COMPARATIVE HISTOMORHOLOGICAL AND HISTOSTEREOLOGICAL TERATOGENIC EFFECTS OF *IN-UTERO* EXPOSURE TO CHLORPROMAZINE AND HALOPERIDOL ON THE DIFFERENTIATION OF THE EPIPHYSIAL GROWTH PLATES OF THE FETAL APPENDICULAR SKELETON IN ALBINO RATS (*Rattus norvegicus)*

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Comparative Histomorphological and Histostereological teratogenic Effects of *In-Utero* Exposure to Chlorpromazine and Haloperidol on the Differentiation of the Epiphyseal Growth Plates of the Fetal Appendicular Skeleton in Albino Rats (*Rattus norvegicus)*

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

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DEDICATION

This work is dedicated to my husband Lawrence and my children Myles and Marya, my Dad Kuria, mum Kairigo, and Mama Mary Gichure for their support and encouragement.

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ABBREVIATIONS / ACRONYMS

AED	Animal Equivalent Dose
ANOVA	Analysis of Variance
ARI	Animal Research Institute
BMPs	Bone Metalloproteinase
BWT	Body Weight
C	Control
CNS	Central Nervous System
CPZ	Chlorpromazine
CRL	Crown Lump Length
D2	Dopamine Two
DNA	Deoxyribonucleic Acid
FDA	Food and Drug Administration of United States of America
FGAs	First-Generation Antipsychotics
G	Grams
GD	Gestation by date
H&E	Haematoxylin and Eosin
HAL	Haloperidol
HDG	High Dose Group

HDHAL G	High Dose Haloperidol Group
HED	Human Equivalent Dose
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KG	Kilogram
Mμ	Micrometer
Mm	Millimeter
OPG	Osteoprotegerin
SEM	Standard Error of Mean
SPSS	Statistical Package of Social Science
TMI	Trimester 1
TMII	Trimester 2
TMIII	Trimester 3
WHO	World Health Organization

DEFINATION OF TERMS

Histomorphology it refers to study of microscopic structures of tissues in this context it means examination of changes in the structures of epiphyseal growth plate following pre-natal exposure to chlor-promazine and haloperidol

Morphometry it's the quantative measurements of the shape and size of an organ and how they change over time. In this context, it means measurements of fetal rat epiphyseal growth plate following prenatal exposure to varied doses of haloperidol and chlor-promazine.

Primary Spongiosa it's a structure which is formed when osteoblasts invade and ossify the calcified cartilage of the fetal epiphyseal growth plate following prenatal exposure to varied doses of haloperidol and chlorpromazine.

ABSTRACT

Chlorpromazine and haloperidol are typical antipsychotics and among the commonly prescribed medicines to manage psychosis in expectant mothers. However, their comparative teratogenic effects on the fetal appendicular skeleton remains unclear. At the same time, whether or not their effects are dose and time dependent is not well elucidated. However, studies have reported a lot of discrepancies with regards to the effects of the two medicines on the fetal bone morphogenesis. The objective of this study therefore was to comparatively evaluate the histomorphological and stereological teratogenic effects of prenatal exposure to varied doses of haloperidol and chlorpromazine on the differentiation of the epiphyseal growth plates of the fetal appendicular skeleton of albino rats. A posttest laboratory-based experimental study design with control only was adopted. The study was carried out at the University of Nairobi, Department of Biological science. A total of two sets of 30 albino rat dams weighing between 200grams to 250grams and from a pure colony of the 3rd series was used as the experimental model. These albino rats were obtained from the lower Kabete veterinary animal house, University of Nairobi. The 30 albino rats were further grouped into two broad categories as follows; 3 control and 27 for treatment group. The treatment group was further divided to evaluate the effects of different doses into three other groups of 9 albino rats, each assigned different doses of haloperidol and chlorpromazine 9 rats for low dose 0.05 mg/kg/3.1 mg/kg, 9 rats for medium dose 1.5mg/kg/11.88 mg/kg and 9 rats for high dose 3.1mg/kg/20.67mg/kg respectively. In order to determine the most venerable time of exposure, the albino rats were further subdivided into three groups of three rats each for trimester one, two and three. The rats were fed with standard rodent pellets and water was given *ad libitum*. Confirmation of pregnancy marked the first day of pregnancy, treatment began, and the animals were sacrificed on gestational day 20 using concentrated carbon dioxide, sustained with ketamine, and fetuses were harvested. Fetal bones of the appendicular skeleton (tibia and humerus) were selected by simple random sampling and histomorphological and histoquantative finding were analyzed. Digital veneer calipers were used for gross morphometric measurement, while histo-photomicrographs were analyzed using swift imaging 3.0 camera 20 mega pixel then exported to swift 3.0 software for data analysis after tissue processing and H&E staining. For histoquantative analysis, data was collected using structured check list stored and coded in excel spread sheet, then analyzed using SPSS version 25 for windows. Data was expressed as means ±SD for all values. The levels of statistical significance of the quantitative Comparative inferential statistics were tested using one-way analysis of variance (ANOVA) for the intragroup, intergroup mean, and multivariate analysis of variance (MANOVA) for interaction, main and pair wise analysis. A p-value of < 0.05 was considered significant. The study found a statistically significant higher fetal weight and crown-lump length for the medium chlorpromazine (11.88 mg/kg) treated group and statistical significant lower fetal parameters for high dose treatment groups of both chlorpromazine and haloperidol (20.67mg/kg, 3.1mg/kg) respectively. In addition, the study found a statistically significant higher percentage surface area of proliferation zone with higher cellular density in this zone for medium dose chlorpromazine treated group from trimester one and statistical significant lower percentage of all the layers of growth plate for haloperidol and chlorpromazine treated groups at high doses more so the chlorpromazine treated group from trimester one as compared to the control. From the study, it can be deduced that the teratogenic effects of chlorpromazine and haloperidol are both time and dose-dependent whereby at high doses and for longer period they had a negative effect on appendicular skeleton. It is recommended high dosages of both chlorpromazine and haloperidol should be avoided especially in first trimester.

CHAPTER ONE

INTRODUCTION

This chapter begins by giving a brief description on the back ground information of chlorpromazine and haloperidol effects on appendicular structure and the existing controversial mechanism of chlorpromazine and haloperidol teratogenicity, statement of the problem, study justification and significant. Then followed by study objectives both broad and specific, study limitation and delimitation, conceptual framework, null and alternative hypothesis.

1.1 The Back Ground Information on the Effects of Chlorpromazine and Haloperidol on the Structures of the Appendicular Skeleton

Chlorpromazine and Haloperidol are among the commonly used medicines in management of maternal psychosis, schizophrenia, intractable hiccups, severe anxiety in pregnancy among others (Ahmed *et al*., 2016). They are both lipophilic and act by blocking dopaminergic receptors with high affinity to dopamine two (D2) receptors (Zamani *et al*., 2015). Studies have shown that these two medicines in class C are associated with perturbations to normal fetal developmental processes in-utero (Rampino et al., 2021). A study by Patton *et al* (2002) showed that perinatal exposure resulted to abnormal lengthening of the limbs while another study showed causation in shortness of stature (Edinoff et al., 2022b).

Haloperidol is a butyrophenone derivative with a molecular weight of 412.3 g/mol and a chemical formula of $C_{21}H_{23}CIFNO_2$ with 90% plasma bound its dopamine two (D2) receptor blocker and its used to manage positive symptoms of schizophrenia such as hallucination and delusion, Tic in Tourette motor syndrome, severe behavioral disorder in children and attention deficit hyperactivity disorder in children (Ostinelli *et al*., 2017). In addition it is used to manage agitation, irritability and mania off label (Hoertel *et al*., 2021). In comparison, chlorpromazine is a dimethyl derivative of the phenothiazine class with a molecular weight of 318.9 g/mol, a chemical formula $C_{17}H_{19}C_1N_2S$ with 98% plasma bound (Ahmed et al., 2016). It is used to manage acute cases of psychosis, positive symptoms of schizophrenia,

bipolar disorder and agitation (Dayabandara et al., 2017).In addition chlorpromazine is used to treat intra-operative nausea and vomiting, chronic hiccups, allay anxiety before surgery and adjunct to treatment of tetanus (Edinoff et al., 2022a).

Worldwide mental and behavioral disorders are among the leading cause of mortality and morbidity (Kulkarni *et al*., 2015). However, morphological effects of FGAs on the appendicular skeleton are riddled with controversy as previous studies carried out on their effects on skeletogenesis showed limb deformity, low birth weight, severe limb reduction defects, phocomelia, cleft palate, and even high birth weight which was attributed to gestational diabetes triggered by the use of antipsychotics (Galbally *et al.*, 2014; Giffin *et al*., 2019). While a study carried out on children born of mothers using chlorpromazine and other neuroleptics for more than two months showed they were taller than the control group (Patton *et al*., 2002).

However, haloperidol is more potent than chlorpromazine in blocking dopaminergic receptors (Al-ghamdi *et al*., 2020). Approximately one-third of pregnant women with psychotic symptoms use antipsychotics at least once; none of the drugs for the treatment of psychosis can be considered entirely safe and are only considered when the benefit outweighs the risk, as even atypical drugs are associated with increased risks of obesity, type 2 diabetes mellitus and dyslipidemia (Ahmed *et al.*, 2016).

Studies are showing that, the two medicines that are in the first-generation antipsychotics (FGAs) are currently being widely prescribed particularly in the management of maternal psychosis in resource-limited countries of Africa, and Kenya included owing to their cost effectiveness, efficacy, and easy accessibility (Ongeri *et al*., 2018,.Edinoff et al., 2022b). These controversies hence prompted this study to establish their comparative histoquantative effects on the differentiation of the epiphyseal growth plates of fetal appendicular skeleton when prenatally exposed in varied doses and at different window periods.

Therefore, understanding *in-utero* exposure histoquantative effects of haloperidol and chlorpromazine at varied doses on the differentiations of the epiphyseal growth plate of the fetal appendicular skeleton in albino rats will be beneficial in selecting therapy.

1.2 The Statement of the Problem

Currently, there is a notable increase in juvenile skeletal disorders globally with an estimated 3million children suffering from skeletal disorders of unknown cause (Al-Mayouf et al., 2021). Approximately 1.71billion people have skeletal disorders worldwide and it's the leading contributor of disability contributing to 17% of all years lived with disability according to WHO (2022). In Kenya, the prevalence of congenital skeletal malformation was estimated at 33.9% (Agot *et al*., 2020). Understanding the risks arising from the antipsychotic medication use in pregnancy particularly in fetal skeletogenesis is becoming an important clinical concern given the current evidence of increasing rate of prescription in the general population for a wide range of disorders (Galbally et al., 2014b). Though, using first generation antipsychotics (FGAs) is thought to contribute to the rising cases of developmental skeletal perturbations in the fetuses very little data exists on their effects on the histomorphological and histostereological on the differentiation of the epiphyseal growth plates of the long bones in the fetus. Increased use of FGAs has been nesseciated by recent rise in cases of mental illness in women of child bearing age with an estimated prevalence rate of 15-29%, and about 1/3 have to use medication (Al-ghamdi, 2020). The ratio of mental illness for females and males is estimated to be 2:1respectively (Ahmed et al., 2016). More so, unwanted pregnancy commonly occurs in women of childbearing age with psychosis (Dar *et al*., 2019). Although there is controversy concerning the effects of chlorpromazine on the fetal appendicular skeleton, while some studies show malformation such as severe limb reduction, micromelia and phocomelia. Other studies found that children born of mothers using chlorpromazine and other neuroleptics in pregnancy tend to be taller than the control group whether they were breastfed or not (Iqbal et al., 2005; Patton et al., 2002) Therefore, this study seeks to establish comparatively the histoquantative effects of *in-utero* exposure to haloperidol and chlorpromazine on the differentiation of the epiphyseal growth plates of the fetal appendicular skeleton when exposed in different gestation periods and at varying doses. Haloperidol and chlorpromazine are used as they are cheap and readily available on prescription; despite them being classified under pregnancy category C that is risk cannot be

adequately ruled out, animal and human studies have shown adverse effects but human studies are inadequate (Ahmed et al., 2016).

1.3 The Justification of the Study

The risk associated with use of chlorpromazine and haloperidol will continue to be a public health problem if the safety of Chlorpromazine (CPZ) and haloperidol (HAL) is not established. The lack of study concerning the histomorphological and histoquantative effects of the drugs on the development of the fetal appendicular skeleton will continue to limit their use in pregnancy. The consequences of not establishing the safety of CPZ and HAL include an increased risk of skeletal disorders in children born to mothers who took CPZ and HAL during pregnancy. This will continue to be a public health concern, as skeletal disorders can have a significant impact on a child's quality of life.

1.4 The Significant of the Study

To quantify the effects of haloperidol and chlorpromazine exposure in utero at different doses and times on the development of fetal appendicular skeleton. In addition, use of CPZ and HAL in pregnancy is a complex issue, with both potential benefits and risks the current study will help to inform clinicians and pregnant women about the risks of CPZ and HAL helps them to make informed decisions in selecting therapy. Furthermore, the data derived from the study can assist the policy maker’s patient and general community and the data finding on CPZ and HAL on the development of the appendicular skeleton can be used as a baseline for more studies on using neuroleptics in bone diseases.

1.5 The Broad Objective

To comparatively evaluate histomorphological and stereological teratogenic effects of prenatal exposure to varied doses of haloperidol and chlorpromazine on the differentiation of the epiphyseal growth plate of the fetal appendicular skeleton in the albino rat (*Rattus norvegicus).*

1.5.1 The Specific Objectives

1. To evaluate the fetal and maternal pregnancy outcomes following prenatal exposure to varying doses of haloperidol and chlorpromazine in Albino rats.
2. To evaluate the histomorphological teratogenic effects of prenatal exposure to varied doses of chlorpromazine and haloperidol on the differentiation of the epiphyseal growth plate on fetal appendicular skeleton in Albino rats.
3. To evaluate the histostereological teratogenic effects of prenatal exposure to varied doses of chlorpromazine and haloperidol on the differentiation of the epiphyseal growth plate on fetal appendicular skeleton in Albino rats.
4. To establish whether the observed teratogenic effects of prenatal exposure to chlorpromazine and haloperidol on developing fetal appendicular skeleton are both time- and dose-dependent.

1.5.2 The Research Questions

1. Do chlorpromazine and haloperidol, taken at different doses during pregnancy, affect the pregnancy outcomes and the health of the mother and babies in albino rats?
2. Do prenatal exposure to varied doses of chlorpromazine and haloperidol influence histomorphological differentiation of the epiphyseal growth plate of fetal rat appendicular skeleton?
3. Do prenatal exposure to varied doses of chlorpromazine and haloperidol influence histoquantative of the differentiating epiphyseal growth plate of fetal rat appendicular skeleton?
4. Do the teratogenic effects (birth defects) observed in the fetal appendicular skeleton exhibit a dose-dependent and time-dependent relationship?

1.6 The Study Assumptions

The research used Albino rats as the model of study, previous study has shown that these breed has close relation to the human biological and functional characteristics (Sengupta, 2010). Thus, the study assumed that the results obtained mimicked a

similar histomorphological and stereological outcomes in the development of human appendicular skeleton.

1.7 The Scope of the Study

This study focused on the comparative histomorphological and histostereological teratogenic effects of prenatal exposure to varied doses of chlorpromazine and haloperidol on the differentiations of epiphyseal growth plates of the fetal appendicular skeleton at different gestational windows in the albino rats. The parameters evaluated include; fetal and maternal pregnancy outcome histomorphological and histoquantative effects of prenatal exposure chlorpromazine and haloperidol on the differentiation of epiphyseal growth plate on fetal appendicular skeleton and whether the observed effects were both dose and time dependent.

1.8.1 Null Hypothesis (Ho)

There is no statistical histostereological teratogenic difference in the differentiation of epiphyseal growth plate of the fetal rat appendicular skeleton following prenatal exposure to varied doses of Haloperidol and Chlorpromazine at different trimesters of exposure.

1.8.2 Alternative Hypothesis (H_1)

There is statistical histostereological teratogenic difference in the differentiation of epiphyseal growth plate of the fetal rat appendicular skeleton following prenatal exposure to varied doses of Haloperidol and Chlorpromazine at different trimesters of exposure.

1.9 Conceptual Framework

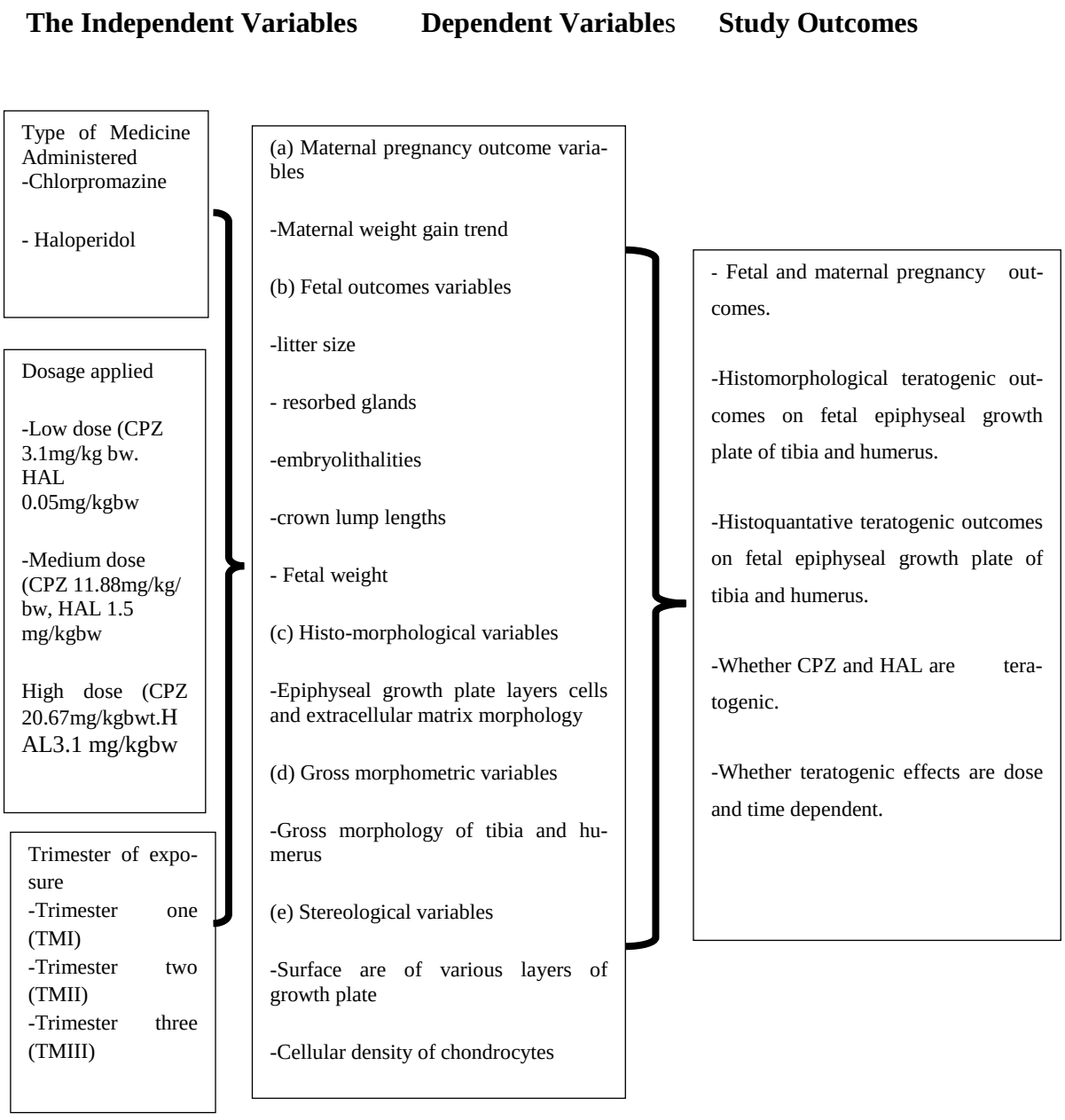


Figure 1.1: Conceptual Frame Work

CHAPTER TWO

LITERATURE REVIEW

This chapter describe the pharmacology of haloperidol and chlorpromazine and their mechanism of causing teratogenicity, the known fetal and maternal pregnancy outcomes following prenatal exposure to FGAs, the known histomorphological teratogenic outcome following prenatal exposure to FGAs and the comparative similarities and differences in the development appendicular skeleton between rats and humans.

2.1 The Pharmacology of Haloperidol and Chlorpromazine

Concerning pharmacology of haloperidol, its first generation antipsychotic which acts by blockage of dopamine receptors especially dopamine 2 (D2) receptors (Donohoe *et al*., 2006). Dopamine is an important neurotransmitter that plays a role in development (Schoretsanitis *et al*., 2020). Haloperidol blocks the dopamine receptors in the mesolimbic and mesocortical system which help alleviate positive symptoms of schizophrenia and bipolar disorder (Wu *et al*., 2022). Haloperidol also antagonizes dopamine receptors in the nigrostriatal pathways causing extrapyramidal movement such as drug induced Parkinsonism akathisia and dystonia. In addition, it blocks D2 receptors in the tuber-infundibula pathway causing hyperprolactenemia (Kulkarni *et al.*, 2015). Haloperidol is a butyrophenone derivative with a molecular weight of 375.9 g/mol and a chemical formula of $C_{21}H_{23}CIFNO_2$ with 90% protein binding capacity (Uguz, 2019). There are various brands in the market, such as Haldol®, Serenace ®, and haloperidol ® (Raha *et al.,* 2012). Haloperidol is used to treat schizophrenia, motor tics in Tourette syndrome, mania in bipolar disorder, delirium, agitation, acute psychosis, and hallucination in alcohol withdrawal, and it is metabolized in the liver and excreted in feces and urine *(*Schoretsanitis *et al.*, 2020). On the other hand, chlorpromazine is a dimethyl derivative of phenothiazine class with a molecular weight of 318.9 g/mol and a chemical formula $C_{17}H_{19}C_1N_2S$ that exacts its action post synaptic blockage of dopamine receptors in the mesolimbic pathway and also antagonize dopamine receptor in the nigrostriatal and tuber-infundibula system causing extra pyramidal

movement disorder and hyperprolactenemia (Wu *et al*., 2022). Some of the common brand names are ,largatil®, Thorazine ®,Hibernal®, Megaphen® and chlorpromazine ® (Alonso-Pedrero *et a*l., 2019). Chlorpromazine is a member of the first-generation typical antipsychotic used to treat schizophrenia, bipolar disorder, and severe behavioral problems in children and control nausea and vomiting during all stages of gestation, including labor and hyperemesis gravid arum (Donohoe *et al.*, 2006). Furthermore, it is used during labor to produce analgesia, amnesia, and sedation (Uguz, 2019). It also relieves prolonged hiccups, anxiety and treats tetanus and acute psychosis (Dar *et al.*, 2019). Haloperidol and chlorpromazine can be given through oral, intramuscular, and intravenous injection routes (Raha *et al.*, 2012). Due to their low molecular weight, they can both close the blood placenta barrier and accumulate in fetal tissue, thus posing a risk of teratogenicity (Ahmed *et al.*, 2016).

2.2 The Fetal and Maternal Pregnancy Outcomes Following In-Utero Exposure to Varied Doses of CPZ and HAL in Albino Rats.

Studies have shown that haloperidol causes teratogenicity by its ability to diffuse the blood placenta barrier due its relatively low molecular weight (418.9 g/mol) and its lipophilic nature (Ahmed *et al*., 2016). Animal studies showed exposure to haloperidol in utero increased the risk of Limb deformity, cleft palate, micromelia, brain and skull malformation, reduced fetal weight gain, and increased fetal death (Furukawa et al., 2014). Haloperidol also causes a severe delay in developing zebra fish embryos when given at a high dose (Lin *et al.*, 2019). Previous studies have shown that chlorpromazine causes teratogenicity due to its lipophilic nature and low molecular weight of 318.9 g/mol which enable it to easily cross blood placenta barrier(Galbally et al., 2014b). A study on the effect of chlorpromazine in fetuses of animals showed an increased risk of congenital malformations such as limb reduction defects, cleft palate, reduced fetal weight, and increased fetal death (Ostinelli *et al*., 2017). Despite inadequate human studies, Altshuler and colleagues noted an increase in the relative risk of developing congenital malformation of about 0.4% compared to the general population (Dudley et al., 2017). The drug also increased perinatal death (Kunimatsu *et al.*, 2010). However, another study showed children born to mothers

who had taken chlorpromazine and other neuroleptics for more than two months were significantly taller than general population (Patton *et al.*, 2002).

2.3 The Morphological Teratogenic Effects of *In-Utero* Exposure to Varied Doses of CPZ And HAL and at Different Trimesters.

Previous animal studies has shown that chlorpromazine when administered at high doses *In-utero,* it associated with increased cases cleft palate, reduced fetal growth, and various congenital limb defects such as phocomelia and micromelia (Iqbal *et al.*, 2005). In addition, kopelman *et al* (2012) reported multiple upper and lower limb defects including phocomelia for children exposed to haloperidol from first trimester. Fluphenazine was noted to cause dilated ventricle of central nervous system, cleft palate, reduction in fetal weight and length (Ahmed *et al.*, 2016).Effects on fetuses of rats and monkeys given at a high dose of chlorpromazine include congenital skeletal malformation, cleft palate, reduced fetal weight gain, and even death(Kulkarni *et al.*, 2015). Kris found no congenital malformation in 14 children he was doing follow-ups for four years whose mothers had been on chlorpromazine at a dose of 50 to 100 mg during pregnancy (Galbally et al., 2014a).

However, another study showed children born of women who had taken neuroleptics for more than two months during pregnancy were taller than the control group whether they were breast fed or not ((Patton *et al.*, 2002)). The use of haloperidol prenatally has been linked with congenital malformations, primarily when used in the first trimester; however, no known congenital malformation has been reported in the third trimester (Ahmed *et al.*, 2016). In contrast, the use of risperidone in the third trimester by 189 women showed a high rate of congenital disabilities (Dudley *et al.*, 2017). In comparison, the other two studies carried out in the third trimester of 142 and 284 pregnant women showed no significant congenital disabilities (Wu et al., 2022). Previous studies has shown that *in-utero* exposure to haloperidol in animals has been linked to causing skull and brain malformation, reduced fetal growth, and even fetal death (MacNeil & Müller, 2016).

While human-controlled studies are inadequate, two isolated cases of severe limb reduction were reported when haloperidol was given concomitantly with other drugs;

other malformations include phocomelia when given in the first trimester, aortic valve defects, multiple upper and lower limb defects and even fetal death (Lin *et al.*2019).Comparatively, chlorpromazine exposure prenatally in animals such as rodents and monkeys has been associated with an increased risk of cleft palate, deformity, reduced fetal weight gain, and increased cases of fetal death (Iqbal et al., 2005). Prenatal exposure to haloperidol in animals has been linked to causing cleft palate, micromelia, skull and brain malformation, reduced fetal growth and even fetal death(MacNeil & Müller, 2016). While, human-controlled studies are inadequate two isolated cases of severe limb reduction were reported when haloperidol was given concomitantly with other drugs other malformations include phocomelia when given in the first trimester, aortic valve defects, fetal death, multiple upper and lower limb defects (Lin et al., 2019). Comparatively, chlorpromazine exposure prenatally in animals such as rodents and monkeys has been associated with increased risk of cleft palate, CNS defects, eye deformity, reduced fetal weight gain and fetal death (Edinoff et al., 2022b).

2.4 The Comparative Similarities and Differences in the Appendicular Skeleton between Rats and Human.

Concerning the comparative similarities and differences in the development of the appendicular skeleton of rats and human, both start in the embryonic period of the intra uterine life with formation of the primary ossification centers in the middle of the diaphysis (DeSesso & Scialli, 2018). Human experience growth plate closure and cessation of bone growth usually occur after sexual maturity while the rats maintain growth plate throughout life though longitudinal bone growth ceases by 26 weeks of age (Roach *et al.*, 2003).

In both, longitudinal bone elongation is by Endochondral ossification process and within the proximal and distal growth plate cartilage which remain functional until proximal distal elongation stops at the opposite end of the shaft of each long bone (Saeidinezhad et al., 2021). Cells in the cartilage of the growth plate elongate the long bones during four successive stages: resting/reserve, proliferation, transformation and ossification (Carney & Kimmel, 2007). These is enabled by

epiphyseal growth plate which is a multilayered cartilaginous structure located between the metaphysis and epiphysis (Burdan et al., 2009). Its composed of actively dividing chondrocyte, extracellular matrix and calcium (De Luca et al., 2001). The cells in resting cartilage are small chondrocyte occurring in pairs or singly and has cytoplasmic vacuole the amount of extracellular matrix is more than the cell number and the mitotic activity of the cells in this layer is slow with notable slow rate of collaged type 11B production (Hallett et al., 2019b).

The groove of Ranvier has chondrocyte progenitor cells and make a collar around the cells in the resting cartilage (Villemure & Stokes, 2009).Zone of proliferation is made of flat chondrocyte organized into columns whereby, mitotic activities occur at the base of the column (Mirtz, 2011). This layer predominantly shows increased synthesis of collagen type II and IX .The hypertrophic zone is below proliferation zone and is subdivided into 2, upper and lower hypertrophic zone, there is reduction of activity in these zone with reduced DNA synthesis and no mitotic division (Hallett et al., 2019a). However, there is increased production of extracellular matrix, phosphate ion and collagen appear in short chain which lead to widening of growth plate. Calcification zone chondrocyte are larger than in other layers and are swollen and terminally differentiated both hypertrophic and calcification zones are called transformation zone (Burdan *et al.*, 2009). The cells nearer to primary spongiosa depicts degenerative features and are enclosed in special vesicle formed by extracellular matrix(Villemure & Stokes, 2009). The phenotypic changes when the chondroblast divide into pre hypertrophic and hypertrophic chondrocyte in which now the volume of cytoplasm increases 10 times and the level of messenger ribonucleic acid rises for early cartilage genes (Abubakar et al., 2019a).

Morphologically the primary spongiosa resemble the lower level of hypertrophic zones but has osteoprogenitor cells. The mineralization has already begun with presence of primary ossified lamella and presence of small blood vessels as illustrated on *figure 2.1 below*. The Collagen type II genes seize to function with introduction of new genes which induce mineralization of extracellular matrix and apoptosis of terminally differentiated chondrocyte which favors occupation by vascular channel and bone marrow stromal cells (Burdan et al., 2009).

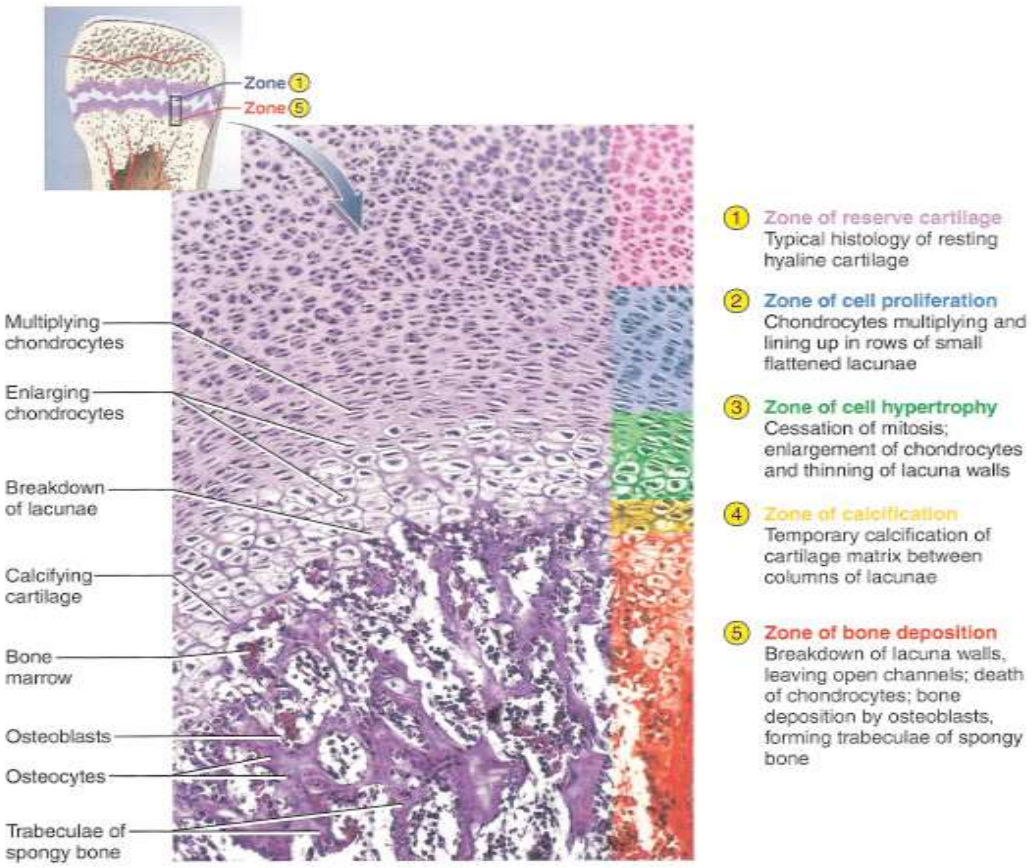


Figure 2.1: Histological Zones of an Epiphyseal Growth Plate (Adapted from Carlson, 2019).

The bone morphogenetic proteins (BMPs) regulate embryonic skeletal development for rat and human. BMPs 2 expressed in growth plate regulate chondrogenesis in growth plate and longitudinal bone growth (De Luca *et al.*, 2001). In addition, in both human and rats endochondral ossification begin with migration and condensation of the mesenchyme cells; these osteochodrogenic cells are differentiated into chondroblast which are key in endochodral bone formation, hence the shaft of long bone is formed in the intrauterine period (Saeidinezhad *et al.*, 2021). While the gestational length in mammals varies greatly the order of occurrence of gestational milestones in mammalian development is almost identical across species (Roellig et al., 2011). The highest rate of longitudinal bone growth of long bones usually occurs during the intrauterine period (Saeidinezhad *et al*., 2021).

The complex process of bone formation may be affected by interference of many factors Involved such as; cell migration and differentiation, oxygen tension and angiogenesis, drugs, hormones, calcium based mineral supply by teratogens which may modify gene expression during mesenchyme differentiation (Hallett *et al.*, 2021; MacNeil & Müller, 2016).The rate of longitudinal bone growth is majorly contributed by two factors within the growth plate; the rate of production of new cells per column and the average size of hypertrophic cells (Villemure & Stokes, 2009) .The height of the growth plate is directly proportionate to the longitudinal bone growth (Hallett et al., 2019b).

CHAPTER THREE

MATERIALS AND METHODS

3.1 The Study Settings

The study was carried out in two settings, where all animals' experimental procedures including breeding, randomization, feeding, weighing, administration of drugs, and harvesting of fetal skeletal structures, were carried in the University of Nairobi (UON) School of biological Sciences Chiromo campus as JKUAT lab was fully occupied and UON laboratory is well equipped with modern weighing machines and cages. While tissue processing and microscopy were done in the histology laboratory, department of Human Anatomy, Jomo Kenyatta University of Agriculture and Technology (JKUAT).

3.2 The Study Design

A post-Test laboratory-based experimental study design with a control only was adopted since the histomorphological and stereological analysis of the fetal epiphyseal growth plate was done after the experimentation with animals and harvesting of the fetal bones.

3.3 The Study Subjects

The study subjects in this study were sexually mature albino rats of pure breed aged between 8-9 weeks and of 3rdth series weighing between 200-230g. They were sourced from 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 large litter 11-16 and have a low incidence of spontaneous congenital anomalies (Shrivastava *et al*., 2011).

3.3.1 The Brief Description of Albino Rats

The albino rats are white rats with pink eyes and long tails. They originated from the breeding of the hooded rat's common in japan in the 19th century. Due to their

relatively docile nature and intelligence, they were domesticated for scientific based research up to date. They are used as model to humans as they possess unique features that makes them ideal for scientific studies such as white fur and pink eye which make it easy to see any changes to the skin or fur and are easy to breed and maintain in laboratory settings. They mature sexually at around the 6^{th} week, have estrous cycle lasting 4 to 5 days in which is time that the rat is sexually active.

3.4 The Sample Size Determination

Sample size determination of female albino rats was done by use of modified resource equation (Arifin & Zahiruddin, 2017). Whereby, if the standard deviation, as well as effect size, is not available, an alternative to power analysis is a resource equation where the degree of freedom (DF) for the error term in the analysis of variance is between 10 to 20 if the value is less than 10, there is need to add more animals to achieve significant result while a value more than 20 increases cost of study and raise ethical issues without much positive changes on the results. Thus, total number of rats (TA) = E (degree of freedom) +Total number of groups (TG).

The formula is n=DF/k+1, n=total number rats where DF= number of independent values that can be estimated in a statistical analysis, k = number of groups (Charon & Kantharia, 2013). Thus, n=20/10=2+1=3 rats per sub group. While total number of groups were 10, therefore 30 is an adequate representative sample size for each study group. In order to determine the number of fetal rats to be used for the experiment. All fetuses per dam were weighed and arranged in an ordinal sequence to eliminate observer biases and ensure objectivity. Three fetuses per rat were chosen by systematic simple random sampling, making a total sample size of 90 fetuses for each study group. The rest were preserved in a 10% formaldehyde solution for future use.

3.5 The Acclimatization of the Rats

Animals were kept in the typical polycarbonate rodent's cage in a humid tropical environment with 12 hours of light-dark cycles at Chiromo animal house for one

week to become accustomed to the experiment before the commencement of research.

3.6 The Feeding and Handling of Rats

The albino rats were fed on standard rodent pellets containing 20% protein 72% carbohydrates and 12% lipid as determined and water was given *ad-libitum* (El Gendy et al., 2015). The investigator was the only one who handled the rats to obtain daily weight, which was done between 0800 am and 0900hrs, and fed them by 0930. All procedures were performed according to the national institute of animal research guidelines on the care of laboratory animal's national research council (Gomez *et al.* (2010).

3.7 The Breeding of the Rats

On breeding of the rats, one sexually mature male Albino rat from the pure colony of the third series was introduced per every cage containing two females overnight and later the males were returned to their separate cages.

3.8 The Procedure Adopted in the Determination of Pregnancy

The determination of pregnancy was done as described by Zamani *et al.* (2015) whereby, a vaginal swab was done and a smear was taken for estrus cytological analysis and observation of sperm on the smear. The female rats were restrained with a gauze holder, and 1ml of saline was introduced into the vaginal cavity using a blunt-tipped disposable pipette; after that, a vaginal swab was done using a cotton-tipped swab moistened with saline buffered with phosphate. The cotton tip was rolled onto a microscope glass slide and was fixed by spraying 95% ethanol, air dried, stained with Giemsa, and observed under a light microscope at a TM X 400. Pregnancy was confirmed by the presence of large cornfield cells, many neutrophils, and scattered epithelial cells, and this was counted as the first day of pregnancy (Zamani et al., 2015). The experimental rats that tested negative for pregnancy were returned in the cages and the males were re-introduced overnight there after the pregnancy was determined.

3.9 The Grouping of the Rats

The 30 rats in each of the two study groups were first randomly assigned into either 3 rats control or 27 rat's treatment group. These 27 rats in the treatment group were then divided in to 3 broad study categories of 9 rats each as follows; 9 low dose group, 9 medium dose group, 9 high dose group. In order to determine the most venerable time of exposure. The 9 albino rats were further subdivided into three groups as follows 3 rats for trimester one, 3 rats for trimester two, and 3 for trimester three for each of the two treatment groups as illustrated on (*Figure 3.1).*

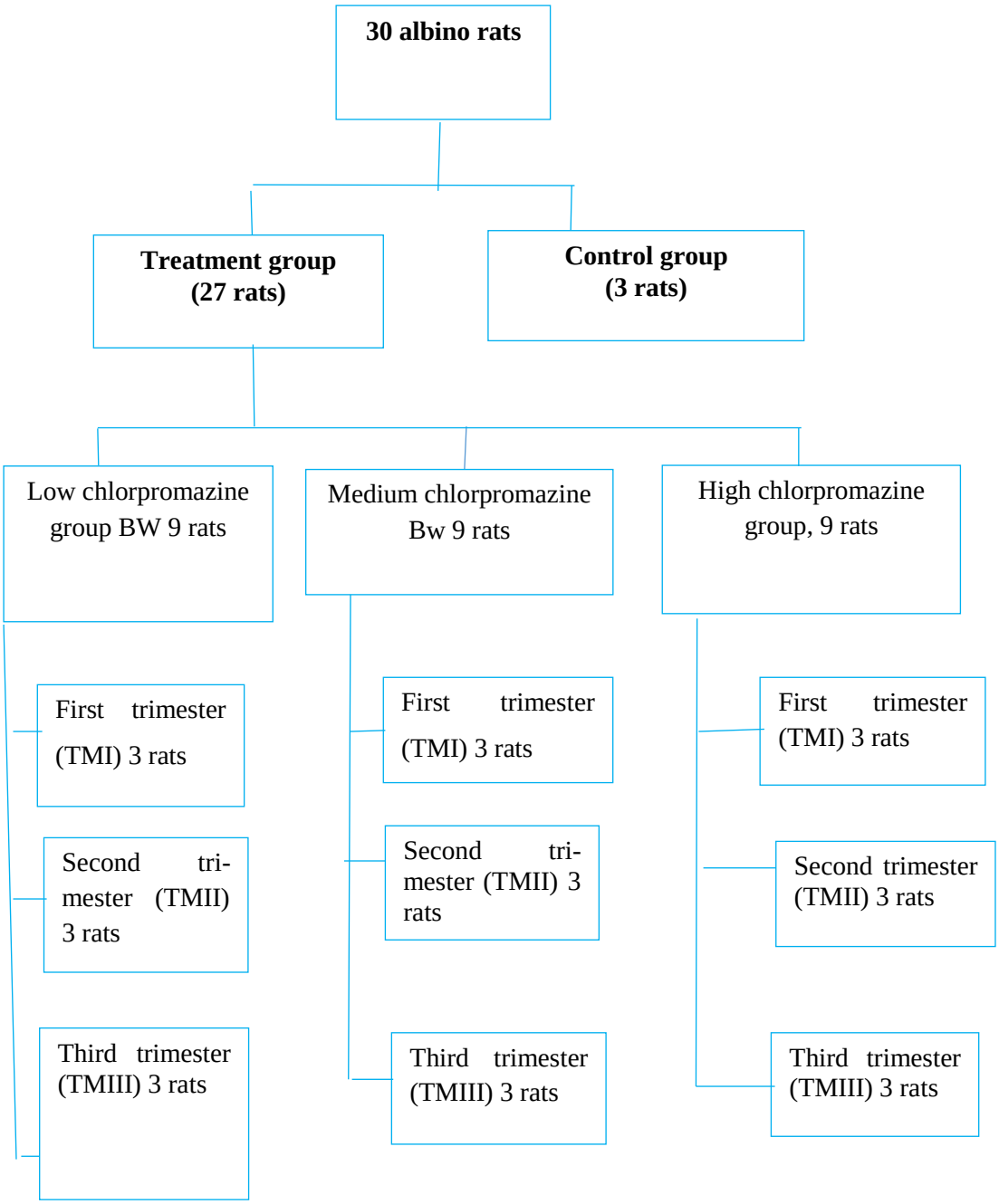


Figure 3.1: showing how the grouping of the rats was done on each of the 30 rats in the two study categories.

3.10 The Selection Criteria

The inclusion and exclusion criteria were as follows: -

3.10.1 The Inclusion Criteria

i. Healthy Albino rat that conceived after being introduced to a male.
ii. All live fetuses at the time of sacrificing of the mothers.

3.10.2 The Exclusion Criteria

i. All albino rats that become sick during experimentation.
ii. All rats that died before the day when sacrificing of the animals was done.

3.11 Determination, Calculation, and Administration of the Haloperidol and Chlorpromazine Doses

The adult chlorpromazine dosage in treating schizophrenia and other psychosis in human range from 30-200 mg per day, while haloperidol ranges between 0.5-30mg per day. These medicines were obtained from government chemist considering their batch numbers and were reconstituted using distilled water.

3.11.1 The Procedure for the Determination of the Haloperidol and Chlorpromazine Doses for the Rats

In order to determine the doses of the two medicines, a simple guide on converting human to animal dosage was applied. It states that Human Equivalent Dose (HED) mg/kg=Animal dose mg/kg multiplied by a constant ratio (Km) 6.2 (Nair & Jacob, 2016).

3.11.2 The Calculation of the Low, Medium and High Doses of Both the Haloperidol and Chlorpromazine Doses for the Rats

The minimum dose of haloperidol in humans is 0.5 mg per day. The medium dose is 15.25 mg. The maximum dose is 30mg. While for chlorpromazine, 30 mg was used as the minimum dose, 115 mg as the medium dose, and 200 mg as the high dose. The

average weight of an adult human is approximately 60 kg. The calculation of rat dosage was as follows;

a) The Determination of the High Haloperidol Doses

Highest dose -30mg

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

b) The Determination of the Medium Haloperidol Doses

Medium- dose -15.25 mg

The average weight of a man-60kg

15.25 mg = 60kg

X=1kg

X=1x15.25/60 =0.2458mg/kg

AED = HED X Km factor

Therefore, 0.2458 mg/kg x 6.2 =1.5241mg/kg

c) The determination of the Low Haloperidol Doses

Lowest dose-0.5 mg

The average weight of a man-60kg

0.5mg = 60kg

X=1kg

X=1x0.5/60 =0.00833mg/kg

AED = HED X Km factor

Therefore, 0.00833mg/kg x 6.2 =0.0517mg/kg

d) The Determination of the High Chlorpromazine Doses

Highest dose -200mg

Average weight of a man-60kg

200mg = 60kg

X=1kg

X=1x200/60 =3.3333mg/kg

AED = HED X Km factor

Therefore, 3.3333mg/kg x 6.2 =20.6665mg/kg

e) The Determination of the Medium Chlorpromazine Doses

Medium- dose -115 mg

The average weight of a man-60kg

115 mg = 60kg

X=1kg

X=1x115/60 = 1.9167mg/kg

AED = HED X Km factor

Therefore, 1.9167 mg/kg x 6.2 = 11.8835 mg/kg

f) The Determination of the Low Chlorpromazine Doses

Lowest dose-30 mg

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 =3.1 mg/kg

g) The Calculation of Specific Rat Dosages for Haloperidol

If, for example, the weight of the rat is 200g and the highest haloperidol dose is 3.1

Mg/kg**,** the calculation is done as follows;

(3.1mg/kg/1000) = 0.03mg/g

0.003mg/g x200g= 6mg

If the haloperidol tablet is 5mg, and reconstitution is done in 10ml of distilled water, then

5mg=10ml

0.6mg=

$\frac{0.6mg \times 10ml}{5mg} = 1.2ml$

3.11.3 The Procedure Adopted for Administering Various Doses of Chlorpromazine and Haloperidol by Use of Gastric Gavage Needle

1) The rat was held carefully on the neck region using the left hand.

2) Then, it was wrapped with a table cloth to avoid soiling the working area and investigator.

3) The rat was positioned against the investigator's body with its mouth facing them.

4) Gavage needle gauge 16 was inserted into the mouth of the rat gently to pass esophageal constrictions and the cardiac sphincter.

5) The bolus was put in the animal's stomach, and the gavage needle was removed gently.

3.12 The Procedure for Euthanizing the Rat, Harvesting, and Fixation of Fetuses

All animals were sacrificed humanely on day 20^{th} by use of concentrated carbon monoxide and sustained by ketamine, and viable fetuses were harvested and fixed.

3.12.1 The Materials Used in Humane Sacrificing of the Rats and Harvesting of the Fetal Bones

I. pregnant rat at gestation day 20
II. cotton wool
III. dissector jar
IV. carbon dioxide cylinder
V. mounting board
VI. mounting pin
VII. pair of scissors
VIII. A pair of toothed forceps
IX. Scalpel with handle
X. Fixative-formaldehyde
XI. Ketamine
XII. Clean gloves
XIII. Magnifying glass
XIV. Ruler
XV. Weighing machine
XVI. Jar for collecting specimen

3.12.2 The Procedure Adopted in Euthanizing and Perfusing the Rats

I. An empty dissector jar was open and wool was introduced
II. The gravid rat was introduced into the heavy bell jar with a tight-fitting lid
III. The rat was first sedated with ketamine then placed in a jar filled with carbon dioxide
IV. The rat was allowed in the jar for 3-5 minutes
V. The rat was removed from the jar and mounted on to board using board using mounting pin with dorsal side on the board.
VI. Using a pair of scissors and forceps, an incision was made in the ventral medial side extending from the xiphoid process to the symphysis pubis
VII. The fetuses were then harvested, body weight, head circumference, crown lump length, upper and lower limb length was measured

3.12.3 The Procedure Adopted In Harvesting of the Fetuses

I. The gravid rat uterine horns were excised along the ant-mesometrial borders using a pair of scissors.
II. To establish whether the fetuses were alive or not, they were touched using a probe and movement confirmed life. All live and dead fetuses were counted and recorded.
III. Resorbed endometrial sites were counted and recorded.
IV. Then, the fetuses were then harvested by cutting the umbilical cord using a pair of scissors to detach each fetus from the mother. Fetal parameters (weight and crown-lump length) were measured using electronic weighing machine and tape measure respectively.

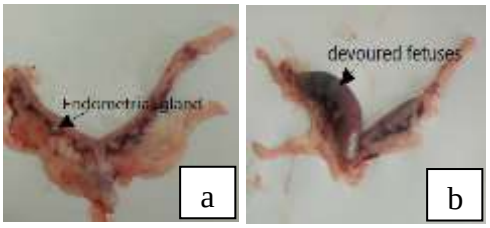


Figure 3.2: Showing (A) Gravid Uterus with Resorbed Endometrial Glands and (B) A Gravid Uterus with Devoured Fetus

Figure 3.3: Showing How Weighing of Fetuses was done

3.13 The Process of Obtaining Bone Specimen for Histoqualitative and Histoquantative Analysis

The process followed to obtain bones of the appendicular skeleton for histoqualitative and histoquantative was as follows; tibia and humerus were chosen after simple random sampling. The soft tissue from the tibia and humerus bones of different fetal rats' groups was removed by placing them in 2% potassium hydroxide for eight days to achieve complete chemical maceration of the soft tissue leaving the bone intact. The Hercules© digital vernier calipers was used to take length of the tibia and humerus upon calibration to the 0.00 mark each time a measurement was made. The measurement was made from the medial malleolus to the tibia inter condyle eminence and, for the humerus from the trochlea to the humeral head.

Limbs from fixed fetuses were harvested at proximal joints, embedded in paraffin wax, and later placed on an electric cold plate for cooling for 24 hours. The tissue blocks were oriented along their long axis and microtome at 5 micrometer thickness for histomorphological and histoquantative analysis by leitz sledge rotatory microtome. Slides were selected using a systemic uniform random sampling technique, and longitudinal sections were obtained. The tissue sections were stained and picked based on the K^{th} value (skip) of 10 calculated as follows: Number of sections made from each tibia and humerus (N) =100 each. A number of desired sections from each tibia and humerus (n) =10 K^{th} or skip value=N/n=100/10=10 every 10^{th} section was picked from section number 10 upon doing simple random sampling to determine the beginning section. To get a representative sample from the right and left tibia and humerus, picked tissue sections from each tibia and humerus (10,10 sections) were subjected to systemic uniform random sampling as follows: Number of sections made from each tibia and humerus (N)=10 number of the desired section from each tibia and humerus (n)=5 K^{th} or skip value =N/n=10/5=2 Every 2^{nd} section was picked beginning from section number 2 upon doing simple random sampling to determine the beginning section to get ten sections from each rat, i.e., five histological section from right and left tibia and five histological sections for right and left humerus. The section 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. 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. 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. Then, they were stained with Eosin for 2 minutes and rehydrated sequentially with 95% and 100% ethanol (3 dips each). Three dips in xylene for 15 seconds and the coverslips were applied and left to dry overnight in the hood.

3.13.1 The Histoquantative Methods of Obtaining the Surface Area of the Various Layers of Epiphyseal Growth Plate,

The following was done ;to determine the beginning and terminal end of the proximal and distal epiphyseal growth plates of the tibia and humerus several criteria were used based on cell size and organization as previously outlined(Alvarez *et al.*, 2000). The length of epiphyseal growth plate zones were identified using an established procedure by Bush *et al* (Bush et al., 2010) .In addition ,epiphyseal growth plate zones were identified manually by eye and marked by drawing a line as described by Abubakar et al (2019a). The junction between the different layers were outlined based on the chondrocyte's morphological characteristics and changes in histological matrix staining. The vertical height of the total epiphyseal growth plates of both proximal and distal tibia and humerus were measured as previously described (Wilsman et al., 2008). The total chondrocyte cell density was determined based on the total cell numbers counted over at least three measured areas of interest (cells/mm^2) per slide. 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. After that, histo- photomicrographs were taken using the optika© light microscope mounted with the swift cam (sc2003) camera at a total magnification of x100 (eyepiece lens x 10, objective lens x10) and x400 (eye piece lens 10, objective x40).

The selected photomicrograph after image capturing was uploaded into STEPanizer stereology analysis software for surface area and area fraction measurements as follows;

(i) Download and install the STEPanizer.

(ii) In the parameters window set magnification, image height, ruler size and test the system

(iii) Import the image to be analyzed.

(iv The counting window scale the image to match the ruler size specified in the parameter Window.

(v) Start counting the points in the image and for the cell density count the chondrocyte cells.

(vi) Export the results to excel spread sheet after counting as illustrated by (*figure 3.4).*

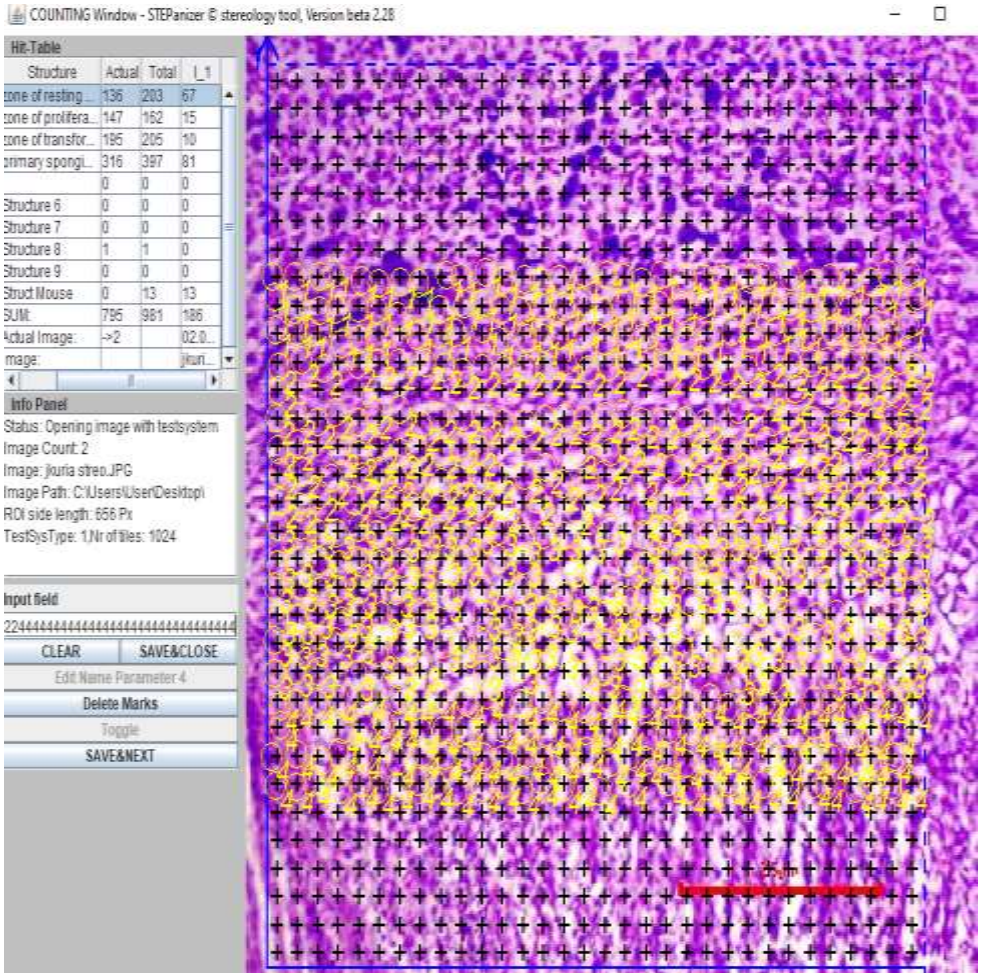


Figure 3.4: The Photograph Showing an Epiphyseal Growth Plate Tissue Section Superimposed on a Point-Counting Grid Using Stepanizer Tool for Stereological Analysis.

3.13.2 The Procedure to Determine the Numerical Density of Chondrocytes

In order to estimate the numerical cellular density of chondrocytes in the layer of epiphyseal growth plates of both proximal and distal humerus and tibia. The systematic sampled section was subsampled by systematic random sampling using the microscope stage veneer with the image been at x400 magnification, the numerical cellular density of the chondrocytes was estimated using a stereological software STEPanizer, by the counting frame the optical dissector counting rule was applied with counting in whole cells found inside the counting frame or allowance

border excluding the forbidden one. The chondrocytes were marked from reference line to lookup section.

$$\mathbf{N}\ (\mathrm{Ch}) = \frac{\sum_{i-n}^{n} Q(ch)}{\sum_{i-n}^{n} p(ref)} \cdot \frac{p}{a-h}$$

Where **N** = the number of chondrocytes displayed hitting the grid

P (ref) = The No, of points hitting the reference space, whole epiphyseal growth plate sections.

P=Represent the total number of points.

a=Represent the area of counting frame.

h=Denote the height of the dissector (Al-Mayouf et al., 2021).

The reference space comprise of all component of epiphyseal growth plate connective tissue was avoided.

3.14 The Study Variables

3.14.1The Independent Variables

Varying doses of haloperidol and chlorpromazine and gestational periods.

3.14.2 The Dependent Variables

The dependent variables include; fetal weight, maternal weight trend, gross length of the tibia, gross length of the humerus, crown-rump length, reserve cartilage percentage surface area and cellular density, proliferation zone percentage surface area and cellular density, transformation zone percentage surface area and cellular density, primary Spongiosa percentage surface area.

3.15 Data Management and Analysis

The histomorphological qualitative data was collected using photomicrographs while continuous data was collected using structured check list stored in excel spread sheets windows version 13 and then exported for analysis to SPSS program for windows version 25. The comparative descriptive analysis of numerical data was computed using ANOVA followed by turkey's post-hoc comparison t-test and MANOVA was carried out to determine main and interaction effects. Data obtained was expressed as mean± standard deviation for all values and the result whose $p< 0.05$ was considered to be statistically significant. The data was presented inform of graphs and tables.

3.16 Ethical Consideration

The ethical approval to carry out the study was sought from the animal ethics and research committee of the University of Nairobi. All procedures for animal handling, feeding, human sacrificing, and harvesting organs were performed 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).With approval from the Animal Ethics Committee University of Nairobi (Ref: FVM BAUEC/2021/326.(Bayne, 1996).

CHAPTER FOUR

RESULTS

The results of this study are presented in line with the study objectives as follows: -

4.1 The Maternal and Fetal Pregnancy Outcomes

4.1.1 Objective 1: To Comparatively Evaluate how the Prenatal Exposure to Varied Doses of Chlorpromazine and Haloperidol Influenced the Maternal and Fetal Pregnancy Out Comes.

The findings of the first objectives are presented at two levels, as follows: -

Level 1: The comparative maternal and pregnancy outcome

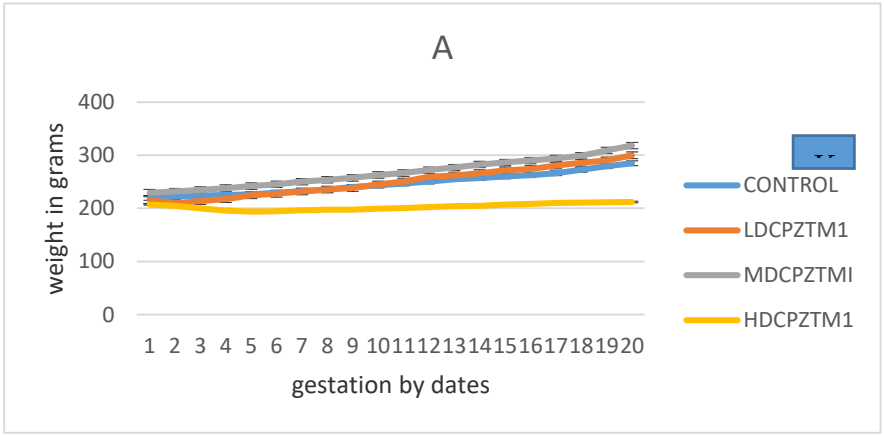
Level 2: The comparative fetal pregnancy outcomes

Level 1: The Comparative Findings on the Maternal Weight Gain Treads between the Chlorpromazine and the Haloperidol Treated Groups at TM_1, TM_2 and TM_3 against the Control.

On the comparative analysis of the daily maternal weight gain trends between the chlorpromazine and haloperidol treatment groups of low, medium and high dose as a point into the extent of the environmental toxicity in which the fetus developed *in- utero* , it was observed that rats in chlorpromazine treated groups had relatively higher daily maternal weight gain more so for medium dose chlorpromazine treated groups from trimester one this could be due to chlorpromazine at lower dose increases production of ghrelin hormone which stimulate appetite in addition it reduce the metabolism leading to storage of fat and weight gain.

However, the rats in high dose chlorpromazine treated groups as well as high dose category in the haloperidol treated groups showed remarkable reduction in weight gain trend as compared with the control (Figure 4.1 to 4.3). This could be attributed to both haloperidol and chlorpromazine effects at high dose are associated with severe drowsiness which interfere with feeding. Though, low and the medium dose

groups for haloperidol treated groups there was a steady weight gain trend that had no remarkable differences with the control. On further observation it was noted that chlorpromazine treatment groups had more negative effects on the maternal weight gains especially for the rats which received high dose from trimester one.



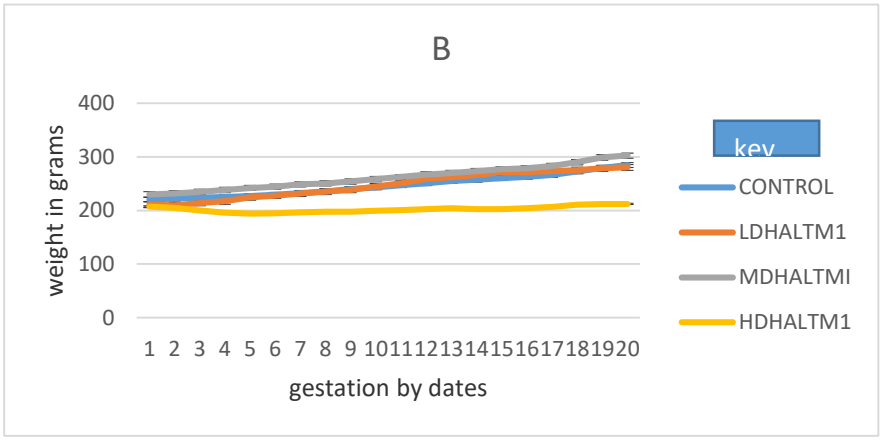


Figure 4.1: The Line Graphs Showing the Comparative Maternal Weight Gain Trends between the Rats Treated with Chlorpromazine and Haloperidol at Trimester One (TMI) Against Control.

KEY*: (A) chlorpromazine treated group*

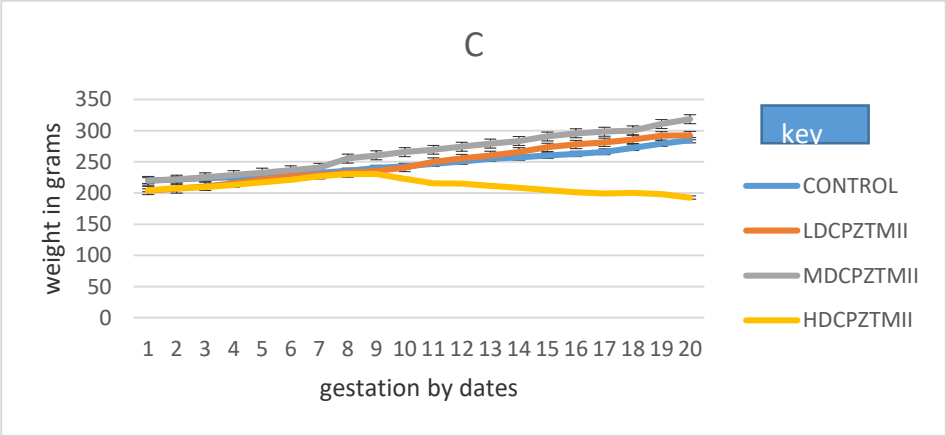
LDCPZTMI=low dose chlorpromazine trimester one
MDCPZTMI= medium dose chlorpromazine trimester one
HDCPZTMI = high dose chlorpromazine trimester one

(B) Haloperidol treated groups

LDHALTMII =*low dose haloperidol trimester one*

MDHALTMII= *medium dose haloperidol trimester one*

HDHALTMII*=high dose haloperidol trimester one*



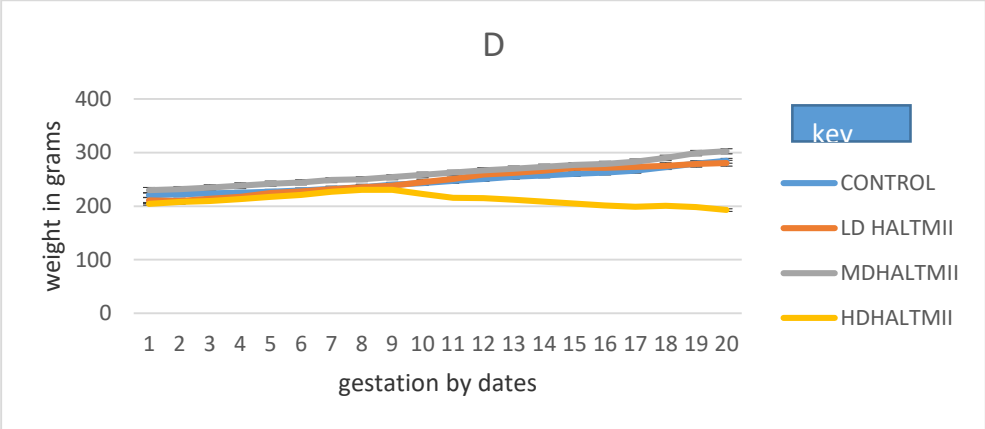


Figure 4.2: The Line Graphs Showing the Comparative Maternal Weight Gain Trends between the Rats Treated with Chlorpromazine and Haloperidol at Trimester Two Against Control.

KEY*: (C) chlorpromazine treated group*

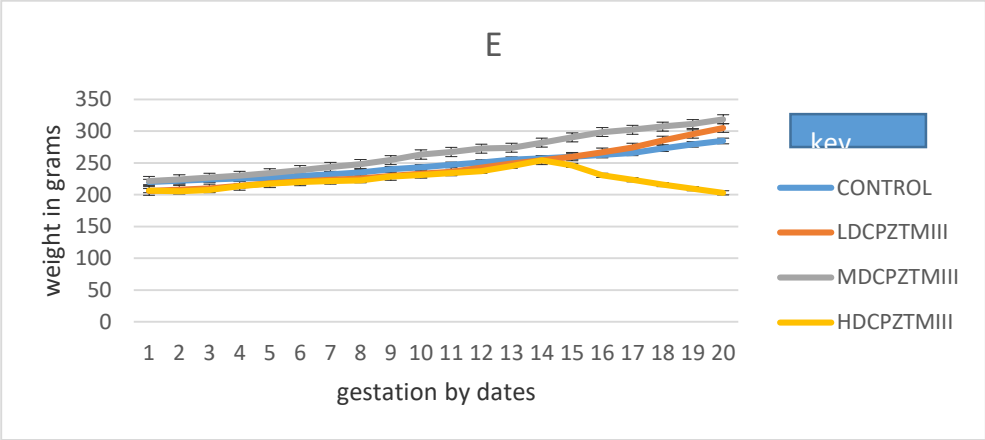
LDCPZTMII=*low dose chlorpromazine trimester two*
MDCPZTMII= *medium dose chlorpromazine trimester two*
HDCPZTMII *= high dose chlorpromazine trimester two*

(D) Haloperidol treated groups

LDHALTMII =*low dose haloperidol trimester two*

MDHALTMII= *medium dose haloperidol trimester two*

HDHALTMII*=high dose haloperidol trimester two*



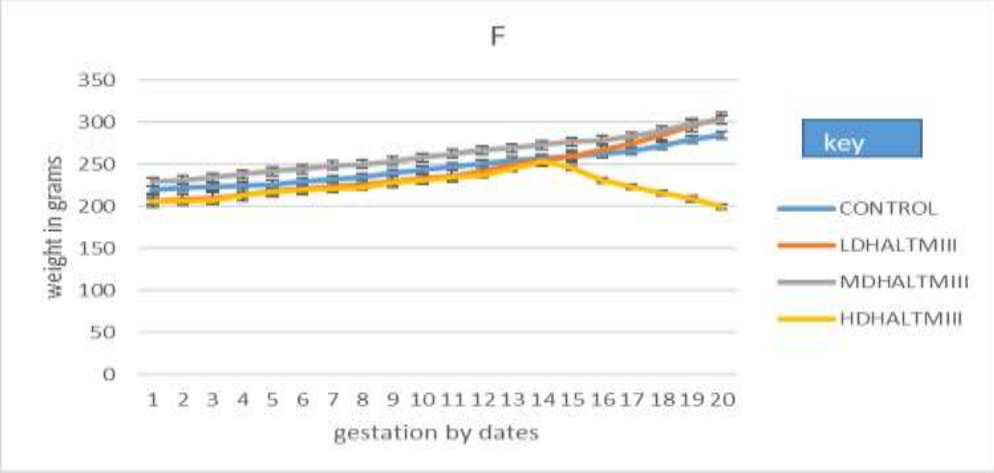


Figure 4.3: The Line Graphs Showing the Comparative Maternal Weight Gain Trends between the Rats Treated with Chlorpromazine and Haloperidol at Trimester Three Against Control.

KEY : (E) Chlorpromazine treated groups

LDCPZTMIII*=low dose chlorpromazine trimester three*
MDCPZTMIII= *medium dose chlorpromazine trimester three*
HDCPZTMIII *= high dose chlorpromazine trimester three*

(F) Haloperidol treated groups

LDHALTMIII *=low dose haloperidol trimester three*

MDHALTMIII= *medium dose haloperidol trimester three*

HDHALTMIII*=high dose haloperidol trimester three*

Level 2: The Comparative Findings on How the Two Medicines Influenced the Fetal Pregnancy Outcomes

The comparative fetal outcomes were assessed in two stages as follows;

Stage 1: The intrauterine fetal pregnancy outcomes of the mean litter sizes, embryolithalities and resorbed glands.

Stage 2: Means of Individual fetal growth and development parameters: fetal weight and Crown-lump Length.

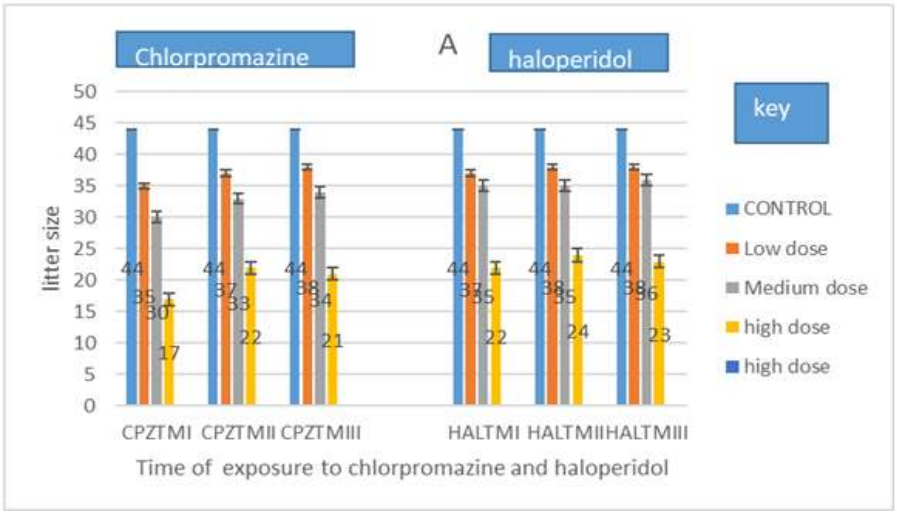
Stage 1. Comparative Analysis of Fetal Outcomes of Mean Litter Sizes, Embryolithalities and Resorbed Glands Following Prenatal Exposure to Haloperidol and Chlorpromazine

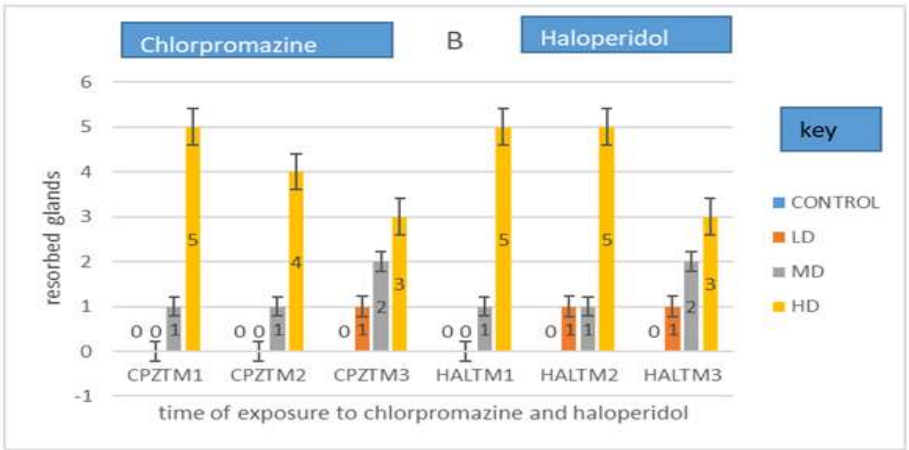
Upon comparative analysis of litter sizes, it was noted that the rats in the control group had the highest number of litter sizes ranging from 11 to 16 per rat with a total of 44. While in the treatment group the number of pups in a litter ranged from 3 to 9 with a total of 27 for chlorpromazine and 4 to 12 for haloperidol across the three dosage groups of low, medium and high with a total of 31 as illustrated in *(Figure 4.4(A)).*

When chlorpromazine was given across all trimesters, the number of resorbed endometrial glands were noted to range from 0 to 16 with the highest number being for high dose from trimester one. While for the haloperidol treatment groups the number ranged from 0 to 18 across all trimesters. There were no resorbed glands observed for the control group. The number of resorbed glands varied with dose and time of exposure, particularly for the rats which received treatment from trimester one (TMI) and two (TMII) as illustrated by *(Figure 4.4 (B))* In addition, the number

of dead fetuses was remarkably high for the two treatment groups as compared to the control and that chlorpromazine had more number of dead fetuses as compared to haloperidol.

On further analysis on how the varying dosages of the two medicines and time of exposure influenced fetal outcomes of litter size,resorbed endometrial glands and dead fetuses,they were noted to have direct dose relation in that increase in amount of the drug lead to decrease in litter size, increase in number of dead fetuses and increase in number of resorbed glands . While there was inverse relationship with the time of exposure as for negative effects were more for the rats which received the drugs from trimester one (TMI) as illustrated in (*Figure 4..4(C)).*





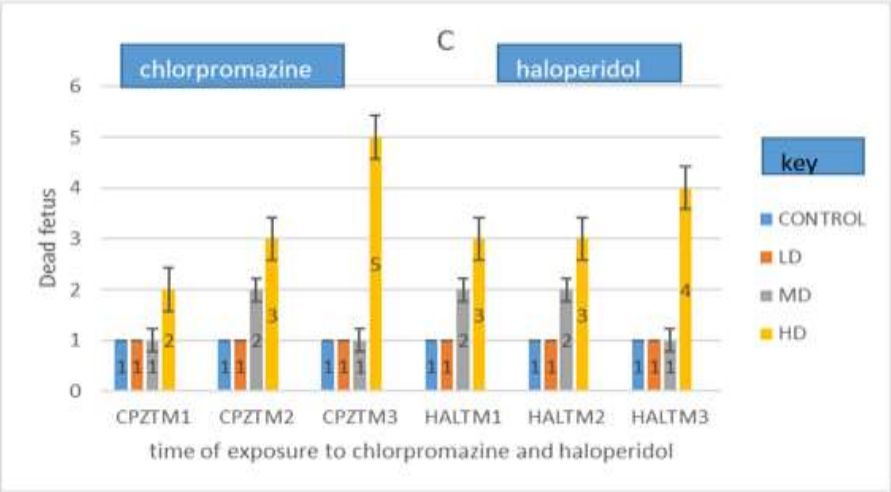


Figure 4.4: The Bar Graphs Showing the Comparative Findings on the Litter Sizes Resorbed Endometrial Glands and Number of Dead Fetuses in Treament Groups at Trimester One ,Trimester Two and Trimester Three against Controls

KEY: *(A) Comparative number of pups in a litter between chlorpromazine and haloperidol treatment groups against control.*

(B) Comparative numbers of resorbed glands between chlorpromazine and haloperidol.

(C) Comparative number of dead fetuses.

Stage 2: The Comparative Findings on How the *In-Utero* Exposure to Haloperidol and Chlorpromazine Influenced Fetal Outcomes of Mean Fetal Weight and Crow Lump Length.

On the comparative analysis of how *in-utero* exposure to the two medicines influenced fetal weight and crown- lump length. The findings were presented in two levels as follows;

Level 1: One way analysis of variance (ANOVA)

Level 2: Multivariate analysis of variance (MANOVA)

Level 1: The ANOVA Findings on How In-Utero Exposure to Chlorpromazine and Haloperidol Influenced the Crown-Lump Lengths and Fetal Weights.

When the overall effects of the two drugs were assessed using one way analysis of variance (ANOVA), without taking into account the dosage or the duration of treatment it was observed that, they both had significant effects on the fetal rat's growth and development as shown by F and P values as follows; fetal weight ($F(18, 38) = 27.283$, $p=0.001$) and crown lump length ($F(18, 38) =70.562$, $p=0.001$) (*Table 4.1*).

On further observation, it was noted that means of fetal weight and crown lump length was greatly affected by the time of exposure for the high dose treated groups of both chlorpromazine and haloperidol and more so chlorpromazine treated group from trimester one. Though, medium dose chlorpromazine treatment group depicted statistically significant increase $p<0.05$ especially for those who received treatment from trimester one as illustrated in (*Table 4.1*).

Table 4.1: The Anova Table Showing the Descriptive Statistics on How the In-Utero Exposure to Varied Doses to Varied Doses of Haloperidol and Chlorpromazine Influenced Means of Fetal Weights and Crown-Lump Lengths.

The study groups	Study groups and dosage levels	The time of exposure to treatment	Mean fetal weight (g)±SD	Mean Crown lump length (mm)±SD
Control	No treatment given(C)	None	6.5667±0.37	5.36±0.14
Chlorpromazine treatment group	Low dose(3.1mg/kg/bw)	(TMI)	6.88±0.15	5.55±0.23
		(TMII)	7.21±0.28	5.73±0.16
		(TMIII)	6.93±0.04	5.59±0.03
	Medium dose (11.88mg/kgbw)	(TMI)	8.16±0.07*	5.92±0.06*
		(TMII)	7.52±0.23	5.78±0.06
		(TMIII)	7.44±0.92	5.62±0.17
	High dose group (20.67mg/kg/bw	(TMI)	3.52±0.34*	3.15±0.08*
		(TMII)	2.98±0.84*	3.14±0.54*
		(TMIII)	3.42±0.47*	2.27±0.22*
Haloperidol treatment groups	Low dose groups	(TMI)	6.41±0.04	5.19±0.13
	(0.05mg/kg/bw	(TMII)	6.39±1.02	5.13±0.09
		(TMIII)	6.66±0.35	5.40±0.06
	Medium dose groups	(TMI)	7.31±1.44*	5.57±0.25*
	1.52mg/kg/bw	(TMII)	6.4±0.46	5.24±0.2
		(TMIII)	5.82±0.73	5.49±0.44
	High dose groups	(TMI)	4.17±0.36*	3.46±0.3*
	3.1mg/kg/bw	(TMII)	3.60±0.28*	3.48±0.26*
		(TMIII)	3.38±0.31*	3.34±0.35*
Overall comparison by Anova F and P values			F(18,38)= 57.283	F(18,38)=70.562
			P=0.001	P=0.001

Key: *values are conveyed as means ± standard deviation of means n=3 per group. (*) the figure bearing the asterisk shows that they are significantly different ($p<0.05$) with the control, using one-way ANOVA with Tukey post hoc multiple comparison t-test.*

Level 2: The Comparative Findings on How *In-Utero* Exposure to the Two Medicines Influenced the Means of Fetal of Weights and Crown Lump Lengths Using Multiple Analysis of Variance (MANOVA).

The comparative analysis on how *in-utero* exposure to varying doses of chlorpromazine and haloperidol influenced the fetal outcomes of weight and crown lump length using MANOVA was done at 3 stages (1, 2 and 3) whereby **Stage 1** results were meant to evaluate whether the observed global effects and their interactions were due to influence of independent variables of drug, dosage and trimesters or due to chance. **Stage 2** Results were meant to evaluate how the individual drug their dosages and the time of exposure influence each of the fetal parameters**. Stage 3** the results were meant to evaluate pairwise comparisons between chlorpromazine and haloperidol at similar dosage levels and time affected the two fetal parameters.

Stage 1: Multivariate Analysis on How Chlorpromazine and Haloperidol Plus their Interactions Generally Influenced Means of Fetal Weights and Crown Lump Length.

Upon carrying out Inferential analysis of the global effects of chlorpromazine and haloperidol on fetal weight and crown lump length showed that the effects were statistically significant in different proportions, overall and due to interactions between drug, dosage, and trimester (see Table 4.2.) below as follows; -

(a) The individual main effects of chlorpromazine and haloperidol (drug) ($F(2,37)=5.210, p<0.01$; Wilks' lambda(λ)=.780;partial Eta squired(η^2 = .220 (dosages)($F(4,74)=90.419, p<0.001$; Wilks' lambda(λ)=.029;partial Eta squired(η^2 = .830 and (trimesters) ($F(4,74)=3.190, p<0.001$; Wilks' lambda (λ)=.727;partial Eta squired(η^2 =.147

(b) Two way interaction drug and dosage ($F(4,74)=11.715, p<0.01$;Wilks' lambda(λ)=.375;partial Eta squired(η^2 = .388 drug and trimester ($F(4,74)=3.417$, $p<0.013$; Wilks' lambda(λ)=.712;partial Eta squired(η^2 = .156 dosage and trimester($F(8,74)=3.679$,

p<0.001; Wilks' lambda (ƛ)=.512; partial Eta squired ($ŋ^2$ = .285 as illustrated in (table *4.2.).*

Though at three way interaction there was no statistical significant difference for drug, dosage and trimester.

Table 4.2: The Stage 1 MANOVA Table on How Chlorpromazine and Haloperidol and their Interaction Globally Influenced Means of Fetal Weight and Crown-Lump Length.

Types of MANOVA evaluation at level 1	**The multivariate statistical tests parameters applied**						
	The comparative global effects Assessed	**The Parameters Used**	**MANOVA test statistics (wilks'labda**	**Statistics (f)**	**Hypothesis Degree of freedom**	**Error Degree of freedom**	**Sig .<0.05**	**The proportion of variance Partial Eta Squared**
The global main effects of drug, dosage and trimesters on mean fetal WT and CRL	Assessment of whether or not the overall effects were due to chance or the drugs	Intercept	.002	8130.677[b]	2.000	37.000	.000	.998
The individual main effects of the drug, dosage and time of exposure on fetal parameters	Assessment of whether or not the observed overall effects were due to chlorpromazine or haloperidol	Drug	.780	5.210[b]	2.000	37.000	.010	.220
	Assessment of whether or not the observed effects were due to low, medium or high dose of either CPZ or HAL	Dosage	.029	90.419[b]	4.000	74.000	.000	.830
	Assessment of whether or not the observed effects were due to differing time of exposure (TMI,TMII,TMIII)	Trimester	.727	3.190[b]	4.000	74.000	.018	.147
Two-way interaction effects on the various layers of proximal tibia and humerus	Assessment of whether or not the observed overall effects were due to interaction between drug and dosage	Drug * dosage	.375	11.715[b]	4.000	74.000	.000	.388
	Assessment of whether or not the observed overall effects were due to interaction between drug and trimester	Drug * trimester	.712	3.417[b]	4.000	74.000	.013	.156
	Assessment of whether or not the observed overall effects were due to interaction between dosage and trimester	dosage * trimester	.512	3.679[b]	8.000	74.000	.001	.285
The three-way interaction effects	Assessment of whether or not the observed overall effects were due to interaction between drug, trimester and dosage	Drug * dosage * trimester	.829	.907[b]	8.000	74.000	.515	.089

***Key** :(*) indicates interaction effects, while (ƀ) indicate exact statistics using MANOVA*

Stage 2: The Manova Level 2 Findings on How the *In-Utero* Exposure to Individual Drug, Dosage, Trimester and their Interactions Influenced Fetal Parameters of Weights and Crown-Lump Lengths

On further analysis of individual effects of chlorpromazine and haloperidol dosages and time of exposure influenced the two fetal growth and development markers it was noted that;-

(i) At individual level the drug, dose and time of exposure depicted varying contribution on means of fetal weight and crown lump length with the dosage contributing the highest followed by the time of exposure as shown by partial Eta squared ($ŋ^2$)(*Table 4.3).*

(ii) At the two-way interaction effects of independent variables (drug and dosage, drug and trimester, dosage and trimester) on fetal weight and crown-lump length it was observed that there was statistically significant main effects and interaction effects with drug and dosage having the highest contribution followed by dosage and trimester of exposure as shown by partial Eta squired ($ŋ^2$) (*Table 4.3).*

(iii) The three-way interaction effects of the independent variables (drug, dosage, and trimester) on the means of fetal weight and crown-lump length were not statistically significant.

Table 4.3: The Manova level 2 Table Showing Comparative Findings on How Individual Drug, Dosages and Time of Exposure Influenced the Fetal Weight and Crown-Lump Lengths.

Test of between subjects						
Independent Variables	**Dependent Variable**	**Df**	**Mean Square**	**The ratio of mean squire for the independent variables to the mean square for the error (Statistics)**	**Sig.**	**Partial Eta Squared**
Corrected Model Wilks' lambda	Fetal weight in grams	18	9.369	27.283	.001	.928
	CRL in cm	18	4.126	70.562	**.001**	.971
Intercept	Fetal wt. in grams	1	1460.089	4252.085	**.001**	.991
	CRL in cm	1	963.354	16474.854	**.001**	.998
Drug	Fetal wt. in grams	1	3.561	10.370	**.003**	.414
	CRL in cm	1	.034	.582	.450	.315
Dosage	Fetal wt. in grams	2	73.424	213.826	**.001**	.918
	CRL in cm	2	34.204	584.936	**.001**	.969
Trimester	Fetal wt. in grams	2	1.826	5.318	**.009**	.219
	CRL in cm	2	.173	2.962	.064	.135
Drug	Fetal wt. in grams	2	3.824	11.138	**.001**	.370
Dosage	CRL in cm	2	1.320	22.578	**.001**	.543
Drug	Fetal wt. in grams	2	.062	.180	.836	.009
Trimester	CRL in cm	2	.320	5.473	**.008**	.224
dosage	Fetal wt. in grams	4	1.113	3.243	**.022**	.254
trimester	CRL in cm	4	.229	3.910	**.009**	.292
Drug	Fetal wt. in grams	4	.199	.579	.680	.057
dosage trimester	CRL in cm	4	.042	.717	.586	.070

Key :(*) indicates interaction effects, while (Ь) indicate exact statistics using MANOVA

Stage 3: The Manova Level 3 Pairwise Comparison on How the Two Medicines Influenced the Mean Fetal Weights, Crown Lump Lengths when Administered at the Same Dosage Levels and Within the Same Trimesters.

Upon carrying out pairwise comparison on how the independent variables influenced the two fetal parameters of weight and crown-lump length in-order to establish how chlorpromazine compared to haloperidol, it was observed that the means were significantly higher for chlorpromazine treatment group in trimester two and significantly lower for trimester three as compared to haloperidol treatment groups as illustrated in ***(Table 4.4).***

Table 4.4: The MANOVA Table on Pairwise Comparisons of How *In-Utero* Exposure to Chlopromazine and Haloperidol Influenced the Means of Fetal Weight and Crown Lump Length When Exposed Within Similar Dosages and Time.

			Pairwise comparison			
Dependent variable	**Dose level**	**The time of treatment exposure**	**Chlorpromazine treatment (CPZ)**	**Haloperidol treatment (HAL)**	**Mean difference between CPZ and Hal treatment**	**Significant <0.05**
Fetal weight	Low Dose	TM1	CPZ	HAL	.478	.336
		TM2	CPZ	HAL	.818	.095
		TM3	CPZ	HAL	.277	.567
	Medium Dose	TM1	CPZ	HAL	1.552*	.002
		TM2	CPZ	HAL	1.119*	.025
		TM3	CPZ	HAL	1.625	.002
	High Dose	TM1	CPZ	HAL	-.654*	.008
		TM2	CPZ	HAL	-.625*	.001
		TM3	CPZ	HAL	-.044*	.028
Crown-Lump Length	Low	TM1	CPZ	HAL	.357	.078
		TM2	CPZ	HAL	.596*	.005
		TM3	CPZ	HAL	.197	.324
	Medium	TM1	CPZ	HAL	.347*	.007
		TM2	CPZ	HAL	.545*	.009
		TM3	CPZ	HAL	.134*	.001
	High	TM1	CPZ	HAL	-.312	.022
		TM2	CPZ	HAL	-.339	.095
		TM3	CPZ	HAL	-.074*	.000

4.1.2 Objective 2: The Comparative Evaluation on How the Prenatal Exposure to Haloperidol and Chlorpromazine on the Histomorphological Effects on Fetal Appendicular Skeleton

The findings on the histomorphological effects of *in-utero* exposure to haloperidol and chlorpromazine on the development of the fetal appendicular skeleton are presented in two levels as follows:

Level 1: The Comparative findings on how the two medicines influenced the histo-cyto architecture of the different layers of both proximal and distal epiphyseal growth plates of the fetal long bones.

Level 2: The Comparative findings on how the two medicines influenced the histological thickness and cellular densities of the different layers of both proximal and distal epiphyseal growth plates of the fetal long bones.

Level 1: The Comparative Findings on How the Two Medicines Influenced the Histocytchitecture of the Different Layers of both Proximal and Distal Epiphyseal Growth Plates of the Fetal Long Bones.

On the comparative findings on how the two medicines influenced histo-cytoarchtecture of the different layers of both proximal and distal epiphyseal growth plate of humerus and tibia, it was generally observed that the cellular and the extra cellular components in both proximal and distal tibia and humerus had no comparable difference.

On assessment of resting cartilage, the chondrocyte cells which are usually inert and majorly give rise to clone of proliferative chondrocytes were noted to maintain their tissue architecture whereby there were small in size sparsely distributed stained darkly with haematoxylin and had substantial amount of cartilage matrix for low and medium chlorpromazine and haloperidol treatment groups across the trimesters (TMI,TMII,TMIII) while for high dose treatment group the cell were more sparsely distributed and some cells lacked nuclei as compared to the control across all trimester (*Figure 4.5,4.9 and 4.13).*

In the zone of proliferation, the chondrocyte cells which are actively dividing forming new column of chondrocytes which push epiphyseal growth plate upward. The cells were noted to increase in size forming columns which resembled stack of coins with increased cytoplasm, darkly stained nuclei and the cartilage matrix surrounded the chondrocytes more so for the two treatment groups at low and medium dose as compared to the control across all the trimesters more so for chlorpromazine treatment groups from trimester one as illustrated on (*Figure 4.6, 4.8, and 4.114).*

In the zone of transformation which majorly allow transition of chondrocytes from actively dividing form to large inactive form. The cells were noted to increase in size with most of them been devoid of nuclei more so for the high dose chlorpromazine groups (HDCPZGs). Though for medium and low dose treatment groups, the cartilage matrix surrounding the chondrocytes increase and appeared whitish and the cells were large with larger lacunae as compared to the control across all trimester (*Figure 4.7, 4.11, and 4.15).* In the zone of primary spongiosa which is the site for the formation of spongy bone, the chondrocyte die and the cartilage matrix calcify creating a network of calcified spicules that are invaded by the blood vessels and osteoblasts which deposit bone matrix on the spicules forming trabecular of bone. The chondrocytes had no nuclei for all the treatment groups and the control while for low and medium dose treatment group was noted to have a greater number of bone trabecular and increased number of osteoblast cells more so for the ones which received treatment from trimester one (Figure *4.8, 4.12, and 4.16).*

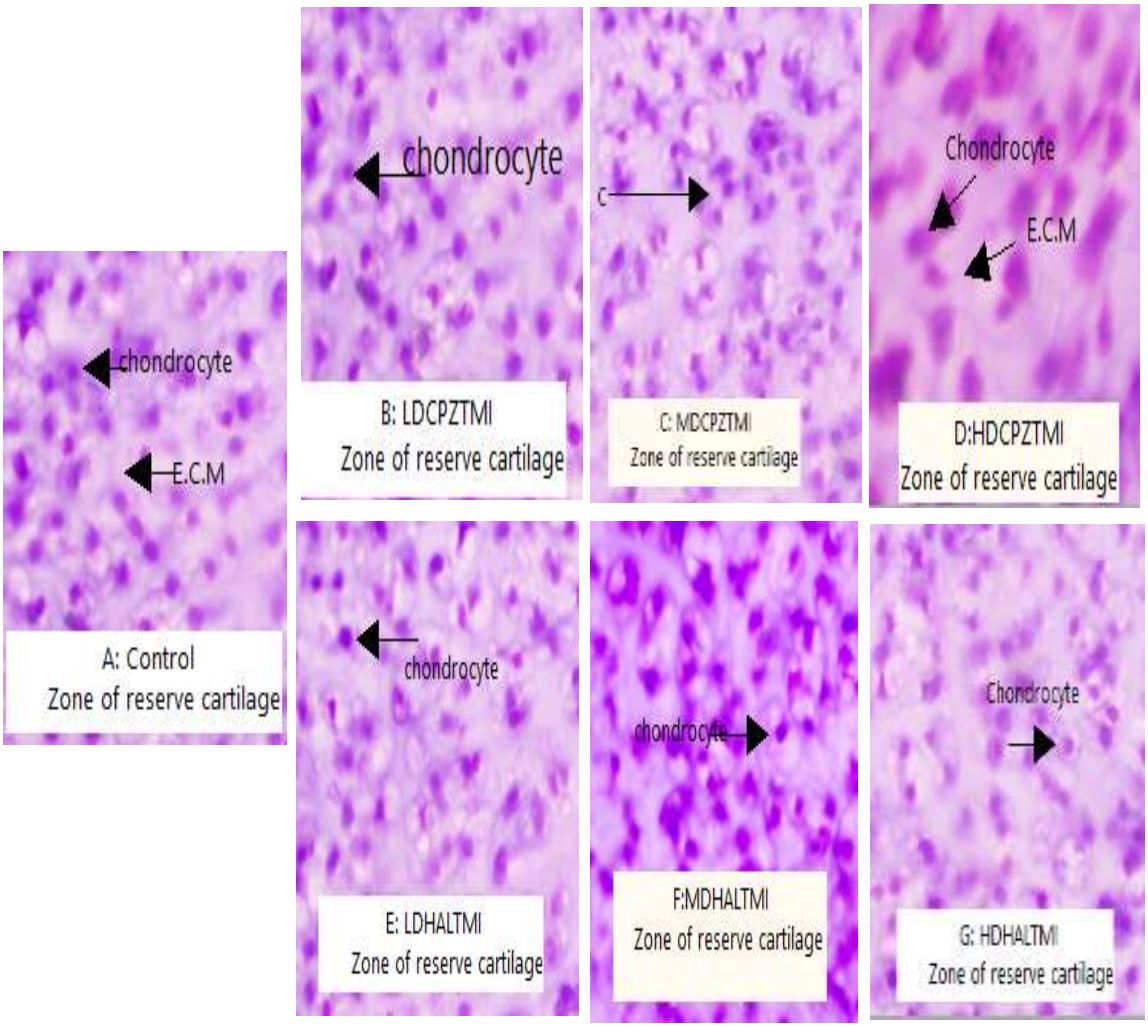


Figure 4.5: The Zone of Resting Cartilage Showing Comparative Chondrocytes Distributions and their Densities in the Treatment Groups at TM1 against the Control.

Key: A *Control -showing densely populated chondrocytes which are small in size E.C.M (extra cellular matrix) B:-LDCPZTMI-Low dose chlorpromazine trimester one showing similar distribution of chondrocyte to the control C:-MDCPZTMI-Medium dose chlorpromazine trimester one D:-HDCPZTMI-High dose chlorpromazine trimester one showing sparsely distributed chondrocytes with more extra-cellular matrix. E:-LDHALTMI-Low dose haloperidol trimester one with similar distribution of chondrocyte to control F:-MDHALTMI- Medium dose haloperidol trimester one with densely populated number of chondrocytes G: - HDHALTMI-High dose haloperidol trimester one showing sparsely distributed chondrocytes magnification is x100.*

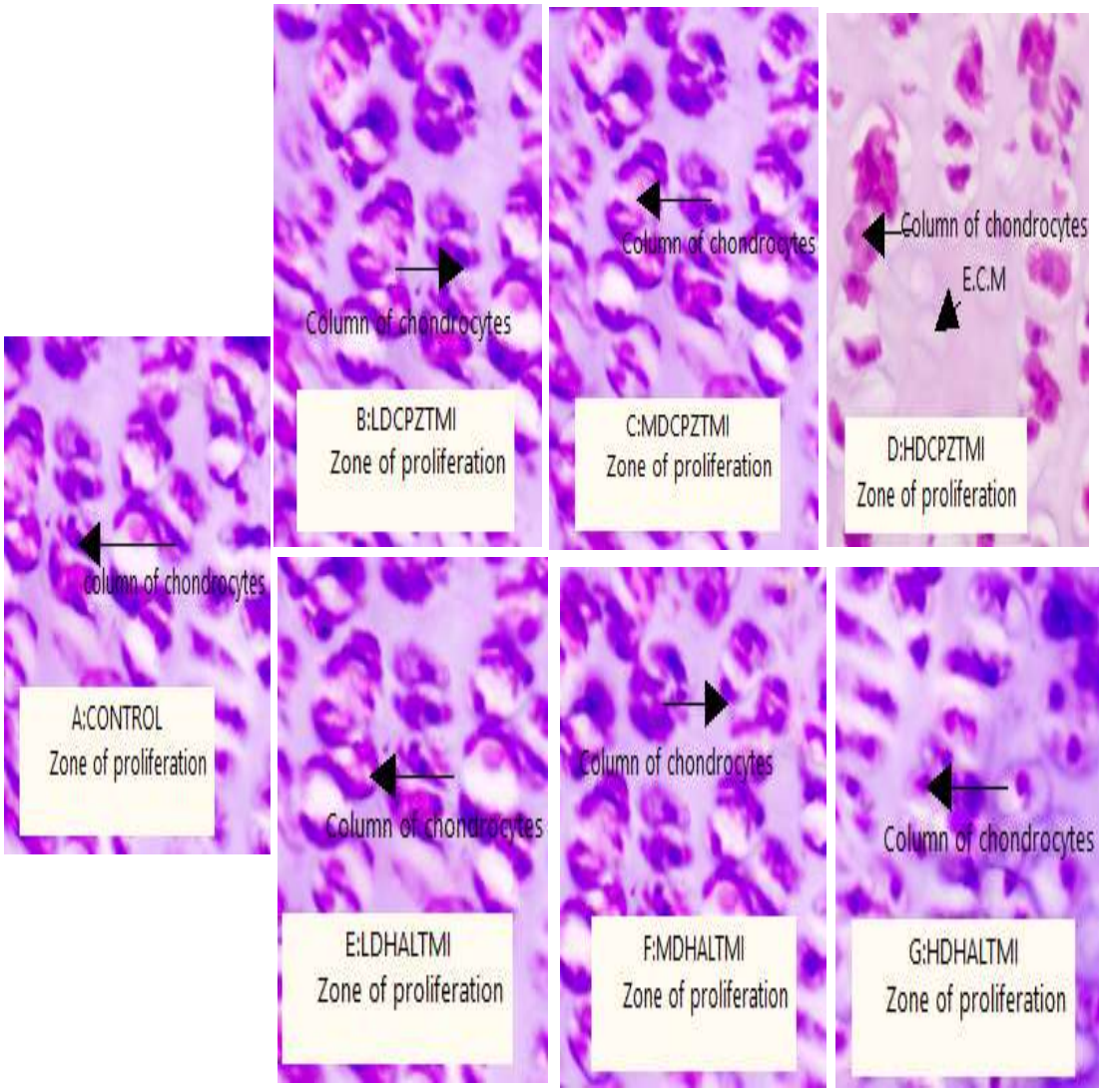


Figure 4.6: The Proliferation Zone Showing the Comparative Distribution of Proliferating Chondrocytes Plus their Densities for the Treatment Groups at TM_1 against Control.

Key: ***A*** *Control- showing column of chondrocytes B: LDCPZTMI-Low dose chlorpromazine trimester one showing similar distribution of chondrocyte to the control C: MDCPZTMI-Medium dose chlorpromazine trimester one D:-HDCPZTMI-High dose chlorpromazine trimester one E:-LDHALTMI-Low dose haloperidol trimester one F:-MDHALTMI- Medium dose haloperidol trimester one G:-HDHALTMI-High dose haloperidol trimester one showing fewer columns of chondrocytes as compared to the control magnification is x400.*

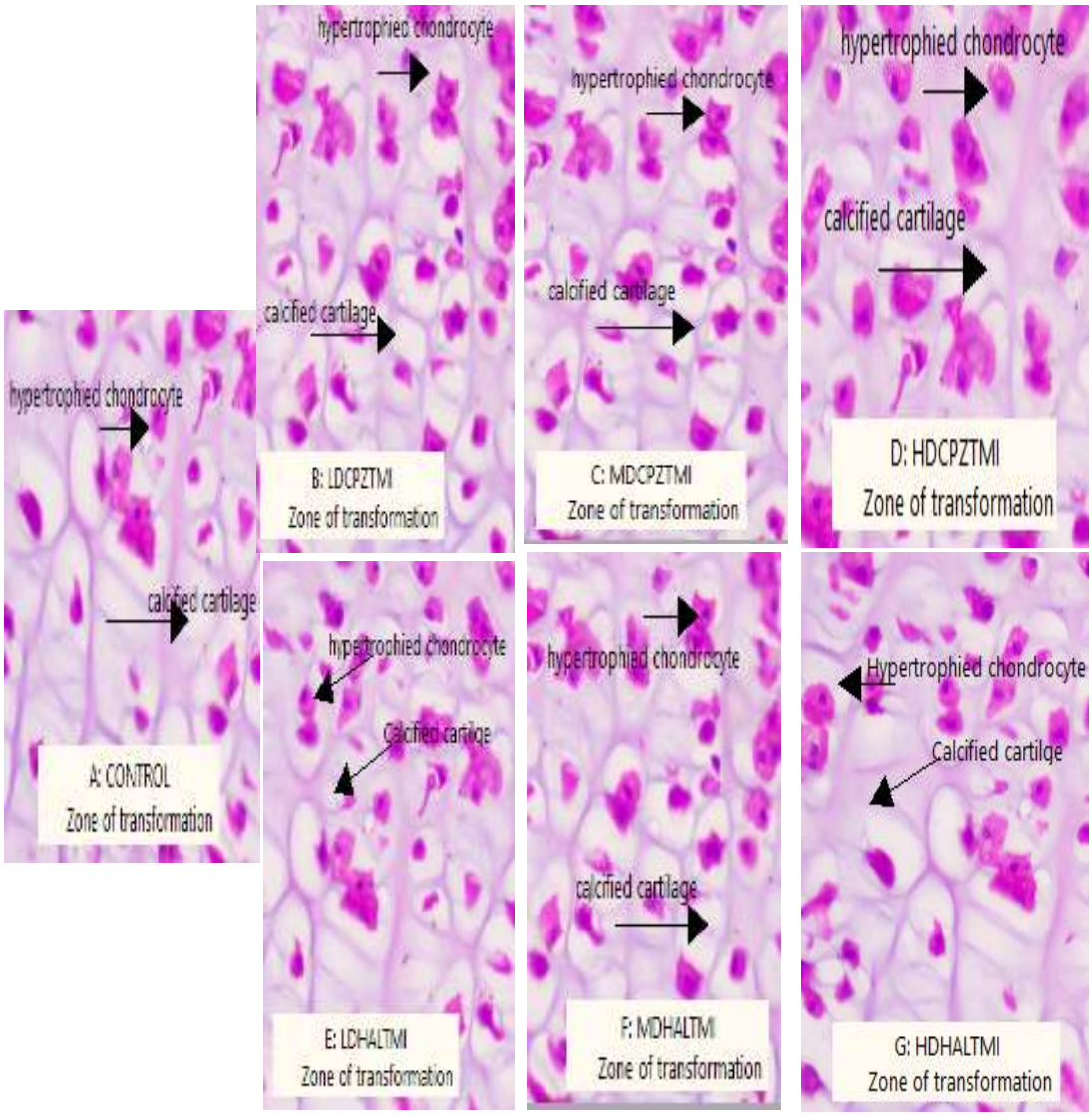


Figure 4.7: The Zone of Chondrocyte Transformation Showing the Comparative Hypertrofication of Chondrocytes in the Treatment Groups against the Control at TM1

Key: A *Control -showing hypertrophied chondrocytes B: LDCPZTMI-Low dose chlorpromazine trimester one similar distribution of chondrocyte trimester one F: - MDHALTMI-Medium dose haloperidol trimester one G:-HDHALTMI-High dose haloperidol trimester one magnification is x400*

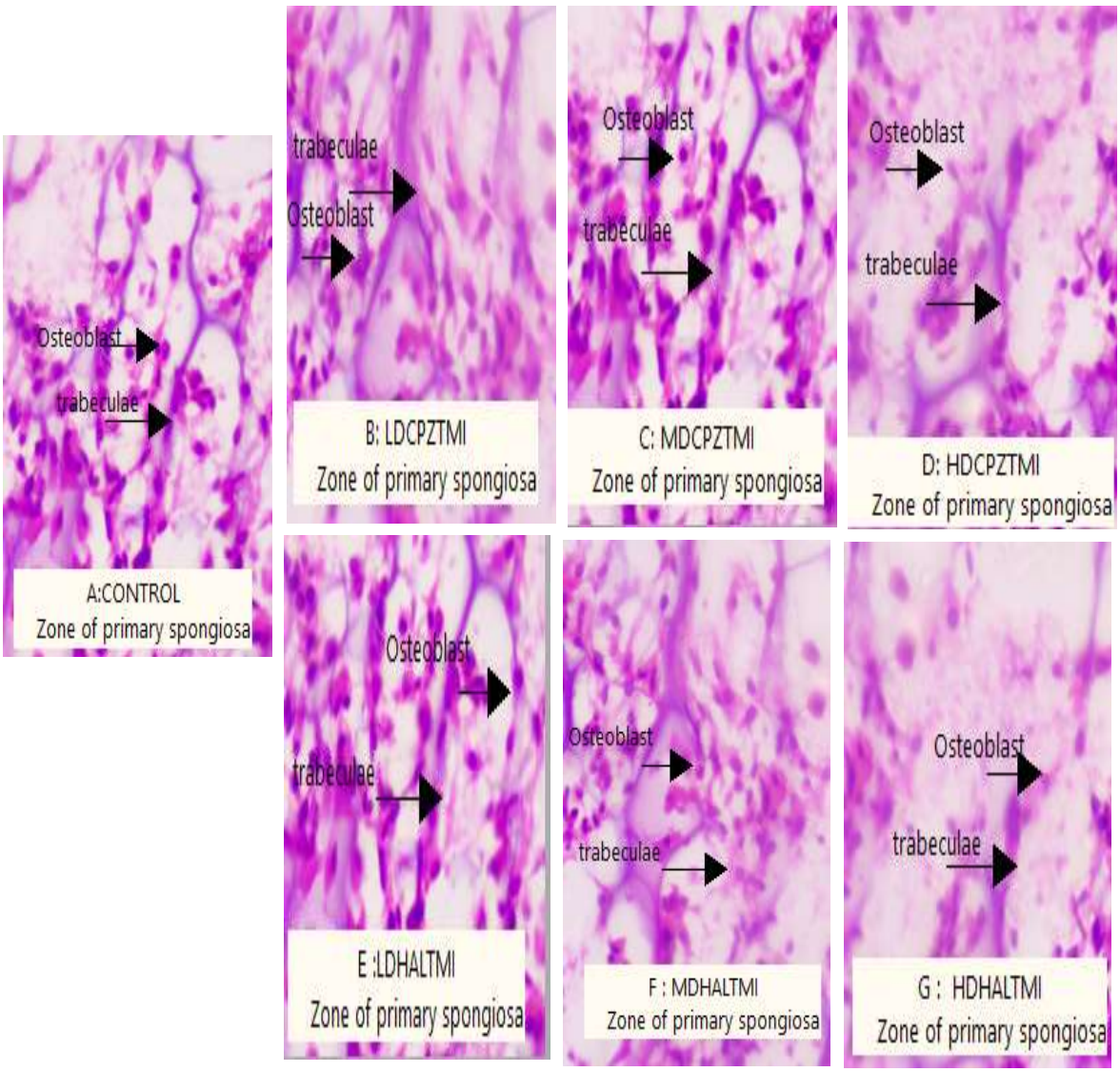


Figure 4.8: The Zone of Primary Spongiosa Showing Trabecular of New Bones and Osteoblast Cells in the Treatment Groups at TM_1 against the Control

Key: A Control- showing new bone trabecular B: LDCPZTMI-Low dose chlorpromazine trimester one C:-MDCPZTMI-Medium dose chlorpromazine trimester one D:- HDCPZTMI-High dose chlorpromazine trimester one E:-LDHALTMI-Low dose haloperidol trimester one F: - MDHALTMI-Medium dose haloperidol trimester one G:- HDHALTMI-High dose haloperidol trimester one magnification is x400

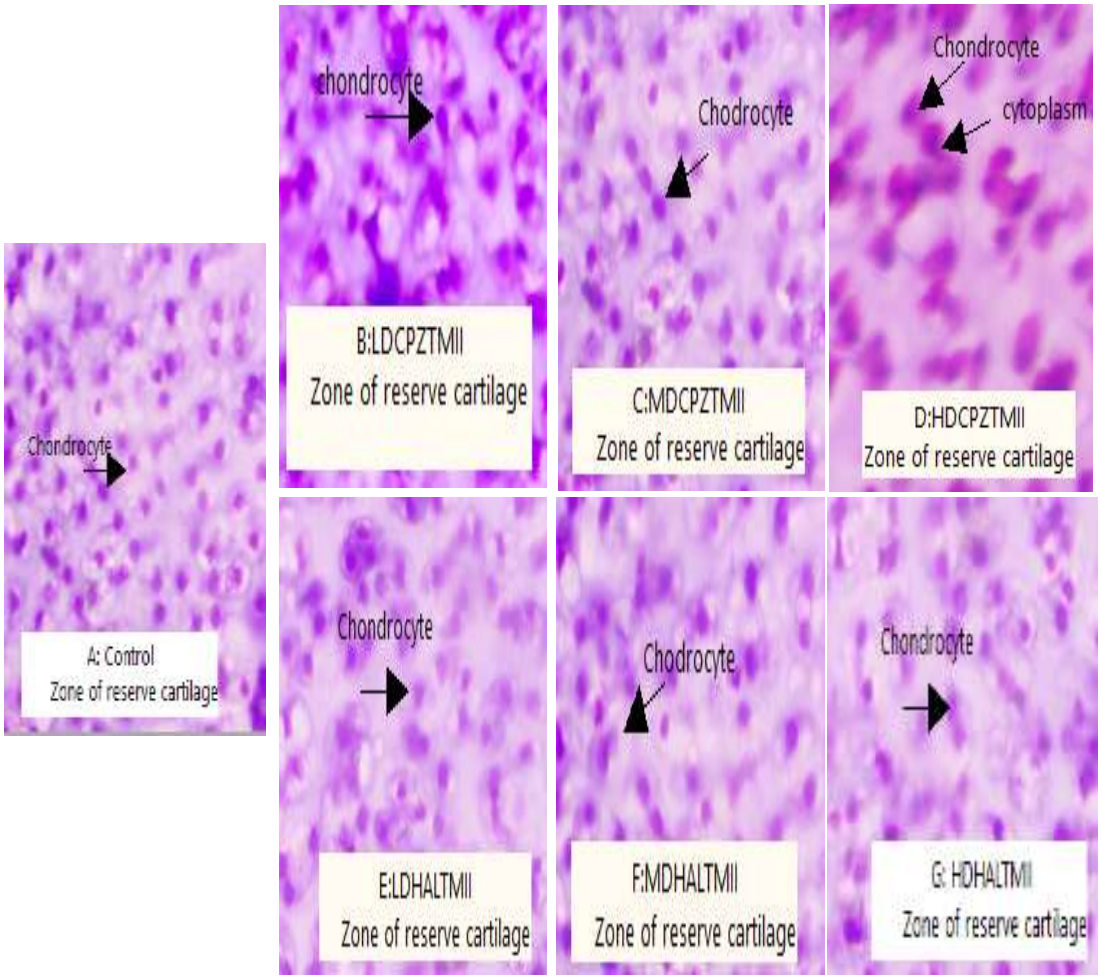


Figure 4.9: The Zone of Resting Cartilage Showing Comparative Chondrocytes Distributions and their Densities in the Rats Treated at TM_2 against the Control.

Key: A *Control- showing column of chondrocytes B:-LDCPZTMII-Low dose chlorpromazine trimester two C:-MDCPZTMII-Medium dose chlorpromazine trimester two D:-HDCPZTMII-High dose chlorpromazine trimester two E:-LDHALTMII-Low dose haloperidol trimester two F: - MDHALTMII-Medium dose haloperidol trimester two G:-HDHALTMII-High dose haloperidol trimester two magnification is x400.*

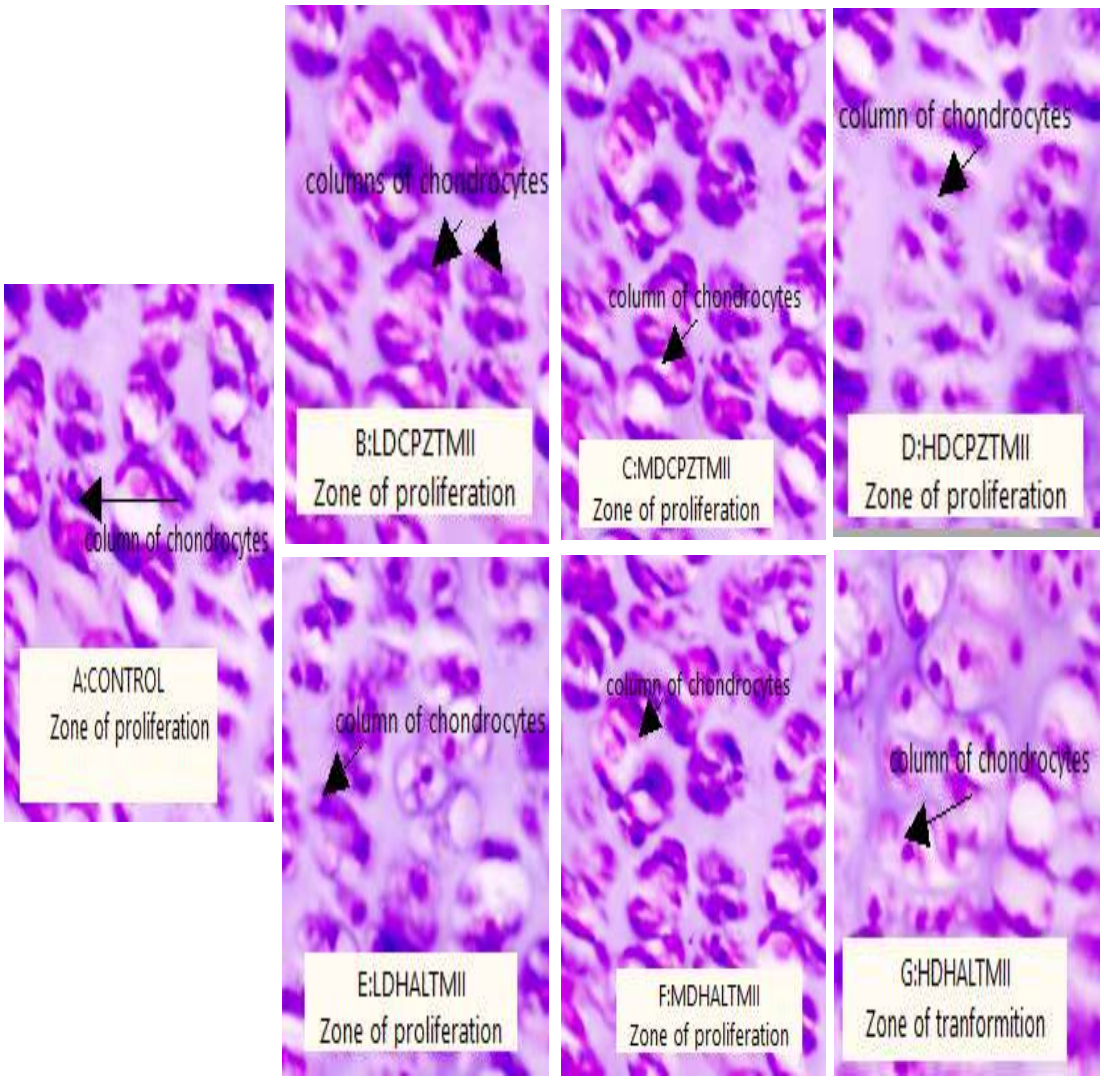


Figure 4.10: The Proliferation Zone Showing The Comparative Distribution of Proliferating Chondrocytes Plus their Densities for the Rats Treated at TM_2 Against Control.

Key: A *Control- showing column of chondrocytes B: LDCPZTMI-Low dose chlorpromazine trimester one showing similar distribution of chondrocyte to the control C: MDCPZTMI-Medium dose chlorpromazine trimester one D:-HDCPZTMI-High dose chlorpromazine trimester one E:-LDHALTMI-Low dose haloperidol trimester one F:-MDHALTMI- Medium dose haloperidol trimester one G:-HDHALTMI-High dose haloperidol trimester one showing fewer columns of chondrocytes as compared to the control magnification is x400.*

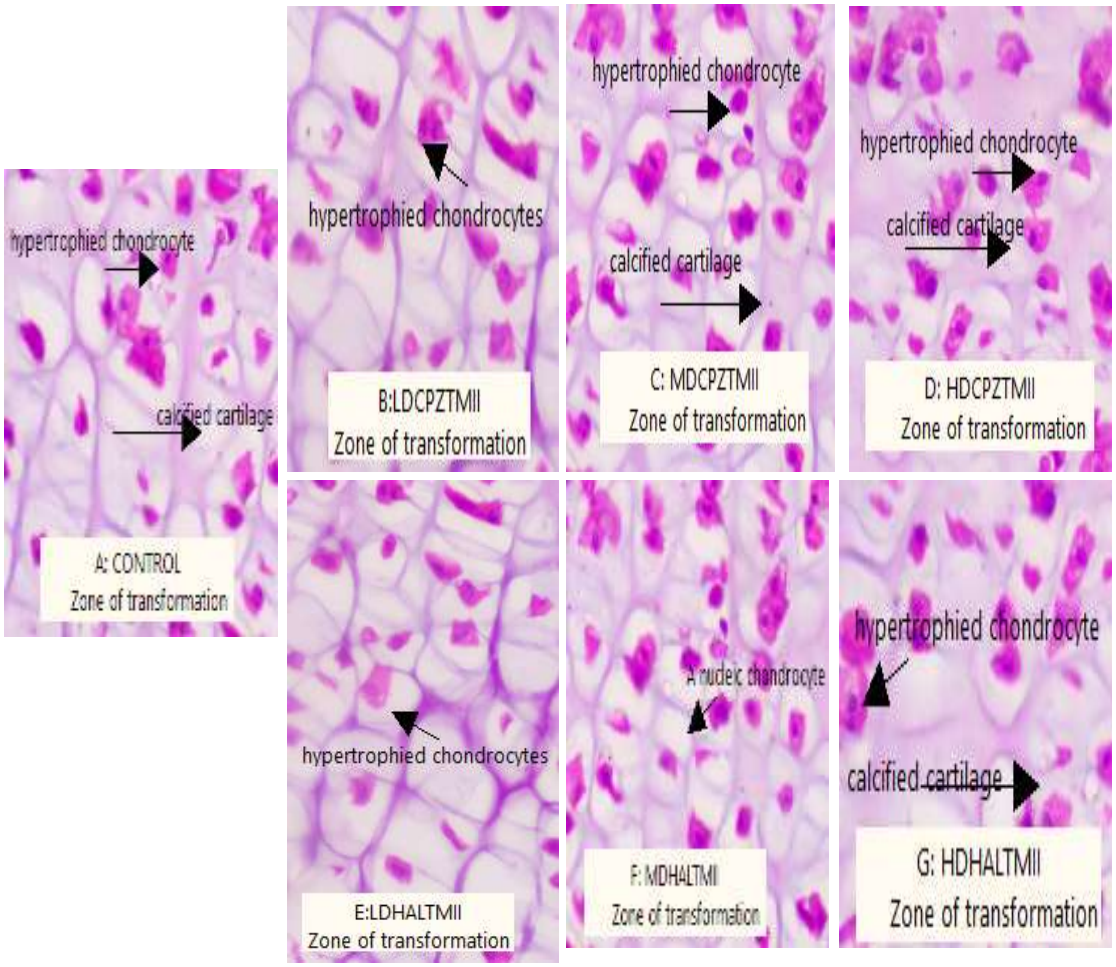


Figure 4.11: The Zone of Chondrocyte Transformation Showing the Comparative Hypertroplication of Chondrocytes in the Treatment Groups against the Control at TM_2.

Key: ***A*** *Control- showing hypertrophied chondrocytes with calcified matrix B:- LDCPZTMII- Low dose chlorpromazine trimester two C: MDCPZTMII-Medium dose chlorpromazine trimester two D: HDCPZTMII-High dose chlorpromazine trimester two E: LDHALTMII-Low dose haloperidol trimester two F: MDHALT-MII- Medium dose haloperidol trimester two G: HDHALTMII-High dose haloperidol trimester two magnification is x400.*

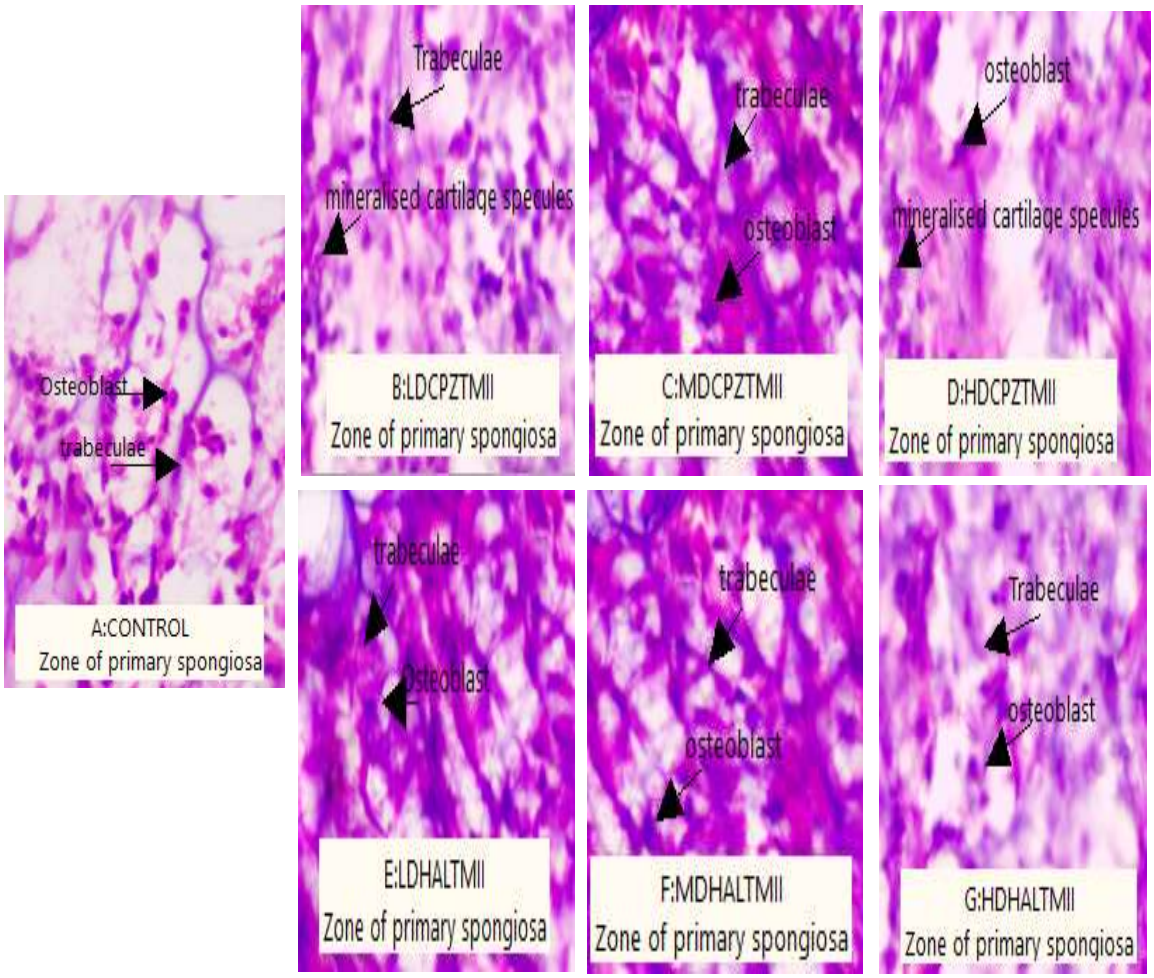


Figure 4.12: The Zone of Primary Spongiosa Showing Trabecular of New Bones and Osteoblast Cells in the Treatment Groups at TM_2 against the Control:

Key: *A Control- showing trabecular of new bone and high number of osteoblast cells B:-LDCPZTMII-Low dose chlorpromazine trimester two C:-MDCPZTMII-Medium dose chlorpromazine trimester two D:-HDCPZTMII-High dose chlorpromazine trimester two E:-LDHALTMII-Low dose haloperidol trimester two F: - MDHALT-MII-Medium dose haloperidol trimester two G:-HDHALTMII-High dose haloperi-dol trimester two magnification is x400.*

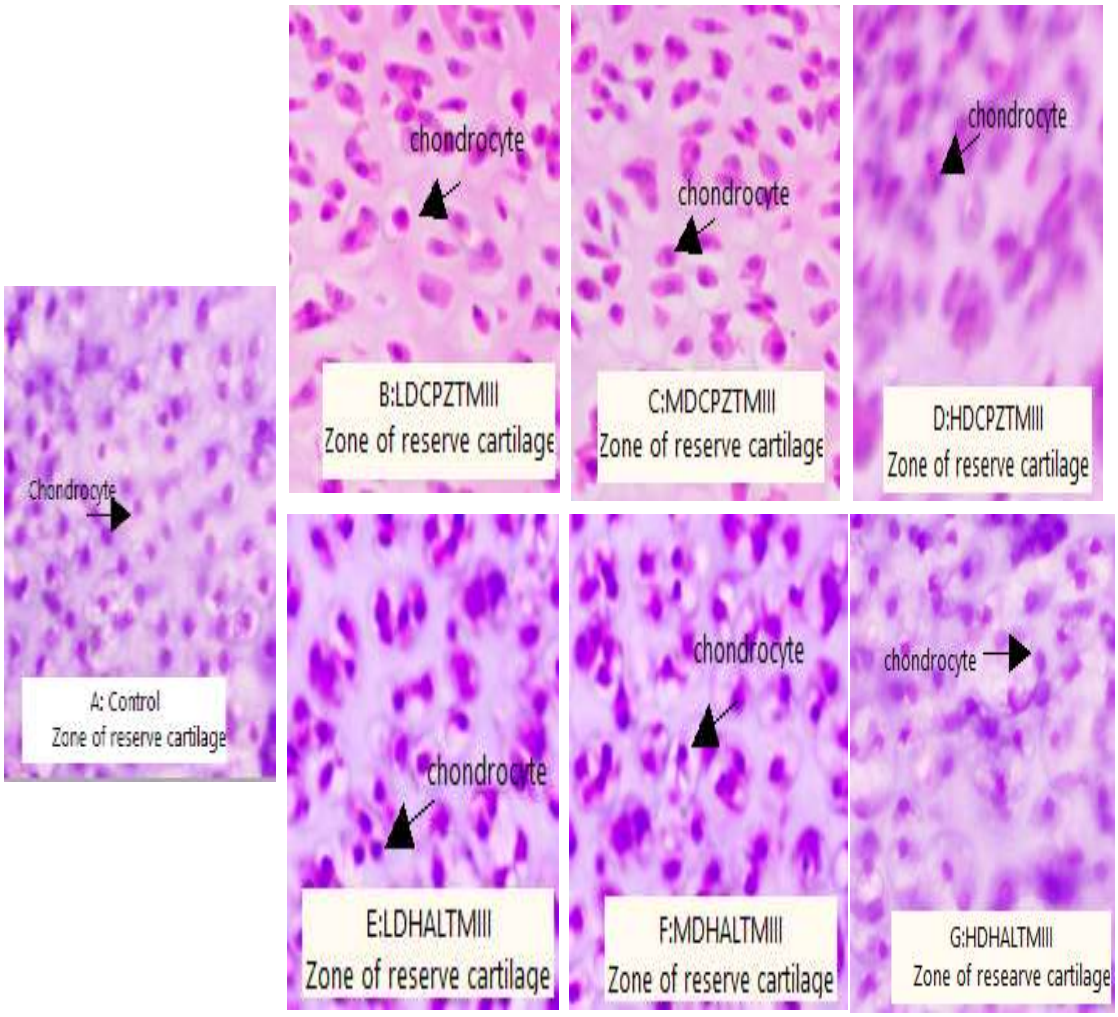


Figure 4.13*:* The Zone of Resting Cartilage Showing Comparative Chondrocytes Distributions and their Densities in the Rats Treated at TM_3 against the Control.

Key: A *Control -showing evenly distributed chondrocyte B:-LDCPZTMIII-Low dose chlor-promazine trimester three C:-MDCPZTMIII-Medium dose chlorpromazine trimester three D:-HDCPZTMIII-High dose chlorpromazine trimester three E:-LDHALTMIII-Low dose haloperidol trimester three F:-MDHALTMIII- Medium dose haloperidol trimester three G:-HDHALTMIII-High dose haloperidol trimester three magnification is x100.*

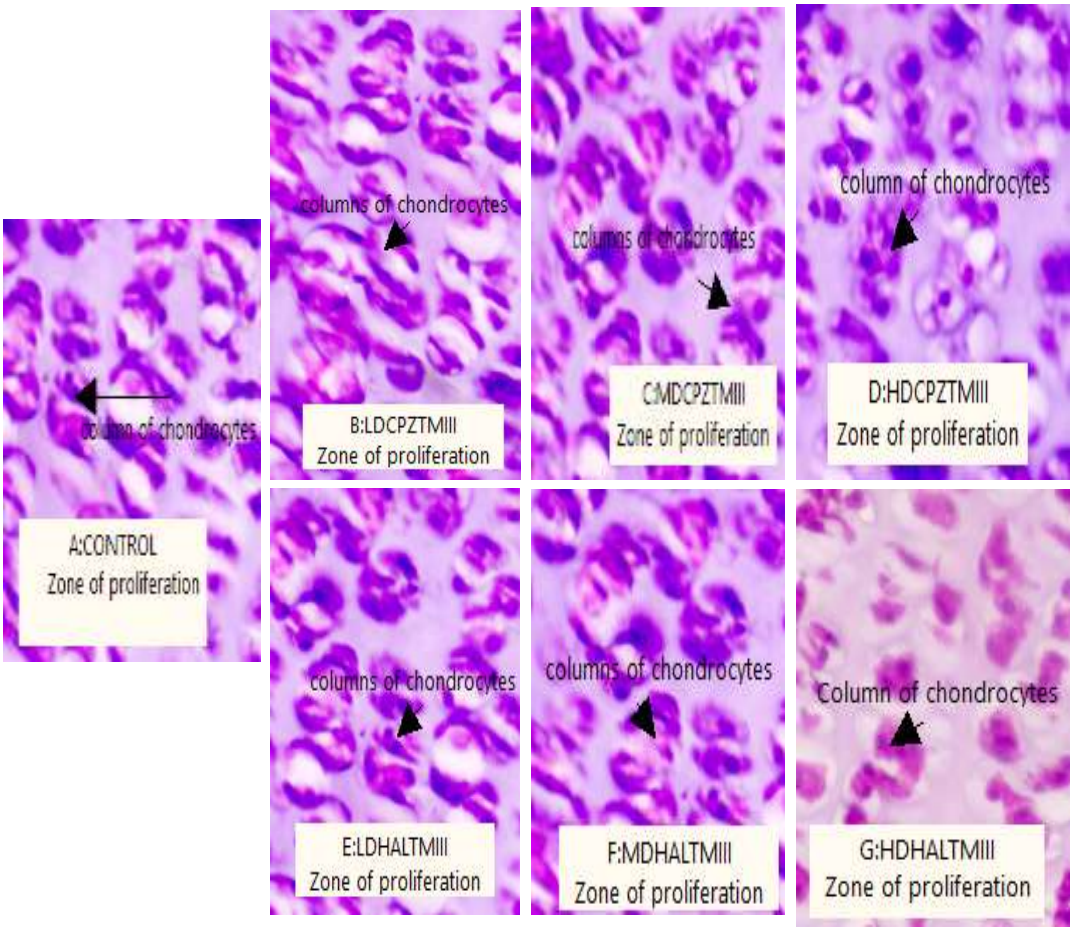


Figure 4.14: The Proliferation Zone Showing the Comparative Distribution of Proliferating Chondrocytes Plus their Densities for the Rats Treated at *TM_3* against Control

Key: ***A*** *Control- showing columns of chondrocytes B:-LDCPZTMIII-Low dose chlorpromazine trimester three C:-MDCPZTMIII-Medium dose chlorpromazine trimester three D:-HDCPZTMIII-High dose chlorpromazine trimester three E:-LDHALTMIII-low dose haloperidol trimester three F: - MDHALTMIII-Medium dose haloperidol trimester three G:-HDHALTMIII-High dose haloperidol trimester three magnification is x100.*

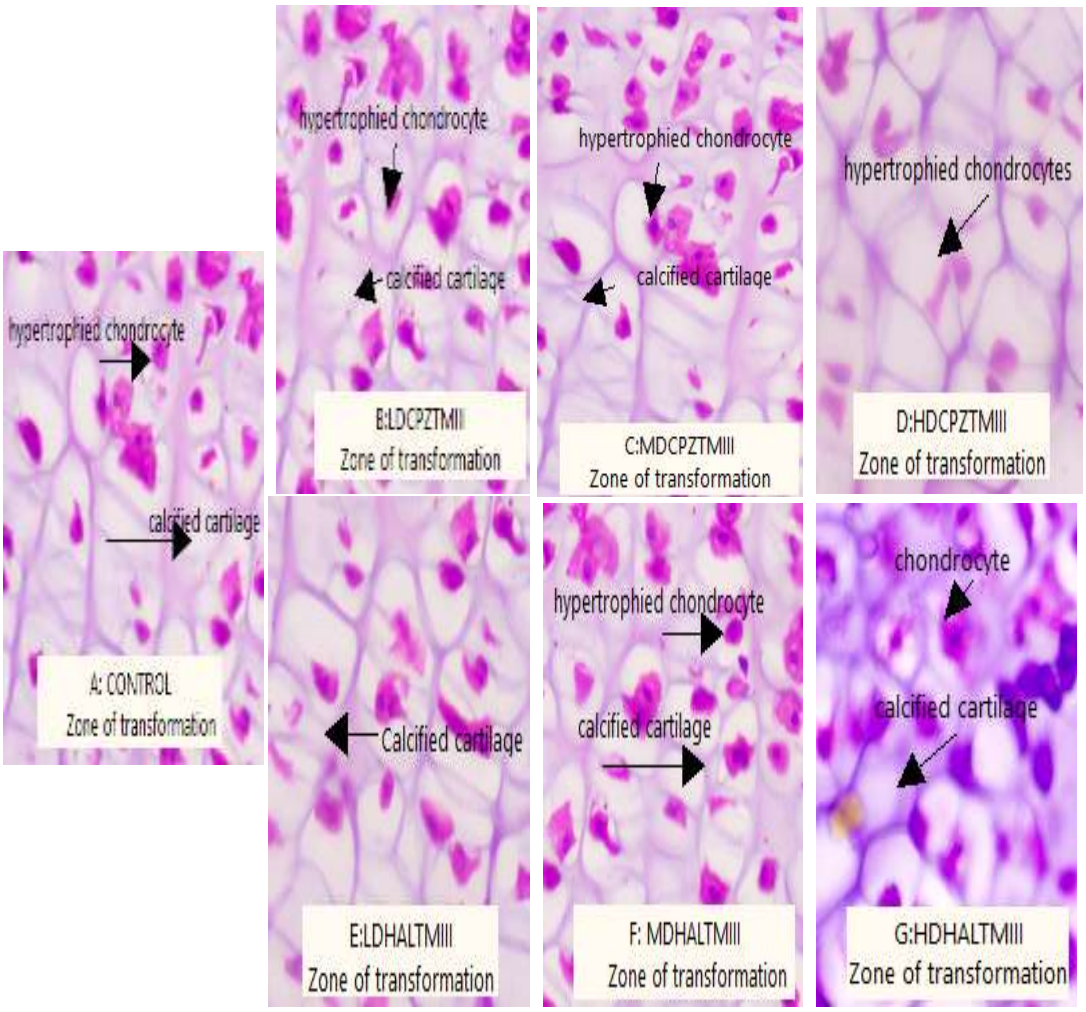


Figure 4.15: The Zone of Chondrocyte Hypertroplication and Transformation Showing the Comparative Hypertroplication of Chondrocytes in the Treatment Groups against the Control at TM3.

***Key**: **A** Control B: - LDCPZTMIII-Low dose chlorpromazine trimester three C:-MDCPZTMIII-Medium dose chlorpromazine trimester three D:-HDCPZTMIII-High dose chlorpromazine trimester three E:-LDHALTMIII-Low dose haloperidol trimester three F: - MDHALTMIII-Medium dose haloperidol trimester three G:-HDHALTMIII-High dose haloperidol trimester three magnification is x100.*

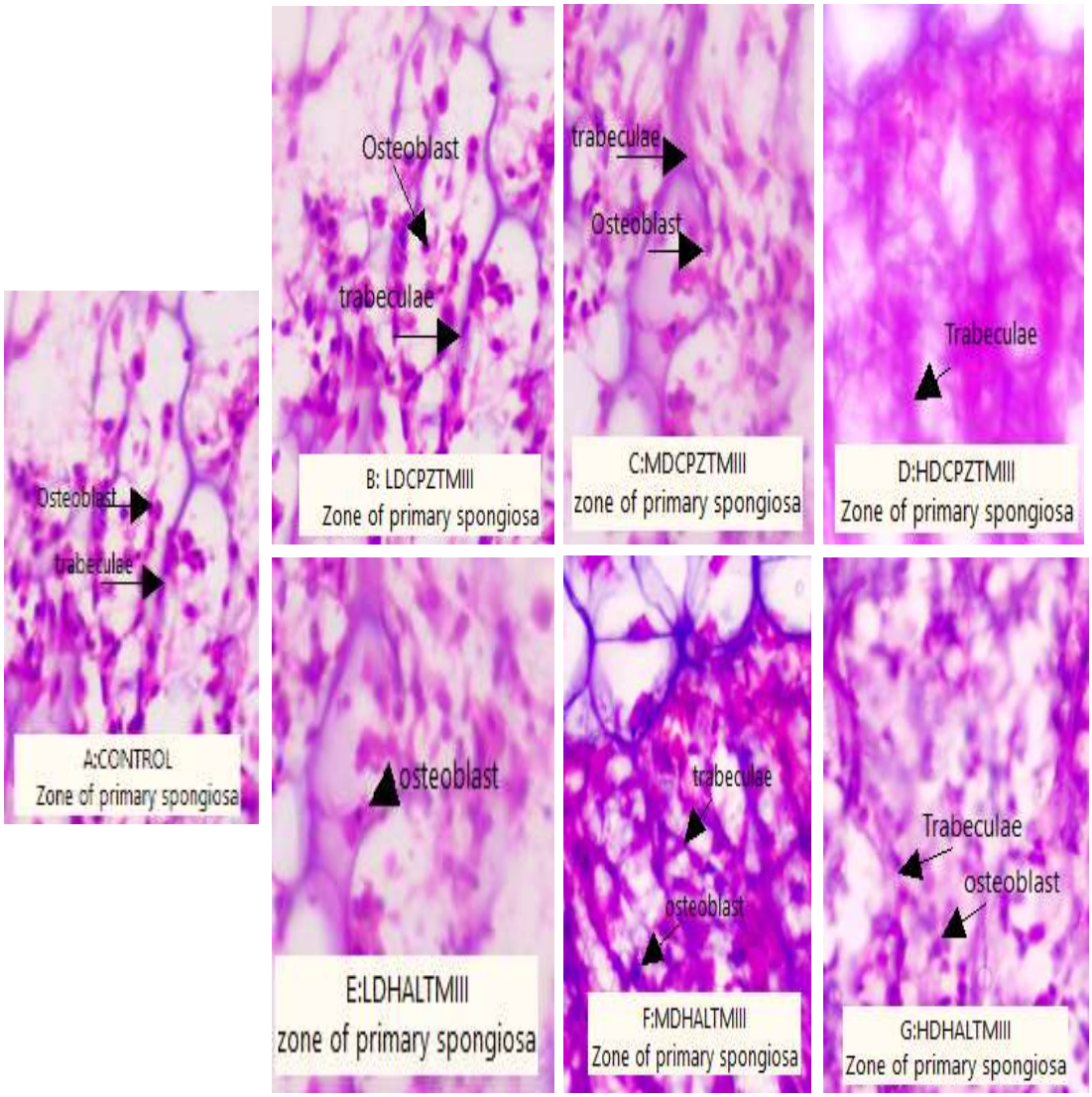


Figure 4.16: The Zone of Primary Spongiosa Showing Trabecular of New Bones and Osteoblast Cells in the Treatment Groups at TM_3. Against the Control:

Key: *A Control B:-LDCPZTMIII-Low dose chlorpromazine trimester three C:-MDCPZTMIII-Medium dose chlorpromazine trimester three D:-HDCPZTMIII-High dose chlorpromazine trimester three E:-LDHALTMIII-Low dose haloperidol trimester three F: - MDHALTMIII-Medium dose haloperidol trimester three G:-HDHALTMIII-High dose haloperidol trimester three magnification is x100.*

Level 2: The Comparative Findings on How the Two Medicines Influenced the Histomorphological Thicknesses and Cellular Densities of the Different Layers of the Proximal and Distal Epiphyseal Growth Plates of the Long Bones.

When both proximal and distal growth plate of fetal tibia and humerus were assessed following pre-natal exposure to haloperidol and chlorpromazine, there was no comparable difference. The comparative thickness of different layers of growth plate were presented according to the time of exposure as follows;- Trimester one (TMI), it was observed that the thickness of the various layers of the growth plate had a direct dose relation at low and medium dose and inverse relation at high dose where by, at low and medium dose treatment groups, the layers and their cellular histo-cyto architecture increased while at high dose they reduced more so, for the chlorpromazine treatment group *(Figure 4. 17).*

At the Trimester two (TMII) similar effects were observed where by, the thickness of the various layers increased at low and medium dose while at high dose the thickness was reduced more so zone of transformation for both chlorpromazine and haloperidol treatment groups. *(Figure 4. 18).*

At Trimester three (TMIII) low and medium dose group had no comparable difference with the control on the various layers, however high dose treatment group depicted reduction in the zone of transformation and zone of proliferation more so, for chlorpromazine treated groups *(Figure 4.19).*

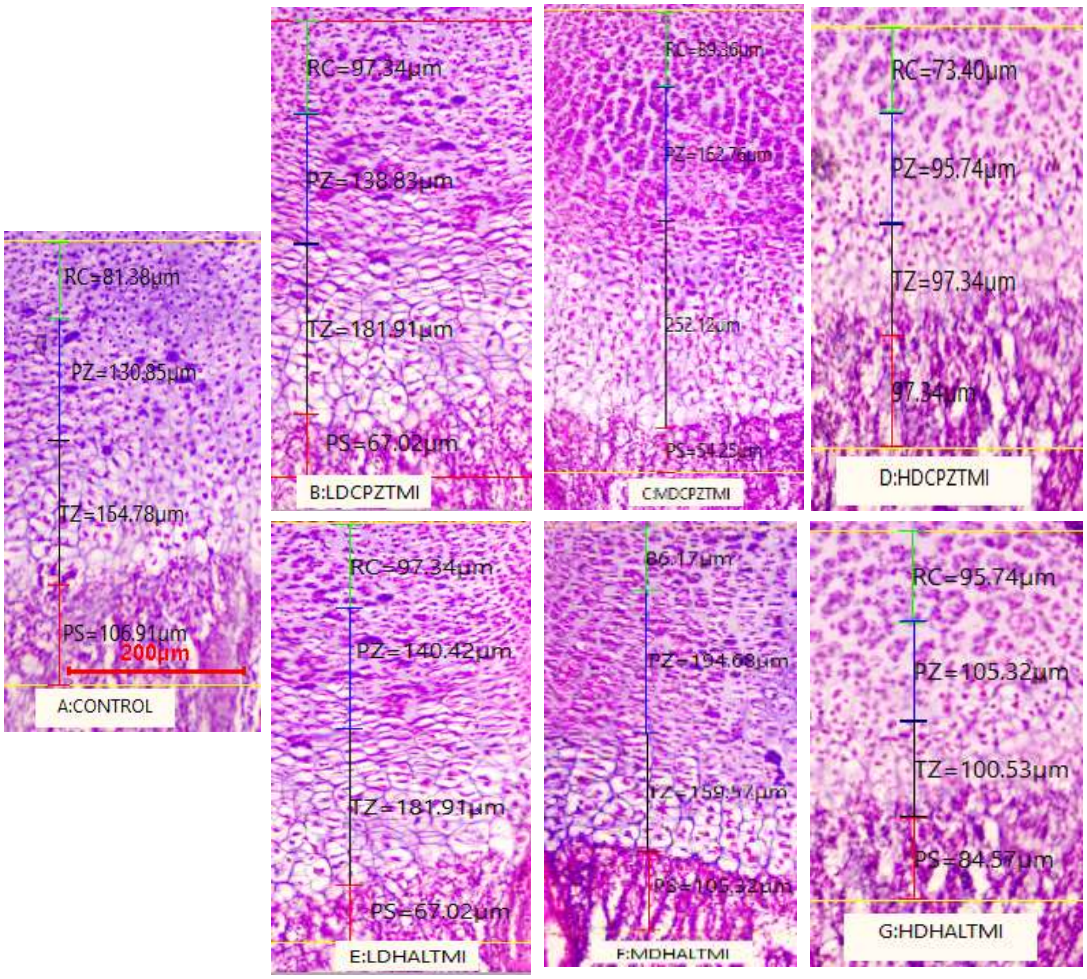


Figure 4.17: Showing the Comparative Thickness of the Various Layers of Epiphyseal Growth Plate in the Treatment Groups against the Control TM_1.

Key; *A Control green (RC)=Reserve cartilage, blue(PZ)=proliferation zone, black(TZ)=transformation zone, red(PS)=primary spongiosa. B: LDCPZTMI-Low dose chlorpromazine trimester one C: MDCPZTMI-Medium dose chlorpromazine trimester one D: HDCPZTMI-High dose chlorpromazine trimester one E: LDHALTMI-Low dose haloperidol trimester one F: MDHALTMI-Medium dose haloperidol trimester one G: HDHALTMI-High dose haloperidol trimester one .Magnification is x100.*

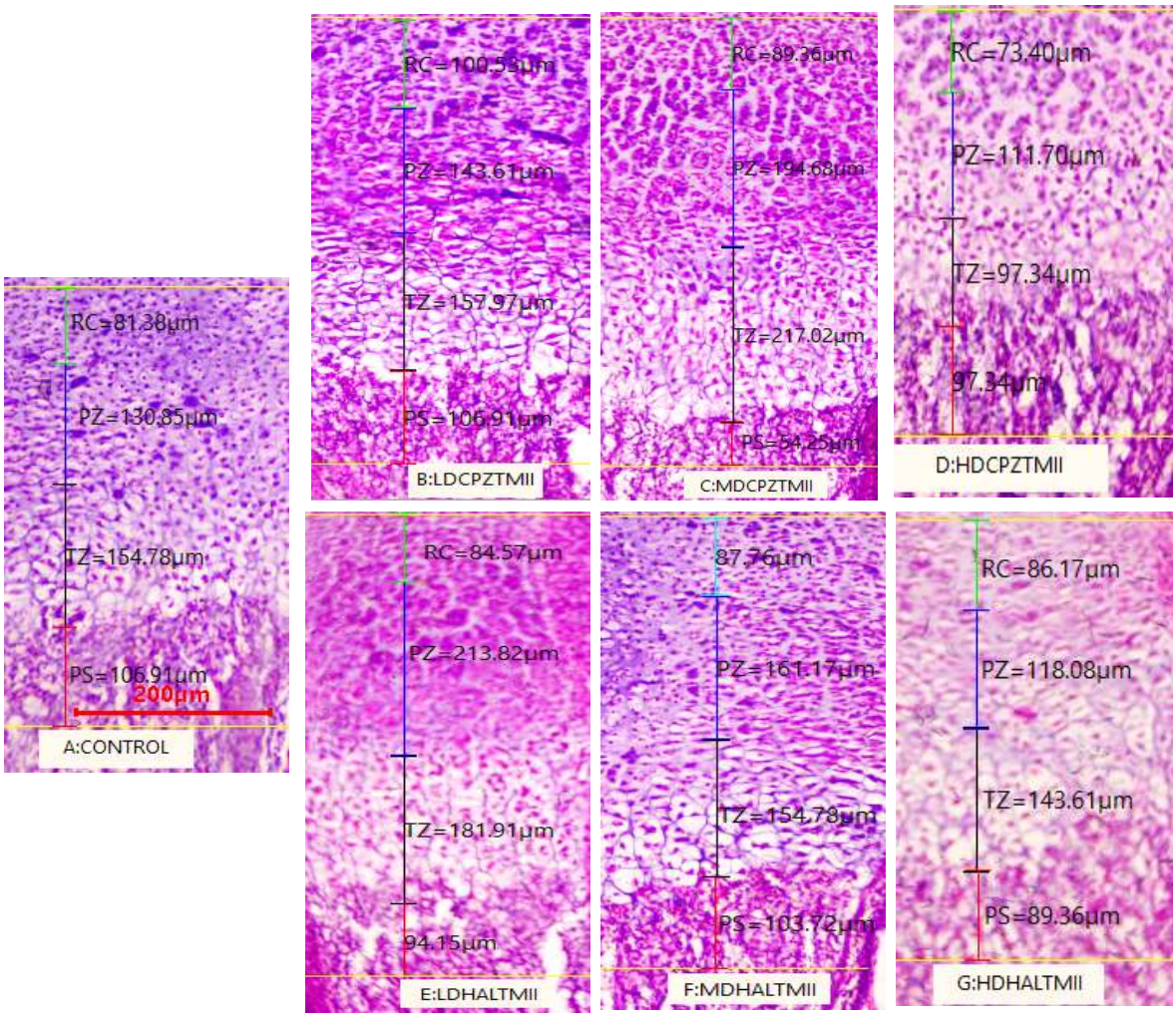


Figure 4.18: Showing the Comparative Thickness of the Various Layers of Epiphyseal Growth Plate in the Treatment Groups against the Control TM2.

***KEY**; A: Control green (RC)=Reserve cartilage, blue(PZ)=proliferation zone, black(TZ)=transformation zone, red(PS)=primary spongiosa. B: LDCPZTMI-Low dose chlorpromazine trimester one C: MDCPZTMI-Medium dose chlorpromazine trimester one D: HDCPZTMI-High dose chlorpromazine trimester one E: LDHALTMI-Low dose haloperidol trimester one F: MDHALTMI-Medium dose haloperidol trimester one G: HDHALTMI-High dose haloperidol trimester one .Magnification is x100.*

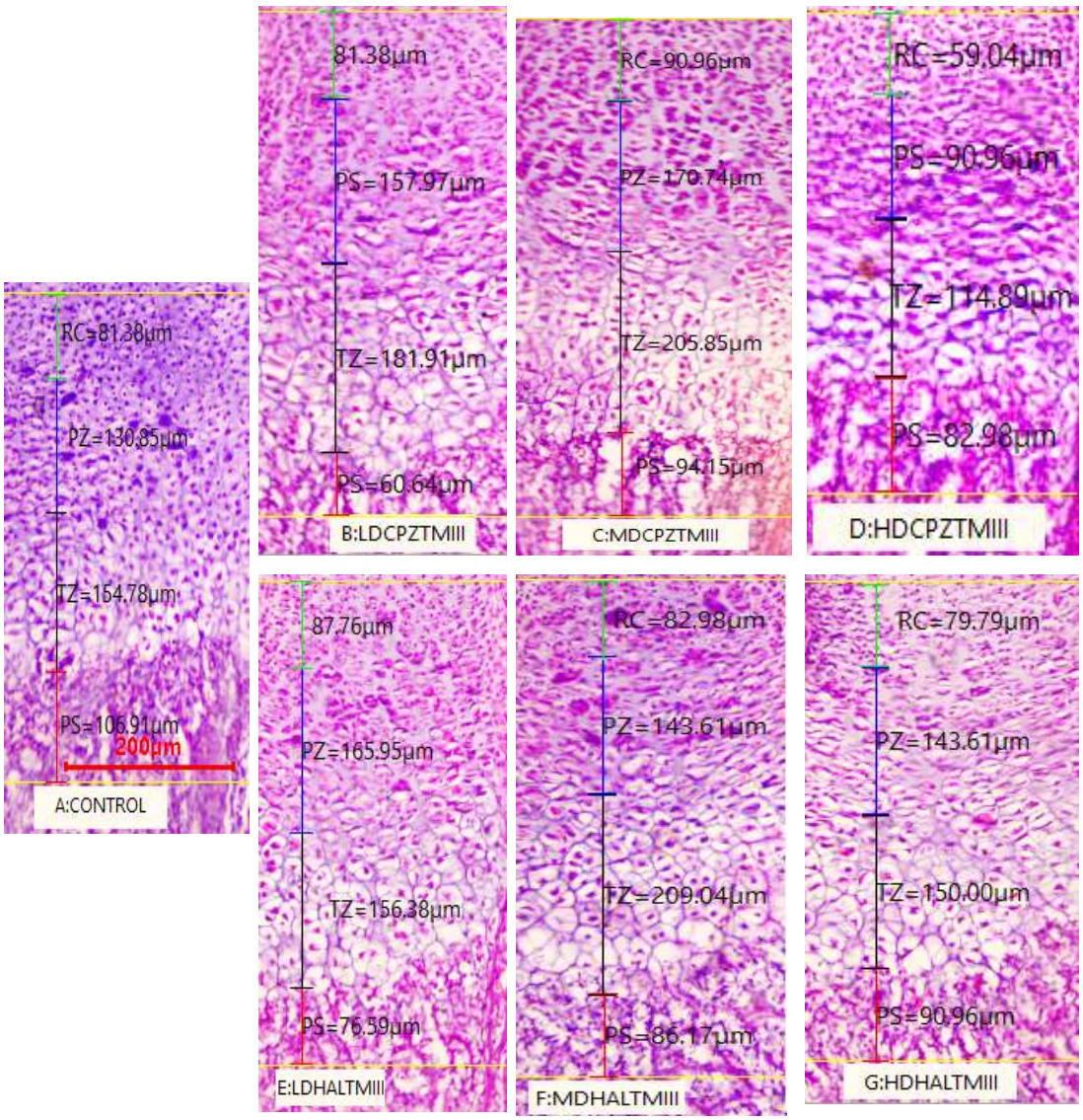


Figure 4.19: Showing the Comparative Thickness of the Various Layers of Epiphyseal Growth Plate in the Treatment Groups against the Control TM_3

***KEY**; A: Control green (RC)=Reserve cartilage, blue(PZ)=proliferation zone, black(TZ)=transformation zone, red(PS)=primary spongiosa. B: LDCPZTMI-Low dose chlorpromazine trimester one C: MDCPZTMI-Medium dose chlorpromazine trimester one D: HDCPZTMI-High dose chlorpromazine trimester one E: LDHALTMI-Low dose haloperidol trimester one F: MDHALTMI-Medium dose haloperidol rimester one G: HDHALTMI-High dose haloperidol trimester one .Magnification is x100.*

4.2 The Histostereological Findings.

4.1.1 Objective 3: The Comparative Evaluation on How the Two Medicines Influenced the Gross Morphometric and Stereological Organization of the Epiphyseal Growth Plate Zones in the Fetal Long Bones.

The findings on the quantative effects of haloperidol and chlorpromazine on the development of the fetal appendicular skeleton are presented in two levels as follows:

Level 1: The comparative findings on how the two medications influenced the gross morphometric measurement of the tibia and humerus.

Level 2: The comparative findings on how the two medications influenced the histostereological changes on the epiphyseal growth plate of a long bone.

Level 1: The Comparative Findings on How the Two Medications Influenced the Gross Morphometric Measurement of the Tibia and Humerus.

Upon in-utero exposure to the two medicines, assessment of gross morphology of fetal tibia and humerus as one of the determinants of histoquantative outcomes was carried out in two stages;

Stage 1: one way analysis of variance (ANOVA).

Stage 2: Multivariate analysis of variance (M ANOVA).

Stage 1: ANOVA findings on how in-utero exposure to chlorpromazine and haloperidol influenced the mean lengths of fetal tibia and humerus.

Upon in-utero exposure to the two medicines, the global effects were assessed without considering dosage and time, as such, both drugs had significant effects on the means of the fetal tibia and humerus length as shown by overall (F) and (p) values as follows-; tibia length ($F(18,38)=68.971, P<0.001$,humerus length ($F(18,38=34.688, P<0.001$.

With regard to the time of exposure, it was observed that medium chlorpromazine treatment group depicted statistically significant increase $p<0.001$ in length of both tibia and humerus,

However, at high dose both chlorpromazine and haloperidol treatment groups depicted statistically significant reduction in length more so, for the rats which received the medicine from trimester one as illustrated on *(table 4.5).*

Table 4.5: The Comparative ANOVA Table on How *In-Utero* Exposure to Chlorpromazine and Haloperidol Influenced the Gross Morphometry of the Mean Length of Tibia And Humerus

The study groups	Study groups and dosage levels	The time of exposure to treatment	Mean fetal Tibia length (mm)±SD	Mean fetal humerus length (mm)±SD
Control	No treatment given(C)	None	5.9±0.26	5.10±0.10
Chlorpromazine treatment group	Low dose(3mg/kg/bw)	(TMI)	6.1±0.05	5.40±0.11
		(TMII)	6.0±0.05	5.10±0.17
		(TMIII)	6.0±0.11	5.13±0.05
	Medium dose (10mg/kgbw)	**(TMI)**	**6.4±0.1***	**5.67±0.05***
		(TMII)	6.2±0.1	5.47±0.05
		(TMIII)	6.1±0.11	5.43±0.21
	High dose group (30mg/kg/bw	**(TMI)**	**4.96±0.05***	**4.43±0.05***
		(TMII)	**4.9±0.158***	**4.61±0.10***
		(TMIII)	**5.03±0.05***	**4.6±0.17***
Haloperidol treatment groups	Low dose groups	(TMI)	5.90±0.1	5.21±0.11
		(TMII)	5.90±0.11	5.03±0.05
		(TMIII)	6.00±0.10	5.11±0.10
	Medium dose groups	(TMI)	6.1±0.1	5.21±0.11
		(TMII)	6.1±0.1	5.33±0.05
		(TMIII)	6.03±0.05	5.26±0.1
	High dose groups	**(TMI)**	**5.03±0.05***	**4.5±0.11***
		(TMII)	**5.00±011***	**4.57±0.57***
		(TMIII)	**5.03±0.05***	**4.47±0.21***
			F(18,38)=68.971	**F(18,38)=34.688**
			P=0.001	**P=0.001**

Key: *values are conveyed as means ± standard deviation of means n=3 per group. (*) the figure bearing the asterisk shows that they are significantly different ($p<0.05$) with the control, using one-way ANOVA with Tukey post hoc multiple comparison t-test.*

Stage 2: The Manova Analysis on How Pre-Natal Exposure to Chlorpromazine and Haloperidol Influenced the Fetal Outcomes of Tibia and Humerus Lengths.

Upon carrying out comparative analysis using MANOVA to evaluate how the two drugs influenced fetal parameter of tibia and humerus length following prenatal exposure to the two medicines, the results were presented in three levels as follows: -

Level 1: How the drug, dosage and time of exposure with their interactions influenced global outcome of the two fetal parameters.

Level 2: How the chlorpromazine and haloperidol individually their dose and time of exposure and their interactions influenced tibia and humerus lengths.

Level 3: Pairwise comparison on how in-utero exposure to chlorpromazine and haloperidol Fetal parameters when exposed at similar dosage and time.

Level 1: The MANOVA Level 1 Findings on How the Drug, Dosage and Time of Exposure with their Interactions Influenced Global Outcome of the Length of Tibia and Humerus of the Fetal Rats.

On analysis of how *in-utero* exposure to chlorpromazine and haloperidol influenced fetal outcome of tibia and humerus length, by checking over all global main effects and interaction effects of drug, dosage and trimester, the three independent variables were found to be statistically significant in different proportions at global and two-way interaction for drug*dosage and dosage*trimester with varying partial Eta squired proportions ($ŋ^2$) as illustrated on *table 4.6* as follows;

i. Individual overall main effects of drug (F (2,37) =14.107, p<0.001; wilks' lambda (ƛ)=0.567, partial Eta squired (ŋ2=.433). Dosage (F (4,74) =131.743, p<0.001; wilks' lambda (ƛ)=0.015, partial Eta squired (ŋ2=.877) and trimester (F (4,74) =2.989, p<0.024; wilks' (F (4,74) =2.989, p<0.024; wilks' lambda (ƛ)=0.741, partial Eta squired (ŋ2=.139).

ii. The two-way interaction effects of drug*dosage (F (4,74) =4.338, p<0.003; wilks' lambda (ƛ)=0.656, partial Eta squired ($ŋ^2$=.190) and dosage*trimester (F (8,74) =3.128, p<0.004; wilks' lambda (ƛ)=0.558, partial Eta squired ($ŋ^2$=.253) though at three-way interaction of drug*dosage*trimester the p value was not statically significant.

Table 4.6: The Level One MANOVA Table on How *In-Utero* Exposure to Chlorpromazine and Haloperidol and their Interactions Globally Influence Fetal Tibia and Humerus Length.

1.Types of MANOVA evaluation at level 1	**The parameter used**	**MANOVA test (Wilks lambda)**	**Statistics(F)**	**Hypothesis degree (df)**	**Error degree of freedom**	**Sig.<0.05**	**Proportion of variance (Partial Eta Squared)**
2.Test on whether the observed effects were due to chance or not	Intercept	.000	110790.934[b]	2.000	37.000	.000	1.000
3.The individual main effects of drug, dosages and trimester	Drug	.567	14.107[b]	2.000	37.000	.000	.433
	Dosage	.015	131.743[b]	4.000	74.000	.000	.877
	Trimester	.741	2.989[b]	4.000	74.000	.024	.139
4.Two-way interaction effects on the fetal dependent variable	Drug Dosage	.656	4.338[b]	4.000	74.000	.003	.190
	Drug and Trimester	.902	.975[b]	4.000	74.000	.427	.050
	Dosage Trimester	.558	3.128[b]	8.000	74.000	.004	.253
5.Three-way interaction effects	Drug and Dosage and Trimester	.767	1.314[b]	8.000	74.000	.250	.124

a. Design: Intercept + DRUG + DOSAGE + TRIMESTER + DRUG * DOSAGE + Drug * Trimester + Dosage * Trimester + Drug Dosage and 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 MANOVA Level 2 Findings on How Individual Drug, Dosage, Trimester of *In-Utero* Exposure, and their Interaction Effects Influenced The Lengths of Fetal Tibia and Humerus.

On further analysis on how the individual drug, dosage and time of exposure influenced fetal outcomes of tibia and humerus length using MANOVA it was observed that dosage contributed the highest followed by the drug while the time of exposure was not statistically significant as illustrated by the varying proportion of partial Eta squired (η^2) (table *4.7).* At two-way interaction there was statistically significant difference between drug*dosage for the two fetal parameters and no three-way interaction effects as illustrated by very low partial Eta values (*Table 4.7).*

Table 4.7: The MANOVA Level 2 Table on How *In-Utero* Exposure to Chlorpromazine and Haloperidol and their Interactions Influenced the Lengths of Fetal Tibia and Humerus Length Individually.

Test of between subjects' effects				
Independent var-iables	**Dependent Variables**	**(F)**	**Sig.**	**Partial Eta Squared**
Drug	tibia	7.741	.008	.269
	humerus	17.713	.000	.318
Dosage	tibia	598.996	.000	.969
	Humerus	282.658	.000	.937
Trimester	tibia	1.791	.008	.186
	humerus	4.034	.026	.175
Drug * Dosage	tibia	3.950	.028	.172
	humerus	4.569	.017	.194
Drug * Trimester	tibia	1.599	.215	.078
	humerus	.275	.761	.014
Dosage trimester	tibia	1.911	.028	.267
	humerus	4.425	.005	.318
Drug * Dosage * Trimester	tibia	1.335	.275	.123
	humerus	1.186	.333	.111

Level 3: Pairwise Comparison Findings on How *In-Utero* Exposure between Chlorpromazine and Haloperidol Influenced the Mean Lengths of Fetal Tibia and Humerus Using MANOVA.

On carrying out the pairwise comparison between the two drugs, in the same dosage group and similar time of exposure in order to find out how they influence fetal parameters of tibia and humerus length, it was observed that:

There was statistically significant difference ($p<0.05$) for the medium dose treated group between the two drugs with chlorpromazine treated group contributing more. However, at high dose the two drugs showed no statistically significant difference in contribution to the deleterious effects as illustrated in (***Table 4.8).***

Table 4.8: The MANOVA Level 3 Table on the Pairwise Comparison between Chlorpromazine and Haloperidol when Exposed in the Same Dosage and the Same Length of a Trimesters on the Lengths of Fetal Tibia and Humerus.

		Multiple/pairwise comparisons				
Dependent variable	**Dose level**	**The time of exposure**	**CPZ**	**HAL**	**Mean difference (CPZ-HAL)**	**Sig <0.05**
Tibia length	Low	TM1	CPZ	HAL	.233	.012
		TM2	CPZ	HAL	.167	.065
		TM3	CPZ	HAL	.067	.453
	Medium	TM1	CPZ	HAL	.300*	**.002**
		TM2	CPZ	HAL	.051*	**.001**
		TM3	CPZ	HAL	.067*	**.05**
	High	TM1	CPZ	HAL	-.017*	**.003**
		TM2	CPZ	HAL	-.033	.007
		TM3	CPZ	HAL	-.32*	**.007**
Humerus length	Low	TM1	CPZ	HAL	.200	.037
		TM2	CPZ	HAL	.067	.475
		TM3	CPZ	HAL	.033	.720
	Medium	TM1	CPZ	HAL	.333*	**.001**
		TM2	CPZ	HAL	.200*	**.037**
		TM3	CPZ	HAL	.233*	**.016**
	High	TM1	CPZ	HAL	-.067*	**.005**
		TM2	CPZ	HAL	-.033	.020
		TM3	CPZ	HAL	.133	.057

Level 2: The Comparative Histostereological Findings on How *In-Utero* Exposure to the Two Medicines Influenced the Epiphyseal Growth Plate of Long Bone.

The histoquantative findings were assessed in two stages. (a) **Level 1** assessed surface area of the various layers of epiphyseal growth plate and (b) **level 2** assessed the cellular densities.

Level 1: Findings on How *In-Utero* Exposure to Chlorpromazine and Haloperidol Influenced Fetal Outcomes of the Percentage Surface Areas of the Different Layers of the Growth Plate.

This was carried out in two stages where by **stage 1 was** the ANOVA findings and **stage 2 the** MANOVA findings

Stage 1: The Comparative ANOVA Findings on How *In-Utero* Exposure to Chlorpromazine and Haloperidol Influenced Percentage Surface Area of Various Layers of the Growth Plates.

Upon carrying out a comparative general assessment on the means of total surface areas of the various layers of epiphyseal growth plate of both the proximal and distal epiphyseal growth plate of tibia and humerus of fetal rats after exposure to the two medicines, it was observed that there was no statistical comparable difference on how the two medicines differed in influencing the global features of the two epiphyseal growth plates.

When the global effects of the two drugs(CPZ&HAL) was assessed without considering dosage and time of exposure it was noted that they had significant effects on all the layers of the epiphyseal growth plate with chlorpromazine depicting more deleterious effects as follows (a) zone of resting cartilage ($F(18,38)=83.817$ (b) zone of proliferation ($F(18,38)=40.102$ (c) zone of transformation ($F(18,38)=50.175$ (d) zone of primary spongiosa ($F(18,38)=49.458$ as illustrated on (*Table 4. 9).*

On further observation, it was noted that the means of the total surface area of various layers of pups' growth plates in the medium-dose chlorpromazine treated

groups were statistically Significant ($p<0.005$) higher than the control group. However, at high dose, both chlorpromazine and haloperidol treated groups depicted a statistically significant decrease ($p<0.05$) on all the layers of the growth plate, more so for the chlorpromazine treatment group from trimester one (*Table 4.9).*

Table 4.9: The Comparative ANOVA Table on How Prenatal Exposure Influenced the Means of the Total Surface Areas of the Various Layers of Epiphseal Growth Plate of a Long Bone against Control.

The study groups	Study groups and dosage levels	The time of expo-sure to treatment	%SA of RC±SD	% SA of PZ ±SD	%SA of TZ±SD	%SA of PS ±SD
Control	No treatment given(C)	None	40.10±0.58	34.66±1.17	26.02±0.06	22.35±0.5
Chlorpromazine treatment group	Low dose	(TMI)	41.05±0.55	36.27±1.19	26.19±0.37	22.13±0.6
		(TMII)	40.05±0.52	34.99±1.24	26.33±0.55	22.03±0.5
		(TMIII)	40.33±0.93	34.56±0.84	26.15±0.78	22.49±0.06
	Medium	(TMI)	42.26±0.44*	36.34±0.1*	27.46±0.39*	23.83±0.11*
		(TMII)	40.65±0.05	36.24±0.15	26.71±0.20	23.30±0.12
		(TMIII)	40.63±0.1	36.13±0.05	26.89±0.60	23.20±0.01
	High	(TMI)	34.30±0.04*	32.0±0.05*	21.89±0.60*	20.46±0.63*
		(TMII)	35.31±0.05*	32.6±0.20*	22.23±1.04*	20.48±0.11*
		(TMIII)	36.45±0.37*	33.26±0.1*	22.43±0.21*	21.640.07*
Haloperidol treatment groups	Low	(TMI)	40.06±0.45	36.40±0.43	26.74±0.25	22.87±0.11
		(TMII)	40.89±1.0	36.18±0.03	26.85±0.04	22.64±0.17
		(TMIII)	40.51±0.26	36.33±0.34	26.14±0.44	23.08±0.56
	Medium	(TMI)	41.22±0.6	36.16±0.53	27.31±0.58	23.36±0.34
		(TMII)	40.39±0.14	35.93±0.48	26.34±0.55	23.48±0.6
		(TMIII)	40.74±0.6	36.04±0.42	25.62±0.48	23.64±0.18
	High	(TMI)	34.67±0.29*	30.9±0.13*	22.56±0.15*	20.32±0.11*
		(TMII)	35.5±0.32*	30.2±0.34*	22.68±0.15*	21.12±0.6*
		(TMIII)	36.85±0.14*	31.3±0.02*	23.41±0.64*	21.36±0.1*
			F(18,38)=83.81	F(18,38)=40.10	F(18,38)=50.175	F(18,38)=49.458
			P=0.001	P=0.001	P=0.001	P=0.001

Key: *values are conveyed as means ± standard deviation of means n=3 per group. All values that bear asterisk (*) are significantly different from the control.*

Stage 2. The Multivariate Analysis of Variance (MANOVA) on How Chlorpromazine and Haloperidol Influenced Mean Surface Area of Various Layers of Fetal Growth Plate.

Upon further analysis on the effects of the two drugs on the surface area and the cell density of the proximal and distal epiphyseal growth plates of humerus and tibia using MANOVA it was noted that there was no comparable difference between them. The MANOVA findings are presented in three levels as follows:

Level 1: *How prenatal exposure to the two drugs influenced the surface area of growth plate globally.*

Level 2: *How the individual drug, their dosages, time of exposure and their interactions influenced various layers of growth plate prenatally.*

Level 3: *Pairwise comparison on how chlorpromazine and haloperidol influenced the surface area of the fetal growth plate when exposed at similar dosage level and time.*

Level 1: **The MANOVA Level 1 Findings on How Prenatal Exposure to Chlorpromazine and Haloperidol Prenatally Influenced the Surface Area of the Fetal Growth Plates Globally.**

On carrying out multivariate analysis to evaluate how the two drugs influenced the percentage surface area of various layers of fetal growth plate globally,the main effects and two ways interaction effects of drug*dosage and dosage*trimester were found to be statistically significant in different proportions (partial Eta squired(η^2) as illustrated in table 4.10 as follow;

(i) The individual main effects of ;(a) drug($F(4,35)=3.597, p<0.015$;wilks lambda(∧)=.709;partial Eta squired (η^2=.291.(b) dosage($F(8,70)=128.153$, $p<0.001$;wilks lambda(∧)=.004;partial Eta squired (η^2=.936 (c) trimester($F(8,70)=3.774, p<0.001$;wilks lambda(∧)=.488;partial Eta squired (η^2=.301.

(ii) The two way combination effects of drug*dosage((F(8,70)=8.934, p<0.001;wilks lambda(∧)=.245;partialEtasquired(η^2=.505. dosage* trimester(F(8,70)=6.420,p<0.001;wilks lambda(∧)=.129;partial Eta squired (η^2=.401.Though there was no three ways interaction effects of drug*dosage*trimester.

Table 4.10: The Manova Level I Table on How *In-Utero* Exposure to Chlorpromazine and Haloperidol and their Interactions Globally Influenced the Surface Area of the Various Layers of Epiphyseal Growth Plate of a Long Bone.

Type of MANOVA evaluated	Parameter used	MANOVA test statistics(Wilks lambda)	Statistics (F)	Hypothesis degree of freedom	Error degree of freedom	Sig<0.05.	Proportion of variance(Partial Eta Squared)
Test on whether the observed overall effects were due to chance or not	Intercept	.000	187735.507[b]	4.000	35.000	**.000**	1.000
The individual main effects of the drug, time of exposure and dosage on %surface area of fetal growth plate of proximal tibia	Drug	.709	3.597[b]	4.000	35.000	**.015**	.291
	Dosage	.004	128.153[b]	8.000	70.000	**.000**	.936
	Trimester	.488	3.774[b]	8.000	70.000	**.001**	.301
Two-way interaction effects on fetal parameters	DRUG * Dosage	.245	8.934[b]	8.000	70.000	**.000**	.505
	DRUG * Trimester	.805	.999[b]	8.000	70.000	.444	.103
	Dosage * Trimester	.129	6.420	16.000	107.564	**.000**	.401
Three ways interaction effects	DRUG*Dosage * Trimester	.504	1.688	16.000	107.564	.060	.157

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.

Key:*(*) indicate interaction effects while (ƀ) indicates exact statistics using MANOVA.*

Level 2: The MANOVA Level 2 Findings on How the Three Independent Variables of the Drug, Dose and Time of Exposure and their Interaction Influenced the Fetal Epiphyseal Growth Plates of Long Bones.

Upon carrying out MANOVA level 2 findings on how the three independent variables of drug, dosage and time of exposure and their interactions either two or three ways influenced fetal parameters of the various layers of epiphyseal growth plate it was noted that

(i) At an individual levels contribution of the drug, dose and trimester of exposure on the various layers of epiphyseal growth plate (i) zone of reserve cartilage, (ii) zone of proliferation, (iii) zone of transformation (iv) zone of primary spongiosa showed a varying proportionate (partial Eta squared (η^2) with dosage having the highest contribution to each of them .

(ii) At two ways interaction effects there was statistical significant difference ($p<0.05$) for the drug and dosage and trimester at varying proportion as illustrated by (Table 4.11) below.

*(iii)*Though at three way interactions of drug, dosage and trimester, there was no interaction effects observed.

Table 4.11: The MANOVA Level 2 Table on How the Three Independent Variables of Drugdosage and Time of Exposure and their Interactions Influenced the Surface reas of the Various Layers of Epiphyseal Growth Plates of A Long Bone.

Test of between subject effects

Independent variables	Dependent Variables	F statistics	Sig.	Partial Eta Squared
Drug	%SA P RC	.159	.692	.104
	%SAPPZ	3.354	.045	.181
	%SAPTZ	1.494	.029	.238
	%SAPPS	8.741	.005	.187
Dosage	%SA P RC	598.357	**.000**	.969
	%SAPZ	286.566	**.000**	.938
	%SA TZ	421.272	**.000**	.957
	%SAPS	395.116	**.000**	.954
trimester	%SA RC	3.015	.061	.137
	%SAPZ	.406	.669	.021
	%SATZ	1.387	.262	.068
	%SA PS	11.470	.000	.376
drug * dosage	%SA RC	.662	**.022**	.034
	%SAPZ	25.460	**.000**	.573
	%SATZ	8.476	**.001**	.308
	%SAPS	8.717	**.001**	.315
drug *trimester	%SA RC	1.137	.331	.056
	%SAPZ	.469	.629	.024
	%SATZ	.928	.404	.047
	%SAPS	1.531	.229	.075
dosage *trimester	%SA RC	20.412	**.000**	.682
	%SAPZ	4.141	**.007**	.304
	%SATZ	6.331	**.001**	.400
	%SAPS	9.553	**.000**	.501
drug	%SA RC	1.797	.150	.159
	%SAPZ	1.299	.288	.120
* dosage * trimester	%SATZ	1.077	.382	.102
	%SAPS	2.398	.067	.202

Level 3: The MANOVA Level Three on the Pairwise Comparison on How Chlorpromazine And Haloperidol Influenced the Surface Areas of the Fetal Growth Plate when Exposed within Similar Dosage Level and the Same Trimesters.

Upon pairwise comparison on how *in-utero* exposure to the two drugs influenced fetal outcomes of various layers of epiphyseal growth plate surface area it was noted that at high dose chlorpromazine had statistically significant more deleterious effects on the zones of transformation and proliferation as compared to haloperidol as illustrated on *(table 4.12).*

Table 4.12: The MANOVA Level 3 Table on Comparative Pairwise Comparison between Chlorpromazine and Haloperidol on the Percentage Means of Total Surface Areas of Various Layers of Tibia the Fetal Epiphyseal Growth Plates .

		Multiple /pairwise comparison				
Dependent	Dose level	The time of treatment exposure	CPZ	HAL	Mean difference(CPZ-HAL)	Significant <0.05
% mean SA of RC	Low dose	TM1	CPZ	HAL	.997	.027
		TM2	CPZ	HAL	-.840	.061
		TM3	CPZ	HAL	.020	.964
	Medium dose	TM1	CPZ	HAL	.040	.927
		TM2	CPZ	HAL	.260	.553
		TM3	CPZ	HAL	-.117	.790
	High dose	TM1	CPZ	HAL	-.297	.499
		TM2	CPZ	HAL	-.183	.675
		TM3	CPZ	HAL	-.400	.363
% mean SA of PZ	LOW	TM1	CPZ	HAL	-.123	.808
		TM2	CPZ	HAL	-1.183	.024
		TM3	CPZ	HAL	-1.767	.001
	MEDIUM	TM1	CPZ	HAL	.153	.762
		TM2	CPZ	HAL	.273	.590
		TM3	CPZ	HAL	.087	.864
	HIGH	TM1	CPZ	HAL	-1.667*	**.002**
		TM2	CPZ	HAL	-2.033*	**.000**
		TM3	CPZ	HAL	-1.930*	**.000**
% mean SA of TZ	LOW	TM1	CPZ	HAL	-.553	.167
		TM2	CPZ	HAL	-.517	.196
		TM3	CPZ	HAL	.007	.987
	MEDIUM	TM1	CPZ	HAL	.150*	**.005**
		TM2	CPZ	HAL	.377	**.044**
		TM3	CPZ	HAL	1.200	**.004**
	HIGH	TM1	CPZ	HAL	-.670	.096
		TM2	CPZ	HAL	-.450	.259
		TM3	CPZ	HAL	-.983	**.017**
% mean SA of PS	Low	TM1	CPZ	HAL	-.743	**.002**
		TM2	CPZ	HAL	-.607	**.009**
		TM3	CPZ	HAL	-.597	**.010**
	Medium	TM1	CPZ	HAL	.467	**.041**
		TM2	CPZ	HAL	.183	.412
		TM3	CPZ	HAL	.440	.054
	High	TM1	CPZ	HAL	.140	.530
		TM2	CPZ	HAL	-.277	.218
		TM3	CPZ	HAL	.280	.213

key: **CPZ =**chlorpromazine
HAL = Haloperidol

Level 2: The Comparative Findings on How the Two Medicines Influenced the Cellular Densities of the Resting Zone, Proliferation Zone and the Transformation Zones Fetal Tibia and Humerus.

Upon assessment of effects of in-utero exposure to chlorpromazine and haloperidol the finding is presented in two stages as follows;

Stage 1: *findings on how in-utero exposure to chlorpromazine and haloperidol influenced the cellular densities of the various layers of epiphyseal growth plate using ANOVA.*

Stage 2: *The multivariate analysis of variance (MANOVA) on how chlorpromazine and haloperidol influenced surface area of fetal growth plate.*

Stage 1: The ANOVA Findings on How *In-Utero* Exposure to Chlorpromazine and Haloperidol Influenced the Cellular Densities of the Various Layers of Epiphyeal Growth Plates.

Upon global assessment of means of cellular density of epiphyseal growth plate of both proximal and distal tibia and humerus of fetal rats following exposure to the two medicines, it was noted that there was no comparable difference. When the global effects of the two drugs (CPZ&HAL) was assessed without considering dosage and time of exposure was statistically significant for all the zones (a)zone of resting cartilage ($F(18,38)=23.904$ (b) zone of proliferation ($F(18,38)= 47.484$ (c) zone of transformation ($F(18,38)= 30.680$ as illustrated on (*table 4.9)* (Lippl et al., 2012)(Spertus et al., 2018).

On further observation it was noted that at the medium dose chlorpromazine treated groups the means of the total surface area of various layers of pups' growth plates were statistically significantly higher than the control group. However, at high dose, both chlorpromazine and haloperidol treated groups depicted statistically significant decrease $p<0.05$ on all the layers of the growth plate more so for the chlorpromazine treatment group from trimester one *(table 4.13).*

Table 4.13: The Comparative ANOVA Table Showing How *In-Utero* Exposure to Chlorpromazine and Haloperidol Influenced the Mean Cellular Densities of the Various Layers of Epiphyseal Growth Plate of a Long Bone against Control.

The study groups	Study groups and dosage levels	The time of exposure to treatment	Cell density of zone of reserve cartilage ±SD	Cell density of zone of proliferation ±SD	Cell density of zone of transformation)±SD
Control	No treatment given(C)	None	53.33±5.77	85±5.00	100±10.00
Chlorpromazine treatment group	Low dose	Trimester one(TMI)	63.00±2.64	93.33±2.88	116.67±5.77
		Trimester two(TMII)	62.33±2.51	88.33±2.88	111.67±10.4
		Trimester three(TMIII)	60.00±8.67	96.00±1.70	121.67±2.88
	Medium dose	Trimester one(TMI)	75.00±5.0*	102.67±6.4*	127.33±2.51*
		Trimester two(TMII)	69.33±2.51	101.67±2.5*	123.33±2.88*
		Trimester three(TMIII)	65.33±2.52	97.67±7.63*	121.67±2.88*
	High dose group	Trimester one(TMI)	31.67±2.88*	55.00±5.0*	63.33±5.77*
		Trimester two(TMII)	38.00±2.64*	58.33±2.8*	71.67±1.60*
		Trimester three(TMIII)	50.00±5.0*	66.67±2.88*	98.67±1.15
Haloperidol treatment groups	Low dose groups	Trimester one(TMI)	61.61±3.05	94.00±3.61	113.33±2.88
		Trimester two(TMII)	65.00±1.0	88.67±3.51	116.67±10.4
		Trimester three(TMIII)	59.67±5.13	95.33±5.00	110.00±1.0
	Medium dose groups	Trimester one(TMI)	66.00±5.29	93.33±5.77	120.00±0.00
		Trimester two(TMII)	60.66±1.15	92.0±2.00	113.33±10.4
		Trimester three(TMIII)	61.±3.605	95.00±5.00	110.00±0.00
	High dose groups	Trimester one(TMI)	40.66±1.15*	61.00±1.73*	69.67±1.52*
		Trimester two(TMII)	40.67±2.08*	67.00±2.65*	71.00±1.73*
		Trimester three(TMIII)	55.33±5.03	71.67±0.58	92.00±5.29
			F(18,38)=23.904 P=0.001	F(18,38)=47.484 P=0.001	F(18,38)=30.680 P=0.001

Key: *values are conveyed as means ± standard deviation of means n=3 per group. (*) the figure bearing the asterisk shows that they are significantly different ($p<0.05$) with the control, using one-way ANOVA with Tukey post hoc multiple comparison t-test.*

Stage 2: The Multivariate Analysis of Variance (MANOVA) on How Chlorpromazine and Haloperidol Influenced the Surface Area of Fetal Growth Plate.

Upon further analysis on the effects of the two drugs on the cellular density of the proximal and distal epiphyseal growth plates of humerus and tibia using MANOVA it was noted that there was no comparable difference between them, the findings are presented in three levels as follows:

Level 1: *How prenatal exposure to the two drugs influenced the cellular densities of the various layers of growth plate globally.*

Level 2: *How the individual drug, their dosages, time of exposure and their interactions influenced cellular density of layers of growth plate prenatally.*

Level 3: *The pairwise comparison on how chlorpromazine and haloperidol influenced cellular densities of the fetal growth plate when exposed at similar dosage level and time.*

Level 1: **The MANOVA Analysis on How Exposure to Chlorpromazine and Haloperidol Prenatally Influenced the Cellular Density of Growth Plate Globally.**

Upon carrying out multivariate analysis to evaluate how the two drugs influenced the cellular density of various layers of fetal growth plate globally,the main effects and two ways interaction effects of drug*dosage and dosage*trimester were found to be statistically significant in different proportions (partial Eta squired(η^2) as illustrated in (*table 4.14**)** as follow*;

(i) The individual main effects of ;(a) drug($F(3,36)=1.249, p<0.006$;wilks lambda(∧)=.906;partial Eta squired ($\eta^2=.294$.(b) dosage($F(6,72)=68.715$, $p<0.001$;wilks lambda(∧)=.022;partial Eta squired ($\eta^2=.851$ (c) trimester($F(6,72)=6.092, p<0.001$;wilks lambda(∧)=.440;partial Eta squired ($\eta^2=.337$.

(ii) The two way combination effects of drug and dosage((F(6,72)=6.864, p<0.001;wilks lambda(∧)=.404;partialEtasquired(η^2=.364.dosage and trimester (F(12,95)=7.115,p<0.001;wilks lambda(∧)=.185;partial Eta squired (η^2=.431.Though there was no three ways interaction effects of drug, dosage and trimester.

Table 4.14: The MANOVA Level 1 Table on How In-Utero Exposure to Chlorpromazine and Haloperidol Globally Influenced the Means of Cellular Densities of Zone of Resting Cartilage, Proliferation Zone and Transformation Zone of Fetal Epiphyseal Growth Plate.

Types of MANOVA evaluation at level 1	The comparative global effects assessed	Parameter used	MANOVA test (Wilks lambda)	F statistics used	Hypothesis df	Error degree of freedom df	Sig.	Partial Eta Squared
	Was the observed effects due to chance	Intercept	.001	11242.515[b]	3.000	36.000	.000	.999
Test on whether theor not observed effects were due to chance or not								
The individual main effects of drug, dosages and trimester	Was the observed effects due to chlorpromazine or haloperidol	Drug	.906	1.249[b]	3.000	36.000	.006	.294
	Was the overall effects due to varied doses of chlorpromazine and haloperidol	Dosage	.022	68.715[b]	6.000	72.000	.000	.851
	Was the observed overall effects were due to differing trimesters	Trimester	.440	6.092[b]	6.000	72.000	.000	.337
Two-way interaction effects on the fetal dependent variable	Were the observed effects due to interaction between drug and dosage	Drug*dosage	.405	6.864[b]	6.000	72.000	.000	.364
	Were the observed effects due to interaction between drug and trimesters	Drug*trimester	.953	.295[b]	6.000	72.000	.938	.024
	Were the observed effects due to interaction between dosage and trimesters	Dosage*trimester	.185	7.115	12.000	95.539	.000	.431
Three-way interaction effects on the fetal dependent variable	Were the observed effects due to interaction between drug, dosage and trimesters	Drug*dosage*trimester	.832	.574	12.000	95.539	.858	.060

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 MANOVA Level 2 Findings on How the Three Independent Variables of Drug, Dosage and Trimesters of Exposure Plus test in the Three Layers of Epiphyseal Growth Plates.

Upon further analysis on how *in- utero* exposure to individual drug, dosage and time of exposure and their interaction using MANOVA influenced cellular densities of the zone of resting cartilage, zone of proliferation and the zone of transformation, it was noted that: -

(i) The individual level contribution of the drug, dosage and trimester of exposure influenced cellular density of the three layers of epiphyseal growth plate of the fetal rat shown a varying partial Eta squired (η^2) to each of them with the dosage showing the highest contribution as illustrated in *(table 4.15).*

(ii) The two ways interaction effects of the drug, dosage and time of exposure to each of the three layers when combined (a) drug and dosage (b) dosage and trimester was found to have statistically significant main effects and interaction effects, with drug and dosage having the highest contribution. Though there were no three-way interaction effects between drug, dosage and trimester.

Table 4.15: The MANOVA Level 2 Table on How *In-Utero* Exposure to Chlorpromazine and Haloperidol Influenced Cellular Density of Various Layers of Epiphyseal Growth Plate at an Individual Level.

Test of between subject effects				
Source	**Dependent Variable**	**F**	**Sig.**	**Partial Eta Squared**
Drug	cell d RC	.000	1.000	.000
	Cell d PZ	.030	.863	.001
	Cell d TZ	3.929	.050	.094
Dosage	cell d RC	166.379	**.000**	**.898**
	Cell d PZ	393.638	**.000**	**.954**
	Cell d TZ	231.416	**.000**	**.924**
Trimester	cell d RC	3.004	**.061**	.137
	Cell d PZ	10.612	**.000**	.358
	Cell d TZ	11.834	**.000**	.384
drug * dosage	cell d RC	9.418	**.000**	.331
	Cell d PZ	13.883	**.000**	.422
	Cell d TZ	2.904	**.047**	.133
drug * trimester	cell d RC	.041	.960	.002
	Cell d PZ	.369	.694	.019
	Cell d TZ	.462	.634	.024
dosage * trimester	cell d RC	16.685	**.000**	.637
	Cell d PZ	4.210	**.006**	.307
	Cell d TZ	12.678	**.000**	.572
drug * dosage * trimester	cell d RC	.956	.443	.091
	Cell d PZ	.189	.943	.019
	Cell d TZ	.682	.609	.067

Key: *cell d RC= cellular densities of zone of resting cartilage*

Cell d PZ= cellular densities of zone of proliferation

Cell d TZ = cellular densities of zone of transformation

Stage 3: The Pairwise Comparison of Histoquantative Percentage Surface Area of Various Layers of Epiphyseal Growth Plate Following *In-Utero* Exposure to Chlorpromazine and Haloperidol Administered at Similar Dosage Level at the Same Trimester.

Upon carrying out pairwise comparison on effects of independent variable on the two fetal parameters to establish how chlorpromazine compared to haloperidol, it was observed that the means were significantly higher for chlorpromazine treatment

group in trimester two and significantly lower for trimester three as compared to haloperidol treatment groups *(Table 4.16).*

Table 4.16: The MANOVA Level 3 Table Showing the Pairwise Comparisons of How In-Utero Exposure to Chlorpromazine and Haloperidol Influenced the Cellular Density of the Various Layers of Epiphyseal Growth Plate W

Multiple/ pairwise comparisons

Dependent variable	Dose level	The time of treatment exposure	CPZ group	HAL group	Mean difference between CPZ and Hal treatment	Significant <0.05
% mean surface area of reserve cartilage	Low dose	TM1	CPZ	HAL	1.333	.688
		TM2	CPZ	HAL	-2.667	.424
		TM3	CPZ	HAL	.333	.920
	Medium dose	TM1	CPZ	HAL	9.000	**.010**
		TM2	CPZ	HAL	4.667	.165
		TM3	CPZ	HAL	4.333	.197
	High dose	TM1	CPZ	HAL	-9.000	**.010**
		TM2	CPZ	HAL	-2.667	.424
		TM3	CPZ	HAL	-5.333	.114
% mean surface area of proliferation zone	LOW	TM1	CPZ	HAL	-.667	.836
		TM2	CPZ	HAL	-.333	.918
		TM3	CPZ	HAL	.667	.836
	MEDIUM	TM1	CPZ	HAL	9.333	**.006**
		TM2	CPZ	HAL	5.667	.085
		TM3	CPZ	HAL	6.667	.044
	HIGH	TM1	CPZ	HAL	-6.000	.069
		TM2	CPZ	HAL	-8.667	**.010**
		TM3	CPZ	HAL	-5.000	.127

CHAPTER FIVE

DISCUSSION, CONCLUSION, AND RECOMMENDATION.

5.1 This Study Discussion is presented in Line with Study Objectives as Follows;

5.1.1 Objective 1: The Comparative Evaluation on How Prenatal Exposure to Varied Doses of Haloperidol and Chlorpromazine Influenced the Maternal and Fetal Pregnancy Outcomes.

Upon evaluating the maternal pregnancy outcomes following prenatal exposure to the two medicines, the current study found that the two medicine had varying effects on maternal weight gain trends across the entire gestation period. In particular, Chlorpromazine at a medium dose caused remarkable maternal weight gain across all trimesters, especially in rats treated from trimester one *(Figures 4.1 to 4.3)*. This could be due to the fact that chlorpromazine at low and medium doses increases ghrelin production (which stimulates appetite), slows metabolism (making it hard to burn calories), and interferes with body composition (increasing fat mass and reducing muscle mass). These findings are consistent with a previous study which found that exposure to antipsychotics for prolonged period of time was associated with weight maternal weight gain (Babu et al., 2015).

In contrast, haloperidol at low and medium dose had no remarkable weight gain difference with control this could be due its reduced interference with metabolism and appetite. These findings concurs with those found by Melek Akar *et al* , (2014). However, the research found that when chlorpromazine and haloperidol were given at high dose there was significant reduction in maternal weight gain more so for the rats which received high dose chlorpromazine from trimester one *(Figure 4.1 to 4.3)* this could be attributed to high dose of both chlorpromazine and haloperidol. More so, chlorpromazine is associated with severe drowsiness which interfere with feeding programme (Meng *et al*., 2018). These findings differ with previous research carried out by Wu *et al* which found that high dose of neuroleptics caused weight gain these difference could be due to the fact that Meta-analysis included the second generation neuroleptics such as clonazepam which causes excess weight gain (Wu *et al*., 2022).

On evaluation of fetal outcomes following prenatal exposure to the two medicines, the current study established that at high dose, both chlorpromazine and haloperidol were associated with increased number of embryolithalities, dead fetus and resorbed gland as compared to control, though chlorpromazine had more cases as compared to haloperidol (*Figure 4.4 (A),(B) and (C)*) this could be due to their ability to cross placenta barrier and interfere with organogenesis mostly chlorpromazine interfere with development of placenta(Furukawa et al., 2014) . In addition, the number of fetuses for the two treatment groups were reduced as compared to the control across all treatment group of high, medium and low dose.

When the fetal pregnancy out comes of mean fetal weight and crown-lump length were evaluated using one way analysis of variance (ANOVA), the current study found that at medium dose chlorpromazine treated group had statistically significant ($p<0.05$) higher mean fetal weight and crown –lump length for the fetuses exposed from trimester one (i) mean fetal weight 7.31±1.44 (ii) crown-lump length 5.57±0.25 as compared to the control *[table 4.1]*. However, at high dose both medicine caused statistical significant ($p<0.05$) reduction in both fetal weight and crown lump length across the trimesters as follows ;(i) mean fetal weight and crown-lump lengths of haloperidol treated group for trimester one 3.38±0.31,3.34±0.36,trimester two 3.60±0.28, 3.48±0.26 and trimester three 4.17±0.36 , 3.46±0.3 respectively.(ii) mean fetal weight and crown-lump length of chlorpromazine exposed group *in-utero* for trimester one 3.42±0.47,2.27±0.22, trimester two 3.48±0.843. 14±0.54, trimester three 3.52±0.34, 3.15±0.08 respectively. In addition the dosage (.918,.969) contributed more to the deleterious effects followed by the time of exposure (.219,.135) and lastly the type of drug (.214,.115) as shown by differing partial Eta squired (η^2) as illustrated on *table 4.2.*This could be attributed to exposure to high dose of both chlorpromazine and haloperidol for a longer period may interfere with normal feeding due to excess drowsiness thus reduced weight gain in both mother and child (Dudley *et al*., 2017).This finding defer with previous study which found that antipsychotics causes high birth weight which may be attributed to gestational diabetes the difference could be attributed to confounding factors such as alcohol, smoking and illicit drug use(Galbally et al., 2014a).

5.1.2 Objective 2. The Comparative Evaluation on How the Prenatal Exposure to Varied Doses of Chlorpromazine and Haloperidol Influenced the Histomorphological Organization of the Epiphyseal Growth Plate.

Regarding the comparative analysis on how *in-utero* exposure to the two medicines influenced fetal outcome of histomorphological differentiation of epiphyseal growth plate of a long bone the current study found that, when chlorpromazine and haloperidol was administered at low doses across all trimester there was no comparative histomorphological difference with the control groups in terms of cellular distribution and tissue cyto- architecture (*fig 4.1. to 4.15).* Though at medium dose for both treatment group the hypertrophic zone had more column of chondrocytes while at high dose the columns of chondrocytes were significantly reduced as illustrated by (*fig 4.2, 4.4 and 4.10).* In addition, when chlorpromazine and haloperidol were given at high doses across all trimesters, there was a comparatively significant reduction in the growth plate length especially zone of transformation and zone of proliferation more so for the chlorpromazine treated groups across all the trimesters *(figure 4.13,4.14 and 4.15)*. This could be attributed to its anticholinergic effects which may inhibit release of growth hormone.in addition dopamine receptor blockage at high rate may lead to reduction in secretion of growth hormone(Noaín *et al*., 2013). This finding agreed with previous study carried out by hanafy and colleagues (Hanafy, S. 2019).

5.1.3 Objective 3: The Comparative Evaluation on How the Prenatal Exposure to Varied Doses of Chlorpromazine and Haloperidol Influenced the Gross Morphometric and Histo-Stereological Differentiation of Epiphyseal Growth Plate.

With regards to the comparative evaluation on how the two medicines influenced the gross morphometric measurements and the histo-stereological differentiation of the epiphyseal growth plates of the long bones, The current study established that, at medium chlorpromazine dose there was statistically significant $p<0.05$ increase of both tibia and humerus for trimester one as follows (i) Tibia length = 6.4±0.1 (ii) Humerus length for =5.67±0.05 *table 4.5.* . In addition, the study found that in-utero exposure to medium dose chlorpromazine influenced the quantitative histological

contribution of all the layers of epiphyseal growth plate thus positively influencing the length of the humerus and tibia (*Table 4.6).*

This could be due to, rats which received medium dose of chlorpromazine had statistically significant higher ($p<0.05$) fetal weight at the point of sacrifice, this may be attributed to, adipose tissue makes hormone leptin whereby ,Endochondral ossification been a complex process, leptin may be an important mediator of 'growth without growth hormone' (Yablonski *et al*,.2007) and leptin stimulate chondrocyte proliferation and differentiation thus playing a role in Endochondral ossification (Khosla, 2002). In addition, the use of FGA in the treatment of psychosis has been associated with a significant increase in serum insulin-like growth factor 1 (IGF-1) which are major contributor to stimulation of clonal expression of proliferation chondrocyte in the epiphyseal growth plate region leading to bone elongation possibly mediated by a reduction in levels of cortisol(Abubakar et al., 2019b; Venkatasubramanian et al., 2010) .Also The rate of longitudinal bone growth is majorly contributed by two factors within the growth plate; the rate of production of new cells per column and the average size of hypertrophic cells .the height of the growth plate is directly proportionate to the longitudinal bone growth (Hallett et al., 2019b). This finding agrees with a previous analysis done by Patton et al. (2002) that found that prenatal exposure to typical antipsychotic medication significantly affected the height of children born of mothers taking chlorpromazine.

However, at high dose both chlorpromazine and haloperidol were noted to cause statistical significant reduction ($p<0.05$) in percentage surface area of various layers of epiphyseal growth plate in all trimesters as follows ;(A) chlorpromazine exposed group(i) zone of resting cartilage TMI,34.30±0.04,TMII 35.31±0.05, TMIII 36.45±0.37 (ii) zone of proliferation TMI 32.06±0.05 TMII 32.76±0.20 TMIII 33.26±0.16 (iii) zone transformation TMI 21.89±0.60 ,TMII 22.23±1.04 TMIII 22.43±0.21 (iv) zone of primary spongiosa TMI 20.46±0.63, TMII 20.48±0.11,TM III 21.64±0.07 (B) Haloperidol exposed group (i) zone of resting cartilage TMI, 34.67±0.29, TMII 35.50±0.32 TMIII 36.85±0.14 (ii) zone of proliferation TMI 30.39±0.13 TMII 30.72±0.34 TMIII 31.33±0.02 (iii) zone transformation TMI 22.56±0.15, TM II 22.68±0.15 TMIII 23.41±0.64 (iv) zone of primary spongiosa

TMI 20.32±0.11TMII 21.12 ±0.6, TM III 21.36 ±0.1.(*Table 4.3.5).* This could be attributed to effects of dopamine blockage at high level which interfere with release of growth hormone this study agrees with a previous study, which found that use of first generation neuroleptics stunted growth leading to overall short stature unless the drug was withdrawn (Wu et al., 2022).there was significant increase in chondrocyte cellular density for medium dose treated group of chlorpromazine treatment group across all trimesters more so for those exposed from trimester one this finding agree with previous study found that the chondrocyte density plays an important role in bone elongation (Abubakar *et al.*, 2019b).

5.2 Conclusion

This study concludes; -Both medicines at low doses has no teratogenic effects across all the trimesters though at medium dose it caused significant fetal & maternal weight gain and at high dose cause reduction in fetal and maternal weight. The fetal rats exposed to MDCPZTMI showed significant increase in column of chondrocytes at the zone of proliferation, thicker zone of transformation and statistically significant increase ($p<0.05$) in percentage surface area of all the zones of epiphyseal growth plate meaning chlorpromazine at that medium dose is a growth promoter. Both medicines at high doses caused statistically significant reduction on the tibia and humerus length, both gross and histostereological parameters evaluated across all trimesters with most severe deleterious effects observed when given from TMI, and especially in the CPZ treated group.The observed injurious effects were both dose and time dependent, with the most toxic teratogenic doses for the 2 been high dose and CPZ having more deleterious effects as compared with HAL.

5.3 Recommendation

This study recommends that;

I. Use of CPZ during pregnancy at medium dose should be avoided as it was noted to cause significant fetal weight gain, which can pause as a problem during delivery.

II. While high doses of both drugs should be avoided especially from TM1 an appropriate alternative should be adopted.

III. Should expectant mothers be on chronic use of both drugs even before conception, the dose should be tapered to a minimum dose and a safer drug may be introduced ensuring maximum maternal benefit without deleterious effects to the growing fetus.

IV. Further studies should be carried out on non-human primates to establish minimum dose level of FGAs which enhance growth without deleterious effects to tap on their positive effects.

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APPENDICESS

Apendix I: Ethical Approval

UNIVERSITY OF NAIROBI
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REF: FVM BAUEC/2021/326

Ms. Jane Wanjiru Kuria.
Dept. Human Anatomy,
JKUA & Technology.
10/11/2021

Dear Jane,

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

Comparative histomorphological and histostereological teratogenic effects of prenatal exposure to varying doses of haloperidol and chlorpromazine during first, second and third trimester on fetal skeletogenesis in Albino rats.

Jane Wanjiru Kuria. HSM301-1487/2020.

We refer to your MSc. proposal submitted to our committee for review and your application letter dated 8th November 2021. We have reviewed your application for ethical clearance for the study. The number of rats, animal husbandry practices and protocols used to assess histomorphological and histostereological teratogenic effects of prenatal exposure to varying doses of haloperidol and chlorpromazine on fetal skeletogenesis meets the minimum standard of the Faculty of Veterinary medicine ethical regulation guidelines.
We hereby give approval for you to proceed with the project as outlined in the submitted proposal.

Yours sincerely,

Kahuwa

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

Appendix II: Publication

IOSR Journal Of Humanities And Social Science (IOSR-JHSS)
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The Noxious Effects Of *Perinatal* Exposure To Different Doses Of Chlorpromazine On Fetal Growth And Development In Albino Rats (*Rattus Norvegicus*).

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ABSTRACT:

The in-utero effect of Chlorpromazine on fetal growth and development has not gotten explained. Thus, this study purposed to evaluate the prenatal effects of this compound on fetal growth and development in albino rats, including the mean fetal weight, the length of the Crown lump, the diameter of the bi-parietal, and the head circumference when applied to albino rats at various doses and gestational periods. The experimental post-test design was used. Thirty albino rat dams were used for the experiment. To evaluate the teratogenic effect of chlorpromazine when exposed in utero. The 30 albino rat dams were split into two major study categories, consisting of 3 control rats and 27 experimental rats. To determine whether the teratogenic implications of chlorpromazine are dose-dependent, the 27 rats were separated into three sub-groups of 9 rats, each based on the dosage: low, medium, and high. The nine rats in the aforementioned experimental groups were subsequently separated into three groups based on the times of exposure (TM1), (TM2), and (TM3) to determine whether the teratogenic effects of chlorpromazine are time-dependent. Standard rodent pellets and water ad-libitum were provided for all the rats. All rats were humanely sacrificed on the gestation day 20th. The fetal growth and developmental indicators were recorded and evaluated for inferential statistics using SPSS version 25. The outcomes statistical significance was determined using a turkey post hoc multiple comparison test, and all values whose $p<0.05$ got considered important. This study illustrated that chlorpromazine is a fetal growth enhancer at low doses and growth inhibiter at high doses when administered in all trimesters compared with the control. However, effects on prenatal development and growth are dose-dependent but not time-dependent. More research on non-human primates is advised to confirm its safety.

Keywords: *in-utero effects, chlorpromazine, teratogenic effects.*

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