variables		Terminal maternal weight	3845.333	2	1922.667	321.384	<.001	.944
	Drugs (Pt, Pb)	Maternal weight gain	.181	1	.181	23.484	<.001	.382
		placental weights.	.008	1	.008	168.250	<.001	.816
		Maternal weight gain	864.000	1	864.000	144.422	<.001	.792
	Trimester (TM1,TM2,TM3)	Maternal weight gain	056	2	.028	584.146	<.001	.968
		Placental weights.	31.719	2	15.860	2059.278	<.001	.991
		Terminal maternal weight	15381.333	2	7690.667	1285.537	<.001	.985
	Doses (Low, Medium, High Dose)* Drugs (Pb, PT)	Maternal weight gain	.001	2	.000	.036	.965	.236
(iv) Two-way interaction effects on each of the maternal dependent variables		Placental weights.	.000	2	.000	2.395	.105	.112
		Terminal maternal weight	.000	2	.000	.042	.102	.119
	Doses (Low, Medium, High Dose)* Trimester (Tm1, Tm2, Tm3)	Maternal weight gain	.001	4	.000	4.135	.007	.303
		Placental weights.	1.409	4	.352	45.723	<.001	.828
		Terminal maternal weight	117.333	4	29.333	4.903	.003	.340
	Drugs *Trimester(Tm1,Tm2,Tm3)	Maternal weight gain	.000	2	.000	.017	.983	.11
		Placental weights.	.000	2	.000	2.266	.118	.107
		Terminal maternal weight	.000	2	.000	.000	.122	.176

Types of MANOVA evaluation At level 2	The group being tested (independent intervention and being compared)	The three t varaibles-dependen dosagevariables.	Type IIIDf Sum of Squares (measure of the amount of variability in the dependent variable after the controlling for the effectsin the model)	Mean Square	F statistics	Sig.	Partial Eta Squared
(v) Three-way interaction effects On each of the maternal dependent variables	Doses (Low, Medium, High Dose) Drugs (Pt, Pb)*Trimester (TM1, TM2, TM3)	Maternal weight gain	.000	4	.000	.010	.113	.211
		Placental weights.	.000	4	.000	1.070	.385	.101
		Terminal maternal weight	.000	4	.000	.000	.761	.424
	Error	Maternal	.293	38	.008			
(vi) Overall inferential statistics on the model results		Weight gain Placental weights.	.002	38	.000			
		maternal weight	227.333	38	5.982			
	Total	Maternal Weight gain	1545.932	57				
		Placental weights.	6.140	57				
		Terminal maternal weight	3878633.000	57				
	Corrected Total	Maternal Weight gain	46.359	56				
		Placental weights.	.135	56				
		Terminal maternal weight	23872.842	56				

Upon carrying out the MANOVA **Level 3** pairwise comparative analysis on how phenobarbital and phenytoin influenced Maternal weight gain, Mean terminal weight, and terminal placental weights when exposed to within the same dosages and the same trimester the study established:

A) Pairwise For Terminal Maternal Weight

i) Pairwise of low doses on terminal maternal weight for TM1, TM2 and TM3 were as follows; (**a)** TM1 mean difference -8.000, p value=0.002 (**b**) TM2 -4.000, p value=0.052 **(c**) TM3 mean difference -2.000, p value=0.054

ii) Pairwise of medium doses on terminal maternal weight: (**a**)TM1 mean difference (-7.000, p value=0.0096) **(b**) Tm2 mean difference (-6.667, p value=0.009) (**c**) TM3 mean difference (-4.000, p. value=0.059)

iii) Pair wise of high doses on terminal maternal weight:(**a**) TM1 mean difference (-8.667, p. value=0.001) (**b**) TM2 mean differences (-8.000, p. value=0.002) (**c**) TM3 mean difference (-8.333, p. value=0.001)

B). Pairwise For Maternal Weight Gain

i) For low doses on maternal weight gain for TM1, TM2 and TM3 were as follows; **a)** TM1 mean difference (-3.667, p value=0.014) **(b**) TM2 mean difference (-3.100, p value= 0.52) (**c)** TM3 mean difference (-2.000, p value=0.054)

ii) Pairwise of medium doses on maternal weight gain: **a**)TM1 mean difference (-5.333, p value=0.001) (**b**) Tm2 mean difference (-5.000, p value=0.001) **c**) mean difference (-2.333, p. value=0.861)

iii) Pair wise of high doses on maternal weight gain: **a**) TM1 mean difference (-5.000, p. value=0.001) (**b**) TM2 mean differences (-5.331, p. value=0.001) **(c**) TM3 mean difference (-3.667, p. value=0.014)

(C). Pairwise for Placenta Weight

i) For low doses on the placenta weight for tm1, tm2 and tm3 were as follows; **a**) TM1 mean difference -.022, p value=0.000) (**b**) TM2 mean difference (-.020, p value= 0.001) (**c**) TM3 mean difference (-.19, p value=0.056)

ii) Pairwise of medium doses on placenta weight: **a**)TM1 mean difference (-.026, p value=0.000) (**b**) TM2 mean difference (-.023, p value=0.000) **c**) TM3 mean difference (-0.0223, p. value=0.000)

iii) Pairwise of high doses on maternal weight gain: **a**) TM1 mean difference (-.43, p. value=0.000) (**b**) TM2 mean differences (-.024, p. value=0.000) (**c**) TM3 mean difference (-.023, p. value=0.000)

upon comparative of pairwise multiple analysis of variance between the phenobarbital and the phenytoin in the same dosage and same time of exposure to establish how the two medicines influenced Maternal weight gain, Mean terminal weight, and terminal placental weights, it was observed to have statistical significance difference in that phenytoin given at the same dosage and the same time of exposure was noted to have minimal effects compared to the phenobarbital and phenytoin (***Table 4.4***).

On mean maternal weight gain, terminal weight, and placental weight across different study groups and dosage levels, it is evident that the influence of

Phenobarbital (PB) and Phenytoin (PT) treatments varies significantly. Maternal weight gain was consistently lower in the PB group across all dosage levels and trimesters. Terminal weight was also notably reduced in the PB group, especially in the low and high-dosage groups. Additionally, placental weight showed consistent reductions in the PB group, highlighting the differential effects of these treatments on maternal and fetal outcomes.

Table 4.4: The MANOVA Level 3: The Pairwise Manova Analysis On How The Phenobarbital And Phenytoin Influenced The Maternal Weight Gain, Mean Terminal Weight, And Terminal Placental Weights In Utero

The comparative mean maternal weight gain, mean terminal weight and placenta weight	Study groups and Dosage levels.	The time of exposure to treatment	Phenobarbital treatment (PB)	Phenytoin treatment (PT)	Mean Difference (PB-PT)	Std. Error	Sig. d	95% Confidence Interval for Differenced	
								Lower Bound	Upper Bound
terminal weight (kg)	Low	TM1	PB	PT	-8.000*	2.418	.002	-12.895	-3.105
		TM2	PB	PT	-4.000*	2.418	.052	-2.895	6.105
		TM3	PB	PT	-2.000*	2.418	.054	-1.895	3.105
	Medium	TM1	PB	PT	-7.000*	2.418	.006	-11.895	-2.105
		TM2	PB	PT	-6.667*	2.418	.009	-11.562	-1.771
		TM3	PB	PT	-4.000*	2.418	.059	-2.895	6.105
	High.	TM1	PB	PT	-8.667*	2.418	.001	-13.562	-3.771
		TM2	PB	PT	-8.000*	2.418	.002	-12.895	-3.105
		TM3	PB	PT	-8.333*	2.418	.001	-13.229	-3.438
Maternal weight gain (g)	Low	TM1	PB	PT	-3.667*	1.427	.014	-6.555	-.779
		TM2	PB	PT	-3.100	1.427	.052	-5.888	.112
		TM3	PB	PT	-2.000	1.427	.069	-4.888	.888
	medium	TM1	PB	PT	-5.333*	1.427	.001	-8.221	-2.445
		TM2	PB	PT	-5.000*	1.427	.001	-7.888	-2.112
		TM3	PB	PT	-2.333	1.427	.861	-5.221	.555
	High.	TM1	PB	PT	-5.000*	1.427	.001	-7.888	-2.112
		TM2	PB	PT	-5.333*	1.427	.001	-8.221	-2.445
		TM3	PB	PT	-3.667*	1.427	.014	-6.555	-.779
placental weight (g)	Low	TM1	PB	PT	-.022*	.006	.000	-.033	-.010
		TM2	PB	PT	-.020*	.006	.001	-.032	-.009
		TM3	PB	PT	-.019*	.006	.056	.030	-.007
	Mediu	TM1	PB	PT	-.026*	.006	.000	-.037	-.014

m	TM2	PB	PT	-.023*	.006	.000	-.034	-.012
	TM3	PB	PT	-.022*	.006	.000	-.034	-.011
High.	TM1	PB	PT	-.043*	.006	.000	-.054	-.032
	TM2	PB	PT	-.024*	.006	.000	-.036	-.013
	TM3	PB	PT	-.023*	.006	.000	-.034	-.011

Key: *- *. The mean difference is significant at the .05 level.*

Stage 2: The comparative findings on how the two medicines [phenytoin and phenobarbital] influenced the fetal pregnancy outcomes

In evaluating how the two medicines influenced the fetal pregnancy outcomes the following parameters were evaluated **(i)** the litter sizes, (**ii**) the number of dead fetuses, and (**iii**) the number of resorbed endometrial glands.

The study established that the highest litter size was in the control group compared to the Phenobarbital and phenytoin treatment groups. In low-dose treated groups and medium-treated groups for both drugs, the litter size was slightly higher as compared to high-dose treated groups. In high-dose treated groups, the litter size was significantly reduced. Comparison between the two treatment groups showed that in the Phenobarbital treatment group, the litter size was reduced compared to those of the phenytoin treatment group across the treatment doses. In addition, litter size was reduced in Phenobarbital and phenytoin treatment groups in trimester one as compared to the control group. It was also established that the litter size in TM3 was higher, followed by the litter size in TM2 with the least number observed in TM1(*Fig 4.1*)

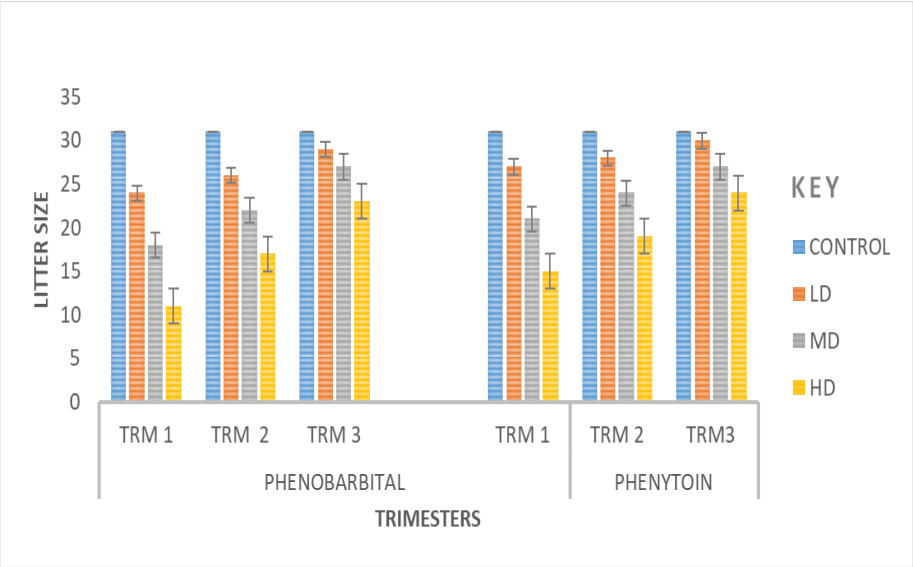

Figure 4.1: The Comparative Findings on How the Two Medicines [Phenytoin and Phenobarbital] Influenced the Litter Size

Upon comparative analysis findings on resorbed glands using a bar graph, the study showed a direct dose-dependent relationship in that the number of resorbed glands increased as the doses increased between the treatment groups. The control group had no resorbed glands observed but for both treatment groups, low-dose treated groups had few resorbed glands, which increased in the medium-dose treated groups, and the highest number of resorbed glands was observed in high-dose treated groups. In terms of trimesters for both treatment groups, it was observed that the treatment groups that ware exposed at TM1 had the highest number of resorbed glands, followed by the treatment group exposed in TM2 with the least resorbed glands observed at TM3. Upon Comparison between the two treatment groups, it was established that the phenobarbital treatment group had a higher number of resorbed glands compared to phenytoin treatment groups across all the trimesters.(*Fig 4.2)*

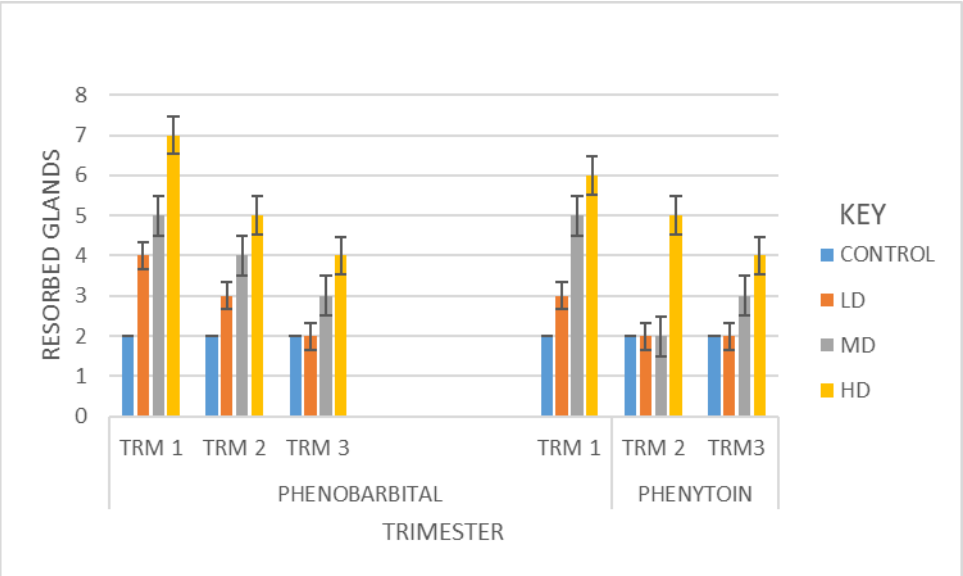

Figure 4.2: The Comparative Findings on How the Two Medicines [Phenytoin and Phenobarbital] Influenced the Fetal Pregnancy Outcome(Resorbed Glands)

Upon the comparative analysis of dead foetuses using a bar graph, the study showed a direct dose-dependent relationship since the number of dead foetuses increased with an increase in the dose as well. No dead foetuses were observed in the control

group but in both the phenobarbital and phenytoin treatment groups, the low-dose treated groups had a few dead foetuses followed by the medium-dose treated groups had a higher number of dead foetuses while the high-dose treated group had the highest number of dead fetuses. According to the trimesters, the highest number of dead fetuses was observed when the treatment groups were exposed to TM1, TM2 was the second highest while TM3 had the lowest number of dead fetuses. Comparison between the two treatment groups indicated that the highest number of dead fetuses was observed in phenobarbital treated group while phenytoin had fewer dead foetuses.

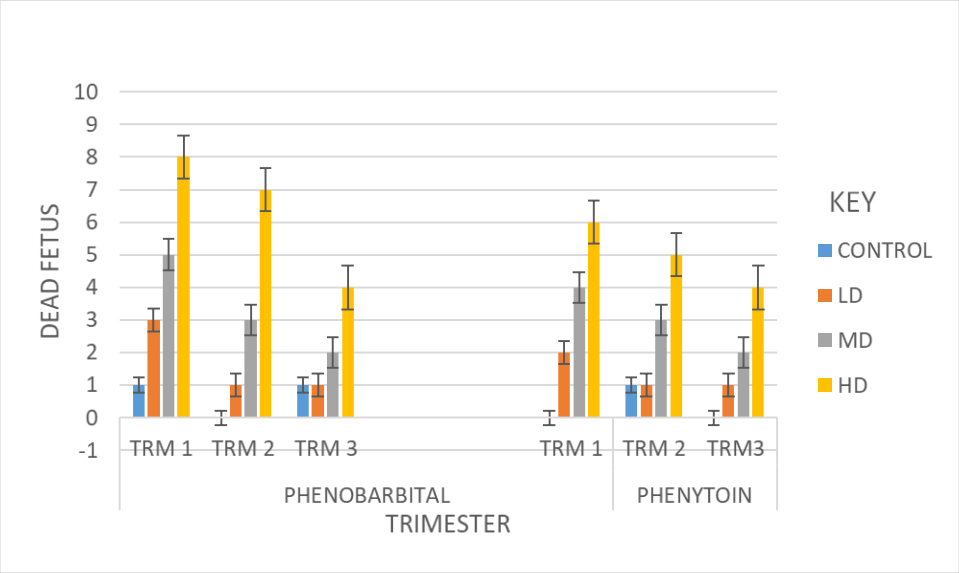

Figure 4.3: The Comparative Findings on How the Two Medicines [Phenytoin and Phenobarbital] Influenced the Fetal Pregnancy Outcome(Dead Fetus)

Stage 3: The Comparative Findings on How the Two Medicines [Phenytoin and Phenobarbital] Influenced the Fetal Growth and Development Parameters

Upon comparing the fetal growth parameters that included the fetal weights, the crown-rump length, the head circumference and the bi-parietal diameters when they were prenatally exposed to varied doses of either phenytoin or Phenobarbital at different trimesters, it was observed that, there was an overall statistical significant reduction in: mean fetal weight ($F(18,38)=16.840$, $P<0.001$), mean crown-rump length ($F(18,38)=19.139$, $P<0.001$), mean fetal head circumference ($F(18, 38)$

=8.936, P<0.001), and mean bi-parietal diameter (F (18, 38) =18.407, P<0.001). The control group was observed to have the highest means (p<0.05) than Phenobarbital and the phenytoin treatment groups.

Upon Evaluating how the two medicines influenced the head circumference, the fetal weight, the bi-parietal diameter and the head circumference, it was established that the reduction in means of the fetal weight, the crown-rump length, the bi-parietal diameter and the head circumference, were dependent on the dosages and time of exposure. The control group was observed to have the highest means of fetal weight, crown-rump length, bi-parietal diameter and head circumference compared to either phenobarbital or phenytoin treatment groups. Both phenobarbital and phenytoin treatment groups denoted that there was a statistically significant difference (P<0.001) in different trimesters with the highest fetal weight, crown-rump length, bi-parietal diameter and head circumference observed in trimester three (TM3), followed by trimester two (TM2) and lastly by trimester one (TM1). Similarly, it was observed that the fetuses from the rats in Phenobarbital and phenytoin treatment groups that received high doses were associated with low fetal weight, low crown-rump length, low bi-parietal diameter and low head circumference, followed by medium dosage groups and lastly by low dosage groups. It was however noted that those exposed to Phenobarbital treatment groups showed that there were statistically significantly lower means of the fetal weight, the crown-rump length, the bi-parietal diameter and the head circumference (p<0.05) as compared to the phenytoin treatment group (***Table 4.5***)

Table 4.5: The ANOVA Table Showing the Comparative Findings on How the Two Medicines Influenced the Four Fetal Growth and Development Parameters When Administered at Varied Doses of Low, Medium and High and at TM1, TM2 And TM3 Compared With The Control.

Drugs	Dosage level	Exposure time	Fetal Weights	Crown-Rump Length	Bi-parietal diameter	Head circumference
	None	Control	6.66667±.22605 3	5.45590±.04926 1	.82457±.02308 9	3.64890±.08332 2
Phenobarbita	Low	Trimester 1	5.3627±.096921	4.32143±.10026 4	.53437±.01070 2	2.99640±.04502 9

l		Trimester 2	5.74720±.059026	5.06733±.051908	.64577±.01842 0	3.13437±.011836
		Trimester 3	6.29889±.178645	5.23047±.096305	.77904±.00527 4	3.42243±.077192
	Medium	Trimester 1	4.81014±.013328	3.95266±.042963	.43437±.01070 2	2.63040±.097455
		Trimester 2	5.37187±.094708	4.68508±.051760	.55368±.01088 8	2.81077±.101509
		Trimester 3	6.14102±.065275	4.95938±.008087	.64130±.03638 0	3.07153±.016363
	High	Trimester 1	2.91940±.034311	2.64143±.121811	.25680±.00871 3	1.90900±.080450
		Trimester 2	3.47920±.219899	3.03987±.012428	.35773±.00782 3	2.08673±.007623
		Trimester 3	3.87299±.119573	4.09466±.069887	.44101±.01387 4	2.28507±.008257
Phenytoin	Low	Trimester 1	5.65365±.079136	4.45434±.089819	.61490±.01184 9	3.11033±.009957
		Trimester 2	5.94833±.013343	5.13281±.009591	.72757±.01338 9	3.25983±.035206
		Trimester 3	6.61182±.138995	5.35667±.061628	.80624±.00023 1	3.52913±.090363
	Medium	Trimester 1	4.9544±.017314	4.08300±.013713	.56140±.00410 7	2.82463±.091108
		Trimester 2	5.73337±.115470	4.83068±.104768	.65830±.02317 2	3.01317±.002136
		Trimester 3	6.55412±.175339	5.09557±.053862	.76037±.00543 5	3.17143±.103894
	High	Trimester 1	3.21169±.082688	3.05299±.020023	.35020±.00405 1	2.01617±.035197
		Trimester 2	3.81372±.099288	3.19785±.056812	.45783±.00767 9	2.17103±.030234
		Trimester 3	4.41402±.183284	4.20065±.075173	.55773±.00782 3	2.43507±.025300
	ANOVA statistic	***F (18,38)***	16.840,	19.139	18.407	8.936
	Significance level	***P VALUE***	P<0.001	P<0.001	P<0.001	P<0.001

Key: Means+SD that bears (*) means that there were statistically significant differences with the control at (p<0.05), While Means+SD of phenytoin that bears (b) means that they are statistically significantly different with phenobarbital at the same dosage level (P<0.05)

4.1.2.2 To Comparatively Evaluate How Phenobarbital and Phenytoin Influenced the Fetal Weight, the Crown-Rump Length, The Bi-Parietal Diameter And The Head Circumference, In Utero Using Manova The Results Were Presented At Three Levels As Follows

Level 1: How phenobarbital and phenytoin and their interactions globally influenced the fetal weight, the crown-rump length, the bi-parietal diameter and head circumference parameters.

Level 2: How phenobarbital and phenytoin, their dosages, time exposure individually and their interaction influenced the fetal weight, the crown-rump length, the bi-parietal diameter and the head circumference in-utero.

Level 3: The pairwise Manova analysis on how the phenobarbital and phenytoin influenced the fetal weight, the crown-rump length, the bi-parietal diameter and the head circumference in utero.

On carrying out a Manova to establish how phenobarbital and phenytoin and their interactions globally influenced the fetal weight, the crown-rump length, the bi-parietal diameter and the head circumference parameters, their interaction effects of drug, dosages and trimester combined, and the interaction effects of the three independent variables of the drug, dosage and time were found to be statistically significant in different proportion (**Table 4.6);**

(i) **At the individual level the effects were as follows a)** Drug F (4,35) =188.182, P<0.001), Wilkis Lambda (Λ=0.44: partial Eta squared(Ƞ2=0.956); **(b)** Dosages F (8,70) =237.177, P<0.001), Wilkis Lambda (Λ=0001: partial Eta squared (Ƞ2=0.964); **(c)** Trimester F (8,70) =117.719, P<0.5), Wilkis Lambda (Λ=0.001: partial Eta squared(Ƞ2=0.931);

(ii) The two-way combination interactions effects of **a)** Drug*trimester F (8,70) =0.788, P<0.001), Wilkis Lambda(Λ=0.727): partial Eta squared(Ƞ2=0.148); **(b)**Drug and dosages F (8,70) =5.514, P<0.001), Wilkis Lambda (Λ=0.376: partial Eta squared (Ƞ2=0.387); **(c)** Trimester and dosage F (16,107) =9.533, P<0.001), Wilkis Lambda (Λ=0.067: partial Eta squared (Ƞ2=0.491);

(iii) The three-way interaction effects among the drug, dosage and trimester; a) drug F (16,107) =1.999, P<0.001), Wilkis Lambda (Λ=0.451: partial Eta squared (Ƞ2=0.180);

Tabl.4.6: The MANOVA Level 1 Table Findings on How the Two Medicines (Phenobarbital and Phenytoin) and Their Interactions Globally Influenced the Four Fetal Parameters: Fetal Weights, Crown-Rump Length, Bi-Parietal Diameter and Head Circumference Parameters

Effect	Wilks' Lambda Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Intercept	.000	90571.834	4.000	35.000	<.001	1.000
Drug	.044	188.182	4.000	35.000	<.001	.956
Dosage	.001	237.177	8.000	70.000	<.001	.964
Trimester	.005	117.719	8.000	70.000	<.001	.931
Drug * Dosage	.376	5.514	8.000	70.000	<.001	.387
Drug * Trimester	.727	1.515	8.000	70.000	.168	.148
Dosage * Trimester	.067	9.533	16.000	107.564	<.001	.491
Drug * Dosage * Trimester	.451	1.999	16.000	107.564	.019	.180

a. Design: Intercept + Drug + Dosage + Trimester + Drug * Dosage + Drug * Trimester + Dosage * Trimester + Drug * Dosage * Trimester

b. Exact statistic

c. The statistic is an upper bound on F that yields a lower bound on the significance level.

Level 2: upon carrying out MANOVA level 2, the comparative multiple analysis results on how the phenobarbital and phenytoin, their doses and the time of exposure plus their interactions influenced the four fetal growth and development parameters ie. fetal weight, the crown-rump length, the bi-parietal diameter and the head circumference in-utero. this study established the following: -

On carrying out a Manova Level 2 to establish how the phenobarbital and phenytoin, their dosages and their interactions globally influenced the fetal weight, the crown-rump length, the bi-parietal diameter and the head circumference parameters, their interaction effects, dosages and trimester combined, the interaction of the three independent variables of the drug, dosage and time were found to be statistically significant in different proportion (***Table 4.7*****);**

The finding of the contribution level of the phenobarbital and phenytoin, their dosage and time of exposure to the four dependent growth and development parameters of **i)** the fetal weight, **ii)** the crown-rump length, **iii)** the bi-parietal diameter and **iv)** the head circumference. The results observed a varying proportion (partial eta squared(Ƞ2) of contribution to each as follows:

At individual effects of how the four dependent variables of the fetal weight, the crown-rump length, the bi-parietal diameter and the head circumference were influenced by either of the following dependent variables of trimester, dosage and drugs.

a) At the individual level the highest contribution of the observed effects was observed at the dosage level with partial eta ranging from 97.7% to 99.3%, followed by trimesters at 90.7% to 98% and lastly drug at 46.35% to 93.9%. as shown in the (***Table 4.7***)

b) At two-way level interaction, when two variables were combined the observed that the effects were highest between doses * trimesters with partial eta ranging from 15% to 88.5% followed by dosages and drugs ranging from 15.1% to 47.2% and lastly drug and trimester ranging from 11.7% to 29.7% on the 4 fetal parameters: the fetal weight, the crown-rump length, the bi-parietal diameter and the head circumference as shown in **(*Table 4.7*)**

c) at three-way interaction, when the three independent variables (drug, dosage and trimester) were combined they were noted to have the highest effects on bi-parietal diameter with partial eta at 29.9%, followed by crown-rump length at 24%, then followed by fetal weight at 11.7 % and finally biparietal diameter at 7.7% as shown in the *(**Table 4.7***)

Table 4.7: The MANOVA Level 2 Table Findings on How the Two Medicines (Phenobarbital and Phenytoin), Their Doses and the Time of Exposure Plus Their Interactions Globally Influenced the Four Fetal Parameters: Fetal Weight, the Crown-Rump Length, the Bi-Parietal Diameter and the Head Circumference Parameter

Tests of Between-Subjects Effects							
Comparative assessment parameter	**Source (independent varaibles- intervention and dosage being compared)**	**Dependent Variable**	**Type III Sum of Squares (measure of the amount of variability in the dependent variable after the controlling for the effectsin the model)**	**Df (k-1)**	**Mean Square (the ratio of the type iiisum of squareof the to thisindependent correspondingvariables to degree ofthe mean freedom) square of error)**	**F-statistic (theratio of the mean square**	**Sig.**	**Partial Eta Squared (measure of the effect size)**
(i) The evaluation of the	Corrected Model	Fetal Weight	76.764a	18	4.265	128.233	<0.001	.984

Tests of Between-Subjects Effects

Comparative assessment parameter	Source (independent varaibles-intervention and dosage being compared)	Dependent Variable	Type III Sum of Squares (measure of the amount of variability in the dependent variable after the controlling for the effectsin the model)	Df (k-1)	Mean Square (the ratio of the type iiisum of squareof the to thisindependent correspondingvariables to degree ofthe mean freedom)	F-statistic (the ratio of the iiimean square square of error)	Sig.	Partial Eta Squared (measure of the effect size)
correctness of the model used for the study		Crown Lump Length	40.143b	18	2.230	503.139	<0.001	.996
		Biparietal Diameter	1.458c	18	.081	390.490	<0.001	.995
		Head Circumference	15.269d	18	.848	220.388	<0.001	.991
(ii) Test on whether the observed results were due to chance	Intercept	Fetal Weight	1170.848	1	1170.848	35205.686	<0.001	.999
		Crown Lump Length	837.823	1	837.823	189017.015	<0.001	1.000
		Biparietal Diameter	15.421	1	15.421	74352.614	<0.001	.999
		Head Circumference	354.079	1	354.079	91989.814	<0.001	1.000
(iii) The Individual independent variable and its effects on each of the three maternal dependent variables	Drug	Fetal Weight	1.091	1	1.091	32.801	<0.001	.463
		Crown Lump Length	.332	1	.332	74.992	<0.001	.664
		Biparietal Diameter	.121	1	.121	581.250	<0.001	.939
		Head Circumference	.234	1	.234	60.711	<0.001	.615
	Dosage	Fetal Weight	54.643	2	27.322	821.518	<0.001	.977
		Crown Lump Length	24.238	2	12.119	2734.153	<0.001	.993
		Biparietal Diameter	.751	2	.375	1809.953	<0.001	.990
		Head Circumference	11.326	2	5.663	1471.253	<0.001	.987
	Trimester	Fetal Weight	12.320	2	6.160	185.229	<0.001	.907
		Crown Lump Length	10.359	2	5.180	1168.536	<0.001	.984
		Biparietal Diameter	.381	2	.190	918.087	<0.001	.980
		Head Circumference	1.490	2	.745	193.595	<0.001	.911
(iv)Two-way interaction effects on each of the maternal dependent variables	Drug * dosage	Fetal Weight	.124	2	.062	1.871	.168	.090
		Crown Lump Length	.033	2	.017	3.764	.032	.165
		Biparietal Diameter	.007	2	.004	16.952	<0.001	.472
		Head Circumference	.008	2	.004	1.011	.373	.051
	Drug * trimester	Fetal Weight	.135	2	.068	2.036	.145	.097
		Crown Lump Length	.031	2	.016	3.523	.039	.156
		Biparietal Diameter	.000	2	.000	.886	.421	.045
		Head Circumference	.001	2	.001	.142	.868	.007
	Dosage * trimester	Fetal Weight	.682	4	.171	5.129	.002	.351
		Crown Lump Length	1.297	4	.324	73.147	<0.001	.885
		Biparietal Diameter	.001	4	.000	.963	.439	.092
		Head Circumference	.008	4	.002	.496	.739	.050
(v) Three-way interaction effects on each of the maternal dependent variables	Drug * dosage * trimester	Fetal Weight	.168	4	.042	1.260	.303	.117
		Crown Lump Length	.053	4	.013	3.005	.030	.240
		Biparietal Diameter	.003	4	.001	4.058	.008	.299
		Head Circumference	.012	4	.003	.796	.535	.077
	Error	FTWT	1.264	38	.033			

Tests of Between-Subjects Effects

Comparative assessment parameter	Source (independent varaibles- intervention and dosage being compared)	Dependent Variable	Type III Sum of Squares (measure of the amount of variability in the dependent variable after the controlling for the effectsin the model)	Df (k-1)	Mean Square (the ratio of the type iii sumof squares to thisindependent correspondingvariables to degree ofthe freedom)	F-statistic (the ratio of the mean square of error)	Sig.	Partial Eta Squared (measure of the effect size)
		Fetal Weight	.168	38	.004			
		Crown Lump Length	.008	38	.000			
		Biparietal Diameter	.146	38	.004			
(vi) Overall inferential statistics on the model results	Total	Fetal Weight	1570.771	57				
		Crown Lump Length	1124.193	57				
		Biparietal Diameter	20.443	57				
		Head Circumference	467.796	57				
	Corrected Total	Fetal Weight	78.028	56				
		Crown Lump Length	40.312	56				
		Biparietal Diameter	1.466	56				
		Head Circumference	15.416	56				

a. R Squared = .984 (Adjusted R Squared = .976)
b. R Squared = .996 (Adjusted R Squared = .994)
c. R Squared = .995 (Adjusted R Squared = .992)
d. R Squared = .991 (Adjusted R Squared = .986)

Level 3 The pairwise Manova analysis on how the phenobarbital and phenytoin influenced the fetal weight, the crown-rump length, the bi-parietal diameter and the head circumference in utero.

A) Pairwise comparison on fetal weight

i) Pairwise of low doses on Fetal weight for TM1, TM2 and TM3 were as follows; **(a)** TM1 mean difference (-.291, p value=0.008 (b) TM2 mean difference (-0.132, p value=0.002) (c) TM3 mean difference (-.313, p value=0.002)

ii) Pairwise of medium doses on fetal weight: (**a**) TM1 mean difference (-.144, p value=0.009) (b) TM2 mean difference (-.361, p value=0.020) (c) TM3 mean difference (-.413, p. value=0.009)

iii) Pair wise of high doses on fetal weight: a)TM1 mean difference (-.292, p. value=0.007) (b) TM2 mean differences (-.335, p. value=0.031) (c) TM3 mean difference (-.541, p. value=0.001)

B) Pairwise comparison on crown rump length

i) Pairwise of low doses on crown-rump length for TM1, TM2 and TM3 were as follows; **(a)** TM1 mean difference (-.133, p value=0.019) (b) TM2

mean difference (-0.065, p value=0.006) (c) TM3 mean difference (-.126, p value=0.026)

ii) Pairwise of medium doses on crown-rump length **a)**TM1 mean difference (-.130, p value=0.022) (**b**) TM2 mean difference (-.130, p value=0.022) (**c**) TM3 mean difference (-.146, p. value=0.011)

iii) Pairwise of high doses on crown-rump length:**a**)TM1 mean difference (-.412, p. value=0.000) (b) TM2 mean differences (-.158, p. value=0.006) (c) TM3 mean difference (-.106, p. value=0.009)

C) Pairwise comparison on bi-parietal diameter

i) Pairwise of low doses on bi-parietal diameter for TM1, TM2 and TM3 were as follows; **(a)** TM1 mean difference (-.081, p value=0.000) (b) TM2 mean difference (-0.082, p value=0.000) (c) TM3 mean difference (-.027, p value=0.026)

ii) Pairwise of medium doses on bi-parietal diameter:TM1 mean difference (-.127, p value=0.000) (b) TM2 mean difference (-.105, p value=0.000) (c) TM3 mean difference (-.119, p. value=0.000)

iii) Pair wise of high doses on bi-parietal diameter: TM1 mean difference (-.093, p. value=0.000) (b) TM2 mean differences (-.100, p. value=0.000) (c) TM3 mean difference (-.177, p. value=0.009)

D) Pairwise comparison on head circumference

i) Pairwise of low doses on head circumference for TM1, TM2 and TM3 were as follows; **(a)** TM1 mean difference (-.114, p value=0.030) (b) TM2 mean difference (-0.125, p value=0.018) (c) TM3 mean difference (-.107, p value=0.042)

ii) Pairwise of medium doses on head circumference: TM1 mean difference (-.194, p value=0.000) (b) TM2 mean difference (-.202, p value=0.000) (c) TM3 mean difference (-.100, p. value=0.006)

iii) Pair wise of high doses on head circumference: **(a)**TM1 mean difference (-.107, p. value=0.001) **(b)** TM2 mean differences (-.084, p. value=0.004) **(c)** TM3 mean difference (-.150, p. value=0.005)

upon comparative finding of pairwise multiple analysis of variance between the phenobarbital and the phenytoin in the same dosage and same time of exposure to

establish how the two medicines influenced fetal weight, the crown-rump length, the bi-parietal diameter and the head circumference it was observed to have statistical significance difference in that phenytoin given at the same dosage and the same time of exposure was noted to have minimal effects compared to the phenobarbital and phenytoin (***Table 4.8).***

Table 4.8: The MANOVA Level 3 Pairwise Table Findings on How the Two Medicines (Phenobarbital and Phenytoin) Influenced the Four Fetal Growth and Development Parameters: The Fetal Weight, the Crown-Rump Length, the Bi-Parietal Diameter and the Head Circumference Parameters in Utero

								95% Confidence Interval for Differenced	
Dependent Variable	**Dosage (Mg/kg bw)**	**Trimesters**	**PB**	**PT**	**Mean Difference(PB-PT)**	**Std. Error**	**Sigd (<.05)**	**Lower Bound**	**Upper Bound**
	Low	Trimester one	PB	PT	-.291	.149	.008	-.592	.011
Fetal Weight		Trimester two	PB	PT	-.132	.149	.002	-.169	.434
		Trimester three	PB	PT	-.313	.149	.002	-.614	-.011
	Medium	Trimester one	PB	PT	-.144	.149	.009	-.446	.157
		Trimester two	PB	PT	-.361	.149	.020	-.663	-.060
		Trimester three	PB	PT	-.413	.149	.009	-.715	-.112
	High	Trimester one	PB	PT	-.292	.149	.007	-.594	.009
		Trimester two	PB	PT	-.335	.149	.031	-.636	-.033
		Trimester three	PB	PT	-.541	.149	.001	-.842	-.240
Crown-Rump Length	Low	Trimester one	PB	PT	-.133	.054	.019	-.243	-.023
		Trimester two	PB	PT	-.065	.054	.006	-.176	.045
		Trimester three	PB	PT	-.126	.054	.026	-.236	-.016
	Medium	Trimester one	PB	PT	-.130	.054	.022	-.240	-.020
		Trimester two	PB	PT	-.146	.054	.011	-.256	-.036
		Trimester three	PB	PT	-.136	.054	.017	-.246	-.026
	High	Trimester one	PB	PT	-.412	.054	.000	-.522	-.302
		Trimester two	PB	PT	-.158	.054	.006	-.268	-.048
		Trimester three	PB	PT	-.106	.054	.009	-.216	.004
	Low	Trimester	PB	PT	-.081	.012	.000	-.104	-.057

Dependent Variable	Dosage (Mg/kg bw)	Trimesters	PB	PT	Mean Difference(PB-PT)	Std. Error	Sigd (<.05)	95% Confidence Interval for Differenced Lower Bound	Upper Bound
		one							
Biparietal Diameter		Trimester two	PB	PT	-.082	.012	.000	-.106	-.058
		Trimester three	PB	PT	-.027	.012	.026	-.051	-.003
	Medium	Trimester one	PB	PT	-.127	.012	.000	-.151	-.103
		Trimester two	PB	PT	-.105	.012	.000	-.128	-.081
		Trimester three	PB	PT	-.119	.012	.000	-.143	-.095
	High	Trimester one	PB	PT	-.093	.012	.000	-.117	-.070
		Trimester two	PB	PT	-.100	.012	.000	-.124	-.076
		Trimester three	PB	PT	-.117	.012	.000	-.141	-.093
	Low	Trimester one	PB	PT	-.114	.051	.030	-.216	-.011
Head Circumference		Trimester two	PB	PT	-.125	.051	.018	-.228	-.023
		Trimester three	PB	PT	-.107	.051	.042	-.209	-.004
	Medium	Trimester one	PB	PT	-.194	.051	.000	-.297	-.092
		Trimester two	PB	PT	-.202	.051	.000	-.305	-.100
		Trimester three	PB	PT	-.100	.051	.006	-.202	.003
	High	Trimester one	PB	PT	-.107	.051	.001	-.210	-.005
		Trimester two	PB	PT	-.084	.051	.004	-.187	.018
		Trimester three	PB	PT	-.150	.051	.005	-.253	-.047

4.2 Objective 2: The Comparative Evaluation of How Prenatal Exposure to Varied Doses of Phenobarbital and Phenytoin Influenced The Histomorphological Findings of Fetal Appendicular Skeleton

The results on how phenobarbital and phenytoin influence the histological changes of epiphyseal growth plate zones when administered at different doses upon exposure at different trimesters.

Level 1: The comparative findings on how the varied doses of phenobarbital and phenytoin influenced the histological thickness of different zones of epiphyseal plate given at different trimesters.

Level 2: The comparative Histomorphological findings on how varied doses of phenobarbital and phenytoin influenced the specific zones of epiphyseal plate when exposed at different trimesters.

Level 1: The comparative findings on how the varied doses of phenobarbital and phenytoin influenced the growth of epiphyseal plate given at different trimesters.

Upon administration of both phenobarbital and phenytoin at different trimester and varied doses, it was established that the The histological architecture of the developing epiphyseal growth plate layers that included the zone of reserve cartilage/the resting zone, the proliferation zone, the zone of chondrocyte hypertrophy, the cartilage degeneration, the zone of calcification and the osteogenic zones were observed to be variably affected by the prenatal exposure to the two medicines in a dose and time-dependent manner as compared to the control.(***Figure 4.4,Figure 4.5 And Figure 4.6***).

Upon exposure to low doses of phenobarbital and phenytoin, it was established that those exposed at TM1 and TM2 showed mild effects on all the layers. Those exposed to low doses at TM3 didn't reveal any much difference as compared to the control. On evaluating the effects of either drug upon exposure to low doses of both phenobarbital and phenytoin, it was established that phenobarbital treatment groups had reduced epiphyseal growth plate layers in TM1 and TM2 as compared to the phenytoin treatment groups. It was however observed that the the groups exposed to low doses at TM3 of both phenobarbital and phenytoin groups had minimal differences when compared to the control(***Figure 4.4***).

Upon administration of medium dosages of both phenobarbital and phenytoin, it was established that the epiphyseal growth plate layers were dose and time-dependent. The exposure to medium doses of both treatment groups indicated that those exposed to TM1 and TM2 showed more effects on all the layers. Those exposed to medium doses at TM3, didn't reveal no much difference as compared to the control. On evaluating the effects of either drug upon exposure to low doses of both phenobarbital and phenytoin, it was established that phenobarbital treatment groups had reduced epiphyseal growth plate layers in TM1 and TM2 as compared to the phenytoin treatment groups. It was however observed that the the groups exposed to

low doses at TM3 of both phenobarbital and pheytoin groups had minimal difference when compared to the control(***Figure 4.5***)

Upon exposure to the high dose of phenobarbital and phenytoin, the zone of reserve cartilage/the resting zone, the proliferation zone, the zone of chondrocyte hypertrophy, the cartilage degeneration, the zone of calcification and the osteogenic zones were observed to be variably affected by the prenatal exposure to the two medicines at high doses as compared to the medium, low and control groups. The high doses at trimester one was noted to have the greatest effects, followed by exposure at tm2 and TM3 respectively. On evaluating which medicine had more effects, it was established that phenobarbital treatment groups had more effects across the three trimesters compared to phentoin treatment groups when exposed at the same time and doses (***Figure 4.6***)

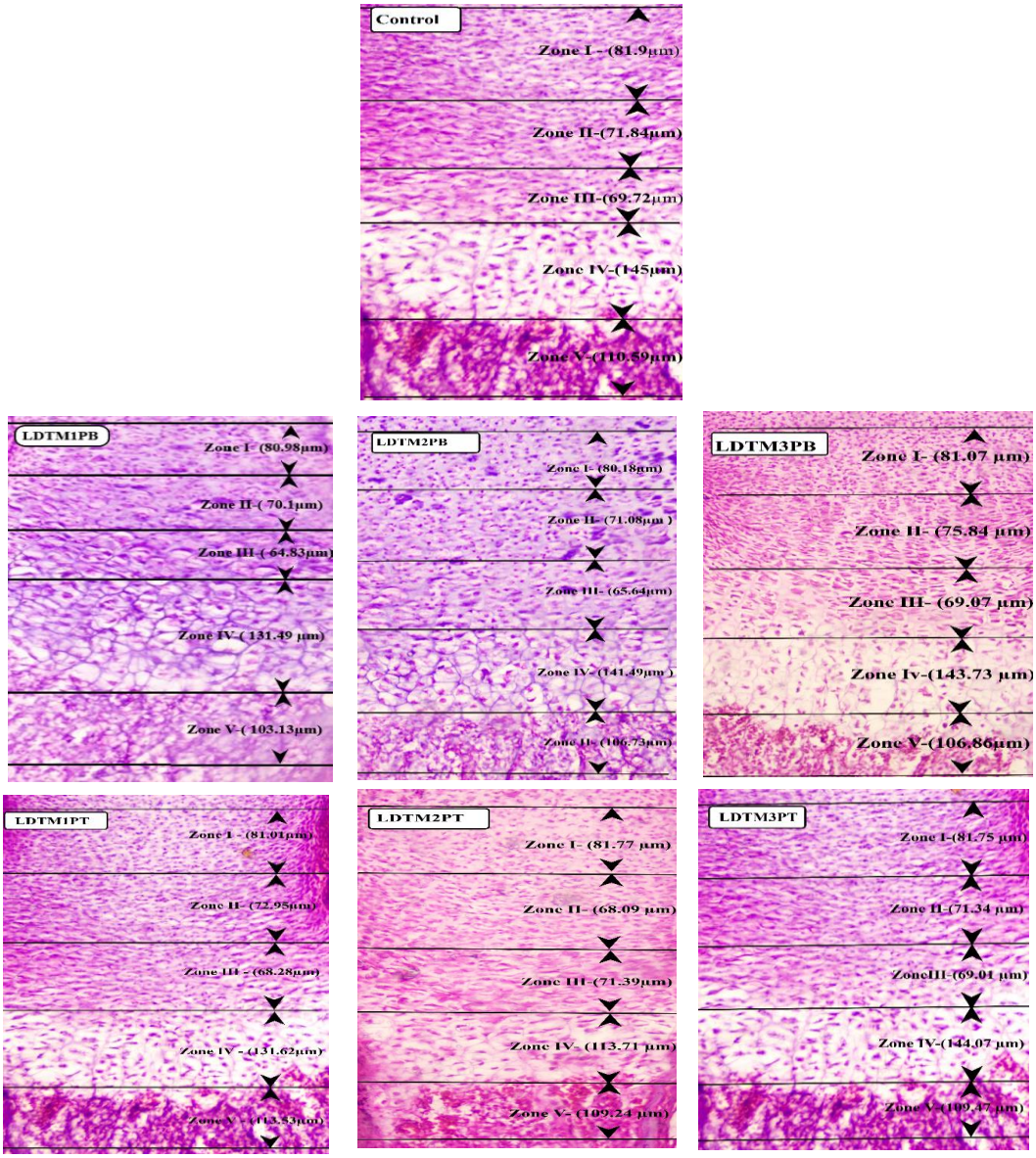

Figure 4.4: The Comparative Photomicrograph Showing Epiphyseal Growth Plate Layers Upon Exposure To Low Doses Of Phenobarbital and phenytoin against control at TM1, TM2, TM3(HE mag x100)

KEY A: Control

B: LDTMIPB-Low dose phenobarbital trimester one showing epiphyseal growth plate layers

C: LDTM2PB- Low dose phenobarbital trimester one showing epiphyseal growth plate layers

D: LDTM3PB - Low dose phenobarbital trimester one showing epiphyseal growth plate layers

E: LDTM1PT - Low dose phenytoin trimester one showing epiphyseal growth plate layers
F: LDTM2PT - Low dose phenytoin trimester two showing epiphyseal growth plate layers
G: LDTM3PT - Low dose phenytoin trimester three showing epiphyseal growth plate layers

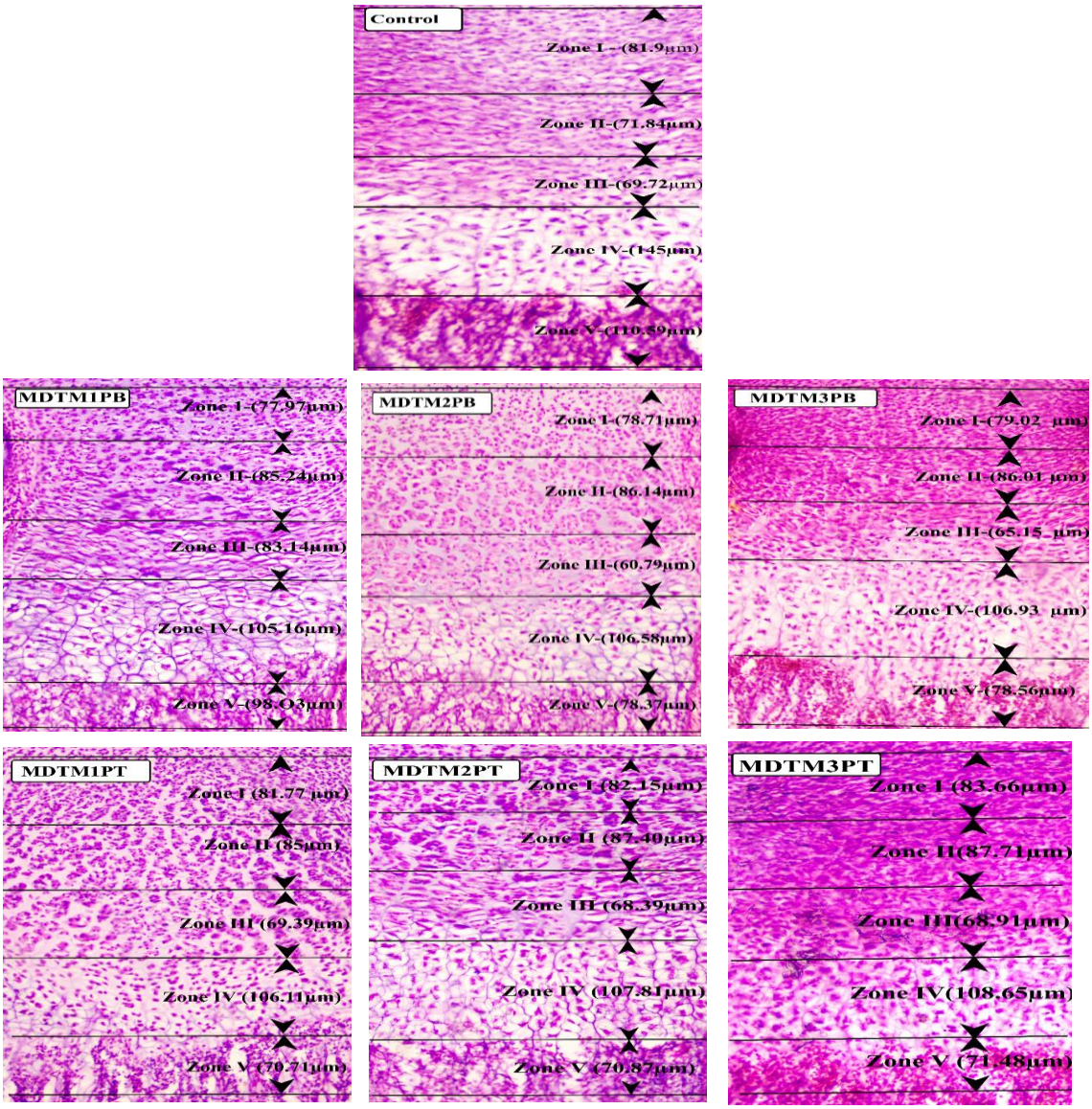

Figure 4.5: The Comparative Photomicrograph Showing Epiphyseal Growth Plate Layers upon Exposure to Medium Doses of Phenobarbital and Phenytoin Against Control at TM1, TM2, TM3(H$E mag x100)

KEY :A: Control

B: MDTMIPB-Medium dose phenobarbital trimester one showing epiphyseal growth plate layers

C: MDTM2PB- Medium dose phenobarbital trimester one showing epiphyseal growth plate layers

D: MDTM3PB - Medium dose phenobarbital trimester one showing epiphyseal growth plate layers

E: MDTM1PT - Medium dose phenytoin trimester one showing epiphyseal growth plate layers

F: MDTM2PT - Medium dose phenytoin trimester two showing epiphyseal growth plate layers

G: MDTM3PT - Medium dose phenytoin trimester three showing epiphyseal growth plate layers

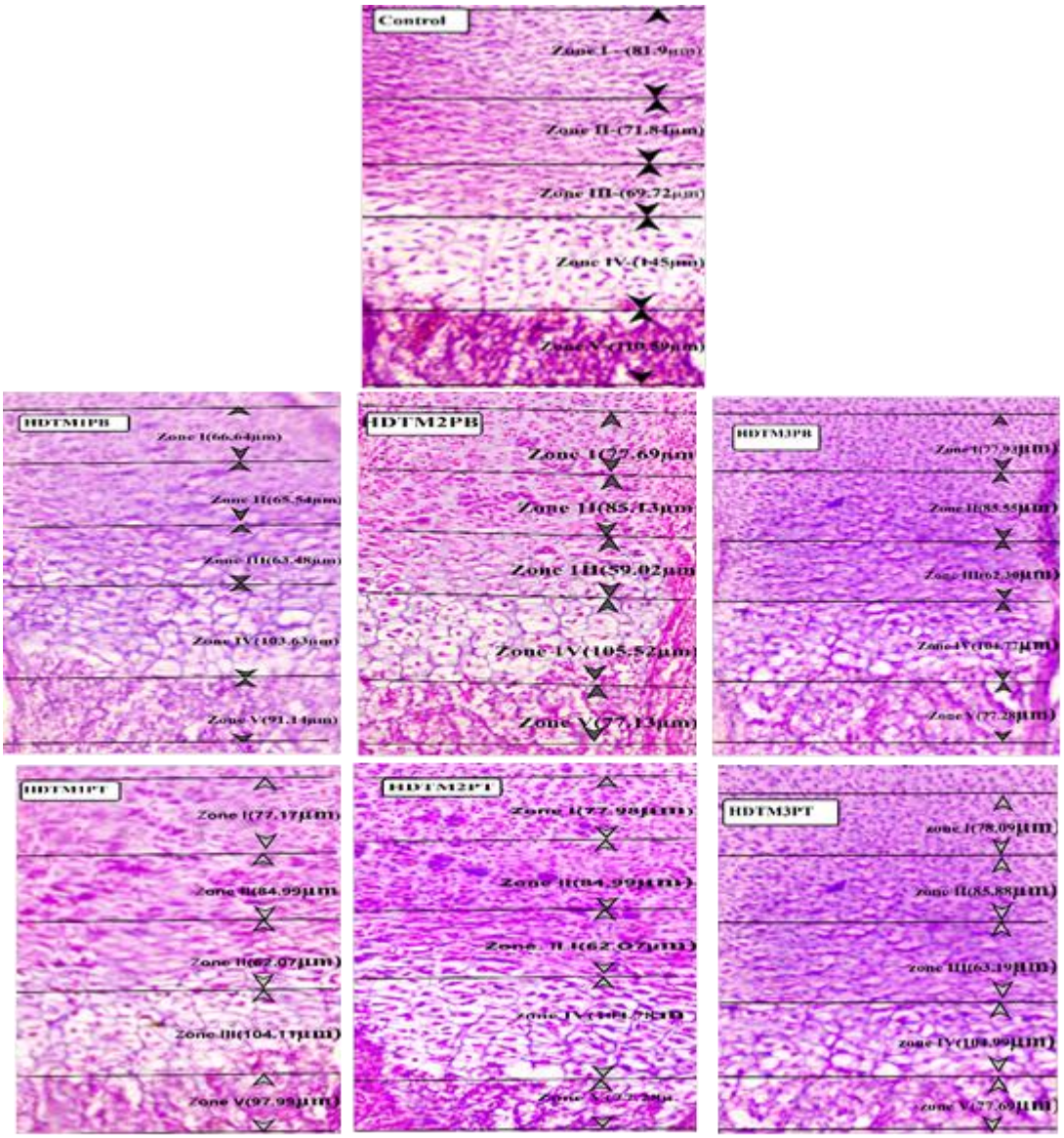

Figure 4.6: The Comparative Photomicrograph Showing Epiphyseal Growth Plate Layers Upon Exposure to High Doses of Phenobarbital and Phenytoin Against Control at TM1, TM2, TM3(H$E mag x100)

KEY *:A: Control*

B: HDTMIPB- High dose phenobarbital trimester one showing epiphyseal growth plate layers

C: HDTM2PB- High dose phenobarbital trimester one showing epiphyseal growth plate layers

D: HDTM3PB - High dose phenobarbital trimester one showing epiphyseal growth plate layers

E: HDTM1PT - High dose phenytoin trimester one showing epiphyseal growth plate layers

F: HDTM2PT - High dose phenytoin trimester two showing epiphyseal growth plate layers

G: HDTM3PT - High dose phenytoin trimester three showing epiphyseal growth plate layers

4.2.2 The Comparative Histomorphological Findings on How Varied Doses of Phenobarbital and Phenytoin Influenced the Specific Zones of the Epiphyseal Plate when Exposed at Different Trimesters

The comparative histomorphological findings on how varied doses of phenobarbital and phenytoin influenced differents zones of epiphyseal growth plate were presented as follows:

4.2.2.1 The Comparative Findings on How the Low Doses of Phenobarbital and Phenytoin Influenced the Reserve Zone of Epiphyseal Growth Plate Given at Different Trimesters (TM1, TM2 and TM3

The resting zone had a comparable distribution pattern when the Low doses of both treatment groups' rats were compared to the control in TM1. The chondrocytes were observed to be sparsely distributed with abundant extracellular matrix in both treatment groups compared to the control. On assessment of reserve cartilage, the chondrocytes were noted to maintain their tissue architecture whereby they were small in size.(TM1, TM2, TM3).It was noted that there was a similar distribution of chondrocytes and extracellular matrix in both treatment groups when compared to the control. (***Figure 4.7)***

4.2.2.2 The Comparative Findings on How the Medium Doses of Phenobarbital and Phenytoin Influenced the Reserve Zone of Epiphyseal Growth Plate Given at Different Trimesters (TM1, TM2 and TM3

The zone of reverse cartilage in TM1 was observed to have maintained their extracellular matrix. The chondrocytes in this zone were observed to be significantly reduced in both treatment groups as compared to the control groups. The chondrocytes in this zone are distributed in an abundance of extracellular matrix. The resting cartilage cells in phenobarbital and phenytoin treatment groups have retained their general connective tissue morphology which happens to resemble that of the control group. The resting cartilage cells and extracellular matrix in the medium dose of phenobarbital and phenytoin treatment groups in TM3 showed similar

morphological characteristics to those of the control group with an abundant and thus accounts for the sparse distribution of chondrocytes(***Figure 4.8***).

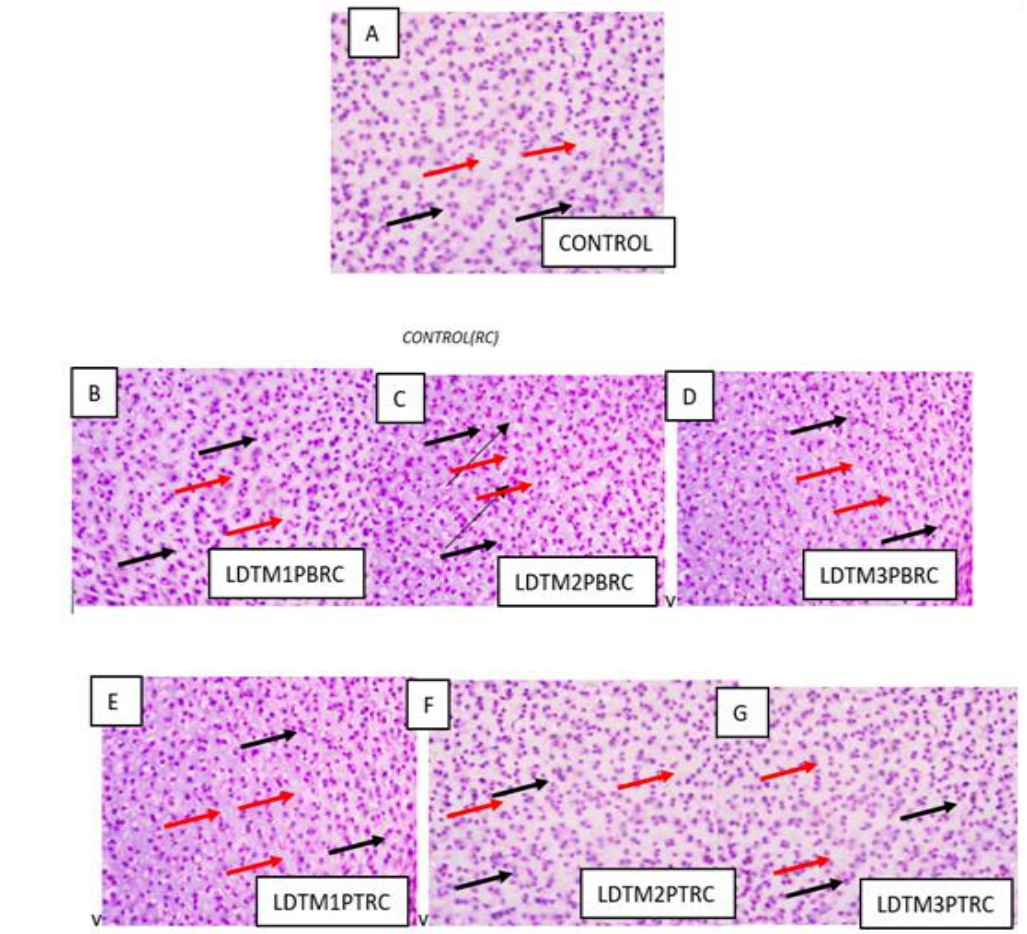

Figure 4.7: The Photomicrograph of the Longitudinal Sections of the Zone of Resting Cartilage Showing Appearances and Distribution of Chondrocytes and Extracellular Matrix Against the Control Treated with Low Doses of Phenobarbital and Phenytoin at TM1, TM2, TM3(H$E mag x100)

***Key**: Arrow: black arrow-chondrocytes, red arrow-extracellular matrix.*
A: Control: showing densely populated chondrocytes which are small in size with less (extra cellular matrix)
B:LDTM1PBRC: Low dose of phenobarbital given at trimester one showing the distribution of chondrocyte and E.C.M
C: LDTM2PBRC-Low dose of phenobarbital given at trimester two showing the distribution of chondrocyte
D: LDTM3PBRC-Low dose of phenobarbital given at trimester three showing the distribution of chondrocyte.
E: LDTM1PBRC-Low dose of phenytoin given at trimester two showing the distribution of chondrocyte
F: LDTM2PBRC-Low dose of phenytoin given at trimester two showing the distribution of chondrocyte
G: LDTM3PBRC-Low dose of phenytoin given at trimester two showing the distribution of chondrocyte.

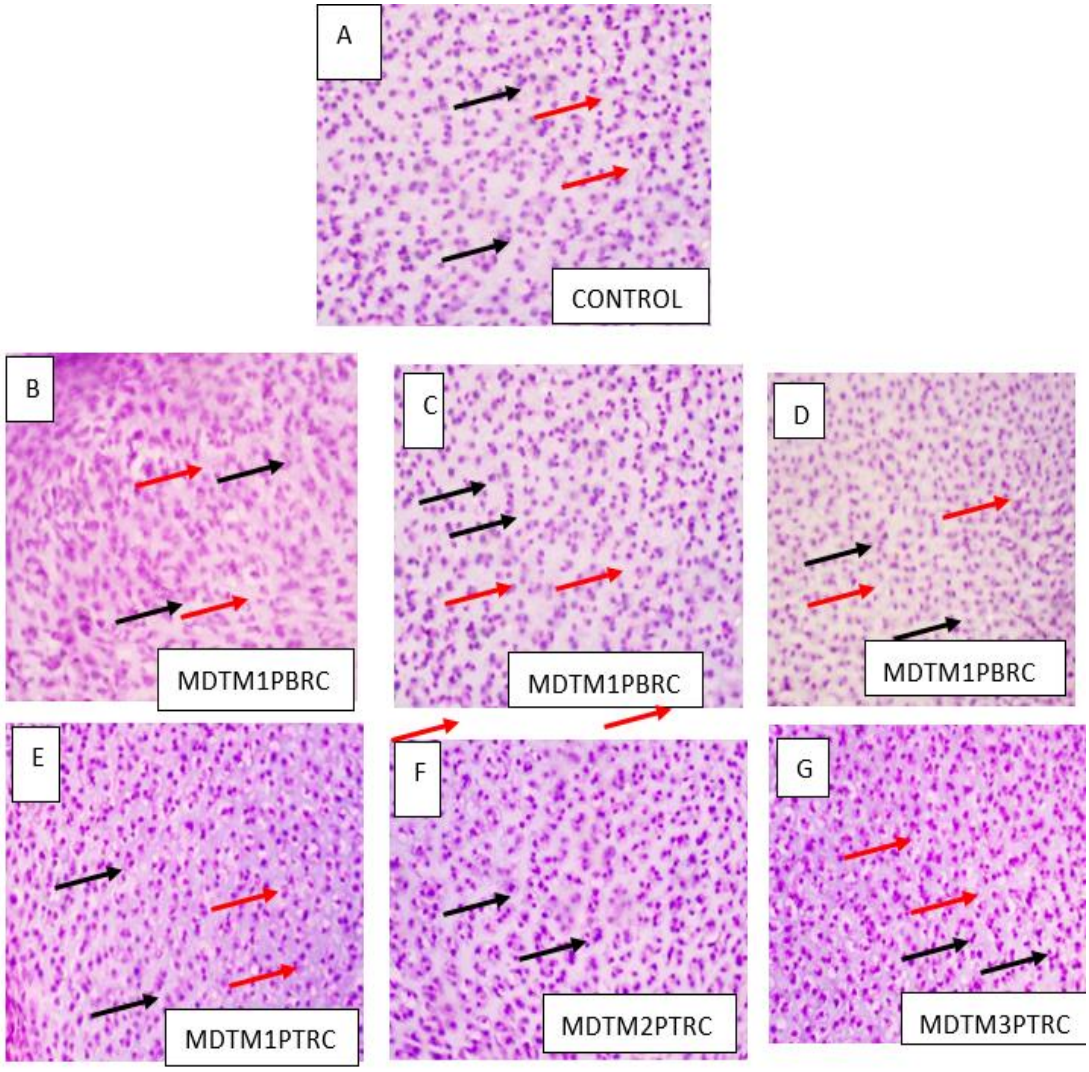

Figure 4.8: The Photomicrograph of the Longitudinal Sections of the Zone of Resting Cartilage Showing Appearances and Distribution of Chondrocytes and Extracellular Matrix Against Control Treated with Medium Doses of Phenobarbital and Phenytoin at TM1, TM2, TM3(H$E mag x100)

Key: Arrow: black arrow-chondrocytes, red arrow-extracellular matrix.
A: Control -showing densely populated chondrocytes which are small in size with less (extra cellular matrix)
B: MDTM1PBRC-Medium dose of phenobarbital given at trimester one showing the distribution of chondrocyte to the control
C: MDTM2PBRC - Medium dose of phenobarbital given at trimester two showing the distribution of chondrocyte
D: MDTM3PBRC - Medium dose of phenobarbital given at trimester three showing the istribution of chondrocyte.
E: HDTM1PTRC - Medium dose of phenytoin given in trimester one showing the distribution of chondrocyte
F: MDTM2PTRC - Medium dose of phenytoin given at trimester two showing the distribution of chondrocyte
G: MDTM3PTRC - Medium dose of phenytoin given at trimester three showing the distribution of chondrocyte.

4.2.2.3 The Comparative Findings on How the High Doses of Phenobarbital and Phenytoin Influenced the Reserve Zone of Epiphyseal Growth Plate Given at Different Trimesters (TM1, TM2 and TM3)

On exposure to high doses of phenobarbital and phenytoin during TM1, it was observed that the cells were more sparsely distributed and some cells lacked nuclei as compared to the control in TM1.

The reverse cartilage shows smaller cells that are intensely staining with Hematoxylin at TM2 as compared to the control. In the reserve cartilage, it was noted that the chondrocytes were smaller in size in all the treatment groups and with smaller nuclei when exposed at TM3 as compared to the control group. (***Figure 4.8***)

4.2.2.4 The Comparative Findings on How the Low Doses of Phenobarbital and Phenytoin Influenced the Proliferative Zone of Epiphyseal Growth Plate Given at Different Trimesters (TM1 TM2 and TM3

In the proliferation zone, the cells were bigger in TM1 than those in the reserve zone and also in control. The zone of proliferation was observed to have smaller cells when administered from trimester two compared to the one in the reverse cartilage in both treatment groups as compared to the control. It was also noted that upon administration of low doses in TM3, the proliferative zone was observed to depict the same size and distribution as the control. (***Figure 4.9)***

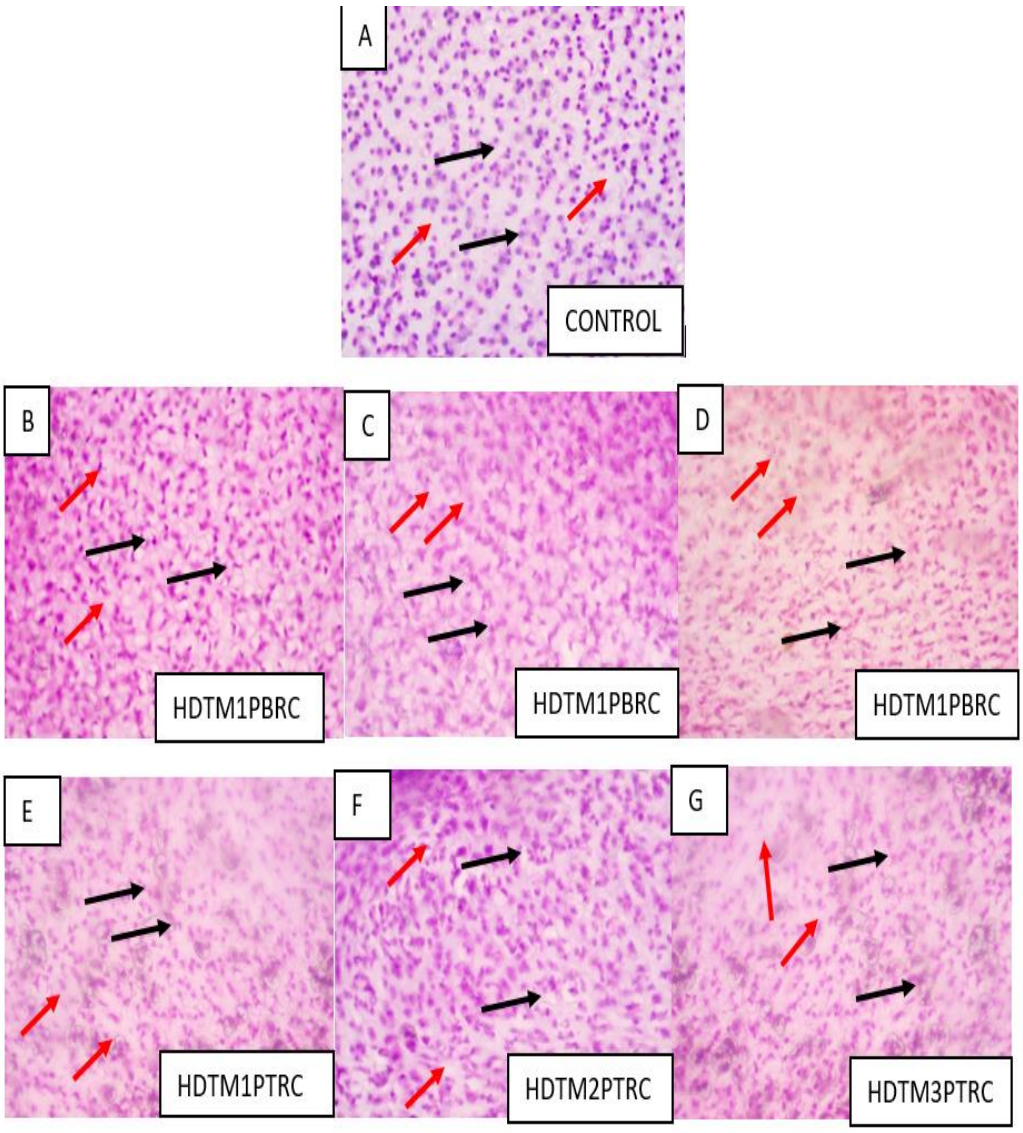

Figure 4.9: The Photomicrograph of the Longitudinal Sections of the Zone of Resting Cartilage Showing Appearances and Distribution of Chondrocytes and Extracellular Matrix against Control Treated at TM1, TM2, TM3(H$E mag x100)

Key: *Arrow: black arrow-chondrocytes, red arrow-extracellular matrix.*

A: Control -showing densely populated chondrocytes which are small in size and E.C.M (extracellular matrix)

B: HDTM1PBRC-High dose phenobarbital given at trimester one showing the distribution of chondrocyte the control

C: HDTM2PBRC -High dose phenobarbital given at trimester two showing the distribution of chondrocyte

D: HDTM3PBRC - High dose phenobarbital given at trimester three showing the distribution of chondrocyte.

E: HDTM1PTRC - High dose phenytoin given at trimester one showing the distribution of chondrocyte

F: HDTM2PTRC - High dose phenytoin given at trimester two showing the distribution of chondrocyte

G: HDTM3PTRC - High dose phenytoin given at trimester three showing distribution of chondrocyte.

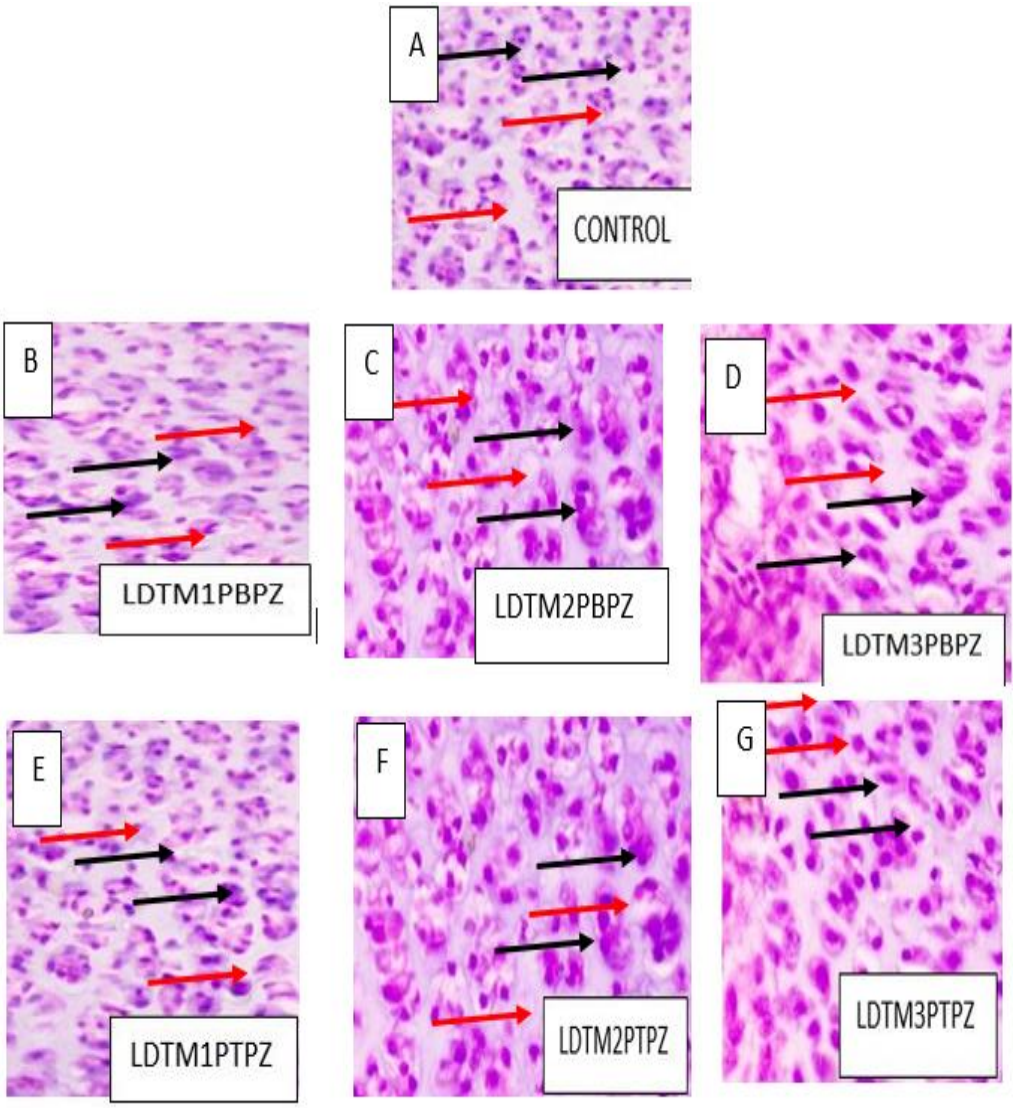

Figure 4.10: The Photomicrograph of the Longitudinal Sections of Proliferative Showing Appearances and Distribution of Chondrocytes and Extracellular Matrix against Control Treated at TM1, TM2, TM3(H$E mag x100)

Key: *Arrow: black arrow-chondrocytes, red arrow-extracellular matrix.*

A: Control -showing densely populated chondrocytes which are small in size E.C.M (extra cellular matrix)
B: -LDTM1PBPZ-Low dose phenobarbital given at trimester one showing distribution of chondrocyte to the control
C: - LDTM2PBPZ – Low dose phenobarbital given at trimester two showing distribution of chondrocyte
D: - LDTM3PBPZ - Low dose phenobarbital given at trimester three showing distribution of chondrocyte.
E: - LDTM1PTPZ - Low dose phenytoin given at trimester one showing the distribution of chondrocyte
F: - LDTM2PTPZ - Low dose phenytoin given at trimester two showing the distribution of chondrocyte
G: LDTM3PTPZ - low dose phenytoin given at trimester three showing the distribution of chondrocyte.

4.2.2.5 The Comparative Findings on How the Medium Doses of Phenobarbital and Phenytoin Influenced the Proliferative Zone of Epiphyseal Growth Plate Given at Different Trimesters (TM1, TM2 and TM3)

In the zone of proliferation at TM1 and TM2 on medium doses of phenobarbital and phenytoin, the cells are bigger with deeply staining nuclei. The zone of proliferation shows large chondrocytes that are intensely in contrast to those in the reserve cartilage(***Figure 4.10***).

4.2.2.6 The Comparative Findings on How the High Doses of Phenobarbital and Phenytoin Influenced the Proliferative Zone of Epiphyseal Growth Plate Given at Different Trimesters (TM1, TM2 and TM3)

Upon exposure to high doses of phenobarbital and phenytoin during TM1, it was observed that the cells were bigger at the zone of proliferation as compared to the control group. The zone of proliferation shows larger cells that are intensely staining with Hematoxylin for cells closer to the groove of Ranvier in comparison to those in the reserve cartilage at TM2. In the proliferative zone, it was noted that the chondroblasts were smaller in size in all the treatment groups and with smaller nuclei when exposed at TM3 as compared to the control group(**Figure *4.11*).**

4.2.2.7 The Comparative Findings on How the Low Doses of Phenobarbital and Phenytoin Influenced the Hypertrophic Zone of Epiphyseal Growth Plate Given at Different Trimesters (TM1, TM2 and TM3)

There were reduced chondrocytes in the hypertrophic zone when phenobarbital and phenytoin groups were exposed to low doses of TM1. The cells were observed to be smaller as compared to the reserve and proliferative zone when exposed to low doses of phenobarbital and phenytoin at TM2. Upon exposure to low doses of phenobarbital and phenytoin at TM3, the chondrocytes in the hypertrophic zone were almost similar in morphology as in the control group(***Figure 4.12***).

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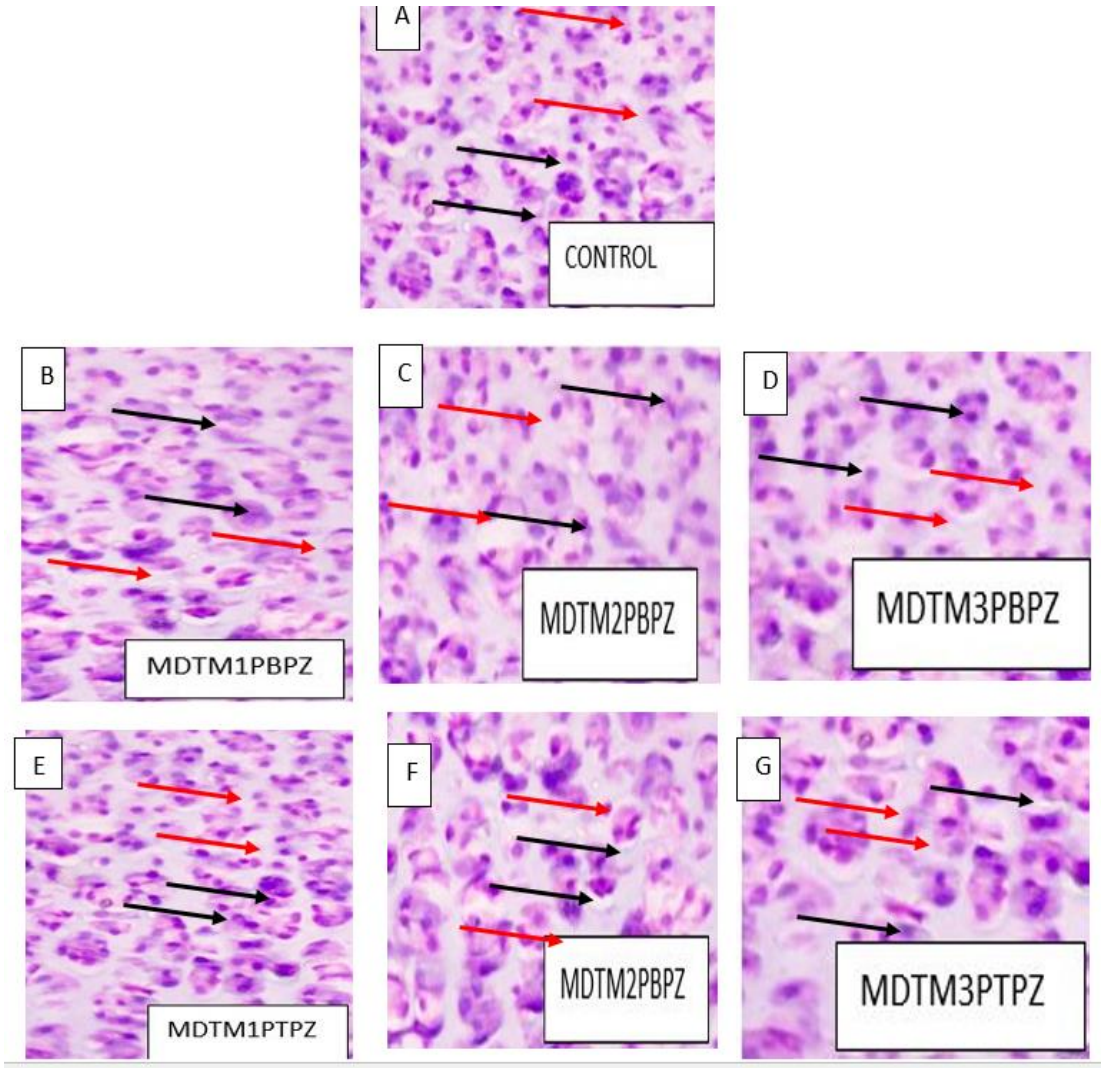

Figure 4.11: The Photomicrograph of The Longitudinal Sections of the Proliferative Zone of Epiphyseal Growth Plate Showing Appearances And Distribution of Chondrocytes and Extracellular Matrix against Control Treated at TM1, TM2, TM3(H$E mag x100)

Key: Arrow: black arrow-chondrocytes, red arrow-extracellular matrix.

A: Control -showing densely populated chondrocytes which are small in size E.C.M (extra cellular matrix)

B: MDTM1PBPZ-Medium dose phenobarbital given at trimester one showing the distribution of chondrocyte and extracellular matrix to the control

C: MDTM2PBPZ - Medium dose phenobarbital given at trimester two showing the distribution of chondrocyte and extracellular matrix

D: MDTM3PBPZ - Mediumdose phenobarbital given at trimester three showing the distribution of chondrocyte and extracellular matrix

E: MDTM1PTPZ - Medium dose phenytoin given at trimester one showing the distribution of chondrocyte and extracellular matrix

F: MDTM2PTPZ - Medium dose phenytoin given at trimester two showing the distribution of chondrocyte and extracellular matrix

G: MDTM3PTPZ - Medium dose phenytoin given at trimester three showing distribution of chondrocyte and extracellular matrix.

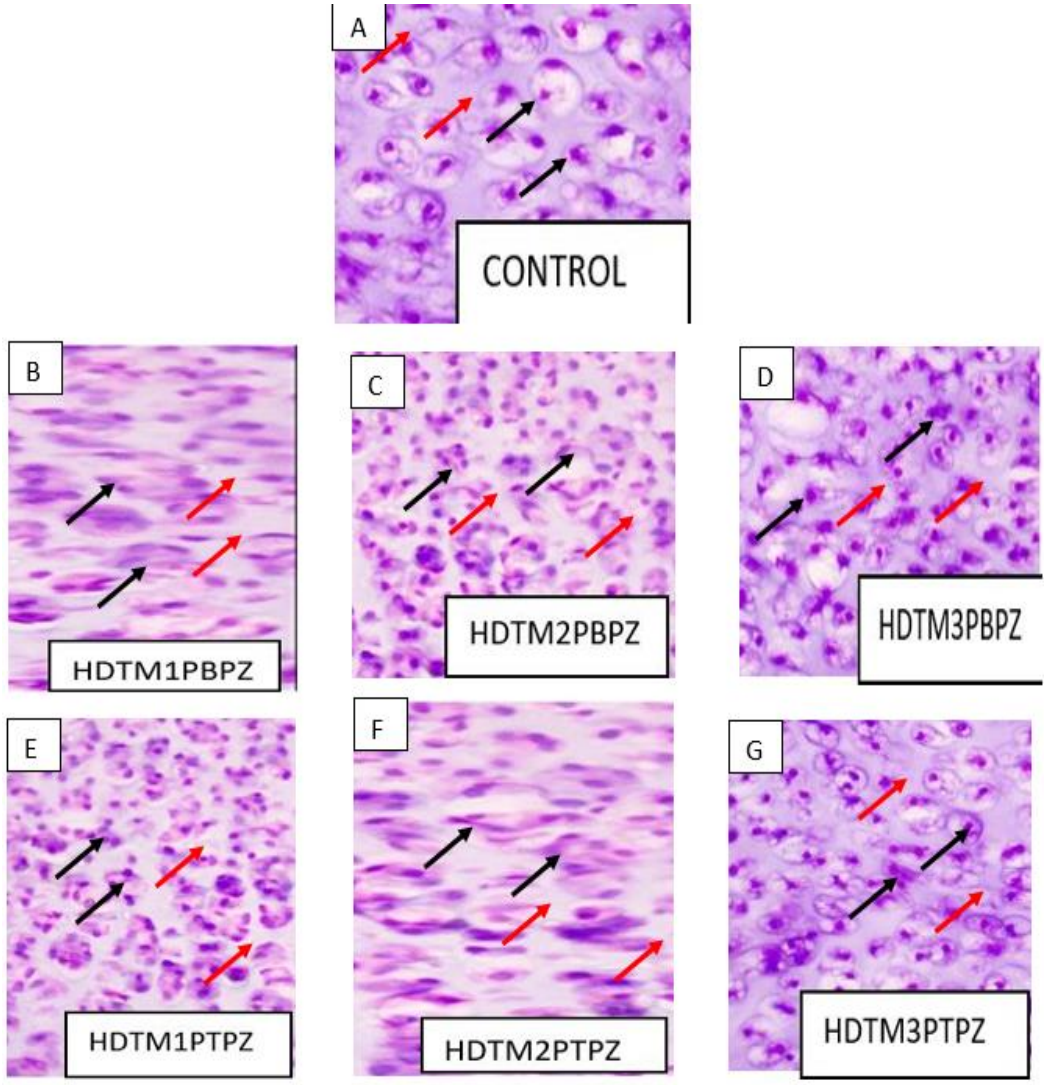

Figure 4.12: The Photomicrograph of the Longitudinal Sections of the Proliferative Zone of Epiphyseal Growth Plate Showing Appearances And Distribution of Chondrocytes and Extracellular Matrix Against Control Treated at TM1, TM2, TM3(H$E mag x100)

Key: Arrow: black arrow-chondrocytes, red arrow-extracellular matrix.

A: Control -showing densely populated chondrocytes which are small in size E.C.M (extra cellular matrix)

B: -HDTM1PBPZ-High dose phenobarbital trimester one showing the distribution of chondrocyte and extracellular matrix to the control

C: - HDTM2PBPZ - High dose phenobarbital trimester two showing the distribution of chondrocyte and extracellular matrix

D: - HDTM3PBPZ - High dose phenobarbital trimester three showing the distribution of chondrocyte and extracellular matrix.

E: - HDTM1PTPZ - High dose phenytoin trimester one showing the distribution of chondrocyte and extracellular matrix

F: - HDTM2PTPZ - High dose phenytoin trimester two showing distribution of chondrocyte and extracellular matrix

G: HDTM3PTRPZ- High dose phenytoin trimester three showing distribution of chondrocyte and extracellular matrix.

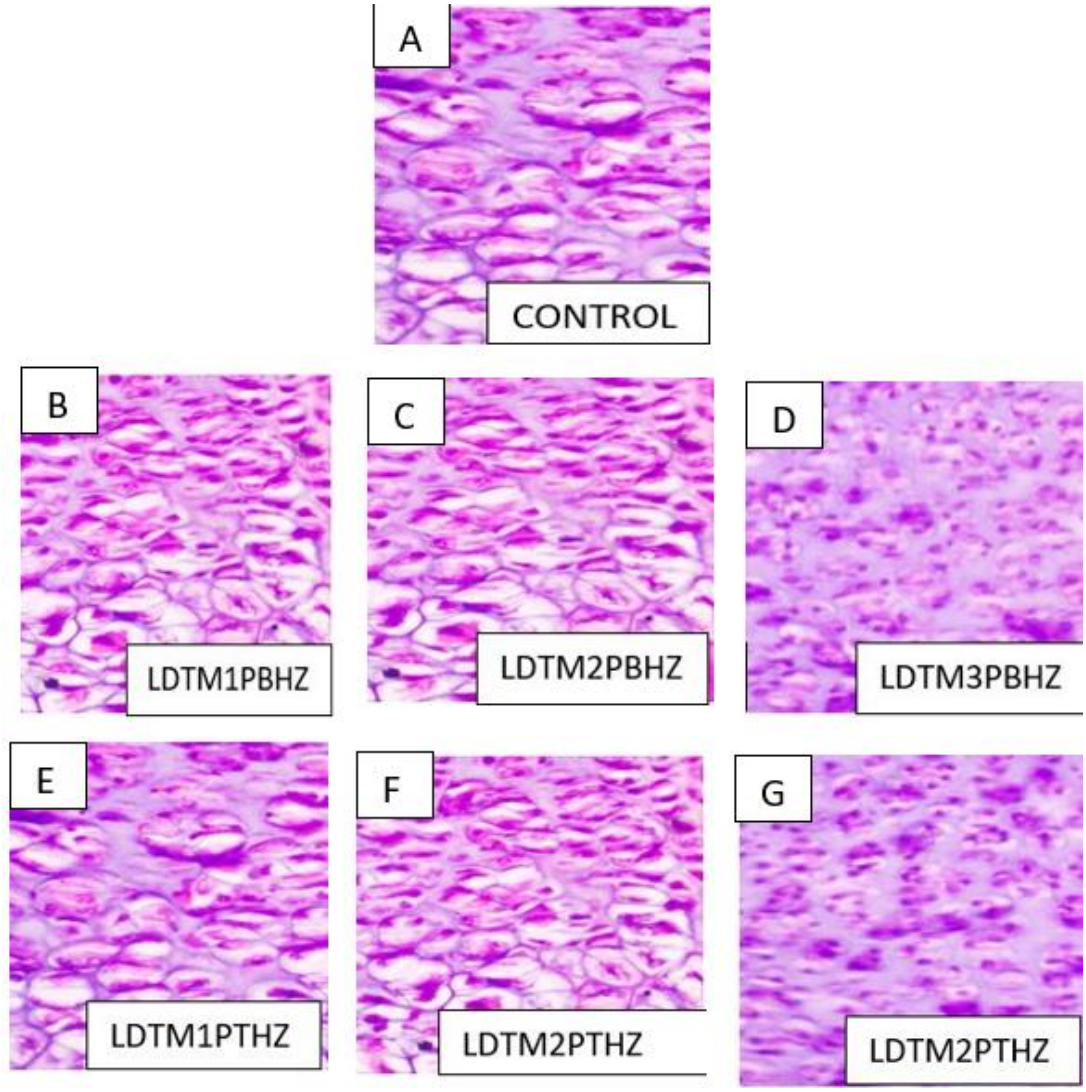

Figure 4.13: The Photomicrograph of the Longitudinal Sections of the Zone of Restingcartilage Showing Appearances and Distribution of Chondrocytes and Extracellular Matrix against Control Treated at TM1, TM2, TM3(H$E mag x100)

Key: Arrow: black arrow-chondrocytes, red arrow-extracellular matrix.

A: Control -showing densely populated chondrocytes which are small in size E.C.M (extracellular matrix)

B: -LDTM1PBPZ-High dose phenobarbital trimester one showing the distribution of chondrocyte to the and extracellular matrix

C: - LDTM2PBPZ – Low dose phenobarbital trimester two showing distribution of chondrocyte and extracellular matrix

D: - LDTM3PBPZ - Low dose phenobarbital trimester three showing the distribution of chondrocyte and extracellular matrix

E: - LDTM1PTPZ - Low dose phenobarbital trimester one showing the distribution of chondrocyte and extracellular matrix

F: - LDTM2PTPZ - Low dose phenobarbital trimester two showing the distribution of chondrocyte and extracellular matrix

G: LDTM3PTPZ - low dose phenobarbital trimester three showing distribution of chondrocyte and extracellular matrix

.4.2.2.8 The Comparative Findings on How the Medium Doses of Phenobarbital and Phenytoin Influenced the Hypertrophic Zone of Epiphyseal Growth Plate Given at Different Trimesters (TM1, TM2 and TM3)

Upon exposure to medium doses of phenobarbital and phenytoin at TM1, it was observed that the chondrocytes in the zone of hypertrophy are smaller compared to the control and TM2 and TM3. The connective tissue matrix between the cells was observed to be abundant. At the zone of hypertrophy, the cells are seen to be larger when exposed to medium doses at TM2 and the cells were observed to be surrounded with a lot of extracellular connective tissue matrix. Chondrocytes in the hypertrophic zone are scattered in an abundance of extracellular matrix and show a large pale staining nucleus with some cells lacking a nucleus which is the same case in the control (***Figure 4.14***).

4.2.2.9 The Comparative Findings on How the High Doses of Phenobarbital And Phenytoin Influenced the Hypertrophic Zone of Epiphyseal Growth Plate Given at Different Trimesters (TM1, TM2 and TM3)

Upon the administration of high doses of phenobarbital and phenytoin when exposed at TM1, it was established that the chondrocytes at the hypertrophic zone were smaller in size as compared to the cells in control, and in treatment groups that were exposed at TM2 and TM3. There is a lot of extracellular matrix as well which accounts for the sparse distribution of the cells in this zone. The chondrocytes in the hypertrophic zone when the phenobarbital and phenytoin were exposed from TM3 were observed to be larger with plenty of extracellular matrix compared to exposure in TM1, TM2 and they are smaller than in the control group (***Figure 4.15***).

4.2.2.10 The Comparative Findings on How the Low Doses of Phenobarbital and Phenytoin Influenced the Degenerative Zone of Epiphyseal Growth Plate Given at Different Trimesters (TM1, TM2 and TM3)

Upon administration of a low dose of phenobarbital and phenytoin in TM1, it was noted that chondrocytes in calcification zones were significantly reduced with few or no nuclei as compared to those exposed in TM2 and TM3. It was however noted that

chondrocytes in phenytoin treatment groups were more populated as compared to the phenobarbital treatment group***(Figure 4.16***)

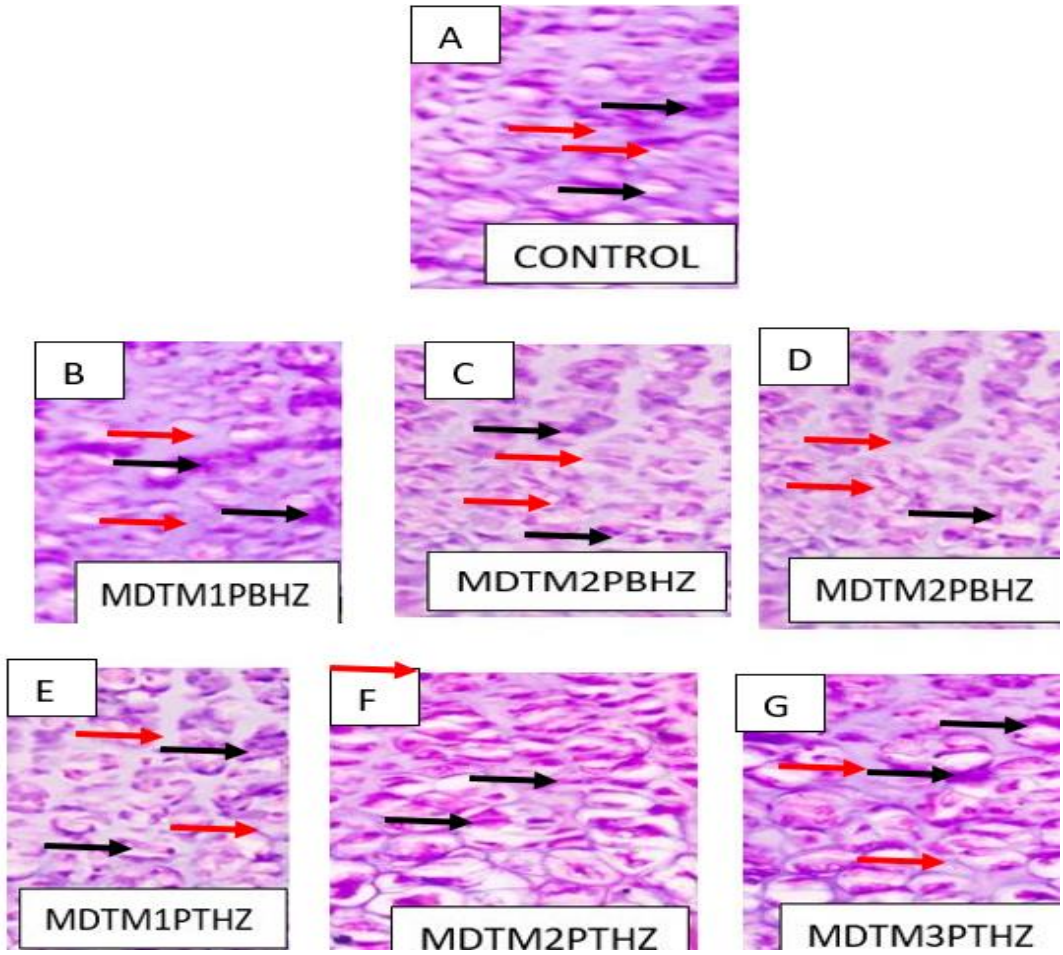

Figure 4.14: The Photomicrograph of the Longitudinal Sections of the Proliferative Zone of Epiphyseal Growth Plate Showing Appearances and Distribution of Chondrocytes and Extracellular Matrix against Control Treated at TM1,TM2,TM3(H$E mag x100)

Key: Arrow: black arrow-chondrocytes, red arrow-extracellular matrix.

A: *Control -showing densely populated chondrocytes which are small in size E.C.M (extracellular matrix)*
B: -MDTM1PBPZ-Medium dose phenobarbital trimester one showing the distribution of chondrocyte and extracellular matrix to the control
C: - MDTM2PBDZ - Medium dose phenobarbital trimester two showing distribution of chondrocyte and extracellular matrix
D: - MDTM3PBDZ - Medium dose phenobarbital trimester three showing the distribution of chondrocyte and extracellular matrix.
E: - MDTM1PTPZ - Medium dose phenytoin trimester one showing the distribution of chondrocyte and extracellular matrix
F: - MDTM2PTPZ - Medium dose phenytoin trimester two showing distribution of chondrocyte
G: MDTM3PTRPZ- Medium dose phenytoin trimester three showing distribution of chondrocyte and extracellular matrix.

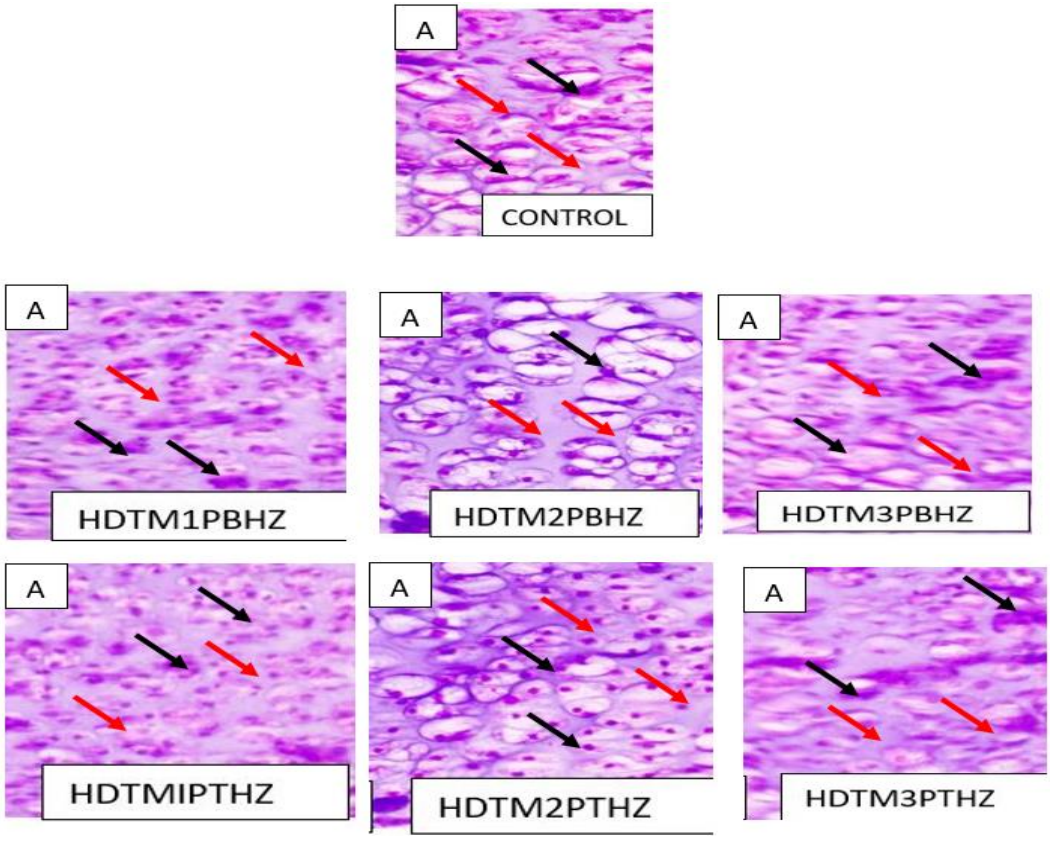

Figure 4.15: The Photomicrograph of the Longitudinal Sections of the Proliferative Zone of Epiphyseal Growth Plate Showing Appearances and Distribution of Chondrocytes And Extracellular Matrix against Control Treated at TM1, TM2, TM3(H$E mag x100)

Key: Arrow: black arrow-chondrocytes, red arrow-extracellular matrix.

A: Control *-showing densely populated chondrocytes which are small in size E.C.M (extracellular matrix)*

B: -HDTM1PBHZ*-High dose phenobarbital trimester one showing the distribution of chondrocyte and extracellular matrix to the control*

C: - HDTM2PBHZ *- High dose phenobarbital trimester two showing distribution of chondrocyte and extracellular matrix*

D: - HDTM3PBHZ *- High dose phenobarbital trimester three showing the distribution of chondrocyte and extracellular matrix.*

E: - HDTM1PTHZ *- High dose phenytoin trimester one showing distribution of chondrocyte and extracellular matrix*

F: - HDTM1PTHZ *- High dose phenytoin trimester two showing the distribution of chondrocyte and extracellular matrix*

G: HDTM1PTRHZ- High dose phenytoin trimester three showing distribution of chondrocyte and extracellular matrix.

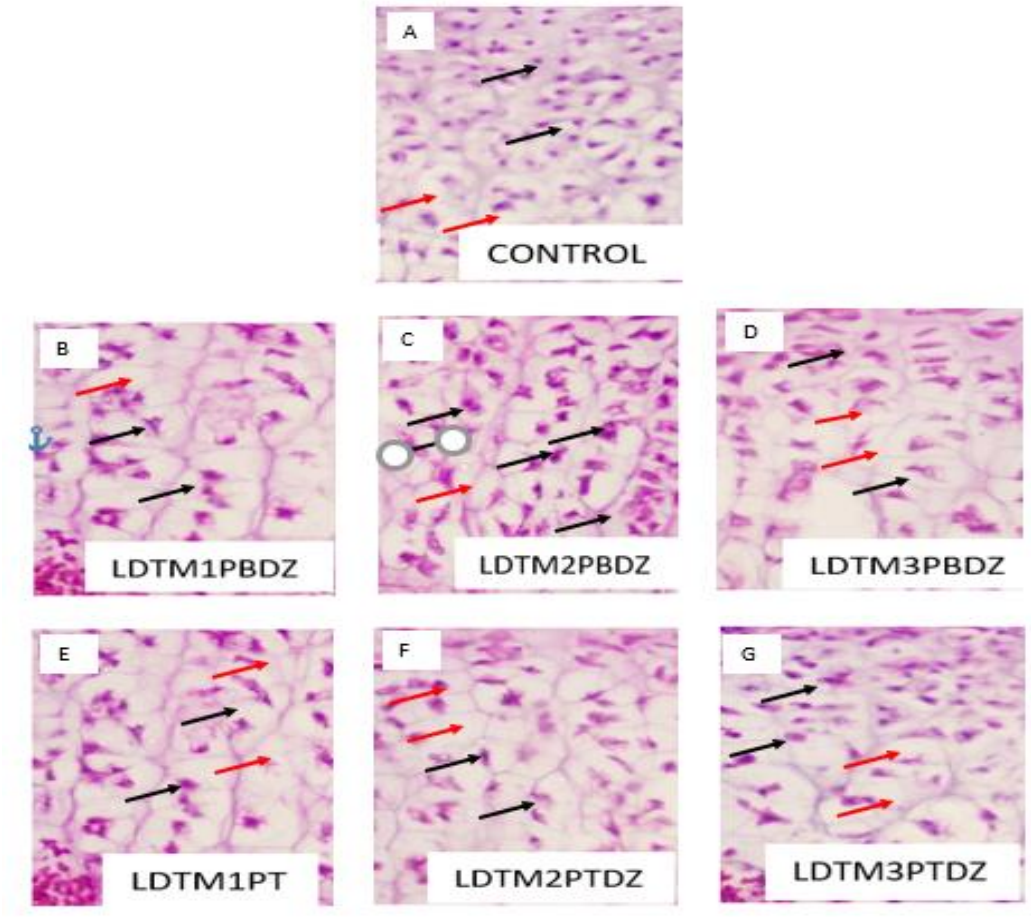

Figure 4.16: The Photomicrograph of the Longitudinal Sections Of The Degenerative Zone of Epiphyseal Growth Plate Showing Appearances and Distribution of Chondrocytes and Extracellular Matrix against Control Treated at TM1, TM2, TM3(H$E mag x100)

Key: Arrow: black arrow-chondrocytes, red arrow-extracellular matrix.

A: Control *-showing densely populated chondrocytes which are small in size E.C.M (extracellular matrix)*

B: -LDTM1PBPZ*-low dose phenobarbital trimester one showing the distribution of chondrocyte and extracellular matrix to the control*

C: - LDTM2PBDZ *- low dose phenobarbital trimester two showing the distribution of chondrocyte and extracellular matrix*

D: - LDTM3PBDZ *- low dose phenobarbital trimester three showing the distribution of chondrocyte and extracellular matrix.*

E: - LDTM1PTDZ *- low dose phenytoin trimester one showing distribution of chondrocyte and extracellular matrix*

F: - LDTM2PTDZ *- low dose phenytoin trimester two showing the distribution of chondrocyte and extra cellular matrix.*

G: LDTM3PTDZ*- low dose phenytoin trimester three showing distribution of chondrocyte and extra cellular matrix.*

4.2.2.11 The Comparative Findings on How the Medium Doses of Phenobarbital and Phenytoin Influenced the Degenerative Zone of Epiphyseal Growth Plate Given at Different Trimesters (TM1, TM2 and TM3)

Upon the administration of medium doses of phenobarbital and phenytoin in TM1, it was observed that the cells in the degenerative zone are smaller in TM1 as compared to TM2 and TM3. It was noted that the connective tissue matrix between the cells was well abundant followed by TM2 and TM3 respectively (***Figure 4.17***).

4.2.2.12 The Comparative Findings on How the High Doses of Phenobarbital and Phenytoin Influenced the Degenerative Zone of Epiphyseal Growth Plate Given at Different Trimesters (TM1, TM2 and TM3)

Upon administration of the high dose of phenobarbital and phenytoin in TM1, it was noted that chondrocytes in calcification zones were significantly reduced with no nucleus as compared to those exposed in TM2 and TM3 in both treatment groups. The connective matrix was also established to be significantly reduced. The chondrocytes and connective tissue matrix were slightly more in the treatment groups that were exposed at TM2 followed by TM3 respectively. Additionally, the chondrocytes and connective tissue were more in control groups compared to all treatment groups. it was however noted that the phenobarbital treatment group had slightly reduced chondrocytes compared to phenytoin treatment groups in TM1, TM2 and TM3 (***Figure 4.18***).

4.2.2.13 The Comparative Findings on How the Low Doses of Phenobarbital And Phenytoin Influenced the Primary Spongiosa Zone of Epiphyseal Growth Plate Given at Different Trimesters (TM1, TM2 and TM3)

The primary spongiosa is characterized by a mixture of terminally anucleated differentiated chondrocytes with calcified connective tissue ***(Figure 4.2.2.13).***

4.2.2.14 The Comparative Findings on How the Medium Doses of Phenobarbital and Phenytoin Influenced the Primary Spongiosa Zone of Epiphyseal Growth Plate Given at Different Trimesters (TM1, TM2 and TM3)

The primary spongiosa is characterized by a mixture of terminally anucleated differentiated chondrocytes with calcified connective tissue. The primary spongiosa, is characterized by a mixture of terminally differentiated chondroblasts and cartilage core that in the treatment group. The chondrocyte dies and the cartilage matrix calcifies creating a network of calcified spicules that are invaded by the blood vessels and osteoblasts which deposit bone matrix on the spicules forming the trabecular bone. The chondrocytes had no nuclei for all the treatment groups and the control while low and medium-dose treatment group was noted to have more number of bone trabecular and an increased number of osteoblast cells more so for the ones which received treatment from trimester one (***Figure 4.19***).

4.2.2.15 The Comparative Findings on How the High Doses of Phenobarbital and Phenytoin Influenced the Primary Spongiosa Zone of Epiphyseal Growth Plate Given at Different Trimesters (TM1, TM2 and TM3)

The zone of primary spongiosa was found to have a mixture of terminally differentiated chondroblats and cartilage core in the treatment groups that received high-dose phenobarbital and phenytoin as compared to the low dose and the control. The hypertrophic percentage surface area of primary spongiosa is large as compared to that of control. The chondrocyte dies and the cartilage matrix calcifies creating a network of calcified cells that are invaded by the blood vessels and osteoblasts which deposit bone matrix forming trabecular bone. The chondrocytes in the phenobarbital and phenytoin treatment group had no nuclei ***(Figure 4.20).***

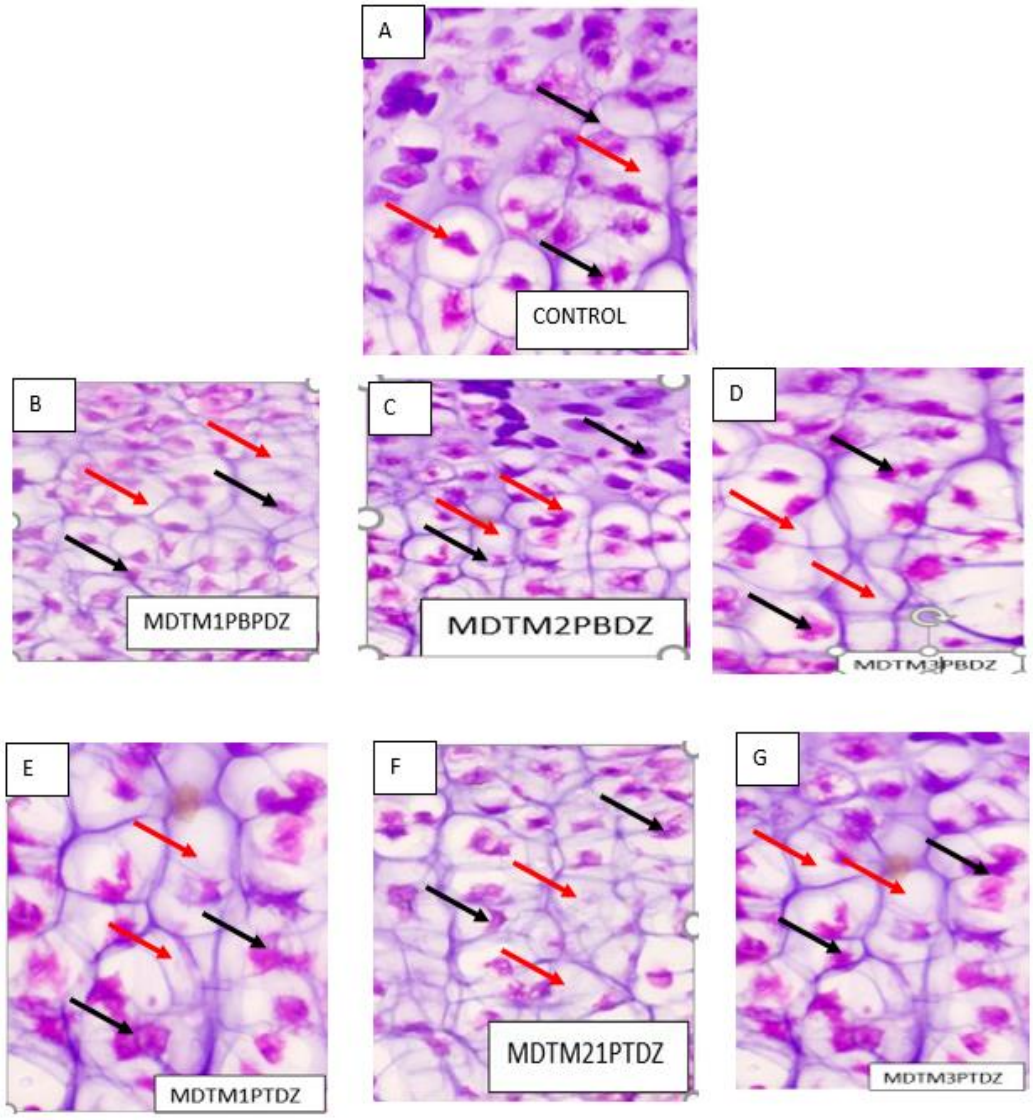

Figure 4.17: The Photomicrograph of the Longitudinal Sections of Proliferative Zone of Epiphyseal Growth Plate Showing Appearances and Distribution of Chondrocytes and Extracellular Matrix against Control Treated at TM1, TM2, TM3(H$E mag x100)

Key: Arrow: black arrow-chondrocytes, red arrow-extracellular matrix.

A: Control *-showing densely populated chondrocytes which are small in size E.C.M (extra cellular matrix)*

B: -MDTM1PBDZ*-Medium dose phenobarbital trimester one showing distribution of chondrocyte and extracellular matrix to the control*

C: - MDTM2PBDZ - *Medium dose phenobarbital trimester two showing distribution of chondrocyte and extracellular matrix*

D: - MDTM3PBDZ *- Medium dose of phenobarbital trimester three showing distribution of chondrocyte and extracellular matrix.*

E: - MDTM1PTDZ - *Medium dose of phenytoin trimester one showing distribution of chondrocyte and E.C.M*

F: - MDTM2PTDZ - *Medium dose of phenytoin trimester two showingdistribution of chondrocyte and extracellular matrix*

G: MDTM3PTRDZ- *Medium dose of phenytoin trimester three showing distribution of chondrocyte and extracellular matrix.*

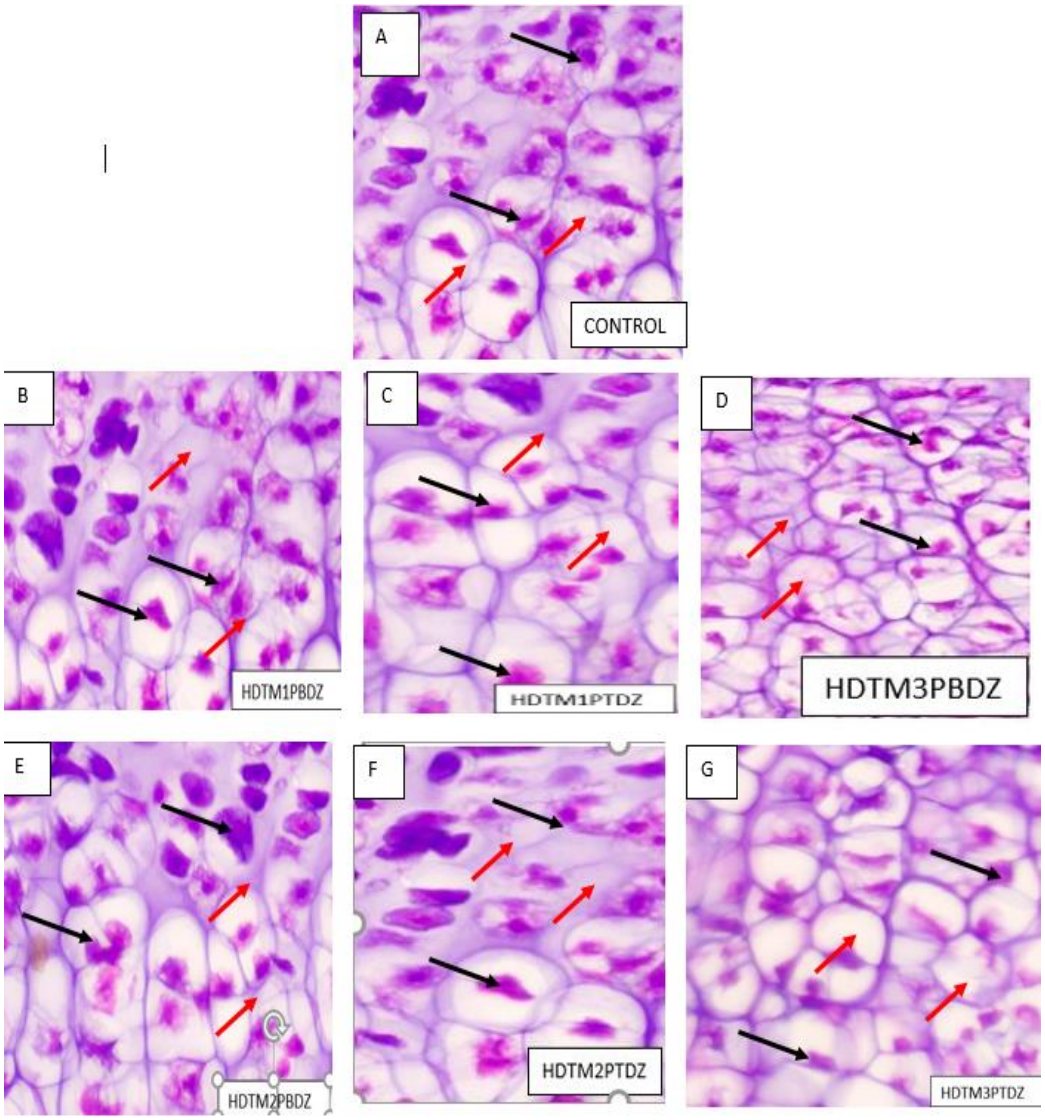

Figure 4.18: The Photomicrograph of the Longitudinal Sections of Degenerative Zone of Epiphyseal Growth Plate Showing Appearances and Distribution of Chondrocytes and Extracellular Matrix against Control Treated at TM1, TM2, TM3(H$E mag x100)

Key: Arrow: black arrow-chondrocytes, red arrow-extracellular matrix.

A: Control *-showing densely populated chondrocytes which are small in size E.C.M (extra cellular matrix)*

B: -HDTM1PBDZ*-High dose phenobarbital trimester one showing distribution of chondrocyte and E.C.M*

C: - HDTM2PBDZ *- High dose phenobarbital trimester two showin distribution of chondrocyte and E.C.M*

D: - HDTM3PBDZ *- High dose phenobarbital trimester three showing distribution of chondrocyte and E.C.M*

E: - HDTM1PTDZ *- High dose phenytoin trimester one showing distribution of chondrocyte and E.C.M*

F: - HDTM2PTDZ *- High dose phenytoin trimester two showing distribution of chondrocyte and E.C.M*

G: HDTM3PTRDZ*- High dose phenytoin trimester three showing distribution of chondrocyte and E.C.M.*

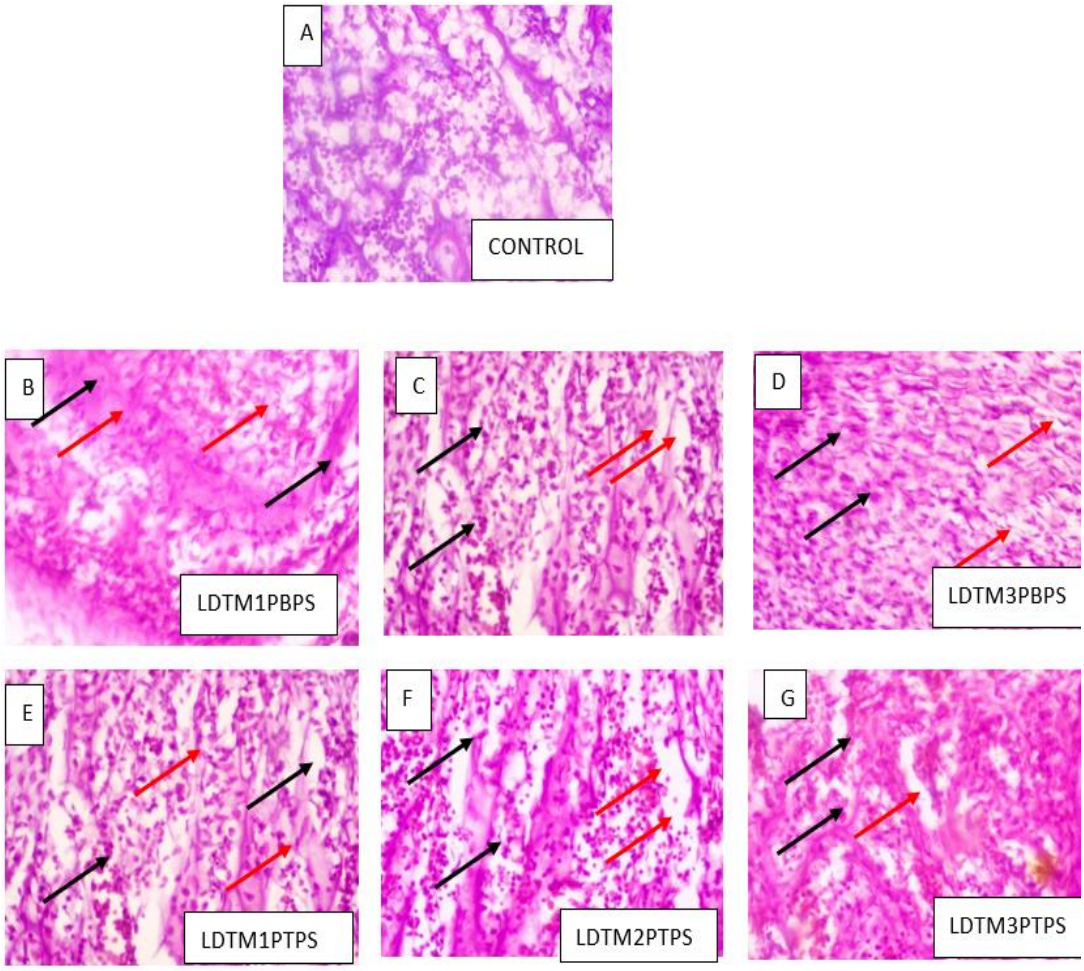

Figure 4.19: The Photomicrograph of the Longitudinal Sections of Primary Spongiosa Showing Appearances and Distribution of Chondrocytes and Extracellular Matrix against Control Treated at TM1, TM2, TM3(H$E mag x100)

Key: Arrow: black arrow-trabeculae, red arrow-cartilage specule.

A: ***Control*** *-showing showing distribution of mineralised cartilage specule, trabeculae and some osteoblast*

B: -LDTM1PBPS- *Low dose phenobarbital trimester one showing distribution of chondrocyt to the control*

C: - LDTM2PBPS *- Low dose phenobarbital trimester two showing distribution of mineralised cartilage specule, trabeculae and some osteoblast*

D: - LDTM3PBPS *- Low dose phenobarbital given at trimester three showing distribution of mineralized cartilage specule, trabeculae and some osteoblast.*

E: - LDTM1PTPS - *Low dose phenytoin givet at trimester two showing distribution of mineralised cartilage specule, trabeculae and some osteoblast*

F: - LDTM2PTPS - *Low dose phenytoin given at trimester two showing distribution of mineralized cartlilage specule,trabeculae and some osteoblast*

G: LDTM3PTPS - *Low dose phenytoin give at trimester two showing distribution of mineralised cartilage specule, trabeculae and some osteoblast*

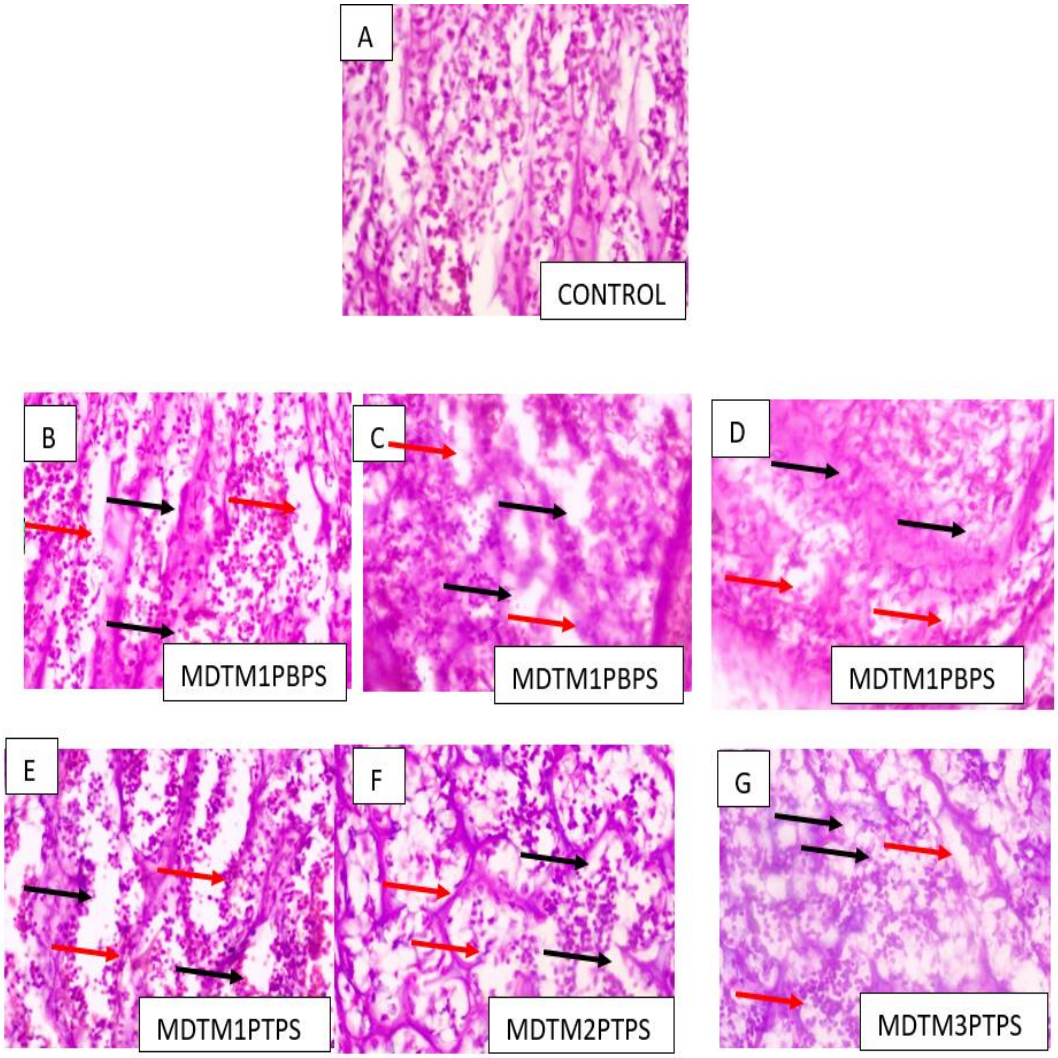

Figure 4.20: The Photomicrograph of the Longitudinal Sections of Proliferative Showing Appearances and Distribution of Chondrocytes and Extracellular Matrix against Control Treated at TM1, TM2, TM3(H$E mag x100)

Key: Arrow: *black arrow-trabeculae, red arrow-cartilage specule.*

A: Control -*showing distribution of mineralized cartilage specule, trabeculae and some osteoblast*

B: MLDTM1PBPS- *Medium dose phenobarbital trimester one showing distribution of mineralized cartilage specule, trabeculae and some osteoblast.*

C: - MDTM2PBPS *- Medium dose phenobarbital trimester two showing distribution of mineralized cartilage specule,trabeculae and some osteoblast*

D: - MDTM3PBPS *- Medium dose phenobarbital trimester three showing distribution of mineralized cartilage specule, trabeculae and some osteoblast.*

E: - MDTM1PTPS *- Medium dose phenobarbital trimester two showing distribution of mineralized cartilage specule, trabeculae and some osteoblast*

F: - MDTM1PTRC *- Medium dose phenobarbital trimester two showing distribution of mineralized cartilage specule, trabeculae and some osteoblast*

G: MDTM1PTRC *- Medium dose phenobarbital trimester two showing distribution of mineralized cartilage specule, trabeculae and some osteoblast*

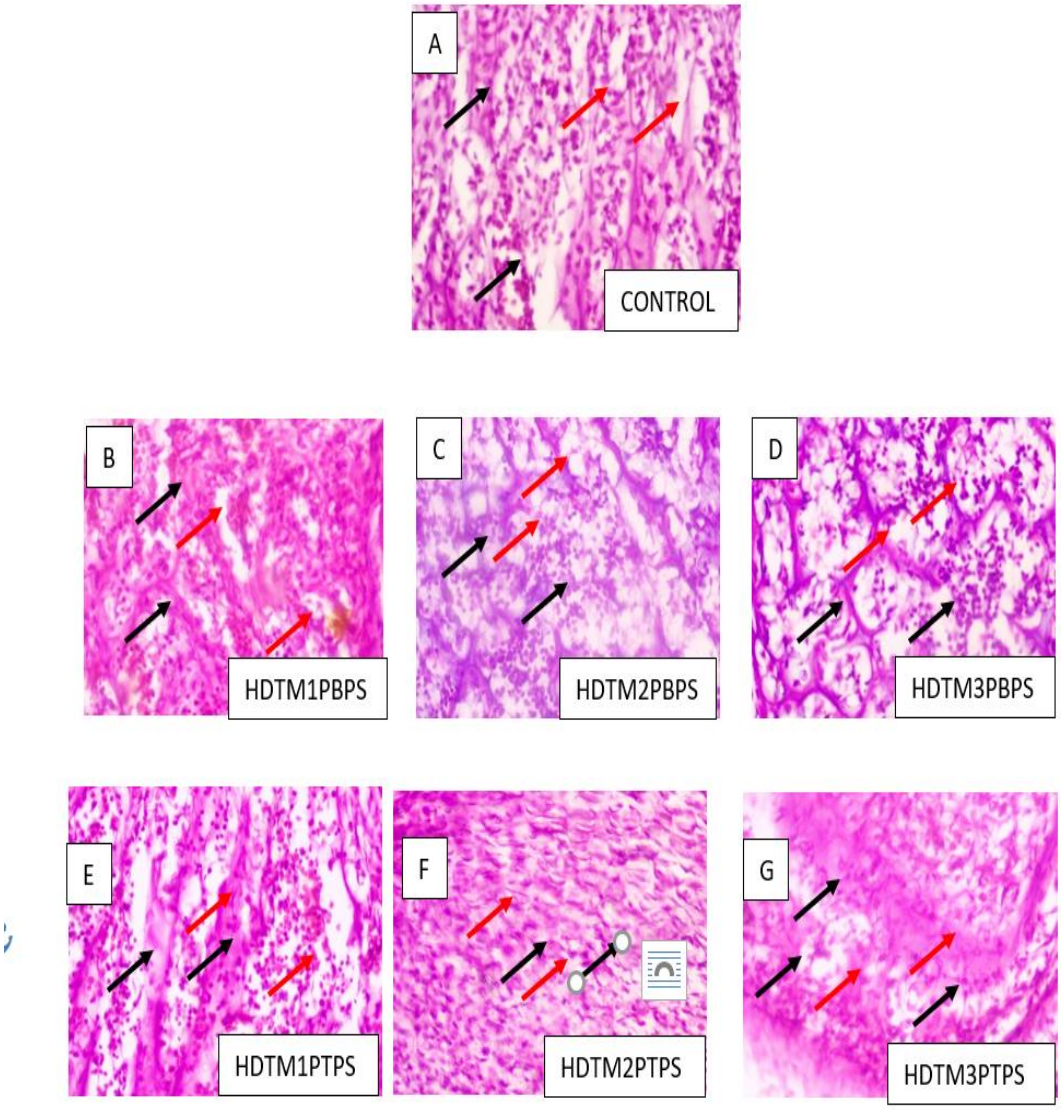

Figure 4.21: The Photomicrograph of the Longitudinal Sections of Primary Spongiosa Showing Appearances and Distribution of Chondrocytes and Extracellular Matrix against Control Treated at TM1, TM2, TM3(H$E mag x100)

Key: Arrow: black arrow-trabeculae, red arrow-cartilage specule.

A: Control *-showing distribution of mineralized cartilage specule, trabeculae and some osteoblast*

B: HDTM1PBPS- *High dose phenobarbital trimester one showing distribution of distribution of mineralized cartilage specule, trabeculae and some osteoblast to the control*

C: - HDTM2PBPS *- High dose phenobarbital trimester two showing distribution of mineralized cartilage specule, trabeculae and some osteoblast*

D: - HDTM3PBPS *- High dose phenobarbital trimester three showing distribution of mineralized cartilage specule, trabeculae and some osteoblast.*

E: - HDTM1PTPS *- High dose phenytoin trimester two showing distribution of mineralized cartilage specule, trabeculae and some osteoblast*

F: - HDTM2PTPS *- High dose phenytoin trimester two showing distribution of mineralized cartilage specule, trabeculae and some osteblast .*

G: HDTM3PTPS *– High dose phenytoin given at trimester two showing distribution of mineralized cartilage specule, trabeculae and some osteblast .*

4.3.1 The Comparative Finding on Gross Morphometric Measurement of Tibia and Humerus Length on Prenatal Exposure to Varied Doses of Phenobarbital and Phenytoin

The finding of comparative length of tibia and humerus following prenatal exposure to phenobarbital and phenytoin established that there were statistically significant differences in the length in treatment groups compared to the control (tibia 5.840±0.1070). This was noted in all the treatment groups that received phenobarbital and phenytoin at medium and high doses in all the trimesters compared to the low-dose treatment group and the control. The treatment groups that were given low dose, was observed to be statistically significantly reduced in trimester 1 and trimester 2 as compared to the treatment group that was exposed to TM3 and the control. The treatment group that was given medium and high doses were observed to have a statistically significant reduction in tibial and humerus length as compared to the low-dose treatment groups and the control. It was however observed that the treatment group that was given low doses in trimester 3 had no statistical significance difference as compared to the control. Upon comparing the mean effect between trimesters, the TM3 treatment group was observed to have a significantly higher mean, followed by TM2 and finally TM1 and upon comparing the effects between the two treatment groups, it was established that, phenytoin treatment groups had a higher means in length of the tibia and humerus ***(Table 4.9).***

Table 4.9: Comparative One-Way Analysis of Variance (ANOVA) Table Showing How Prenatal Exposure to Varied Doses of Phenobarbital and Phenytoin Influenced the Means of the Tibia and the Humerus

Drug	Dosage	TRIMESTER	TIBIA	HUMERUS
Control	Control	CONTROL	5.840±.107	5.169±.027
Phenobarbital	Low dose	TM1	4.973±.050*	4.775±.008*
		TM2	5.120±.012*	4.874±.013*
		TM3	5.635±.034	5.044±.008
	Medium dose	TM1	4.541±.038*	4.360±.041*
		TM2	4.635±.046*	4.493±.002*
		TM3	4.759±.030*	4.555±.035*
	High dose	TM1	4.222±.075*	4.212±.058*
		TM2	4.382±.067*	4.368±.045*
		TM3	4.558±.012*	4.458±.036*
Phenytoin	Low dose	TM1	5.099±.012*	4.830±.029*
		TM2	5.265±.032*	4.983±.001*
		TM3	5.760±.058*	5.091±.008*
	Medium dosed	TM1	4.633±.017*	4.468±.058*
		TM2	4.732±.022*	4.686±.001*
		TM3	4.888±.005*	4.703±.001*
	High dose	TM1	4.354±.057*	4.345±.028*
		TM2	4.502±.059*	4.452±.038*
		TM3	4.632±.047*	4.588±.005*

NOTE: The means followed by a starlet(*) denotes that the mean is statistically significant with the means.

KEY: %TSA-percentage of Total surface area –reserve cartilage

4.3.2 The Multivariate Comparative Analysis on How the Two Medicines Influenced Lengths of Tibia and Humerus

In doing a multivariate comparative analysis to evaluate how the two medicines influenced the length of the tibia and humerus, the results are represented in three levels:

Level 1: How the two medicines and their interactions influenced the length of fetal bones when exposed prenatally

Level 2: How the individual drug, dosage and time of exposure plus their interactions influenced the length of the two bones when exposed prenatally.

Level 3: The pairwise comparison results on how the two medicines influenced the lengths of the tibia and humerus when exposed at the same time and in the same trimesters.

Level 1: On carrying out a multivariate analysis(manova) to assess how phenobarbital and phenytoin influenced the length of tibia and humerus when exposed prenatally by checking their global (main effects) and interaction effects of drug, doses and time were found to be statistically significant in different proportions (partial Eta squared(Ƞ2)

The individual overall effects of a) Drug (Wilks Lambda ∧=.139, F (2, 37) =114.818 P<0.0001 partial (η2 .861). b) Dosage (Wilks Lambda ∧=.008, F (4, 74) =189.131, P<0.0001, partial eta squared (η2 .911) and c) trimester Wilks lambda ∧=.003 (F (7,74) =334.736, P<0.001; partial eta squared (η2 .948) (***Table 4.10***).

The two-way combination interaction effects of a) drug and dosage, (Wilks ∧=.914, F (4, 74) =0.847, P<0.0001 partial eta squared (η2 .044) b) Drug and trimester (Wilks Lambda∧=.703, F (4, 74) =3.571, P<0.0001, partial eta squared (η2 .162) and c) Dosage and trimester, (Wilks Lambda ∧=.126, F (8, 74) =16.782, P<0.0001, partial eta squared (η2= .645)(***Table 4.10***).

The three-way combination, when all three variables are combined i.e. the three-way interactions among the drug, dosage and trimesters (Wilks Lambda∧=.743, F (4, 74) =1.484, P<0.0001 partial eta squared (η2= .138) ***(Table 4.10).***

Table 4.10: Comparative Multivariate Analysis of Variance (Manova) Level 1 Table Showing How Prenatal Exposure to Varied Doses of the Two Medicines and Their Interactions Globally Influenced the Lengths of the Tibia And Humerus

Multivariate Tests

Effect		MANOVA TEST STSTISTIC (Wilks' Lambda Value)	STATISTICS F	Hypothesis DEGREE FREEDOM df	Error DEGREE OF FREEDOM df	Sig.<0.5	PROPORTION OF VARIANCE (Partial Eta Squared)
	Intercept	.000	631088.485	2.000	37.000	.000	1.000
	Drug	.139	114.818	2.000	37.000	.000	.861
	Dosage	.008	189.131	4.000	74.000	.000	.911
	Trimester	.003	334.736	4.000	74.000	.000	.948
	Drug * Dosage	.914	.847	4.000	74.000	.500	.044
	Drug * Trimester	.703	3.571	4.000	74.000	.010	.162
	Dosage * Trimester	.126	16.782	8.000	74.000	.000	.645
	Drug * Dosage * Trimester	.743	1.484	8.000	74.000	.178	.138

a. Design: Intercept + DRUG + DOSAGE + TRIMESTER + DRUG * DOSAGE + DRUG * TRIMESTER + DOSAGE * TRIMESTER + DRUG * DOSAGE * TRIMESTER

b. Exact statistic

c. The statistic is an upper bound on F that yields a lower bound on the significance level.

Level 2: The comparative findings on how the drug, dosage and time of exposure plus their interaction influenced the lengths of tibia and humerus on prenatal exposure.

On carrying out the Manova level two analysis on individual drugs, dose and time of exposure and their interaction to establish how they influenced the lengths of tibia and humerus when prenatally exposed, it was observed that:

a) At the individual level the highest contribution of the observed effects was observed at the dosage level with partial eta ranging from 98.8% to 98.9%, followed by trimesters at 94.4% to 95.6% and lastly drug at 66.9% to 77.4%. as shown in the (***Table 4.3.1.3***).

b) At two-way level interaction, when two variables were combined the observed effects were highest between doses * trimesters with partial eta ranging from 68.6% to 81.2% followed by drug and trimester ranging from 3%% to 27.2% and lastly drug and dosages ranging from 2% to 27.1% on the tibia and humerus length. (***Table 4.11).***

c) at three-way interaction, when the three independent variables (drug, dosage and trimester) were combined they were noted to have the highest effects on tibia length with partial eta at 15.1 % followed by the length of the humerus with partial eta at 4.4% as shown in the (***Table 4.11***).

Table 4.11: The MANOVA Level 2 Table Findings on How the Two Medicines (Phenobarbital And Phenytoin), Their Doses and the Time of Exposure Plus Their Interactions Influenced the Lengths of the Tibia and Humerus on Prenatal Exposure

Tests of Between-Subjects Effects							
Source	**Dependent Variable**	**Type III Sum of Squares**	**Df**	**Mean Square**	**F**	**Sig.**	**Partial Eta Squared**
Corrected model	Tibial	12.191a	18	.677	289.283	<.001	.993
	Humerus	5.601b	18	.311	302.645	<.001	.993
Intercept	Tibial	1029.664	1	1029.664	439807.373	<.001	1.000
	Humerus	906.962	1	906.962	882193.326	<.001	1.000
Drug	Tibial	.179	1	.179	76.644	<.001	.669
	Humerus	.134	1	.134	129.876	<.001	.774
Dosage	Tibial	7.137	2	3.568	1524.236	<.001	.988
	Humerus	3.571	2	1.786	1736.840	<.001	.989
Trimester	Tibial	1.502	2	.751	320.761	<.001	.944
	Humerus	.841	2	.421	409.259	<.001	.956
Drug * dosage	Tibial	.002	2	.001	.390	.679	.020
	Humerus	.015	2	.007	7.056	.002	.271
Drug * trimester	Tibial	.000	2	.000	.063	.939	.003
	Humerus	.011	2	.005	5.323	.009	.219
Dosage * trimester	Tibial	.383	4	.096	40.925	<.001	.812
	Humerus	.085	4	.021	20.784	<.001	.686
Drug * dosage * trimester	Tibial	.004	4	.001	.439	.780	.044
	Humerus	.007	4	.002	1.689	.173	.151
Error	Tibial	.089	38	.002			
	Humerus	.039	38	.001			
Total	Tibial	1364.211	57				
	Humerus	1224.833	57				
Corrected total	Tibial	12.280	56				
	Humerus	5.640	56				

A. R Squared = .993 (Adjusted R Squared = .989)
B. R Squared = .993 (Adjusted R Squared = .990)

Level 3: The Manova pairwise comparison results on how the phenobarbital and phenytoin influenced the length of the tibia and humerus when exposed to phenobarbital and phenytoin within the same dosages and the same trimester

upon comparative pairwise MANOVA analysis between the phenobarbital and the phenytoin in the same dosage and same time of exposure to establish how the two medicines influenced the lengths of the tibia and humerus, it was observed to have a statistically significant difference in that phenytoin given at the same dosage and the same time of exposure, it was noted to have minimal effects compared to the phenobarbital.

(**I**)Pairwise of low doses on tibia length for TM1, TM2 and TM3 were as follows;

i)Pairwise of medium doses on tibia length

(**a**) TM1 mean difference (-.014, p value=0.000 (**b**) TM2 mean difference (-0.08, p value=0.002) (**c**) TM3 mean difference (-.009, p value=0.001)

ii) Pairwise of medium doses on tibia length:

a)TM1 mean difference (-.048, p value=0.000) (**b**) TM2 mean difference (-.008, p value=0.002) (**c**) TM3 mean difference (-.016, p. value=0.002)

iii) Pairwise of high doses on tibia length:

a)TM1 mean difference (-.011, p. value=0.000) (**b**) TM2 mean differences (-.009, p. value=0.001) (**c**) TM3 mean difference (-.014, p. value=0.000)

(**II**)Pairwise of low doses on humerus length TM1, TM2 and TM3 were as follows;

(**a**) TM1 mean difference (-.012, p value=0.000) (**b**) TM2 mean difference (-0.009, p value=0.000) (**c**) TM3 mean difference (-.007, p value=0.002)

ii) Pairwise of medium doses on humerus length:

a)TM1 mean difference (-.014, p value=0.000) (**b**) TM2 mean difference (-.011, p value=0.000) **(c**) TM3 mean difference (-.013, p. value=0.000)

iii) Pairwise of high doses on humerus length:

a)TM1 mean difference (-.016, p. value=0.000) (**b**) TM2 mean differences (-.014, p. value=0.000) (**c**) TM3 mean difference (-.009, p. value=0.000)

Upon comparative of pairwise multiple analysis of variance between the phenobarbital and the phenytoin in the same dosage and same time of exposure to establish how the two medicines influenced tibia length and the humeral length, it was observed to have statistically significant difference in that phenytoin given at the same dosage and the same time of exposure was noted to have minimal effects compared to the phenobarbital and phenytoin.

Table 4.12: The MANOVA Level 3 Pairwise Table Findings on How the Two Medicines (Phenobarbital And Phenytoin) Influenced the Length of the Tibia and Humerus When Exposed to Phenobarbital and Phenytoin within the Same Dosages and the Same Trimester in Utero

								95% Confidence Interval for Difference	
Dependent Variable	**Dosage (Mg/kg bw)**	**Trimesters**	**phenobarbital**	**phenytoin**	**Mean Difference (PB-PT)**	**Std. Error**	**Sigd (<.05)**	**Lower Bound**	**Upper Bound**
Tibia Length	Low	Trimester one	PB	PT	-.014	.002	<.001	-.018	-.009
		Trimester two	PB	PT	-.008	.002	.002	-.013	-.003
		Trimester three	PB	PT	-.009	.002	<.001	-.014	-.004
	Medium	Trimester one	PB	PT	-.048	.002	<.001	-.053	-.043
		Trimester two	PB	PT	-.008	.002	.002	-.013	-.003
		Trimester three	PB	PT	-.016	.002	<.001	-.021	-.011
	High	Trimester one	PB	PT	-.011	.002	<.001	-.016	-.006
		Trimester two	PB	PT	-.009	.002	<.001	-.014	-.004
		Trimester three	PB	PT	-.014	.002	<.001	-.019	-.009
Humeral Lengt	Low	Trimester one	PB	PT	-.012	.002	<.001	-.016	-.007
		Trimester two	PB	PT	-.009	.002	<.001	-.013	-.005
		Trimester three	PB	PT	-.007	.002	.002	-.012	-.003
	Medium	Trimester one	PB	PT	-.014	.002	<.001	-.018	-.010
		Trimester two	PB	PT	-.011	.002	<.001	-.016	-.007
		Trimester three	PB	PT	-.013	.002	<.001	-.018	-.009
	High	Trimester one	PB	PT	-.016	.002	<.001	-.020	-.011
		Trimester two	PB	PT	-.014	.002	<.001	-.018	-.009
		Trimester three	PB	PT	-.009	.002	<.001	-.013	-.004

4.3.3 The Comparative Effects of the Percentage Surface Areas of Different Zones of Epiphyseal Growth Plate Upon Exposure to Varied Doses of Phenobarbital and Phenytoin

The comparative effects on surface area epiphyseal growth plates of the fetal tibia of Rattus norvegicus that include the following zones: reserve cartilage zones, proliferative zones, hypertrophic zone, calcification zone and primary spogiosa zone

established that there was a statistically significant difference($p<0.05$) between phenobarbital and phenytoin treatment groups as compared to the control groups.

Upon exposure to the low dose of phenobarbital and phenytoin, it was observed to be statistically significantly different when drugs were administered in the first and second trimesters as compared to control in all epiphyseal growth plates zones (***Table 4.13***).

It was however established that when medicine was introduced in trimester three at low dosages there was no statistically significant difference when compared to the control group (***Table 4.13***).

Upon exposure to the medium dosages of phenobarbital and phenytoin at different trimesters, it was noted to be statistically significant as compared to the control. The percentage of total surface area of reverse cartilage of epiphyseal growth plate as well as the proliferation zone percentage of total surface area of epiphyseal growth plate showed statistically significant differences in all the three trimesters when the Medium dosages of phenobarbital and phenytoin Groups were compared to the control group ***(Table 4.13***).

In addition, the total surface areas of the hypertrophic zone and calcification zone, and the primary spongiosa percentage of the epiphyseal growth plate, the percentage of their surface area showed a statistically significant difference in both medium treatment groups in the trimesters (tm1, tm2, and Tm3) as compared to control as shown ***(Table 4.13).***

Upon exposure to the high dosages of phenobarbital and phenytoin at different trimesters, it was noted to be statistically significant as compared to the control. The percentage of total surface area of reverse cartilage of epiphyseal growth plate as well as the proliferation zone percentage of total surface area of epiphyseal growth plate showed statistically significant differences in all three trimesters when the high dosages of phenobarbital and phenytoin Groups were compared to the control group ***(Table 4.13).***

Table 4.13: The Comparative One-Way Analysis of Variance (ANOVA) Table Showing How Prenatal Exposure to Varied Doses of Phenobarbital and Phenytoin Influenced the of the Percentage Surface Areas of Different Zones of Epiphyseal Growth Plate

DRUG	DOSAGE	TRIMESTER	% TSA OFTRC	%TSA OF TPZ	% TSA OF THZ	%TSA OF TCZ	%TSA OF PS
Control	Control	CONTROL	37.36±.015	23.53±.02	25.51±.41	21.47±.153	54.81± .06
Phenobarbital	Low Dose	TM1	35.63±.015*	19.90±.067*	22.46±.40*	17.92±.056*	47.84±.08*
		TM2	36.14±.139*	20.18±.1*	23.52±.288*	18.34±.113*	49.76±.109*
		TM3	37.11±.015	22.19±.026	24.89±.125	20.20±.116	52.2±.072
	Medium Dose	TM1	29.63±.02*	16.51±.191*	18.5±.078*	15.11±.01*	44.703±.071*
		TM2	30.55±.072*	17.48±.05*	19.67±.546*	17.21±.015*	46.96±.023*
		TM3	31.33±.131*	19.037±.021*	21.66±.199*	19.17±.05*	47.06±.030*
	High Dose	TM1	26.4±.075*	15.19±.056*	16.68±.10*	14.19±.059*	45.3±.036*
		TM2	27.38±.081*	16.16±.158*	17.01±.02*	16.29±.04*	46.31±.055*
		TM3	28.33±.14*	17.02±.01*	18.34±.042*	17.95±.053*	48.55±.314*
Phenytoin	Low Dose	TM1	35.96±.026*	20.43±.399*	23.17±.299*	19.29±.156*	49.54±.14*
		TM2	36.62±.012*	21.1±.075*	24.85±.049*	19.90±.075*	50.39±.344*
		TM3	37.22±.01	23.56±.061	25.81±.133	22.28±.055	53.69±.389
	Medium Dosed	TM1	31.28±.232*	20.92±.765*	19.84±.22*	17.21±.105*	47.57±.025*
		TM2	32.65±.032*	19.42±.025*	21.81±.162*	18.72±.045*	48.54±.136*
		TM3	33.46±.026*	21.37±.157*	23.12±.066*	21.18±.053*	49.29±.055*
	High Dose	TM1	29.94±.021*	17.39±.136*	18.80±.081*	16.16±.060*	47.43±.129*
		TM2	30.80±.081*	18.26±.167*	19.55±.131*	18.21±.090*	48.96±.076*
		TM3	31.37±.038*	19.06±.129*	20.75±.067*	19.17±.006*	50.22±.130*

NOTE: The means followed by a starlet denotes that the mean is statistically significant with the means.
KEY: %TSA-percentage of Total surface area; RC –reserve cartilage

Level 1: the surface areas of the epiphyseal growth plate when exposed prenatally by checking their global (main effects) and the interaction effects of drug, doses and time were found to be statistically significant in different proportions (partial Eta squared(Ƞ2)

i)The individual overall effects of **a**) Drug (Wilks Lambda ∧=.071, F (2, 37) =4494.942, P<0.0001 partial (η2 .929) **b)** dosage (Wilks Lambda ∧=.007, F (4, 74) =196.321, P<0.0001, partial eta squared (η2 .914) and **c**) trimester wilks ∧=.035 (F (7,74) =79.831, P<0.001; partial eta squared (η2 .812) (***Table 4.14*****)**

ii)The two-way combination interaction effects of **a)** drug and trimester, (Wilks Lambda ∧=.335, F (4, 74) =13.443, P<0.0001 partial eta squared (η2 .421) **b**) Drug and dosage (Wilks lambda∧=.358, F (4, 74) =12.430, P<0.0001, partial eta squared (η2 .402) and **c**) Dosage and trimester, (Wilks Lambda ∧=.047, F (8, 74) =33.551, P<0.0001, partial eta squared (η2= .784). (***Table 4.14***)

iii)The three-way combination, when all three variables are combined ie the three ways interactions among the drug, dosage and trimesters (Wilks Lambda ∧=.253, F (4, 74) =9.148, P<0.0001 partial eta squared (η2= .497) (***Table 4.14***)

Table 4.14: Comparative Multivariate Analysis of Variance (MANOVA) Level 1 Table Showing How Prenatal Exposure to Varied Doses of the Two Medicines and Their Interactions Globally Influenced the Surface Area of the Epiphyseal Growth Plate of the Tibia

Multivariate Tests							
Effect		**Value**	**F**	**Hypothesis df**	**Error df**	**Sig.**	**Partial Eta Squared**
Intercept	Wilks' Lambda	.000	4494.942b	2.000	37.000	<.001	1.000
Drug	Wilks' Lambda	.071	242.355b	2.000	37.000	<.001	.929
Trimester	Wilks' Lambda	.035	79.831b	4.000	74.000	<.001	.812
Dosage	Wilks' Lambda	.007	196.321b	4.000	74.000	<.001	.914
Drug * Trimester	Wilks' Lambda	.335	13.443b	4.000	74.000	<.001	.421
Drug * Dosage	Wilks' Lambda	.358	12.430b	4.000	74.000	<.001	.402
Trimester * Dosage	Wilks' Lambda	.047	33.551b	8.000	74.000	<.001	.784
Trimester * Dosage*Drug	Wilks' Lambda	.253	9.148b	8.000	74.000	<.001	.497

a. Design: Intercept + Drug + Trimester + Dosage + Drug * Trimester + Drug * Dosage + Trimester * Dosage + Drug * Trimester * Dosage
b. Exact statistic
c. The statistic is an upper bound on F that yields a lower bound on the significance level.
Pairwise of % surface area

Level 2: The comparative findings on how the drug, dosage and time of exposure plus their interaction influenced the surface area of the epiphyseal growth plate of tibia on prenatal exposure to phenobarbital and phenytoin.

On carrying out the Manova level two analysis on individual drugs, dose and time of exposure and their interaction to establish how they influenced the surface area of different zones of the epiphyseal growth plate of the tibia when prenatally exposed, it was observed that:

a) At the individual level the highest contribution of the observed effects was observed at the dosage level with partial eta ranging from 68.7% to 99.9%, followed by trimester with partial eta ranging from 37.3.4% to 99.8% and lastly drug ranging

from 44.2% to 99.4% on the percentage of surface areas of different zones of the epiphyseal growth plate of tibia. as shown in the (***Table 4.15***)

b) At two-way level interaction, when two variables were combined the observed effects were highest between doses * drug with partial eta ranging from 11.5% to 98.7% followed by dosage and trimester ranging from 17.1% to 95.8% and lastly drug and trimester ranging from 11.8% to 47.7% on the percentage of surface areas of different zones of the epiphyseal growth plate of tibia ***(Table 4.15***).

c) at three-way interaction, when the three independent variables (drug, dosage and trimester) were combined they were noted to have the highest effects on the percentage of surface areas of different zones of the epiphyseal growth plate of the tibia with partial eta at 15.3 % on the surface area of the hypertrophic zone, followed by 16.4% of the surface area of proliferative zone, then partial eta of 58.4% of reserve cartilage, followed by partial eta of 67.7% of primary spongiosa zone and finally partial eta of calcification zone of partial eta of 82.7% of the surface area of the epiphyseal growth plate of tibia bone as shown in the (***Table 4.15).***

Table 4.15: The MANOVA Level 2 Table Findings on How the Two Medicines (Phenobarbital and Phenytoin), Their Doses and the Time of Exposure Plus Their Interactions Influenced the Surface Area of the Epiphyseal Growth Plate of the Tibia on Prenatal Exposure

Tests of Between-Subjects Effects							
Source	**Dependent Variable**	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**	**Partial Eta Squared**
Corrected Model	%SA T RC	678.823a	1837.712		5145.063	<.001	1.000
	%SATPZ	314.146b	1817.453		9.877	<.001	.824
	%SATHZ	443.235c	1824.624		468.530	<.001	.996
	%SATCZ	243.136d	1813.508		2070.820	<.001	.999
	%SATPS	371.359e	1820.631		803.862	<.001	.997
Intercept	%SA T RC	45405.471	1	45405.471	6194619.029	<.001	1.000
	%SATPZ	16410.823	1	16410.823	9287.005	<.001	.996
	%SATHZ	19785.562	1	19785.562	376465.277	<.001	1.000
	%SATCZ	14585.026	1	14585.026	2236004.525	<.001	1.000
	%SATPS	101490.817	1	101490.817	3954457.955	<.001	1.000
Drug	%SA T RC	47.003	1	47.003	6412.524	<.001	.994
	%SATPZ	53.143	1	53.143	30.074	<.001	.442
	%SATHZ	37.383	1	37.383	711.305	<.001	.949
	%SATCZ	41.309	1	41.309	6332.972	<.001	.994
	%SATPS	47.808	1	47.808	1862.794	<.001	.980
Dosage	%SA T RC	513.041	2	256.520	34996.810	<.001	.999
	%SATPZ	147.536	2	73.768	41.746	<.001	.687
	%SATHZ	285.645	2	142.823	2717.525	<.001	.993
	%SATCZ	64.254	2	32.127	4925.320	<.001	.996
	%SATPS	109.518	2	54.759	2133.616	<.001	.991

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Trimester	%SA T RC	24.931	2	12.466	1700.685	<.001	.989
	%SATPZ	39.886	2	19.943	11.286	<.001	.373
	%SATHZ	57.297	2	28.648	545.101	<.001	.966
	%SATCZ	101.119	2	50.559	7751.184	<.001	.998
	%SATPS	86.938	2	43.469	1693.704	<.001	.989
Drug * Dosage	%SA T RC	20.658	2	10.329	1409.149	<.001	.987
	%SATPZ	8.758	2	4.379	2.478	<.097	.115
	%SATHZ	4.184	2	2.092	39.801	<.001	.677
	%SATCZ	.110	2	.055	8.458	<.001	.308
	%SATPS	2.551	2	1.275	49.697	<.001	.723
Drug * Trimester	%SA T RC	.134	2	.067	9.158	<.001	.325
	%SATPZ	1.221	2	.610	.345	.710	.118
	%SATHZ	.865	2	.432	8.228	<.001	.302
	%SATCZ	.055	2	.027	4.196	.023	.181
	%SATPS	.889	2	.445	17.324	<.000	.477
Dosage * Trimester	%SA T RC	.641	4	.160	21.848	<.000	.697
	%SATPZ	5.134	4	1.283	.726	.579	.171
	%SATHZ	3.432	4	.858	16.325	<.000	.632
	%SATCZ	5.682	4	1.421	217.785	<.001	.958
	%SATPS	11.021	4	2.755	107.356	<.001	.919
	%SA T RC	.391	4	.098	13.351	<.001	.584
Drug * Dosage * Trimester	%SATPZ	4.610	4	1.153	.652	.629	.164
	%SATHZ	.112	4	.028	.533	.712	.153
	%SATCZ	1.183	4	.296	45.347	<.001	.827
	%SATPS	2.041	4	.510	19.881	<.001	.677
Error	%SA T RC	.279	38.007				
	%SATPZ	67.149	381.767				
	%SATHZ	1.997	38.053				
	%SATCZ	.248	38.007				
	%SATPS	.975	38.026				
Total	%SA T RC	61210.139	57				
	%SATPZ	21847.009	57				
	%SATHZ	26463.849	57				
	%SATCZ	19579.963	57				
	%SATPS	136673.260	57				
Corrected Total	%SA T RC	679.102	56				
	%SATPZ	381.295	56				
	%SATHZ	445.232	56				
	%SATCZ	243.384	56				
	%SATPS	372.334	56				

a. R Squared = 1.000 (Adjusted R Squared = .999)
b. R Squared = .824 (Adjusted R Squared = .740)
c. R Squared = .996 (Adjusted R Squared = .993)
d. R Squared = .999 (Adjusted R Squared = .998)
e. R Squared = .997 (Adjusted R Squared = .996)

Level 3: The Manova pairwise comparison results on how the phenobarbital and phenytoin influenced the surface area of the epiphyseal growth plate of the tibia on prenatal within the same dosages and the same trimester

Upon comparative of pairwise MANOVA level three analysis between the phenobarbital and the phenytoin in the same dosage and same time of exposure to establish how the two medicines influenced the surface area of the growth plate of the tibia, it was observed to have a statistically significant difference in that phenytoin given at the same dosage and the same time of exposure, it was noted to have minimal effects compared to the phenobarbital.

(**A**)Pairwise of low doses on the surface area of the reserve zone in the epiphyseal growth plate of the tibia bone for TM1, TM2 and TM3 were as follows; (**a**) TM1 mean difference (- 0.333, p value=0.000 **(b**) TM2 mean difference (-0.477, p value=0.000) (**c**) TM3 mean difference (-.107, p value=0.135)

ii) Pairwise of medium doses on the surface area of reserve cartilage:**a**) TM1 mean difference (-1.653, p value=0.000) (**b**) TM2 mean difference (-2.103, p value=0.000) (**c**) TM3 mean difference (-2.127, p. value=0.001)

iii) Pairwise of on the high dose surface are of reserve cartilage: **a**) TM1 mean difference (-3.543, p. value=0.001) **(b**) TM2 mean differences (-3.413 **(c)** TM3 mean difference (-3.037, p. value=0.001)

i)Pairwise of low doses on the proliferative zone for TM1, TM2 and TM3 were as follows; (**a**) TM1 mean difference (-.527, p value=0.003) (**b**) TM2 mean difference (-0.920, p value=0.002) **(c**) TM3 mean difference (-1.370, p value=0.002)

ii) Pairwise of medium doses on proliferative zone:(**a**)TM1 mean difference (-4.413, p value=0.001) (**b**) TM2 mean difference (-1.947, p value=0.038) (**c**) TM3 mean difference (-2.337, p. value=0.081)

iii) Pairwise of high doses on the surface area of the proliferative zone:**(a**)TM1 mean difference (-2.207, p. value=0.049) (**b**) TM2 mean differences (-2.100, p. value=0.060) (**c**) TM3 mean difference (-2.037, p. value=0.068)

(**C**)Pairwise of low doses on the surface area of the hypertrophic zone of epiphyseal growth plate for TM1, TM2 and TM3 were as follows; (**a**) TM1 mean difference (-.713, p value=0.003) (**b**) TM2 mean difference (-1.330, p value=0.002) (**c**) TM3 mean difference (-.927, p value=0.002)

ii) Pairwise of medium doses on proliferative zone:**a**) TM1 mean difference (-1.347, p value=0.000) (**b**) TM2 mean difference (-2.137, p value=0.038) **(c**) TM3 mean difference (-1.463, p. value=0.081)

iii) Pairwise of high doses on the surface area of the proliferative zone:**a)**TM1 mean difference proliferative zone: (-2.120, p. value=0.004) (**b**) TM2 mean differences (-2.540, p. value=0.060) **(c**) TM3 mean difference (-2.400, p. value=0.008)

(D) Pairwise of low doses on the surface area of the calcification zone in the epiphyseal growth plate of the tibia bone for TM1, TM2 and TM3 were as follows; (**a**) TM1 mean difference (- 1.370, p value=0.000 **(b**) TM2 mean difference (-1.557, p value=0.000) (**c**) TM3 mean difference (-2.080, p value=0.002)

ii) Pairwise of medium doses on the surface area of the calcification zone:**a)**TM1 mean difference (-2.100, p value=0.000) **(b**) TM2 mean difference (-1.517, p value=0.000) (**c**) TM3 mean difference (-2.010, p. value=0.000)

(**iii**) Pairwise of on the high dose surface are of calcification zone: **a**) TM1 mean difference (-2.122, p. value=0.000) (**b**) TM2 mean differences (-2.540, p value=0.009) (**c**) TM3 mean difference (-2.400, p. value=0.000)

(**E**)Pairwise of low doses on the surface area of primary spongiosa of epiphyseal growth plate for TM1, TM2 and TM3 were as follows; (**a**) TM1 mean difference (-1.700, p value=0.003) (**b**) TM2 mean difference (-0.620, p value=0.002) (**c**) TM3 mean difference (-1.487, p value=0.002)

ii) Pairwise of medium doses on the surface area of primary spongiosa of epiphyseal growth plate:**(a)**TM1 mean difference (-2.863, p value=0.000) (**b**) TM2 mean difference (-1.587, p value=0.001) **(c)** TM3 mean difference (-2.237, p. value=0.081)

iii) Pairwise of high doses surface area of primary spongiosa of epiphyseal growth plate:(**a)**TM1 mean difference (-2.127, p. value=0.049) (**b**) TM2 mean differences (-2.650, p. value=0.007) (**c**) TM3 mean difference (-1667, p. value=0.008)

Upon comparative of pairwise multiple analysis of variance between the phenobarbital and the phenytoin in the same dosage and same time of exposure to establish how the two medicines influenced surface areas of zones of epiphyseal growth plate of tibia namely: reserve zone, proliferative zone, hypertrophic zone, degeneration (calcification) zone and primary spongiosa zone. It was observed to

have a statistically significant difference in that phenytoin given at the same dosage and the same time of exposure was noted to have minimal effects compared to the phenobarbital.

Table 4.16: The MANOVA Level 3 Pairwise Table Findings on How the Two Medicines (Phenobarbital and Phenytoin) Influenced the Surface Areas of the Epiphyseal Growth Plate of Tibia on Prenatal Exposure

								95% Confidence Interval for Differenced	
Dependent Variable	**Dosage (Mg/kg bw)**	**Trimesters**	**(PB)**	**(PT)**	**Mean Difference (PB-PT)**	**Std. Error**	**Sigd (<.05)**	**Lower Bound**	**Upper Bound**
	Low	Trimester one	PB	PT	-.333	.070	<.001	-.475	-.192
		Trimester two	PB	PT	-.477	.070	<.001	-.618	-.335
		Trimester three	PB	PT	-.107	.070	.135	-.248	.035
%SA RC	Medium	Trimester one	PB	PT	-1.653	.070	<.001	-1.795	-1.512
		Trimester two	PB	PT	-2.103	.070	<.001	-2.245	-1.962
		Trimester three	PB	PT	-2.127	.070	<.001	-2.268	-1.985
	High	Trimester one	PB	PT	-3.543	.070	<.001	-3.685	-3.402
		Trimester two	PB	PT	-3.413	.070	<.001	-3.555	-3.272
		Trimester three	PB	PT	-3.037	.070	<.001	-3.178	-2.895
	Low	Trimester one	PB	PT	-.527	1.085	.630	-2.724	1.671
%SATPZ		Trimester two	PB	PT	-.920	1.085	.402	-3.117	1.277
		Trimester three	PB	PT	-1.370	1.085	.215	-3.567	.827
	Medium	Trimester one	PB	PT	-4.413	1.085	<.001	-6.611	-2.216
		Trimester two	PB	PT	-1.947	1.085	.081	-4.144	.251
		Trimester three	PB	PT	-2.337	1.085	.038	-4.534	-.139
	High	Trimester one	PB	PT	-2.207	1.085	.049	-4.404	-.009
		Trimester two	PB	PT	-2.100	1.085	.060	-4.297	.097
		Trimester three	PB	PT	-2.037	1.085	.068	-4.234	.161
	Low	Trimester one	PB	PT	-.713	.187	<.001	-1.092	-.334
		Trimester two	PB	PT	-1.330	.187	<.001	-1.709	-.951
%SATHZ		Trimester three	PB	PT	-.927	.187	<.001	-1.306	-.548
	Medium	Trimester one	PB	PT	-1.347	.187	<.001	-1.726	-.968
		Trimester two	PB	PT	-2.137	.187	<.001	-2.516	-1.758
		Trimester three	PB	PT	-1.463	.187	<.001	-1.842	-1.084
	High	Trimester one	PB	PT	-2.120	.187	<.001	-2.499	-1.741
		Trimester two	PB	PT	-2.540	.187	<.001	-2.919	-2.161
		Trimester three	PB	PT	-2.400	.187	<.001	-2.779	-2.021
	Low	Trimester one	PB	PT	-1.370	.066	<.001	-1.503	-1.237
		Trimester two	PB	PT	-1.557	.066	<.001	-1.690	-1.423
%SATCZ		Trimester three	PB	PT	-2.080	.066	<.001	-2.213	-1.947
	Medium	Trimester one	PB	PT	-2.100	.066	<.001	-2.233	-1.967
		Trimester two	PB	PT	-1.517	.066	<.001	-1.650	-1.383
		Trimester three	PB	PT	-2.010	.066	<.001	-2.143	-1.877
	High	Trimester one	PB	PT	-2.122	.066	<.001	-2.632	-1.936
		Trimester two	PB	PT	-2.540	.187	<.001	-2.919	-2.161
		Trimester three	PB	PT	-2.400	.187	<.001	-2.779	-2.021
	Low	Trimester one	PB	PT	-1.700	.131	<.001	-1.965	-1.435
		Trimester two	PB	PT	-.620	.131	<.001	-.885	-.355
%SATPS		Trimester three	PB	PT	-1.487	.131	<.001	-1.751	-1.222
	Medium	Trimester one	PB	PT	-2.863	.131	<.001	-3.128	-2.599
		Trimester two	PB	PT	-1.587	.131	<.001	-1.851	-1.322
		Trimester three	PB	PT	-2.237	.131	<.001	-2.501	-1.972
	High	Trimester one	PB	PT	-2.127	.131	<.001	-2.391	-1.862
		Trimester two	PB	PT	-2.650	.131	<.001	-2.915	-2.385
		Trimester three			-1.667	.131	<.001	-1.931	-1.402

4.4 To Comparatively Establish Whether the Histomorphological and Stereological Effects of Prenatal Exposure to Phenobarbital and Phenytoin Are Both Time and Dose-Dependent

The results showed that there was a shortening of long bones in all the treatment groups as compared to the control. It was however noted that those animals that received medium and high dosages had much more effects as compared to low dosages and control. The number of chondrocytes was seen to be reduced in all the treatment groups as compared to control. The high dosages treatment groups in both treatment categories indicated more effects, followed by medium dosages and lastly low dosages. In terms of timing, those exposed during trimester one were highly affected followed by those exposed in group two and finally trimester one. Phenobarbital and phenytoin enlarge the zone of hypertrophic and suppresses the proliferative zone. Consequently. The proliferative zone in the treatment groups was statistically reduced than in the control group. Additionally, the treatment group that was introduced to phenytoin and phenobarbital treatment from the first trimester showed more reduction followed by medium dosages and finally low dosages. Additionally, those who were subjected to phenobarbital showed slightly higher means compared to phenytoin among the two treatment groups.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

Introduction:

The current study aimed to comparatively evaluate the histomorphometric and histostereological teratogenic effects of prenatal exposure to varied doses of phenobarbital and phenytoin on the development of fetal skeleton in Albino rats (*Rattus norvegicus*). The discussion is presented along the study objectives as follows:

5.1 Comparative Teratogenic Foetal and Maternal Pregnancy Outcome Following Prenatal Exposure to Phenobarbital And Phenytoin At Different Doses Administered At Different Trimesters

This study established that there was mean reduction in fetal weight, crown-rump length, bi-parietal diameter and head circumference in the Phenobarbital and phenytoin treatment groups as compared to the control group. Additionally, the mean reduction in crown-rump length, fetal weight, bi-parietal diameter and head circumference were observed to be time and dose-dependent. Statistically significant lower means (P=0.001) were associated with medium and high dosage groups as compared to low dosage groups and control groups. Further, statistically significantly lower means of fetal body weights, crown-rump length, bi-parietal diameter and head circumference were observed in trimester one (TM_1) and trimester two (TM_2) as compared to lastly by trimester three (TM_3).(***table 4.1***)

The current study results concur with those of Matalon *et al.*,(2002) on teratogenic morphological effects on the development of the fetal skeleton which showed that carbamazepine which is in the same class as the drugs of this study caused detrimental effects on fetal growth parameters. Additionally, the findings on the reduction of fetal growth parameters agree with those of another study that observed, exposure to lamotrigine, carbamazepine, lamotrigine, and valproic acid, which has the same mode of action as phenobarbital and phenytoin when administered at varied dosages and gestation period, (Anatomy, 2020; Kilic *et al*., 2014; Lavu *et al.*, 2021).

This observation on reduction in the fetal growth indicators may have been attributed to the fact that phenobarbital and phenytoin cross the placenta blood barrier and are known to cause bradycardia resulting in reduced blood flow and subsequently reduced oxygen and nutritional supply leading to slow growth and development (Danielsson *et al*., 2003).

It was however noted that the phenobarbital-treated group had slightly lower fetal weight, crown-rump length, head Circumference and bi-parietal diameter compared to all phenytoin-treated groups, across the different times of exposure. This differs from another study that established that phenytoin treated group reduced fetal growth parameters compared to phenobarbital treatment (Fleeman *et al*., 2023).

On comparative analysis of how prenatal exposure to the two medicines(phenobarbital and phenytoin) influenced the maternal pregnancy outcome that included; (i) maternal weight gain, (ii) mean terminal weight, and (iii) placental weights established that there were statistically significant reductions ($P<0.001$) in both Phenobarbital and phenytoin treatment groups as compared to the control group (***Table 4.1*)**. Further, the means in maternal pregnancy outcomes were observed to be time and dose-dependent in that statistically significant higher means ($P<0.001$) were associated with low and medium-dose groups as compared to high-dose groups. Additionally, statistically significant ($p<0.001$) higher means of maternal pregnancy outcome were observed in trimester three (TM_3), followed by trimester two (TM_2) and lastly by trimester one (TM_1)**(*Table 4.1*).** Additionally, it was also found that phenytoin treatment groups had a higher means of maternal parameters than the phenobarbital treatment group (a) terminal placental weights, ($F(18,38) = 156.082$ $P= 0.001$), (b) Mean terminal weight ($F(18,38) = 13.639$ $P= 0.042$), (c) Mean maternal weight gain ($F(18,38) = 33.963$ $P= 0.049$) ***(Table 4.4*)** These findings concur with Carol *et al* (2020) whose finding established that phenytoin lowers maternal parameters such as the placental weights irrespective of the dosage. This could have been attributed to the fact that they interfere with maternal cardiovascular functions resulting in reduced placenta oxygen supply leading to placenta ischaemia

This study however differ from a previous study that established that the use of antiepileptic drugs prenatally is not associated with adverse maternal outcome(Razaz et al., 2017).

5.2 Comparative Teratogenic Histomorphological Findings Following Prenatal Exposure to Phenobarbital and Phenytoin at Different Doses Administered at Different Trimesters

On comparative analysis of how prenatal exposure to the two medicines(phenobarbital and phenytoin) influenced the epiphyseal growth plate of a long bone, this study established that low doses of phenobarbital and phenytoin have no comparative histomorphological differences in cell morphology and distribution (as shown in figure...) with the control groups. At medium and high doses for both phenobarbital and phenytoin treatment groups, the epiphyseal growth plates had a reduced number of chondrocytes as compared to the low doses and control. These findings concur with Ozekin *et al.,(*2020) who found that high doses of phenobarbital will interfere with the growth and development of the long bone. This could have been caused by the fact that the two drugs are ion channel blockers (phenytoin inhibits voltage–gated sodium channels while phenobarbital inhibits $GABA_A$ receptors). This will cause changes in cell membrane potentials that will eventually influence epiphyseal growth plate development (Ozekin *et al*., 2020). In addition, the two medicines cause decreased vitamin D, calcium and phosphate deposition as well as increase the level of parathyroid hormone in the blood. This causes direct effects on chondrocyte development(Alexander *et al*., 2016).

5.3 The Comparative Gross Morphometric Measurements of the Length of the Tibia and Humerus Upon Prenatal Exposure to Varied Doses of Phenobarbital and Phenytoin in

This study found that there was a statistically significant reduction in the length of groups that were given medium and high doses of phenobarbital and phenytoin in all the trimesters as compared to the control group. It was however observed that the treatment groups that received low doses in trimester 3 had no statistically significant difference as compared to the low dose in trimester one and trimester two. Upon

comparing the effects between the two treatment groups, it was established that phenytoin treatment groups had a higher length of the tibia and humerus. This study agrees with another study on the effects of gabapentin and valproic acid which belong to the same class as phenobarbital and phenytoin, when exposed prenatally on fetal outcome during the first trimester(Etemad et al., 2012a). The current study found that, at medium and high doses of phenobarbital and phenytoin, there was a statistically significant $p<0.05$ reduction of both the tibia and humerus.

On the comparative evaluation of the teratogenic histostereological effects of prenatal exposure to the two medicines on the fetal epiphyseal growth plate of a long bone, the study found that prenatal exposure to medium and high doses of the phenobarbital and phenytoin influenced the histoquantitive contribution of the zones of epiphyseal(Yan et al., 2016). This could have been attributed to impaired vascular invasion leading to inhibition of chondrocyte proliferation, delaying mineralization and mesenchymal differentiation in the two treatment groups(Yan et al., 2016). This finding agrees with brown *et al* (2010) study on carbamazepine, an antiepileptic drug with a similar mode of action to the two medicines that found that it causes reduction of the long bones by reducing mineralization of the bone. This could have been attributed to the fact that phenobarbital and phenytoin treatment affect the invasion of blood vessels in the cartilage(Yan et al., 2016).

5.4 To Establish Whether the Teratogenic Effects on Developing Fetal Appendicular Skeleton are Time- and Dose-Dependent

The study established that both phenobarbital and phenytoin when given at low doses over a short period, there was no statistically significant difference as compared to the control. It was however observed that both treatment groups when exposed at medium and high doses at prolonged duration may cause a reduction in epiphyseal growth plates. This could be caused by high doses of phenobarbital and phenytoin that are associated with increased episodes of nausea and drowsiness which interfere with feeding habits (Zhang et al., 2011).

5.5 Conclusion

i) The findings of this study established that both Phenobarbital and Phenytoin at varied doses, leads to remarkable decrease in fetal parameters for growth when exposed prenatally. The mean decrease in these fetal parameters was observed to be time and dose dependant. It was also established that the mean reduction was more in Phenobarbital treatment groups as compared to the phenytoin treatment group. This evidence indicates that Phenobarbital has more detrimental effects as compared to phenytoin.

ii) With regards as to the comparative evaluation of how the varied doses of the phenobarbital and phenytoin influence maternal and fetal outcomes the study concluded that the medium and high doses of the two medicines and especially when given during the first and second semester have detrimental effects on maternal and fetal outcomes With regards to the comparative evaluation of which intervention is the most effective in restoring the normal lipid profile level in obese subjects, the study concludes the combined interventions of exercise training and caloric restriction, administered at medium to high dosage levels, was the most effective in resolving hyperlipidemia, followed by medium-dose caloric restriction and lastly low dosages of all the interventions.

iii) Concerning the comparative histomorphological effects of the varied doses of phenobarbital and phenytoin on the cellular architectures it was established that , the two drugs when given during the first and second semester will have a significant effects while at medium and higher doses will have remarkable effects on the cells thus affecting epiphyseal growth plate

iv) Concerning the comparative evaluation of histo-stereological effects of the varied doses of phenobarbital and phenytoin, it was established that the two drugs affected the epiphyseal growth plate especially when exposed during first and second semester. It was however noted that both drugs at low doses would have minimal effects. On the epiphyseal growth plate

5.6 Recommendation

i) The study recommend that the use of phenobarbital and phenytoin at medium and high doses should be avoided especially during TM1 and TM2

ii) It also recommend that in te circumstances where pregnant mothers requires anticonvulsive medicines, phenytoin is safer as compared to phenobarbital

iii) The expectant mothers to use minimum effective dose to achieve maximum convulsion control.

iv) The study therefore recommends for further follow up studies and clinical trials to be carried out in species close to human.

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APPENDICES

Appendix I: Data Capture Sheets

Data Capture Sheet For Expectant Albino Rats

Albino Rat

Identity…….

Initial.....Weight……..Dose Calculation……

DATE	WEIGHT IN GRAMS	PHENOBARBITONE DOSE (mg/kg)	PHENYTOINDOSE (mg\kg)	GENERAL CONDITION OF RAT

Appendix II: Certificate of Ethical Approval

UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

P.O. Box 30197,
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Ext. 2300
Direct Line. 4448648

REF: FVM BAUEC/2021/332

Mr. Joseph Wachira
Dept. Human Anatomy,
JKUA & Technology.
26/11/2021

Dear Joseph,

RE: Approval of proposal by Faculty Biosafety, Animal use and Ethics committee

Comparative morphometric and histostereological teratogenic effects of prenatal exposure to phenytoin and phenobarbital on fetal skeleton in albino rats.
Joseph Macharia Wachira HSM301-1196/2020

We refer to your MSc proposal submitted to our committee for review and your application letter dated 13th November 2021. We have reviewed your application for ethical clearance for the study. The number of rats, animal husbandry practices and the proposed protocol that will be used for comparative morphometric and histostereological teratogenic effects of prenatal exposure to phenytoin and phenobarbital on fetal skeleton meets the minimum standard of the Faculty of Veterinary medicine, Biosafety and Animal use and Ethical committee regulation guidelines.
We hereby give approval for you to proceed with the project as outlined in the submitted proposal.

Yours sincerely,

Kaluwa

Dr. Catherine Kaluwa, Ph.D
Chairperson, Biosafety, Animal Use and Ethics Committee,
Faculty of Veterinary Medicine,
University of Nairobi

Appendix III: Publication

IOSR Journal Of Humanities And Social Science (IOSR-JHSS)
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The Comparative Teratogenic Effects Of Prenatal Exposure To Varied Doses Of Phenobarbital And Phenytoin On Fetal Growth In Utero In Albino Rats (Rattus Norvegicus)

[1]Joseph Macharia Wachira, [2]Joseph Kariuki Kweri, [3]James Mwangi Kanyoni, [4]Ann Wairimu Mwangi, [5]Walter Rono,[6] Caroline Sigei, [7]Anne Nyaga, [8]Jane Kuria, [9]Cyrus Kamau, [10]Jennifer Segut
Department of human anatomy, Jomo Kenyatta University of Agriculture and Technology, Kenya.

ABSTRACT
The in-utero exposure to phenytoin or Phenobarbital during pregnancy in the management of maternal neurological disorders like epilepsy, bipolar diseases, seizures among other has been associated with a wide range of fetal congenital malformation ranging from musculoskeletal, neurological and organ system disorders. However, their comparative teratogenic effects in terms of fetal growth have not been well elucidated hence the basis of this study. Therefore, this study seeks to comparatively evaluate fetal effects when exposed to phenytoin or Phenobarbital in development of fetal skeleton in albino rats. In carrying out the study a total of 57 nulliparous female Albino Rats dams from a pure colony/breed 3rd generation was used. The 57 albino rats were categorized into 54 rat experimental groups and 3 rats control. Further, the 54 experimental were broadly categorized into two major groups of 27 rats each based on the drug administered. The first group received phenytoin while second group received phenobarbital. Each of the Experimental groups were further divided into 3 groups of 9 rats based on the trimester i.e. one, two and three respectively. To evaluate the effect of dosage level the 9 rats were further subdivided into 3 groups of low dosage, medium and high dosage each composed of 3 rats. The Comparative effects on growth parameters between phenobarbital and phenytoin on fetal weight, bi-parietal diameter (BD), head circumference and crown-rump length (CRL) were evaluated. Excel spread sheet were used for data entry and SPSS version 25 was used for analysis and tables were used to present finding.
The result of this current study demonstrated that the timing of drug exposure significantly influenced fetal growth and development parameters and the dosage administered for both phenobarbital and phenytoin. Particularly, exposure during the 1st and 2nd trimesters, especially at medium to high doses, had more pronounced effects on fetal growth. The study revealed that mean fetal weights, crown-rump lengths, bi-parietal diameters, and fetal head circumferences decreased with increasing dosages and longer exposure times. Moreover, phenytoin exhibited greater effects on growth parameters compared to Phenobarbital throughout all trimesters.
In conclusion, this research establishes that both Phenobarbital and Phenytoin, at varying doses, lead to a significant decrease in fetal growth parameters when exposed prenatally. The magnitude of this decrease was dependent on both the timing of exposure and the dosage used. Therefore, it emphasizes the critical importance of considering the dose and timing of exposure to these drugs during pregnancy. Therefore is need for further research to elucidate the potential mechanisms responsible for the observed effects of these drugs and to identify appropriate dosages for both Phenobarbital and phenytoin. Such insights will contribute to better management of maternal neurological disorders while safeguarding fetal development.

Date of Submission: 02-08-2023 Date of Acceptance: 12-08-2023

I. INTRODUCTION

Studies have demonstrated that anticonvulsant medications used in management of maternal neurological conditions including, epilepsy, seizures, and bipolar diseases, have been associated with teratogenic effects during organogenesis. Due to these potential risks to the developing fetus, the U.S. Food and Drug Administration (FDA) classify these drugs as class C medicines, indicating that there is evidence of adverse effects in animal studies, but there may be situations where the benefits of using the medication in pregnant women outweigh the potential risks (Gedzelman & Meador, 2012). Phenobarbital and phenytoin are some of the most commonly used medicines in the management of maternal neurological conditions during pregnancy in the developing countries like