COMPARATIVE MORPHOLOGICAL AND HISTO-STEREOLOGICAL TERATOGENIC EFFECTS OF PHENOBARBITAL AND PHENYTOIN ON THE DEVELOPING FETAL KIDNEYS IN ALBINO RATS (*Rattus norvegicus*)

JENNIFER CHEPKEMOI SEGUT

MASTER OF SCIENCE

(Human Anatomy)

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Comparative Morphological and Histo-stereological Teratogenic Effects of Phenobarbital and Phenytoin on the Developing Fetal Kidneys in Albino Rats (*Rattus norvegicus*)

Jennifer Chepkemoi Segut

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DECLARATION

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

Signature……...……. …….Date……...…….

Jennifer Chepkemoi Segut

This thesis has been submitted for examination with our approval as University Supervisors

Signature…… Date…….

Dr. Joseph Kweri, Ph.D

JKUAT, Kenya

Signature……..… Date…….

Dr. Ann Mwangi, PhD

JKUAT, Kenya

Signature……..… Date…….

Dr. James Mwangi Kanyoni, PhD

JKUAT, Kenya

Signature…… Date…….

Walter Rono, PhD

JKUAT, Kenya

DEDICATION

A special feeling of gratitude to my loving parents late Mr. Nehemiah K. Segut and Mrs. Nancy C. Segut for giving a very good foundation academically. I also wish to dedicate this work to my beloved brothers, and sisters namely:- Janet C. Segut, Geoffrey K. Ngetich, Jescah C. Segut, and Philemon K. Ngetich, for their immeasurable support throughout this research process. Above all, glory to God.

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ABBREVIATIONS AND ACRONYMS

AED	Anti-Epileptic Drug
ANOVA	Analysis of variance.
BAUEC	Biosafety, Animal Use and Research Ethics Committee
BCL2	B-cell Lymphoma 2
BMS	Bownan's Space
$C_{12}H_{12}N_2O_3$	Phenobarbital molecular formula
Ca^{2+}	Calcium ions
CAKUT	Congenital anomalies of the kidney and urinary tract
CD	Cortical Density
CKD	Chronic Kidney Disease.
CRL	Crown Rump Length
CV	Cavalieri Volume
DCT	Distal Convoluted Tube
Df	Degree of freedom
DPH	Phenytoin.
DPX	Digital Picture Exchange
ESRD	End-Stage Renal Disease.

et al	Is a latin word which means and “others”
FW	Fetal Weight
GABA	Gamma-Aminobutyric Acid
GABAA	Gamma-Aminobutyric Acid A receptor
GD	Gestational Dates
GD1	Gestation Day One
GD14	Gestation Day Fourteen
GD20	Gestation Day Twenty
GD7	Gestation Day Seven
GT	Glomeruli Tuft
H&E	Hematoxylin and eosin
HC	Head Circumference
HD	High Dose
HED	Human Equivalent Dose
Ho	Null hypothesis
In-utero	Is a latin word meaning "in the womb" or "in the uterus."
IUPAC	International Union of Pure and Applied Chemistry
JGA	Juxta Glomeruli Apparatus
JKUAT	Jomo Kenyatta University of Agriculture and Technology

JPEG Joint Photograph Expert Group

KV Kidney Volume

LD Low Dose

LPB Low Dose Phenobarbital

LPT Low Dose Phenytoin.

MANOVA Multivariate Analysis of Variance

MC Mesangial Cells

MCKD Medullary cystic kidney disease

MCMs Major Congenital Malformations.

MD Medullary Density

MD Medium Dose

Na+ Sodium ions

P450 (**CYP**) Cytochrome p- 450 enzymes

Pax-2 Paired box gene 2

PB Phenobarbital

PCO2 Partial pressure of carbon dioxide.

PO_2 Partial pressure of oxygen.

PT Phenytoin

SAFARI Small Animal Facility for Research and Innovation

SD Standard Deviation

SE Standard Error

SPSS Statistical Package for the Social Sciences

TM Trimester

TM_1 Trimester one

TM_2 Trimester two

TM_3 Trimester three

UON University of Nairobi

DEFINITION OF TERMS

Agenesis Failure of all or part of an organ to develop during embryonic growth

Degeneration of tubules Deterioration and loss of function in the cells of renal tubules

Embryopathies A developmental abnormality of an embryo or fetus especially when caused by maternal disease or drug exposure.

Gastric-gavage needle This is a tubular needle like gadget that is fitted into a calibrated syringe and was used to administer phenobarbital and phenytoin to the experimental animals through the mouth into the stomach.

Histo-morphology This is the histological technique applied to study and analyze the histo-morphological shapes of cells and non-cellular constituents of tissues that include distribution of cells per field, shapes of specific histological components, and micrometric measurements of specific cells to establish their ratio in relation to the connective to stromal tissue of the kidney.

Histo-Stereology This is the process of quantifying cells and tissues by applying developed standard micrometric and quantitative methods to estimate the sizes and quantities of cells, tissues, volume densities of microscopic constituents of tissues/organs when viewed under the microscope using specified magnifications for parameters like such as cellular numbers, cellular densities, ratios, length, total volume, and volume densities.

Hypertrophy The increase in the volume of an organ or tissue due to the enlargement of its component cells

Malformations An abnormally formed part of the body.

Nephrogenesis The process of formation of the nephrons**.**

Perturbation Deviation from normal for example abnormal development of the kidneys.

Teratogenicity The ability of a substance for example a drug to cause defects in a developing fetus

ABSTRACT

The prenatal teratogenicity exposures to phenobarbital and phenytoin on the morphology and the histo-stereological structures of the developing fetal kidneys remains largely unclear. At the same time, it is also not clear whether or not the teratogenic effects of the two medicines are dose and time dependent. In carrying out this study, post-test only control experimental study design was adopted. A resource equation for one-way analysis of variance (ANOVA) was used to arrive at a sample size of 30 albino rats *(Rattus norvegicus)* for each of the two-treatment groups. The rats were nine weeks old and weighed between 150-250 mg. These 30 albino rats in each of the study categories of phenobarbital and phenytoin were first randomly assigned into two broad study groups of three rats control and the 27 rats treatment category. To first evaluate the intrauterine effects of varied doses, the 27 rats in each of the treatment category were further subdivided in to three sub-groups of nine rats each as follows: nine rats for low dose, nine rats for medium and nine for high dose groups. To further evaluate the effects on the time of exposure, the nine rats in each of the three doses categories of low, medium and high was were further sub-divided into three sub-groups of three rats according to the trimesters of exposures as follows; three rats for Trimester I (TM_1); three rats for trimester II (TM_2) and three rats for trimester III (TM_3). At gestation day 20, all the rats were humanely sacrificed, three fetuses from each rat were selected and their kidneys harvested for histo-morphological and stereological analysis. The data was collected using a structured check list, then coded and entered into the computer using an excel spreadsheet for windows version 10, it was then exported to the Statistical package for the Social Scientist (SPSS) version 25 for statistical analysis. The inferential statistical significance levels were determined by use ANOVA and MANOVA and all values whose $P<0.05$ were considered significant. This study established that the prenatal exposure of both phenobarbital and phenytoin in all doses had a statistical significant reduction ($p<0.05$) to all the maternal and fetal growth parameters that included placental weights, maternal weight gain, birth weights, bi-parietal diameters as well as crown lamp lengths, when exposed at TM_1 and TM_2. At trimester three (TM_3), only the high doses were seen to negatively influence the outcomes. The histo-stereological finding revealed that *in-utero* exposure to either of the two medicine has a deleterious effect on the developing fetal kidneys including significant decrease ($p<0.05$) in the kidney volumes and volume densities. Widening of the bowman's spaces, renal tubules and the increase in cellular densities per glomeruli was also observed in a dose and time dependent manner. In conclusion all doses of both phenytoin and phenobarbital are teratogenic to the developing fetal kidneys at TM_1 and TM_2 and whenever possible the mothers should then be advised not to use these two medicines in their early pregnancy.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Phenobarbital and phenytoin are two anticonvulsant medicines commonly prescribed in the management of maternal convulsive disorders during pregnancy (Moussa *et al*., 2015). Phenobarbital is derived from a barbiturate compound (Mifsud, 2010). It's molecular weight is 232.24 g mol-1(Martinez *et al*., 2018), while phenytoin that is derived from the hydantoin compound has a molecular weight is 252.27mmol/l (Patocka *et al*., 2020b). All anticonvulsant medicines have been shown to have some degree of teratogenicity according to the American food, drug authority (FDA). Thus among the medicines should be used with caution during pregnancy (Conover *et al*., 2021), though both phenobarbital and phenytoin have remained the (front-runners medicines) commonly prescribed because of their low cost, effectiveness, and easy accessibility, (Ilangaratne *et al*., 2012). Their teratogenic effects on the histo-morphology and stereology of the developing fetal kidneys remains largely unclear.

In addition to phenytoin being used to treat epilepsy, it is also used to treat a variety of diseases, including migraine, dizziness, hiccups, myocardial infarction, burns, and in promoting wound healing (Wlodarczyk *et al*., 2012). Phenobarbital when used in the treatment of maternal health issues during pregnancy (like maternal convulsive disorders) it crosses the maternal placental barrier and associated with some form of abnormal development of fetal structures leading to malformations and deformities in newborns (Tetro *et al*., 2018).

Previous studies have shown that all types of antiepileptic medicines when used during pregnancy in the management of maternal epilepsy increases the chances of congenital malformations (Meador, 2020). Some recent studies have reported that the prevalence of anticonvulsant embryopathies like fetal malformations, obstructive urinary congenital anomalies, renal agenesis, neural tube defects, hypospadias, growth retardation, and hypoplasia of the face (Conover *et al*., 2021), were reported

to increase by three-fold in infants exposed to antiepileptic drugs in fetal life compared with unexposed infants (Saeed *et al*., 2020).

Maternal and fetal pregnancy outcomes are important teratogenic parameters as these elucidate the relationship between the fetal growth environment *in-utero* and the subsequent influence on the fetal organogenesis (Cunha *et al*., 2015).

Further studies done on fetuses from laboratory animals exposed to phenytoin in utero showed that, these fetuses had defects like stunted growth, impairment in the ossification of the sternum, immaturely developed cerebral ventricles and other cardiovascular deformities (Wlodarczyk *et al*., 2012).

Though the comparative nephron-teratogenic effects of phenytoin and phenobarbital has not been well elucidated, studies done on first-generation antiepileptic like carbamazepine that are in the same generation with phenytoin and phenobarbital showed mal-development of the fetal kidneys in the treatment groups. The observed histological changes included the atrophy of glomeruli, expansion of bowman's space, degeneration of partial layer of bowman's capsule, glomerular accumulation of cells, hemorrhage, congestion and detachment of tubular epithelial lining cells from basement membrane in the cortical region and also degeneration of renal tubules and collecting ducts in renal medulla represented by loss of nucleus, cell swelling and cell death (Al-bakri *et al.*, 2016).

In some other studies done on sodium valproate which has the same mode of action as phenobarbital report changes in the kidney that included:- interstitial hemorrhage, cloudy swelling of renal tubules, hyper-cellular glomeruli, blood vessels congestion, proliferation of mesangial cell and hydropic changes on the proximal and distal convoluted tubules (El-Shenawy & Hamza, 2016). Though both medicines have been associated with some congenital anomalies, the effects of phenobarbital and phenytoin use during pregnancy on the development of fetal kidneys (in terms of their deleterious effects on the histomorphology, the histo-cytoarchitecture and the stereological effects) remains unclear.

The aim of this study therefore was to establish baselines of histo-morphological and histo-stereological teratogenic effects of phenobarbital and phenytoin when exposed prenatally at varied doses and at different gestational periods. This was with a view to having some baseline data to guide other future studies in higher non-human primates that have similar genomic relationship to humans.

The results of this study have potential to guide clinicians in the management of maternal convulsive disorders like epilepsy among others in future and unravel some intrauterine causes of renal disease and abnormalities in children.

1.2 Statement of the Problem

Whether or not the nephro-teratogenic effects of the two medicines are dose and time dependent is largely undocumented. This coupled with the rising cases of both juvenile and adulthood renal failures of unknown causes, the need to start tracing the probable *in-utero* causes like the use of anticonvulsant medicines during pregnancy cannot be overemphasized.

Though phenytoin and phenobarbital are known to cause congenital defects in the developing fetal viscera, their comparative histosteriological and histomorpholocal nephro-teratogenic effects on the developing fetal kidneys remains unclear

Lack of this histomorphological and histostereological data on developing fetal kidneys also exist in a situation where, today there is also insufficient data on congenital kidney diseases in pediatric that is associated with prenatal renal teratogenicity exposures from phenytoin and phenobarbital even from the renal prenatal nephron teratogenic registries (Hattori *et al.,* 2015). This is despite previous studies showing that most of the common causes of end-stage kidney failures witnessed in children and adolescents emanate from a congenitally induced malfunction of the urinary tract and kidneys representing a broad range of disorders affecting humans (Ashuntantang *et al*., 2017). At the same time, chronic kidney diseases (CKD) of unknown are also on the increase world-wide and are currently among the leading causes of death and disability worldwide (Paidi et al., 2021).

Globally, Kidney diseases are currently on the increase and are among the leading cause of death *(*Hasan *et al.,* 2018*)* - WHO report of 2021 - 11-13% *(*Masalskienė *et al.,* 2021). Sub Saharan Africa and reported an overall prevalence of 15·8% (Matsha & Erasmus, 2019) with 3.7% (2.7–5.1%) in Kenya (Muiru *et al.,* 2020).

Phenobarbital and phenytoin has been associated with acute interstitial nephritis leading to development of acute kidney inflammations (Perazella & Rosner, 2022). Transient renal failure was reported with the use of phenytoin. (PHT), tubular injury, were reported with chronic use of phenytoin (Hamed, 2017a). Numerous case reports have reported hypersensitivity reactions associated with initiation of carbamazepine and phenytoin that have led to multisystem signs and symptoms, which also involves the kidneys. Other case reports describe renal teratogenicity in patients using phenobarbital (Mahmoud *et al*., 2020).

This study aim was to establish baselines of histo-morphological and quantitative nepro-teratogenic effects of phenobarbital and phenytoin on the developing fetal kidneys when exposed prenatally at varied doses and at different gestational periods.

1.3 Justification of the Study

Both phenobarbital and phenytoin may continue posing a risk to the developing fetal kidneys and their teratogenic risks will continue being unknown. Therefore, there is need to comparatively evaluate their relative teratogenic risks on histomorphological and histostereological effects on the developing fetal kidneys. Such data would be useful in determining the extent to which doses of these two medicine can be down regulated for the mothers using the them during pregnancy. This is due to the fact that they cannot be withdrawn therefore, there is need to find out the relative risks between the two medicines. The albino rats were chosen due to the fact that they have a low incidence of spontaneous occurring congenital malformation and they are resilient and can withstand a wide range of study medicine

1.4 Significance of the Study

The data generated will form basis for guidelines on their applications during pregnancy. Therefore, reducing the risks of exposure which in the long run lead to reduction in cases of renal anomalies. Data would be useful in determining the extent to which doses of these two medicine can be down regulated for the mothers using the them during pregnancy. It will form a foundation for carrying out further studies on higher non-human primates that have a close genetically relations to humans.

The Nephro-teratogenic histostereological and histomorphological data generated from this study on the prenatal renal teratogenicity exposures to phenobarbital and phenytoin will form a foundation for carrying out further studies that would help the clinicians in making an informed choice between the two medicines when needed during pregnancy. The findings of this study will also assist in retracing the embryological origin of some renal kidney disorders of unknown causes seen in adolescents and adulthood.

When further studies are done using higher primate on these medicines, the histo-quantitative data obtained on the comparative teratogenic effects of phenobarbital and phenytoin will be helpful to health policymakers in the country in developing guidelines on their applications during pregnancy.

1.5 Broad Objective

To comparatively evaluate the histo-morphological and histo-stereological teratogenic effects of prenatal exposures to phenobarbital and phenytoin on the developing fetal kidneys in Albino Rats (*Rattus norvegicus*).

1.5.1 Specific Objectives

1. What are the effects of prenatal exposure to varied doses of phenytoin and phenobarbital on the fetal and maternal pregnancy outcomes in albino rats (*Rattus norvegicus*).
2. To comparatively evaluate the effects of prenatal teratogenicity exposures to varied doses of phenytoin and phenobarbital on the histo-morphological

organization of the developing fetal kidneys in albino rats *(Rattus norvegicus).*

3. To comparatively evaluate the effects of prenatal teratogenicity exposures to varied doses of phenytoin and phenobarbital on the stereology of the histological structure of the developing fetal kidneys in albino rats *(Rattus norvegicus).*
4. To comparatively evaluate whether or not the observed histo-morphological and histo-stereological teratogenic effects of prenatal teratogenicity exposures to phenytoin and phenobarbital on the developing fetal kidneys are dose and time-dependent.

1.5.2 Research Questions

1. To comparatively evaluate the effects of prenatal exposure to varied doses of phenytoin and phenobarbital on the fetal and maternal pregnancy outcomes in albino rats (*Rattus norvegicus*)?
2. What are the effects of prenatal teratogenicity exposures to varied doses of phenytoin and phenobarbital on the histo-morphological organization of the developing fetal kidneys in albino rats *(Rattus norvegicus)?*
3. What are the effects of prenatal teratogenicity exposures to varied doses of phenytoin and phenobarbital on the stereology of the histological structure of the developing fetal kidneys in albino rats *(Rattus norvegicus)?*
4. Are the observed histo-morphological and histo-stereological teratogenic effects of prenatal teratogenicity exposures to phenytoin and phenobarbital on the developing fetal kidneys dose and time-dependent?

1.5.3 Null Hypothesis (Ho)

There are no remarkable significant differences in the histo-morphological and histo-stereological teratogenic effects on the developing fetal kidneys when prenatally exposed to phenobarbital and phenytoin administered at varied doses and in different window periods in albino rats (*Rattus norvegicus*).

1.5.4 Study Limitations

1. Performing the histo-stereological and histo- morphological teratogenic effects were only done using light microscope due to unavailability of electron microscope.
2. The soft-ware used for histo-stereology were limited to first order stereology, because of lack of stereology microscope.

1.5.5 The Study Delimitations

1. The study only involved pre-natal exposure of phenobarbital and phenytoin and their effects were not studied post-natally in this study.
2. The study also was only limited to effects of phenobarbital and phenytoin on the kidneys and their effects were not studied on other organs though the drugs also affects these organs like the brain.

1.6 The Conceptual Frame Work

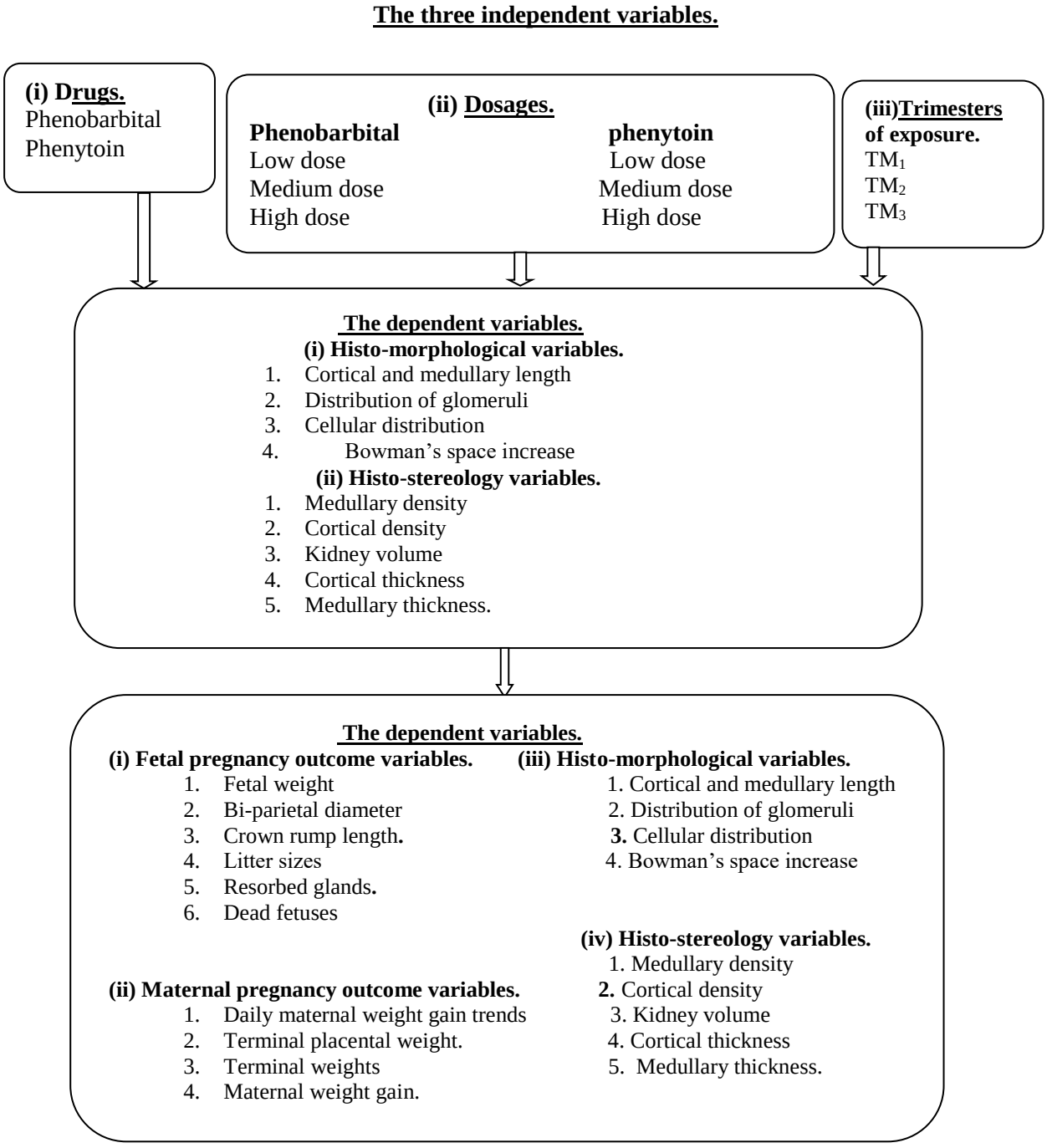


Figure 1.1: A conceptual framework for the relationship between dependent and independent variables

Adopted from (Varpio et al., 2020).

CHAPTER TWO

LITERATURE REVIEW

2.1 A Brief Description of Phenobarbital and Phenytoin Pharmacological Properties

Phenobarbital whose molecular mass is 232.24 g mol-1 with a chemical formula of $C_{12}H_{12}N_2O_3$ is a barbiturate compound called phenobarbital, whose International Union of Applied and Pure Chemistry (IUPAC) name it as 5-ethyl-5-phenyl-1,3-diazinane-2,4,6trione. It is a commonly prescribed drug to treat epilepsy though it is also used to primarily treat other conditions like anxiety disorder and also as a hypnotic drug (Martinez *et al*., 2018). Phenobarbital can be administered intravenously by injection and orally through solutions. It has also been shown that it can be administered subcutaneously (Hosgood ., 2016). Phenobarbital, is an effective antiepileptic drug that has been used since the early twentieth century (Ilangaratne *et al*., 2012).

Phenobarbital is relatively low cost and favorable cost-efficacy ratio, which is lower than that of any other antiepileptic drug in current use, makes the drug affordable and suitable for use in low and middle-income countries, where cost-effectiveness often supersedes other priorities (Ilangaratne *et al*., 2012). Phenobarbital together with other barbiturates bring in to play their antiepileptic mechanism by primarily by facilitating gamma aminobutyric acid (GABA) mediated inhibition by interacting with receptors of GABAA (Löscher & Rogawski, 2012). Barbiturates also activates of GABAA receptors resulting in an increase in chloride influx hyperpolarizes, thus slowing down the epileptic activity transmission (Löscher & Rogawski, 2012).

Phenytoin on the other hand is a hydantoin derivative with molecular weight of 252.27mmol/l (Patocka *et al.*, 2020b). It is used as an anticonvulsant and also classified as antiarrhythmic (Class I.B.) (Mathews *et al*. 2019). It is commonly used to treat partial and tonic-clonic seizures. For the last eight years, it has been under

clinical evaluation and has a greatly selective inhibitory effect on the cerebral cortex (motor area) (Patocka *et al*., 2020; and Abou-khalil, 2019) has a small therapeutic range, and a total concentration in the serum that is more than 80 µM is associated with clinically relevant toxicity for several patients (Desai, 2014); It's metabolism occurs in the liver by hepatic enzymes and excretion is through the kidneys therefore, phenytoin can accumulate in the body, causing toxicity in the circumstances whereby a person has renal failure (Patocka *et al*., 2020a). It is 90% albumin-bound, and therefore hemodialysis does not remove phenytoin (Maheshwari *et al*., 2020). However, the cellular modes of action of PHT remain unknown. Majority of studies show that PHT inhibits calcium (Ca+) and sodium (Na+) fluxes, modifies Na +, potassium (K+) transport, increases brainy aminobutyric acid (GABA) level, and potentiates GABA mediated postsynaptic inhibition (Keppel & David, 2017). The World Health Organization advocates a first-line agent for focal and tonic-clonic seizures in resource-restricted countries in all age groups. This is because of its broad-spectrum activity, recognized efficacy, low cost, and ease of use with once-daily dosing (Ilangaratne *et al*., 2012). Phenobarbital also remains a relatively commonly prescribed drug in many high-income countries despite its sedative and behavioral effects (Mifsud, 2010).

Antiepileptic drugs that also includes phenytoin and phenobarbital have been known to have teratogenic effects to a wide range of fetal organs including nervous system, musculoskeletal system, Urogenital system among others (Tomson & Battino, 2012). As such, mothers suffering from epilepsy have higher chances of getting infants with malformations (Koo & Zavras, 2013). Prevalence of major malformations in newborns whose mothers are epileptic using any of the anti-epileptic drugs is from four to six percent whereas in general population on the other hand is only two to four percent (Obstetrics, 2011). Antiepileptic drugs (AEDs) have the capacity to affect the development fetus throughout pregnancy but despite this women are advised to continue with their antiepileptic medication because on comparing the risk caused by the anticonvulsant drugs and the convulsions, the latter cause many risks to the child and the mother compared to the drugs (Wlodarczyk *et al*., 2012; and Farghaly *et al.,* 2017*)*. Though antiepileptic drugs have been known to be teratogenic to developing fetal kidneys, less is known about the comparative safety of

Antiepileptic drugs exposure in utero (Veroniki *et al*., 2017). There is a lot of inadequacy on how to manage epileptic mothers because the anatomical teratogenic risks for majority of anticonvulsants remain insufficient or are entirely not known ; therefore the clinicians have no proof to direct the choice of the medications in child bearing women (Meador, 2020). This is because use of more than one type of anti-seizure is more harmful than using one (Nie *et al*., 2016). It is therefore advisable to use one drug during pregnancy where it is possible prompting the need to know their comparative safety in pregnancy (Koo & Zavras, 2013).

Previous teratogenic studies have also shown that the most common cause of end-stage kidney disease in adolescents and children are related to teratogenic congenital malfunction of the urinary tract and kidney, representing a broad range of disorders affecting humans *(*Hattori *et al.,* 2015*).* Diversity of the malformations summarized by CAKUT is high and there are numerous associated syndromes. The genetic background of these malformations remains unknown in most of the cases (Isert *et al*., 2020). Other studies by (Masalskienė *et al*., 2021) have also shown that the common cause of CKD among children is congenital anomalies of the kidney and urinary tract (CAKUT) and with progressing CKD, various complications occur including End-stage renal disease (ESRD).

2.2 The Established General in-Utero Teratogenic Mechanisms of Phenytoin and Phenobarbital

The existing literature has shown that the prenatal exposures to both phenobarbital and phenytoin *in-utero* is linked both major and minor congenital anomalies of the fetal viscera that includes:- hypospadias, neural tube defects, cardiac malformations, cleft palate and cleft lip among others (Tomson & Battino, 2012).

The *in-utero* teratogenic mechanism of phenytoin is effected by the fact that phenytoin readily crosses the blood placental barrier (Patocka *et al.,* 2020c). Like PHT, P.B. was also shown to cross the placenta readily (Rubinchik-Stern & Eyal, 2012). Some studies have hypothesized that it is (phenytoin) DPH which is teratogenic (Nie *et al.,* 2016), while others have suggested that fetal malformations are caused by the interaction effect of DPH on folate metabolism (Reynolds &

Green, 2020). It was demonstrated changes in liver microsomal P-450 could partly explain an elevation in serum DPH levels perceived in pregnant animals. DPH is metabolized by hydroxylation and appears to be rate-limited (Franco & Perucca, 2015). Once the drug-metabolizing enzymes become saturated, additional DPH accumulates in the serum.

Pregnant animals receiving DPH were compared with non-pregnant animals and it was observed that there was a significant decrease in P-450 at days 7 and 14 in the pregnant group (Whelehan & Delanty, 2019). The blood pressure and the heart rate for the mothers treated with phenytoin decreased by approximately 15% which resulted in reduction of PO2 and increase in PCO2 indicating that phenytoin cause teratogenicity by inducing hypoxia to the fetus. This leads to disruption of blood vessels and then necrosis of forming and developing structures (Ritchie *et al*., 2021).

Other studies have also shown that essential mediators for the teratogenic action of phenytoin are as follows:- The sensitivity to develop malformations is caused by the excessive arrhythmia-related generation of reactive oxygen species as indicated by high activities of antioxidant enzymes rather than impaired antioxidant defense (Rubinchik-Stern & Eyal, 2012).

Phenytoin has been under clinical evaluation for around eight decades. It is primarily used to treat partial and tonic-clonic seizures (Sj *et al*., 2016) because of its selective inhibitory effect on the motor area of the cerebral part of the brain (Patocka *et al*., 2020).

The therapeutic range for phenytoin is narrow, and a total serum concentration greater than 80 µm is, for many patients, linked to clinically relevant toxicity (Desai, 2014). The kidneys typically excrete phenytoin after hepatic enzymes have metabolized it. However, a toxic accumulation of phenytoin can present itself in the context of people with renal failure (Hamed, 2019). Since phenytoin is 90% albumin-bound it cannot be removed by hemodialysis (Maheshwari *et al*., 2020).

Concerning teratogenicity of phenobarbital, different active signals: - Paired box gene 2 (pax-2) and B-cell lymphoma 2 (BCL-2) are thought to promote

nephrogenesis and induce renal dysplasia. B-cell lymphoma 2 is a protein typically expressed in developing kidneys that protect cells from apoptotic death. In transgenic mice, and overexpression of B-cell lymphoma 2 leads to renal cytogenesis through a high apoptotic rate. Vineyard *et al*. B-cell lymphoma 2 is consistently and ectopically denoted in dysplastic kidney epithelial. Statistics suggest that cystic formation and development can be modulated by B-cell lymphoma 2 overexpression, or it can result from a decreased ability of renal cells to acquire terminal differentiation. Phenobarbital elevates BCL-2 levels, causing suppression or reduction of programmed cell death. Phenobarbital therapy taken antenatally could lead to nephrogenesis abnormality causing multicystic dysplastic kidney (MCDK) through an abnormal regulation of BCL-2 (Hamouda & Shaban, 2016).

2.3 The Comparative Mode of Phenobarbital and Phenytoin Teratogenicity

Existing literature has shown that both phenobarbital and phenytoin are known to be teratogenic (Tomson *et al*., 2019). Though, the teratogenic mechanisms of phenobarbital remain in largely unclear, some studies have shown that rise in blood pressure associated with use of phenobarbital upregulates cytochrome P450s and produces oxidative stress through the generation of superoxide radicals, leading to the production of hydroxyl radicals, resulting in the formation of 8-oxodeoxyguanine that results in guanine, cytosine to thymine, adenine transversions. These findings demonstrate that that PB-induced oxidative stress may be the one that causes the observed developmental defects in the fetus (Tomson & Battino, 2012).

Phenytoin on the other hand once ingested by the mother is metabolized to a toxic reactive intermediate metabolite (arene oxide) that is, accountable for the detected teratogenic effects proposed by one of the favored theories out of the several different hypotheses that have been set forth. More specifically, the actual teratogenic molecule may be an arene oxide metabolite produced enzymatically during the bio activation of PHT by the cytochromes (Wlodarczyk *et al*., 2012)

2.4 Development of the Fetal Kidneys in Albino Rats Vis A-Vis the Humans

Existing literature has shown that the humans, the non-human primates as well as in rats the developmental stages of the kidneys depict similar developmental stages (Publication, 2017). Other study by (Cullen-McEwen *et al.*, 2015) also found out that kidneys of the rats follow similar developmental pathways and also that they have the same morphology. Their cell culture studies using nephrons were also noted to exhibit similar genetic expression of patterns that can be related to time scales in morphogenesis (Frazier, 2017). Thus, animal studies provide critical information that aids in the understanding of renal development across species, including primates such as humans (Little & McMahon, 2012). As such, the three main phases of fetal kidney development *in-utero* for both rats and in humans include, the pronephritic stage, mesonephros, and metanephros stage (Bueters *et al*., 2020). The pronephrons and metanephric stages are temporary stages where the developing primitive kidney structures in both humans and rats soon regresses after formation following the differentiation of metanephrons to adult kidneys (Frazier, 2017). The first stage of kidney development is marked by the development of transient pronephric ducts in both rats and humans that regresses soon after their formation. This appears approximately around the 22^{nd} day and 11^{th} day of gestation in humans and rats, respectively. Metanephric kidney develops as an outgrowth and branching of ureteric bud into metanephric mesenchyme as mesonephrons regress leading to the formation of nephrons (McMahon, 2016). Since most developmental abnormalities or drug-related injury occurs during nephron formation, the process of development of nephrons or "nephrogenesis" is essential to understand *(Luyckx et al*., 2017).

In humans, nephrogenesis occurs in very different contexts compared to other *species* (McMahon, 2016). This is because the renal morphologic development occurs exclusively *in- utero*, with organogenesis and nephrogenesis occurring from the 6^{th} week of gestation to 36^{th} weeks. After 36 weeks, nephrogenesis is accomplished and each kidney has a whole complement of nephrons (Hoy *et al*., 2010). While nephrogenesis begins in the embryo and undergoes a discrete developmental series, it is completed in humans before delivery while in the rats, it

continues postnatally and is not completed until day 11^{th} -15^{th} day of postnata*l* life (Cullen-McEwen *et al*., 2015).

It has been known that the capacity to concentrate urine develops postnatally. A marked increase in the capacity to concentrate urine occurs in the first 3.5 weeks of life due to the production of vasopressin and an expression of a receptor in the kidneys that is vasopressin receptor (Kriz & Kaissling, 2008).

2.5 Comparative Gross Anatomy and the Histology of Rat and Human Kidneys.

On the gross location of the kidneys, both the rat and human kidneys are retroperitoneal organs in that they lie behind the parietal peritoneum attached to the posterior wall of the abdomen (Hoy *et al*., 2010). Similarly, the kidneys of the rat are found anteriorly and lateral to the spinal cord behind the peritoneum, resembling humans (Cullen-McEwen *et al*., 2015). Human and rat kidneys share the same morphological features in that distinctive features and some of these features consist of the existence of the renal pyramids and renal pelvis and a clearly identifiable medulla and cortex (Drucker & Oster, 2015). Both in human and rats, the kidney has the cortex on the outer part which covers the medulla on the inner aspect and medulla further bisected to form renal pyramids which are triangular in shape and their tips face the minor calyx whereby they empty the urine (Kriz & Kaissling, 2008).

Histologically, the rodent kidney has only one papilla which extends all the way to the pelvis of the renal and sometimes even to the proximal part of the ureter; although they have a single papillae, their anatomical zones are similar to that of humans (McMahon, 2016). While a human has several papillae whereby each papilla projects into a cup-like minor calyx which is an extension of the renal pelvis (McBride, 2016). Nephrons are the specific structural units of the kidney and both rats and humans share similar morphological features with the rats; every kidney has approximately 30,000 to 35,000 nephrons; every human kidney has an estimated one million, but significant inter-individual differences exist (Kriz & Kaissling, 2008). Every nephron has glomeruli which is an anastomosing tuft of capillaries generated from afferent arteriole and drains into tubules of the kidney and the glomerulus are invested by podocytes (Drucker & Oster, 2015). The glomerulus invaginates

bowman's capsule and the urinary space together with the proximal tubule have cuboidal epithelial lining with a lot of mitochondria and brush border (Levey *et al*., 2005).

Majority of nephrons form in mice and rats after birth and only before the term in human fetuses, this directly accentuates essential differences in the optimal environment for nephrogenesis between species (McMahon, 2016). In utero, the fetal organs including the kidneys develops in a relatively hypoxic environment with a sudden increase in blood oxygen concentrations occurring at the time of birth. In addition, there is a marked increase in systemic blood pressure at birth (McMahon, 2016), and renal blood flow is also increased. Hence, the final complement of nephrons formed within the kidneys is totally dependent on the in utero conditions in the case of the human infant except in the cases of premature birth (McMahon, 2016).

The renal tissues of rats are known to show similar metabolic and histological patterns with human renal tissues therefore histomorhological changes caused by a certain drug in rats are just the same at the ones likely to be seen in human beings (El-Shenawy & Hamza, 2016).

2.6 Teratogenic Effects of Antiepileptic Medicines on the Fetal Development.

Existing literature suggested that antiepileptic drugs were responsible for the increased risk of congenital abnormalities when exposed prenatally in offspring of women with epilepsy and not the underlying disease (Tomson *et al*., 2018). A study done by (Obstet et al., 2015) demonstrated that anticonvulsants embryopathy prevalence i.e. obstructive urinary congenital anomalies, renal agenesis, neural tube defects, hypospadias, retardation of growth and face hypoplasia had 3-4 folds increase when the infants were exposed to anti-epileptic medicines compared to infants who were not exposed and this hypothesis has been supported by several studies.

In a recent study prevalence of anticonvulsant embryopathy, for example, malformations like obstructive urinary congenital anomalies, renal agenesis, neural

tube defects, hypospadias, growth retardation, and hypoplasia of the face, was reported to increase three-fold in infants exposed to antiepileptic drugs in fetal life compared with unexposed infants and several studies have supported this hypothesis (Obstet *et al*., 2015).

Anticonvulsants appeared to increase incidences of congenital malformations, for example, cleft palate, by 30-fold, and to increase the incidence of skeletal anomalies, congenital heart disease, mental retardation, central nervous system abnormalities, and anomalies of genitourinary system compared to the average population (Tomson *et al*., 2018). Other studies have also shown that the most common major congenital malformations MCMs observed in children whose mothers were managed with AEDs for example urogenital malformations, cardiac defects, skeletal abnormalities, craniofacial defects, and are matching with major congenital malformations (MCMs) observed in the general population (Ban *et al.*, 2015).

The food and drug administration (FDA) classified the fetal risk of phenobarbital in the pregnancy category D, though there are controversial outcome concerning the teratogenic sequela of phenobarbital (Ashtarinezhad *et al*., 2016). Phenobarbital taken antenatally by epileptic mothers has been associated with a 3-4-fold increase in the occurrence of significant abnormalities like defects in the urinary tract, facial defects, heart defects, and minor abnormalities (Güveli *et al*., 2017). However, other studies have reported a rise in the occurrence of congenital disabilities neither for Phenobarbital or phenytoin which was not significant (Bastaki *et al*., 2018).

Phenytoin was also classified the fetal risk of pregnancy category D (Ramoz & Patel-Shori, 2014). Previous study by Tomson & Battino, (2012) have suggested that phenytoin (the commonly used anticonvulsant medication) may be teratogenic in humans. Phenytoin has been associated malformations including ectopic kidney, cryptorchidism, and cardiac defects such as ventricular septa1 defects and right-sided ductus arteriosus or aortic arch (Običan & Scialli, 2011). (Güveli *et al*., 2017) also showed that Phenytoin defects included cleft palate, hydronephrosis, hydrocephalus, and shortening of long fetal bones of the appendicular skeleton.

Other anomalies of soft tissues seen in the fetuses whose mothers were exposed to phenytoin prenatally were malformed kidney, cerebral hemorrhage, curled tail, fused digit, connective tissue infiltration of the cerebral hemispheres, ectrodactyly, deformed hind paw, and cleft lip (Yoshioka *et al*., 2021).

Whether malformations occurring in the offspring of epileptic mothers on treatment are due to epilepsy or anticonvulsants or are coincidental remains controversial. Still, some studies have indicated that mothers taking anticonvulsants during pregnancy run a two to threefold increased risk of having malformed offspring, compared with the average population (Conover *et al*., 2021).

2.7 Histomorphological Teratogenic Effects Antiepileptics on the Fetal Kidneys

Existing literature has shown that long-term use of antiepileptic drugs (AEDs) was found to have detrimental effects on the kidneys (Hamed, 2017b). According to studies which were previously done on carbamazepine which are in the same generation with phenytoin and phenobarbital and has the same mode of action with phenytoin, the rats which were in treatment group kidneys showed histological changes like, atrophy of glomeruli, expansion of space, degeneration of partial layer of bowman's capsule, glomerular accumulation cells, hemorrhage, congestion and detach of tubular epithelial lining cells from basement membrane in the cortical region and also degeneration of renal tubules and collecting ducts in renal medulla represented by loss nucleus, cell swelling and cell death *(*Al-bakri *et al.,* 2016*)*. Other studies done on sodium valproate which is also from first-generation showed some changes in the kidney including interstitial hemorrhage, cloudy swelling of renal tubules, proliferation of mesangial cells, hypercellular glomeruli and blood vessels congestion, hydropic changes on the proximal and distal convoluted tubules (El-Shenawy & Hamza, 2016).

Histological changes on other antiepileptic drugs, for example, the second generation oxcarbazepine, whereby the control group showed standard histological structure *(Hamdi et al.*, 2016a). On the other hand, kidney sections acquired from fetuses whose mothers were managed with oxcarbazepine 108 mg/kg from day 17 to day 20 of gestation showed changes histologically which included edema between glomeruli

and the tubules, bowman's capsular spaces were widened, glomeruli were shrunk, vacuoles in the cytoplasm and deterioration of the outer borders of cell lining the tubules (Hamdi *et al*., 2017). These results are in agreement with the study of administration of levetiracetam caused acute cellular swelling in lining epithelium of the tubules at the cortex, glomeruli shrinkage and hydropic degeneration in the kidney (Hamdi *et al*., 2017). In the kidney histological changes were as follows, the fetuses whose mothers were treated with levetiracetam 600mg/kg showed focal hemorrhages in between the tubules and blood vessels congestion and (Tekcan *et al*., 2017). In the kidney, the kidney of neonates whose mothers were treated with levetiracetam 300mg/kg on the 7^{th} day of lactation showed hydropic degeneration in the kidney, detached cell of tubules, shrinking in glomeruli and pyknotic nuclei (Al-Ibrahimi & Al-Bakri, 2017).

For the first time, the applied AEDs-treatment showed a potent teratogenicity characterized by dense peri-glomerular cellular granulomatous lesions associated with degeneration of epithelial cells lining renal tubules (El-Gaafarawi & Abouel-Magd, 2015). The glomeruli appeared swollen and increased in cellularity and their bowman's space was missing (El-Gaafarawi & Abouel-Magd, 2015). Treating the epileptic mother with used AEDs, increased the damage of renal tubules. The glomeruli become swollen, lobulated and occluded almost of the bowman's capsule space. Peri-glomerular renal cell infiltration was also detected (El-Gaafarawi & Abouel-Magd, 2015).

Previous studies done by (Jassim, 2013) showed that when valproic acid was prescribed, the histological appearance of the kidneys showed the tubules particularly distal and proximal showed hydropic changes and their glomeruli were hyper cellular. On the other hand, fetuses maternally injected with gabapentin exhibited degeneration of renal corpuscles in the form of shrunken or absent glomeruli along with increased periglomerular space (Badawy *et al*., 2019).

From light microscopy micrograph, the experimental epileptic mother rats showed mild peritubular inflammatory cellular infiltration associated with degeneration of tubular lining epithelial cells and reduction of tubular lumina. Few numbers of renal

tubules were completely enclosed by hyaline casts. The glomeruli showed a marked increase of cellularity, lobulated and filling almost of the bowman's capsule space (El-Gaafarawi & Abouel-Magd, 2015)

This study aims to comparatively evaluate the histo-morphological and the histostereological effects of phenobarbital and phenytoin on development of fetal kidneys, following prenatal subjection to varied doses and at different gestational periods as this data is generally lacking.

CHAPTER THREE

MATERIALS AND METHODS

3.1 The Study Site/Area

This study was carried out in two different locations. The first study site was the Animal house in the school of biological sciences in the University of Nairobi (UON) where the acquisition of the animals, daily animal feeding, drug administration, daily maternal weights recording, harvesting of fetuses and recording of maternal and fetal pregnancy outcomes were done. The second study site was the department of Human anatomy Jomo Kenyatta University of Agriculture and Technology (JKUAT) where the second phase of this research was carried out including histological processing and histo-stereological analysis.

3.2 Study Design

A post-test only control experimental study design was adopted in conducting the study. In this study, outcome of phenobarbital and phenytoin was measured only once after the administration in order to determine their effects.

3.3 The Study Sample

A pure colony of 30 nulliparous Albino rat dams (*Rattus norvegicus)* of the third-generation breed at University of Nairobi (UON) animal house was used for each drug in the study model. This species of rats was chosen as the experimental model based on the following known facts on albino rats;(i) they have low prevalence of spontaneously occurring congenital malformation to their fetuses, (ii) they usually have large litter size of between 3 to16, (iii) Their gestation period is relatively short compared with other experimental animals as its 21 days (Martignoni *et al*., 2006).

3.4 Sampling Size Determination

In determining the sample size of 30 albino rats for either the phenytoin or phenobarbital study groups, the resource equation by Arifin and Zahiruddin 2017, whose formula is n=DF/k + 1 was used. In this formula, n represented the total number of rat dams per group. DF was the degree of freedom while k represented the total number of subgroups. In this research, there were 10 sub-groups for each of the treatment groups (10 for phenobarbital and 10 for phenytoin treatment groups). Based on this research equation, the acceptable range of degrees of freedom (DF) was taken to be between 10 to 20. However, since a value less than ten has been shown in other studies not yield significant results, in this case a DF of 20 was considered sufficient and hence a total number of 30 rats was obtained in each of the treatment group. That is 30 rats for phenobarbital and 30 rats for phenytoin.

Hence n =20/10 +1 = 3 (subjects per group).

Therefore 10 groups x 3 subjects per group = 30 dams in each of the treatment group.

3.5 Breeding of the Rats

One male albino rat sexually mature from a pure colony was put into a cage with two female rats at 1530 hours (+/-30 minutes).

Then the male rats were removed the following morning at 0930 hours (+/- 30 minutes) and returned to their separate cage.

3.6 Selection Criteria of the Rats

This was done as follows: -

3.6.1 The Criteria for Inclusion

i. All animals that tested positive after pregnancy test was done. This served as a sign of having conceived on their first day after staying with a male in the same cage overnight.

ii. All fetuses that were found to be alive at the point of sacrificing the pregnant rats on the gestational day 20.

3.6.2 The Exclusion Criteria

i. All animals that tested positive for pregnancy but showed any signs of sickness at the beginning of the experiment.
ii. All pregnant animals that showed any signs of sickness at the beginning of the experiment.
iii. All live fetuses that showed any signs of sickness at the beginning of the experiment.

3.7 Pregnancy Determination

The following were the pregnancy detection materials:

a) 10mls blunt-tipped disposable pipettes

b) 0.85%phosphate buffered saline

c) Microscope slides

d) Papanicolaou stain or Giemsa stain

e) Cotton tipped swab

f) Ethanol (95%)

3.7.1 Confirmation of Mating

The spermatozoon from the animals after mating, was present when vaginal smear was done and viewed under microscope and this confirmed coitus or possible fertilization.

3.7.2 The Procedure for Confirmation of Fertilization

a) The animals were restrained using a gauze holder against the body.

b) 1 millimeter of saline were introduced into the vaginal cavity using a blunt-tipped disposable pipette (vaginal wash).
c) Swabs that had cotton at the tips were moistened using phosphate-buffered saline were then inserted gently in to the vaginal canal.
d) Before withdrawing the swabs, they were slightly rotated.
e) The moist swab was withdrawn and rolled onto a clean glass microscope slide.
f) After withdrawing the moist swab, it was then rolled on to a clean microscope slide.
g) The specimen was then sprayed fixed using 95% ethanol
h) The slides were then allowed to air dry.
i) The slides were then stained using Giemsa stain.
j) The slides were then viewed using the B.P. Olympus microscope

3.7.3 Confirmation of Pregnancy or the First Day of Gestation

Pregnancy was ascertained when vaginal wash was done 24 hours after mating, the existence of polyhedral cornified epithelial cells and neutrophils on the smear was used to establish estrous changes, which was indicated as gestational day one (GD1) (Martignoni *et al*., 2006).

3.8 The Grouping of the 30 Albino Rats in Each of the Phenobarbital and Phenytoin Study Groups.

The 30 rats in either of the phenobarbital or the phenytoin study groups were first randomly assigned into two broad study groups of three rats (control) and 27 rats (experimental). To evaluate the effects of intrauterine exposure to varied doses of either phenobarbital or phenytoin, the 27 rats in each of the experimental category were then subdivided in-to three sub-groups of nine rats each according doses as follows: nine low dose rats, nine medium dose rats and nine high dose rats.

To evaluate the effects of *in-utero* exposure to the two medicines based on the time of exposure, the nine rats in each of the dosage groups were further subdivided into three subgroups of three rats each according to the time of exposure as follows three

for trimester one (TM_1), three rats for trimester 2 (TM_2) and three rats for trimester three (TM_3) ***(Figure 3.1).***

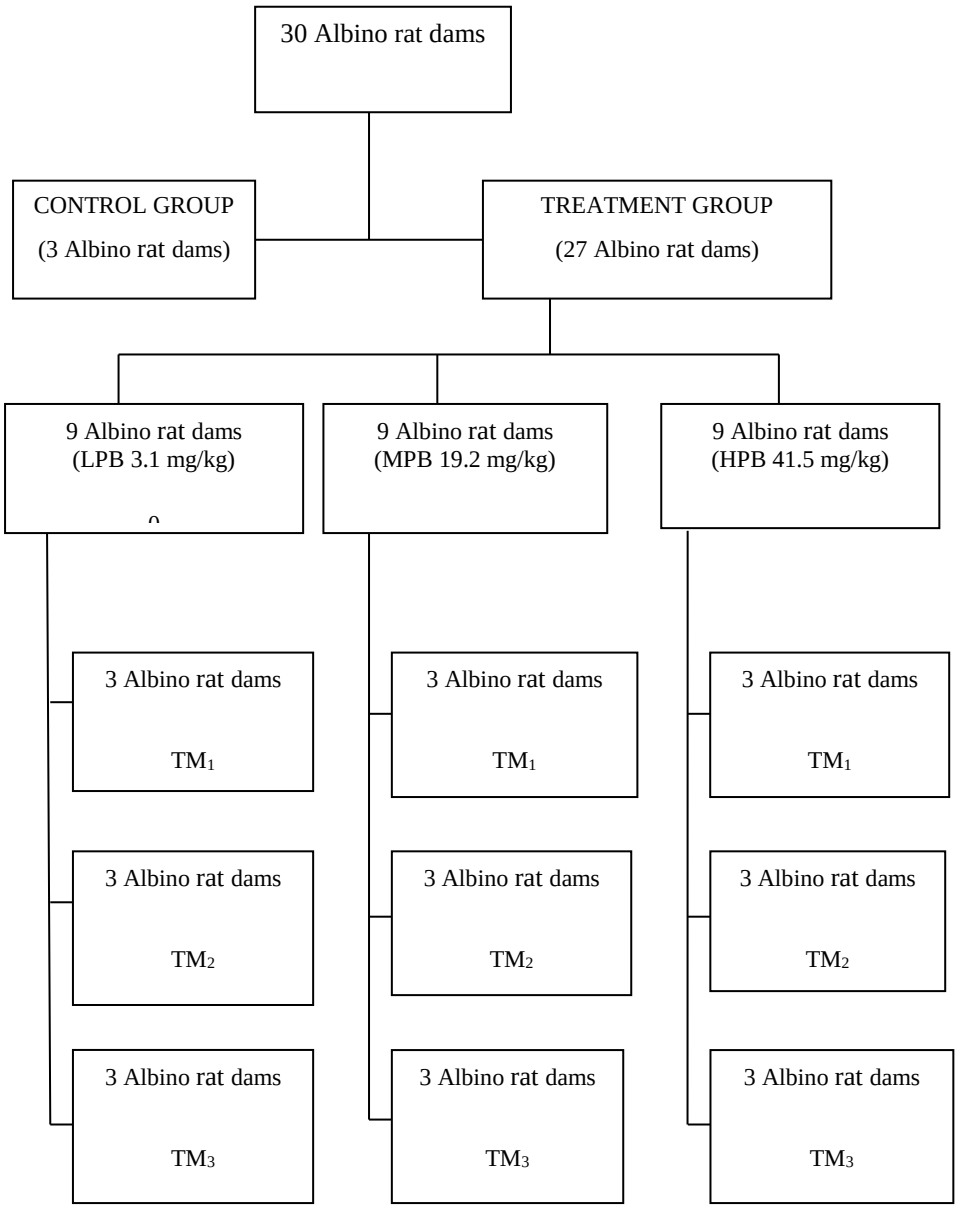


Figure 3.1: Shows How the 30 Albino Rats in Each of the two Study Groups (The Phenytoin and the Phenobarbital Study Group) Were Organized in Control and the Experimental Subgroups Selection of Fetuses Used in Histo-Morphology and Stereology.

In this study, the number of litter sizes ranged from three to fourteen per rat. Three fetuses were then selected from each rat using simple random sampling method for histo-morphological and stereological evaluation resulting in a total sample size of

180 fetuses that is three fetuses from each of the 60 rats, which is the same as 180 fetuses. For stereological and histo-morphological evaluation, their kidneys were harvested for use. The rest of the kidneys from other fetuses were preserved in zenkers solution to be used later.

3.9 The Feeding of the Study Rats.

All rats were fed on standard rodent pellets obtained from Unga feed Limited. It contained by weight (g/100g); 4% cellulose, 68% starch, 20% protein, and 5% lipid (corn oil), but as follows by calories: - 12% lipids, 72% carbohydrates, 20% proteins, and 54mg/kg zinc plus water *ad-libitum*. The feeds were put on these cages for the rats to feed on and this was done every morning at 0830 hours as outlined by (Martignoni *et al*., 2006) ***(Figure 3.2).***

Figure 3.2: Showing How the Polycarbonated Plastic Cages Used in the Study.

The control groups received the standard diet plus water *ad libitum* only for the entire gestation period from gestational day 1(GD_1) to gestational day 20 ($GD_{20.}$ For the rats in the treatment groups, they were fed on the standard rodent diet plus water *ad-libitum*, then they received their treatment doses for either phenobarbital or phenytoin according to their study categories of either low dose, medium dose or high dose and according to the trimesters of exposure as either TM_1, TM_2 and TM_3. All animals however received folic acid supplementation and medicines were administered at 0930 every morning using a gastric gavage needle.

3.10 The Daily Weighing of the Rats and Drug Administration during Experimentation.

It is only the investigator who handled the rats when taking the daily weights and in administering the treatments with the gastric gavage needle that was done daily between 0700 and 0830 hours and 0900-0945 hours respectively. All procedures were performed were carried out according to the care of laboratory animal guidelines (Retnam *et al*., 2016)

3.11 Determination of the Phenobarbital and Phenytoin Doses, Reconstitution, and Administration.

This was done as follows: -

3.11.1 Determination of Phenytoin and Phenobarbital Doses:

Phenobarbital tablets from Hikma Pharmaceuticals in USA batch number NSC 9848 and Phenytoin from Aurobindo Pharma - Milpharm Ltd in India batch numbers AUST R 297268 bought from government chemist in Nairobi. A simple guide for converting animal dosages from human dosages by Nair and Jacob, (2016) was applied, which states that dose is equally related to body weight. The minimum dose of phenobarbital in human is 30 mg/day, the medium dose is 185 mg/day, and the maximum dose is 400 mg/day while for phenytoin, the minimum dose was n human is 300 mg/day, the medium dose is 600 mg/day, and the maximum dose is 1200 mg/day. To determine human equivalent dose (HED) for the phenobarbital and Phenytoin, average body weight of a human being that is 60 kg was used. These doses were divided by 60kg to obtain HED.

The following HED were therefore obtained: -

0.5 mg/kg/bw, 3.1 mg/kg/bw 6.7 mg/kg/bw for phenobarbital and 5 mg/kg/bw, 10 mg/kg/bw and 20 mg/kg/bw for phenytoin were obtained for low, medium and high dose respectively.

After obtaining the human equivalent dose HED, animal equivalent dose (AED) was arrived at by multiplying human equivalent dose (HED) by Km factor which is 6.2 which is equivalent 3.1mg/kg/bw for the low phenobarbital dose group, 19.2 mg/kg/bw for the medium phenobarbital dose group and 41.5 mg/kg/bw for high phenobarbital dose and 31mg/kg/bw for the low phenytoin dose group, 62mg/kg/bw for the medium phenytoin dose group and 124mg/kg/bw for high phenytoin dose.

3.11.2 Reconstitution of Phenobarbital and Phenytoin

Phenobarbital (30mg) and phenytoin (100mg) which were obtained in form of tablets were dissolved in 10 millimeters of distilled water. The dissolved phenobarbital and phenytoin were then administered to the rats guided by their weights and specific dosage.

3.11.3 The Procedure Followed in Drug Administration Using Gastric Gavage

1. The animal was grasped carefully from the neck region using left hand.
2. The animal was then covered with the table linen to prevent the animal from soiling the investigators attire.
3. The rat was then laid against the body with the mouth facing the investigator.
4. The gavage needle was then gently introduced into the mouth of the animal, turning it gently beyond the esophageal constrictions and cardiac sphincter.
5. The phenobarbital/ phenytoin dose was then introduced in the stomach of the animal.
6. The gavage needle was then gently removed.

3.12 Sacrificing the Animals and Harvesting of Fetuses.

All the pregnant rats were humanely sacrificed on the gestational day 20 (GD_{20}) using concentrated carbon dioxide between 0900 hours and 1100 hours (Rai & Kaushik, 2018). After five minutes of carbon dioxide exposure, the rats were

mounted on a dissection board and the anterior abdominal wall of the mother was opened from the xiphisternal joint to the symphysis pubis along the linea alba. The full extent of both uterine horns exposed promptly.

Before opening either horn, fetal positions within the horns as well as the number of live and dead fetuses, as indicated by their movement following a gentle pressure, were recorded (total litter size). Also, the number of the "devoured endometrial glands," characterized by yellowish nodules found along the mesometrial margin of the uterine horns that marks any original implantation site, were counted and recorded. Thus, the material glands unoccupied by living or recently dead fetuses represented the number of prior resorptions.

The uterine horns were then cut along the anti-mesometrial border to expose the fetuses, placentas and embryonic membranes, using a pair of forceps, the fetuses were then removed gently together with their placentas. The fetuses together with their placentas were then weighed, general fetal morphology assessed and noted down before kidney harvesting done for fixation. The fetal crown rump lengths were carried out by taking measurements from the snout to the base of the tail recorded in centimeters

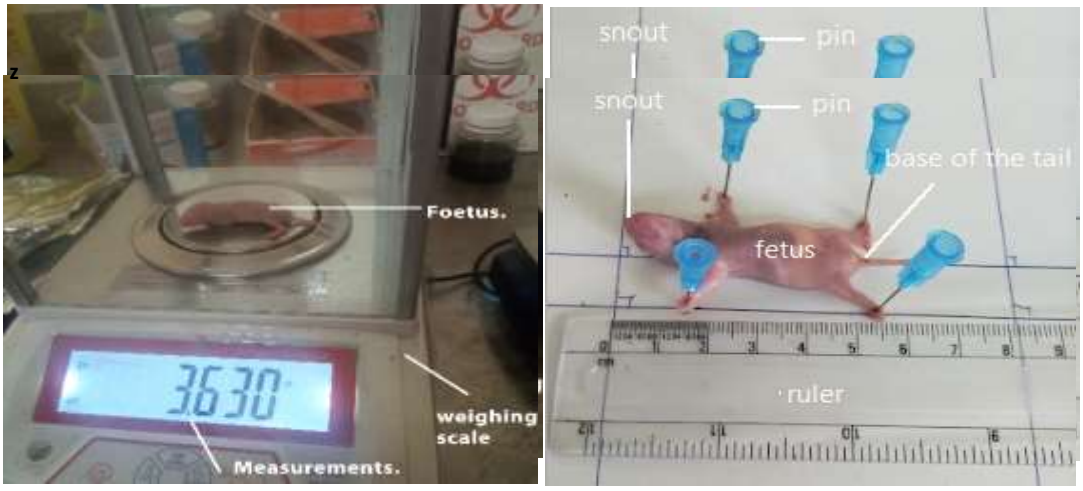


Figure 3.3: An Illustration of How the Fetal Parameters Were Measured.

KEY:-

(A) How the fetal weights (FW), were taken using electronic weighing scale using Scout pro model SPU 4001.

(B) How the crown rump lengths (CRL) were taken from the snout to the base of the tail using a ruler.

3.12.1 Determination of Vulnerable Periods of Phenytoin and Phenobarbital Teratogenesis on the Fetal Kidneys

To determine the vulnerable periods of phenytoin and phenobarbital teratogenesis, they were administered at different times of the gestation period. For TM_1 treated groups, these drugs were administered daily starting from day 1 (GD1) until day 20 (GD20). For TM_2, these drugs were administered from day 7 (GD7) until day 20 (GD20). For TM_3 treated groups, these drugs were administered from day 14 (GD14) until day 20 (GD20).

In every trimester, three out of the nine rats either received low dose phenytoin or phenobarbital, the other three rats received either medium dose phenytoin or phenobarbital and the remaining three rats either received high dose phenytoin or phenobarbital respectively. This was aimed at determining teratogenicity in terms of dosage administered.

3.12.2 Grouping of Fetuses for Light and Histo-Stereological Analysis for Phenytoin and Phenobarbital

After sacrificing the mothers, a total of 180 fetuses were chosen; that is, three fetuses

After sacrificing the mothers, a total of 180 fetuses were acquired. Three fetuses were selected from each rat using simple random sampling method for histo-morphological and stereological evaluation resulting in a total sample size of 90 fetuses that is three fetuses from each of the 30 rats, which is the same as 90 fetuses for each of the treatment group making the total fetuses to be 180.

A total number of 9 fetuses were then obtained from the control group while the treatment group contributed to a total number of 81 fetuses for each treatment group – making a total of 90 fetuses per treatment group.

In the treatment group, a total number of 27 fetuses were obtained for each dose groups that is low, medium and high dose groups for each of the treatment groups.

3.12.3 Humane Sacrificing of the Fetuses and Harvesting of the Fetal Kidneys

Materials; scalpel blade, scalpel blade handle, concentrated carbon dioxide, bell or dissector jar, cotton gauze or cotton wool, pair of scissors, mounting board, mounting pins, a pair of forceps (toothed), hypodermic needle gauge 20, gloves (surgical), magnifying glass, ruler, electronic weighing machine, collection bottles fixatives-10% formaldehyde solution and specimen.

3.12.4 The Procedure for Anesthetizing and Perfusing the Fetuses.

1. Concentrated carbon dioxide was connected into the bell jar with a tight-fitting lid.
2. The fetuses were then put into the bell jar for 3-5 minutes to euthanize.
3. They were then removed from the bell jar and mounted onto the board using mounting pins with dorsal sides on the board.
4. The fetus was mounted on the board using mounting pins (dorsal side facing the board).
5. Abdominal layers were dissected at the middle to expose the abdominal viscera
6. The kidneys were then identified in the posterior abdominal wall retroperitoneally.
7. The kidneys were then excised at the level of the renal pelvis.
8. The kidneys were then immersed in the preferred fixative (formaldehyde) to enable perfusion to proceed with processing either for light or for 24 hours ***(Figure 3.4).***

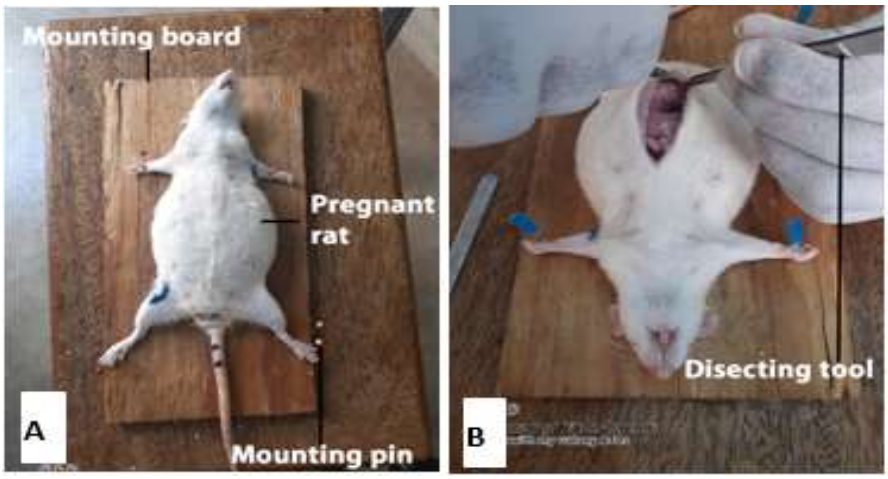


Figure 3.4: An Illustration on How the Abdominal Walls was opened to Exposes the Fetuses in the Uterine Horns for Harvesting.

KEY:-

(A) A pregnant rat mounted on a dissection board (B) how abdominal layers were dissected at the middle.

3.13 The Procedure Adopted for Processing Slides for Light Microscopy.

Staining Materials; Specimen bottles, distilled water, Zenker's solution (1 liter), Digital picture exchange (DPX) mountant, Hematoxylin and eosin, glass slides and coverslips, glass staining square jars, microtome knives, paraffin wax, rotary microtome, slide holders, heater and water bath container, xylene, distilled water, formaldehyde 10% concentration, glassware for preparing dilutions, alcohol, woodblocks, beakers, cedarwood oil, egg albumin, dropper, toluidine solution.

3.13.1 Procedure that was used for processing the Fetal Kidneys Specimens for Light Microscopy

1. The kidneys were fixed in formaldehyde solution for 24 hours
2. They then were dehydrated in an ascending concentration of alcohol (50%, 60%, 70%, 80%, 90%, 95%, and 100% (absolute) each for one hour.
3. The kidneys were cleared with xylene.

4. Then infiltrated with paraffin wax for 12 hours at 56^0c
5. The kidneys tissue was then orientated in the longitudinal axis
6. It was then embedded in paraffin wax on the wooden blocks
7. Excess wax was trimmed off till the entire length of the kidney tissue is exposed
8. 5µm thick longitudinal sections were cut from the head to tail regions with Leitz sled rotary microtome.
9. The cut sections were then floated in water at 37^0 Celsius to spread the tissue
10. The sections were then stacked onto glass slides, applied as a thin film with a micro-dropper.
11. The slides then dried in an oven at 37^0 Celsius for 24 hours.
12. They were then stained with Hematoxylin and eosin (H&E).

3.14 Stereological Analysis

3.14.1 Estimation of the Total Kidney Volume Using Archimedes Method.

The Archimedes' principle was used to obtain an independent kidney volume. The Archimedes volume was estimated by inserting the whole kidney tissue into graduated beakers containing normal saline, and the displacement was measured. The normal saline displaced by the kidney represented the actual kidney volume (Bai *et al*., 2019). These Archimedes volumes was then used as the reference volumes. This method was compared to the other methods, and the mean an d standard deviation of the measurements was calculated.

3.14.2 Preparation of Kidney Tissues for Stereology.

The fetal kidney for stereological analysis were removed quickly, placed in cold saline solution and trim of adipose tissue, weighed, and immersed in formaldehyde solution for 24 hours at room temperature (23^0c) to allow for proper fixation. The samples of the fetal kidney tissue were then processed using graded alcohol, xylene, and paraffin blocks were used for embedding. Each kidney was exhaustively

sectioned into 5µm thick sections both transversely and longitudinally from one end of the paraffin block using a rotary microtome model Leica RM 2135, Germany.

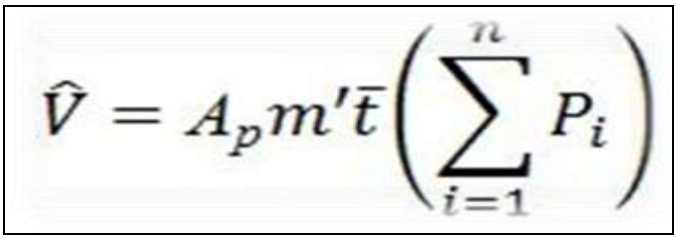
3.14.3 Staining Method

Hematoxylin and eosin was used to stain the kidney sections (Ahmed, *et al* 2016).

3.14.4 Determination of Stereological Total Kidney Volume and Volume Densities Using Cavalieri Point Counting Methods.

The digital images of the kidney tissue were captured using stereological sampling rules with same magnification and saved in the jpeg (joint photograph expert group) file format at adequate resolution. Images taken (microscope digital camera) both for the experimental and control groups were organized appropriately and saved in one folder. A calibrated scale bar was added to one image of a batch to define the real dimensions of the structures under investigation, and placed on left hand side. Where stereological estimation required the use of a guard area it was set and were not be changed in the course of the whole experiment to obtain consistent results. The stereological measurements were performed as previously described by (Mwangi *et al*., 2023) ***(Figure 3.14.4.2).***

Using both the cavalieri point-counting method, total stereological kidney volumes and the estimation of the volume densities for both the cortical and medullary layers of the kidney structures were determined. The following steps were followed (a) Preparation of kidney cavalieri sections of 5µm thick. (b) Selection of the spacing for the point probe. (c) The point probe was then tossed randomly onto each section. (d) STEPanizer stereology tool was used to count the number of points that hit the region of interest. (e) All sections were processed keeping a tally of counts per section. (f) The volume was then calculated by applying the below formula ***(Figure 3.5).***



$$\hat{V} = A_p m' \bar{t} \left(\sum_{i=1}^{n} P_i \right)$$

Figure 3.5: The Formula used to Calculate the Cavalieri Kidney Volume

Where;

A_P: is the Area associated with a point

m': Is the section evaluation interval

t bar: Is the mean section cut thickness

p_i: Are the points counted on the grid

Twenty sections of 5µm thickness from each longitudinal kidney section were selected by systematic uniform random sampling (Mostafa & Ahmad, 2018). The researcher then used the microscope's stage Vernier to view the images at magnification of X40 and X100. The volume of the kidney was then obtained by multiplying the number of points that hit the region of interest (kidney) X the area per point and the slice thickness (5 micrometers).

Volume = no of points x area per point x slice thickness.

STEPnizer software was used to do the point counting.

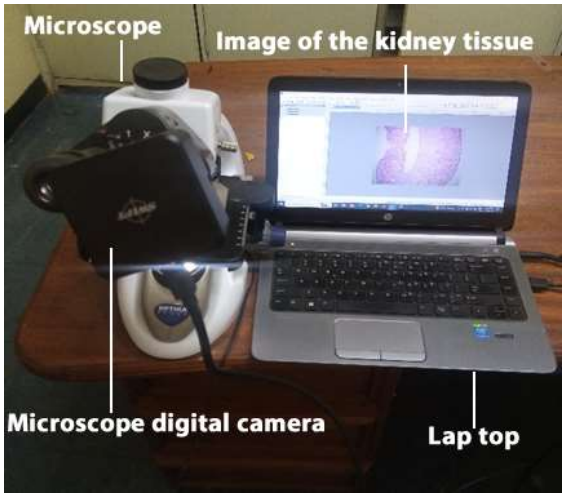


Figure 3.6: Illustration on how Digital Camera Mounted onto the BH-Olimpus Microscope was used to take the Fetal Kidney Histological Images.

3.14.5 Correction of the Kidney Tissue Shrinkage.

The following method was applied to quantify shrinkage caused by histological procedures and fixation. Archimedes displacement method was used to calculate the volume of the removed harvested kidney. After tissue processing and exhaustively sectioning, the kidney volume was estimated with cavalieri method. The kidney volume shrinkage was then calculated as per procedure described by (Baldelomar *et al*., 2018) as follows:-

$$\text{Shrinkage} = \frac{(\text{Volume before}) - (\text{Volume after})}{(\text{Volume before})}.$$

The volume before was the kidney volume that was determined through the water immersion method, while volume after was the volumes determined through the cavalieri method. The final volume of the kidneys was corrected after estimating the shrinkage.

3.15 Procedure Followed in Taking Photomicrographs.

1. Histological slides were mounted on the stage of the microscope.
2. The focus was adjusted until the image to be photographed is in focus

3. The field was magnified appropriately.
4. Photographs of the regions were taken as they were viewed best under the focus of the microscope.
5. Photographs were then transferred to the computer using a flash disc.
6. The photographs were then uploaded and labeled using the Adobe fireworks software.

3.16 Statistical Analysis

Statistical package for social sciences (SPSS) for Windows Version 25 Chicago Illinois was used to analyze data. The histostereological parameters was analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison tests. The multivariate analysis between groups on how the three independent variable of the drug, dose and the time of exposure influenced both the maternal, fetal and the histostereological parameters assessed was done by using Multiple Analysis of Variance (MANOVA). All results with a significance F-value ($p < .05$) was considered as significant.

3.17 Ethical Approval

The experimental protocol was approved by University of Nairobi – Faculty of Biosafety, Animal use and Research Ethics Committee (BAUEC). The protocol followed the guidelines for the care of laboratory animals (Mostafa & Ahmad, 2018).

CHAPTER FOUR

RESULTS

The findings of this study are aligned with study objective. However, the findings of Objective 4 on whether or not the observed histo-morphological effects are dose and time dependent are integrated in the findings of the other three objectives.

The Maternal and Fetal Pregnancy Outcomes.

4.1 Objective One: The Comparative Evaluation on How the Two Medicines Influenced the Maternal and Fetal Pregnancy Outcomes

The findings of this first objective are presented at two levels as follows: -

Level 1: The comparative maternal pregnancy outcomes

Level 2: The comparative fetal pregnancy outcomes

4.1.1 The Comparative Effects on How the Two Medicines Influenced the Maternal Pregnancy Outcomes.

In evaluating the maternal pregnancy outcomes, to determine the level of intrauterine toxicity of phenobarbital and phenytoin on foetal growth and development *in-utero*, the following parameters were evaluated; (i) the terminal placental weights, (ii) the mean terminal weight, (iii) mean total weight gain. This was done by doing the univariate, bivariate and multivariate regression analysis by use of both ANOVA and MANOVA. The overall global effects of both phenobarbital and phenytoin on maternal pregnancy outcome was assessed by the univariate and bivariate analysis using ANOVA. At the global level, the study established that the administration of varied doses of both phenobarbital and phenytoin during pregnancy caused a significant ($P<0.05$) deleterious reduction to all the three maternal pregnancy outcomes parameters assessed when compared with the control (***Table 4.1)***. Phenobarbital was further noted to have more harmful deleterious effect on maternal

pregnancy outcome parameters as compared to phenytoin as shown in the ANOVA results of table (***Table 4.1)*** as follows:- [(a) terminal placental weights,= (F(18,38)=156.082 P= 0.001), (b) mean terminal weight (F(18,38)= 13.639 P= 0.042), (c) mean maternal weight gain= (F(18,38)= 33.963 P= 0.049) (***Table 4.1).***

Concerning how the varying doses and the time of exposure influenced the maternal pregnancy outcomes, it was further noted that in both treatment groups of phenytoin and phenobarbital, the three maternal pregnancy outcomes were highly influenced when the treatments were initiated at TM_1 and TM_2 that recorded the worst detrimental outcomes noted when high doses were administered (***Table 4.1).***

Table 4.1: The ANOVA Comparative Findings on How the Two Medicines Globally Affected the Maternal Pregnancy outcomes Parameters between the Treatment Groups Compared with the Controls.

The study groups	**Study groups and dosage levels.**	**The time of exposure**	**The comparative mean terminal weight, terminal maternal weight gain and placental weight for various study groups**		
			Mean terminal weight (g)± SD)	**Mean maternal weight gain(g) ± SD)**	**Mean placental weight (g) ± SD)**
Control.	Control (C)				
	No treatment	None.	292.1923±. .0287	98.000 ±.0007	0.4378±.0003
	Low dose	TM_1	247.1205± .0215	51.2717±.0033	0.2906±0.0028
	treatment group	TM_2	264.2559± .0938	57.2645±.0048	0.3410 ±0.0036
	(LPB)-	TM_3	293.0472± .0033	87.2643±.0019	0.3760 ±0.0135
	[3.1 mg/kg/bw)				
	Medium dose	TM_1	**243.2458±.0868***	**45.3050±.00326***	**0.2959 ±0.0032***
The	treatment	TM_2	249.2110± .0646	55.2890±.0012	0.3239 ±0.0046
Phenobarbital	group(MPB)-	TM_3	260.1454± .0312	69.2835±.00165	0.3691 ±0.0007
treatment groups	[19.2mg/kg/bw)				
	High dose	TM_1	**243.1873± .0513***	**37.3350±.0011***	**0.2318±0.0012***
	treatment group	TM_2	**244.1703± .7589***	**50.3262±.0005***	**0.2777 ±0.0034***
	(HPB) (41.5	TM_3	267.1454± .0312	59.3126±.0008	0.3165 ±0.0012
	mg/kg/bw)				
	Low dose	TM_1	257.2136± .9245	56.00 ±.0001	0.3111 ±0.0011
The phenytoin	treatment group	TM_2	277.3776± .0744	62.2795±.0018	0.3627 ±0.0031
treatment groups	(LPT)-(31	TM_3	303.2914± .0315	92.2672±.0033	0.3949 ±0.0007
	mg/kg/bw).				
	Medium dose	TM_1	255.0318± .0979	50.3179±.0068	**0.2906 ±.0003***
	treatment group	TM_2	259.0939± .0131	60.2925±.0017	0.3239±.0003
	(MPT)-[62	TM_3	271.2283± .0753	74.2882±.0024	0.3691 ±.0032
	mg/kg/bw).				
	High dose	TM_1	**253.3111± .0135***	**42.3666±.0025***	**0.2747 ±.0034***
	treatment	TM_2	**254.5431± .0223***	**55.3553±.0033***	**0.3020 ±.0040***
	group(HPT)	TM_3	278.0696± .0072	64.3446±.0038	0.3392 ±.0012
	(124 mg/kg/bw).				
			F(18,38)= 13.639 P= 0.042	F(18,38)= 33.963 P= 0.049	F(18,38)=156.082 P= 0.001

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

Upon carrying out the MANOVA level II analysis to find out how the three independent variables of the **individual drug**, **dose** and **the time of exposure** plus their interactions influenced each of the three maternal outcome parameters this study found out that: -

a) At individual level, it was observed that the three independent variables had a significant role to play in influencing the three maternal pregnancy outcome parameters but in varying proportions as indicated by the values of wilk's labda Partial Eta squared (η^2) ***(Table 4.2).*** From this table it can be seen at individual level the type of drug had a statistical significant contribution at (P<.05) contributed more with Partial Eta squared (η^2) of 99.1 % on the placental weights, 98.5 % on terminal maternal weight and 96.8 on maternal weight gain ***(Table 4.2).***
b) At two-way level interaction effects it was observed that the element of the drug and dosages, had the worst effects followed by the combination of time of exposure and drug then lastly the combinations of time of exposure in combination with dosages as shown in the three variables of the mean total of maternal weight gain, the mean placental weights and the terminal maternal weight with Partial Eta squared (η^2) of 82.8%, 11.2 %, and 10.7% on the placental weights ***(Table 4.2).***
c) At three -way level interaction effects of doses, drugs and the trimesters, the placental weights had the highest contribution with Partial Eta squared (η^2) of 10.1 % ***(Table 4.2).***

Table 4.2: The Level 2 MANOVA Results on How the Individual Drug, Dose and the Time of Exposure plus Their Interactions Influenced Each of the Three Maternal Outcome Parameters Prenatally.

Types of MANOVA evaluation At level 2	The groups being tested	The three dependent variables.	Type III Sum of Squares	Df	Mean Square	F statistics	Sig.	Partial Eta Squared
(i) The evaluation on the correctness of the model used for the study	The Corrected: - The wilks labda Model)	Maternal Weight gain	46.067[a]	18	2.559	332.303	<.001	.994
		Placental weights.	.134[b]	18	.007	156.082	<.001	.987
		Terminal maternal weight	23645.509[c]	18	1313.639	219.582	<.001	.990
(ii) Test on whether the observed results were due to chance	Intercept (grand total)	Maternal weight gain	1162.152	1	1162.152	150897.516	<.001	1.000
		placental weights .	4.772	1	4.772	100378.178	<.001	1.000
		Terminal maternal weight	2875148.613	1	2875148.613	480596.689	<.001	1.000
(iii) The Individual independent variable and its effects on each of the three maternal dependent variables	DOSES (Low, medium, high	Maternal weight gain	.028	2	.014	294.149	<.001	.939
		Placental weights .	7.113	2	3.556	461.786	<.001	.960
		Terminal maternal weight	3845.333	2	1922.667	321.384	<.001	.944
	DRUGS (PT,PB)	Maternal weight gain	.056	2	.028	584.146	<.001	.968
		Placental weights .	31.719	2	15.860	2059.278	<.001	.991
		Terminal maternal weight	15381.333	2	7690.667	1285.537	<.001	.985
	TRIMESTER (TM_1,TM_2,TM_3)	Maternal weight gain	.181	1	.181	23.484	<.001	.382
		placental weights .	.008	1	.008	168.250	<.001	.816
		Maternal weight gain	864.000	1	864.000	144.422	<.001	.792
(iv) Two- way interaction effects on each of the maternal dependent variables	DOSES (Low, medium, high dose)* DRUGS (PB,PT)	Maternal weight gain	.001	4	.000	4.135	.007	.303
		Placental weights .	1.409	4	.352	45.723	<.001	.828
		Terminal maternal weight	117.333	4	29.333	4.903	.003	.340
	DOSES (Low, medium, high dose)* TRIMESTER (TM_1,TM_2,TM_3)	Maternal weight gain	.000	2	.000	.017	.983	.001
		Placental weights .	.000	2	.000	2.266	.118	.107
		Terminal maternal weight	.000	2	.000	.000	1.000	.000
	DRUGS * TRIMESTER (TM_1,TM_2,TM_3)	Maternal weight gain	.001	2	.000	.036	.965	.002
		Placental weights .	.000	2	.000	2.395	.105	.112
		Terminal maternal weight	.000	2	.000	.000	1.000	.000
(v) Three-way interaction effects On each of the maternal dependent variables	DOSES (Low, medium, high dose) DRUGS (PT,PB)* TRIMESTER (TM_1,TM_2,TM_3)	Maternal weight gain	.000	4	.000	.010	1.000	.001
		Placental weights .	.000	4	.000	1.070	.385	.101
		Terminal maternal weight	.000	4	.000	.000	1.000	.000
	Error	Maternal Weight gain	.293	38	.008			
		Placental weights .	.002	38	.000			
		maternal weight	227.333	38	5.982			
	Total	Maternal Weight gain	1545.932	57				
		Placental weights .	6.140	57				
		Terminal maternal weight	3878633.000	57				
	Corrected Total	Maternal Weight gain	46.359	56				
		Placental weights.	.135	56				
		Terminal maternal weight	23872.842	56				

*Key:- *Indicates interaction effects among the independent variables.*

Upon carrying out the MANOVA pairwise comparison to establish how the two medicines differed in influencing the three maternal pregnancy outcomes in the same

dosage levels this study found out that phenobarbital had severe effects to the maternal pregnancy outcome as compared to phenytoin ***(Table 4.3).***

Table 4.3: The Level Three MANOVA Pairwise Comparison Table on How the Two Medicines Influenced Three Maternal Outcome Parameters when Exposed Within the Same Dosage Level and Duration of Treatment.

The comparative mean maternal outcome parameters for various study groups	Study groups and dosage levels.	The time of exposure to treatment	Phenobarbital treatment (PB)	Phenytoin treatment (PT)	Mean Difference (PB-PT)	Std. Error	Sig.[d]	95% Confidence Interval for Difference[d] Lower Bound	Upper Bound
terminal weight (kg)	Low	TM_1	PB	PT	-8.000*	2.418	.002	-12.895	-3.105
		TM_2	PB	PT	-4.000*	2.418	.052	-2.895	6.105
		TM_3	PB	PT	-2.000*	2.418	.054	-1.895	3.105
	Medium	TM_1	PB	PT	-7.000*	2.418	.006	-11.895	-2.105
		TM_2	PB	PT	-6.667*	2.418	.009	-11.562	-1.771
		TM_3	PB	PT	-4.000*	2.418	.059	-2.895	6.105
	High.	TM_1	PB	PT	-8.667*	2.418	.001	-13.562	-3.771
		TM_2	PB	PT	-8.000*	2.418	.002	-12.895	-3.105
		TM_3	PB	PT	-8.333*	2.418	.001	-13.229	-3.438
Maternal weight gain (g)	Low	TM_1	PB	PT	-3.667*	1.427	.014	-6.555	-.779
		TM_2	PB	PT	-3.100	1.427	.052	-5.888	.112
		TM_3	PB	PT	-2.000	1.427	.069	-4.888	.888
	medium	TM_1	PB	PT	-5.333*	1.427	.001	-8.221	-2.445
		TM_2	PB	PT	-5.000*	1.427	.001	-7.888	-2.112
		TM_3	PB	PT	-2.333	1.427	.861	-5.221	.555
	High.	TM_1	PB	PT	-5.000*	1.427	.001	-7.888	-2.112
		TM_2	PB	PT	-5.333*	1.427	.001	-8.221	-2.445
		TM_3	PB	PT	-3.667*	1.427	.014	-6.555	-.779
placental weight (g)	Low	TM_1	PB	PT	-.022*	.006	.000	-.033	-.010
		TM_2	PB	PT	-.020*	.006	.001	-.032	-.009
		TM_3	PB	PT	-.019*	.006	.056	.030	-.007
	Medium	TM_1	PB	PT	-.026*	.006	.000	-.037	-.014
		TM_2	PB	PT	-.023*	.006	.000	-.034	-.012
		TM_3	PB	PT	-.022*	.006	.000	-.034	-.011
	High.	TM_1	PB	PT	-.043*	.006	.000	-.054	-.032
		TM_2	PB	PT	-.024*	.006	.000	-.036	-.013
		TM_3	PB	PT	-.023*	.006	.000	-.034	-.011

*Key:- * The mean difference of less than 0.05 is significant and was denoted by asterisk (*)*

4.1.2 The Comparative Fetal Pregnancy Outcomes

On the fetal pregnancy outcomes, the findings are presented in two levels: -

(i) Level 1: The intrauterine fetal outcomes parameters.

The parameters examined included comparative (i) litter sizes, (ii) resorbed endometrial glands, (iii) dead fetuses and they were examined while the fetuses

were still inside the uterine horns immediately after opening the maternal anterior abdominal wall.

(ii) Level 2: The fetal growth and development outcome parameters.

The following parameters were used; - (i) fetal weight (FW) (ii) Bi-parietal diameter (BPD), (iii) Head circumference (HC) and (iv) crown rump length (CRL)

4.1.2.1 The Comparative Litter Sizes, Resorbed Endometrial Glands, Dead Fetuses.

In evaluating the comparative dose response relationship between the phenytoin and the phenobarbital treated groups on the number of resorbed glands and dead fetuses to assess their level of toxicity in utero, it was observed that the teratogenic effects on the maternal resorbed endometrial glands and the number of dead fetuses depicted a direct doses response relationship across the trimesters in that when the doses were increased, the number of resorbed glands and the number of dead fetuses also increased ***(Figure 4.1B, Figure 4.1C).*** The mean litter sizes on the other hand was observed to depict an inverse dose relationship in that when the dose was increased the number of litter size decreased. More effects were observed in the high treatment groups in that they had the least number of litter size, highest numbers of both dead fetuses, and the number of resorbed glands. To the contrary these teratogenic outcomes were observed to be least at low doses on both medicines and particularly lowest when treated at trimester three (TM_3) ***(Figure 4.1*** **A).**

It was however notable that despite the two medicines showing similar treads in all the maternal pregnancy outcomes when evaluated in terms of dosages and the time of exposures there was slight variations between the phenytoin and the phenobarbital treated groups in that that phenobarbital depicted a higher teratogenic influence in all the maternal pregnancy outcomes compared to both the phenytoin treated groups and the control (***Figure 4.1A, Figure 4.1B*** and ***Figure 4.1C).***

Concerning how phenobarbital and phenytoin influenced the three fetal parameters in relation to varied doses and differing trimesters, the two medicines were noted to have inverse proportion in relation to time as well as direct dose relationship. The litter sizes, were observed to be low, while the resorbed endometrial glands, dead fetuses were noted to be high in both phenobarbital and phenytoin treatment groups as compared to control (***Figure 4.1A, Figure 4.1B*** and ***Figure 4.1C).***

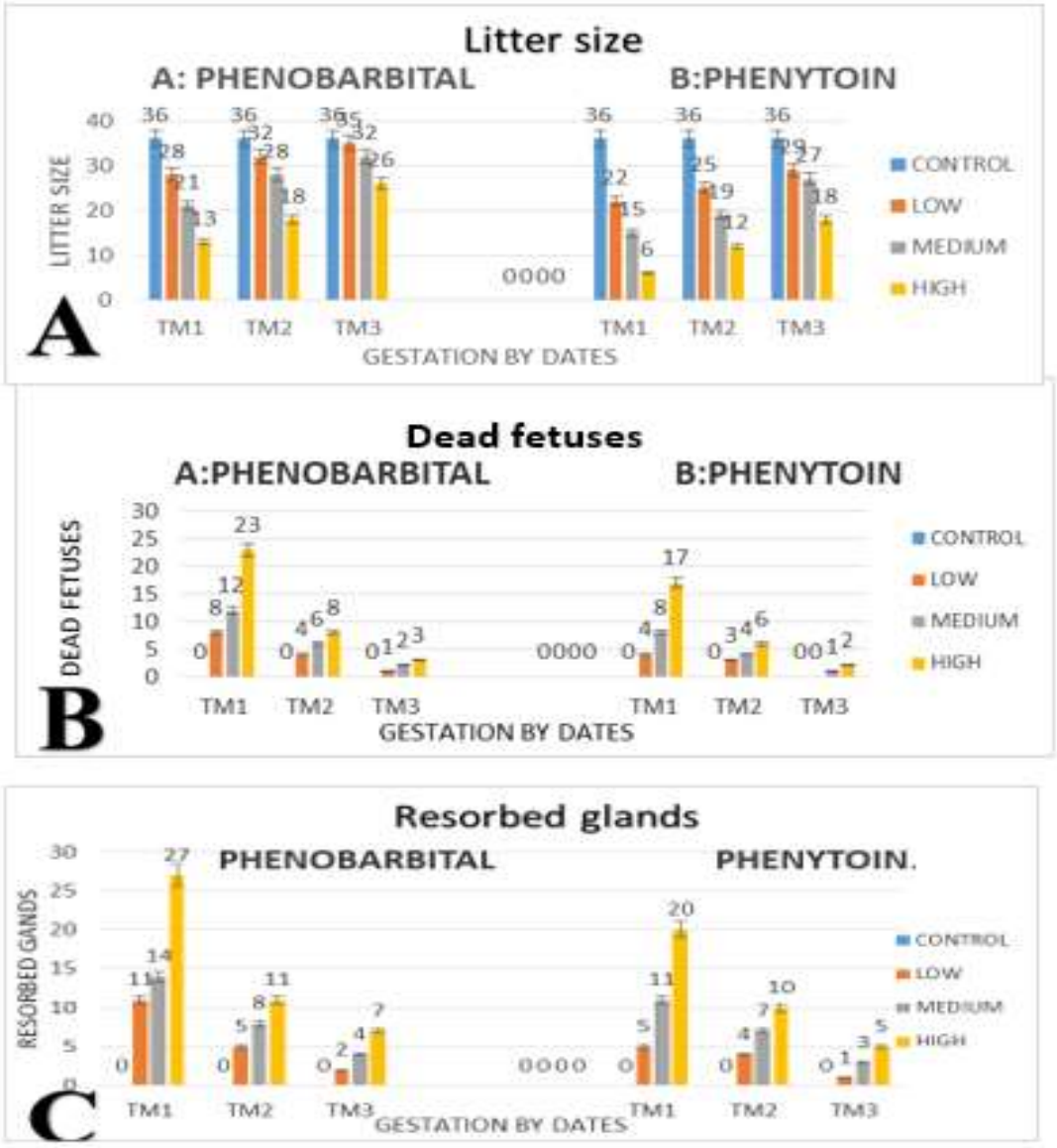


Figure 4.1.: The Bar Graphs Showing Comparative Litter Size, Dead Fetuses and Resorbed Glands.

KEY: -

A-The comparative litter sizes between phenobarbital and phenytoin treated groups.
B- The comparative dead fetuses between phenobarbital and phenytoin treated groups.
C-The comparative resorbed glands between phenobarbital and phenytoin treated groups.

4.1.2.2 The Individual Fetal Pregnancy Outcomes

Upon evaluating how the three independent variables which included doses, drugs and trimesters influenced the four foetal pregnancy outcomes i.e. (i) Mean fetal weight (FW), (ii) Mean bi-parietal diameter (BPD), (iii) Mean head circumference (HC) and (iv) Mean crown rump length (CRL) using univariate, and bivariate by use of both (ANOVA), it was observed that there was significant difference between the control and the treatment groups, this current study found that at the global level, significant detrimental reduction in all the four fetal pregnancy outcomes parameters assessed was observed at ($P<0.05$) on the administration of varied doses of both phenobarbital and phenytoin during pregnancy when comparison was done with the control (***Table 4.4)***.

When comparison was made between the two medicines, Phenobarbital was further noted to have more injurious (detrimental) effect on maternal pregnancy outcome parameters as compared to phenytoin as shown in the ANOVA results on table (***Table 4.1.2.2.1)*** as follows:- i) Fetal weight $F(18,38)= 332.303$ $P= 0.00$ (FW), (ii) Bi-parietal diameter (BPD) $F(18,38)= 178.154$ $P= 0.03$, (iii) Head circumference $F(18,38)= 95.816$ $P= 0.01$ (HC) and (iv) Crown rump length CRL) $F(18,38)= 37.390$ $P= 0.00$ (***Table 4.4)***.

Concerning the time of exposure and the dosages, it was further noted that in both the two treatment categories of phenobarbital and phenytoin, the four foetal growth and development outcomes parameters were highly affected when the treatments were initiated at TM_1 and TM_2 with the worst detrimental outcomes when high treatment dose were administered (***Table 4.4)***.

Table 4.4: Comparative Findings on Foetal Outcome for Phenobarbital and Phenytoin at Different Doses Administered at Different Trimesters against the Control using ANOVA.

The study groups	Study groups and dosage levels.	The time of exposure to treatment	The comparative mean fetal weight, bi-parietal diameter, head circumference and crown rump length widths for various study groups			
			Mean fetal weight (FW)(g) ± SD)	Mean bi-parietal diameter (BPD) (cm) ± SD)	Mean head circumference (HC) (cm) ± SD)	Mean crown rump length CRL) (cm) (g) ± SD)
Control.	Control (C)					
	No treatment	None.	6.4643±.1022	1.559±.05508	4.464±.1028	4.291±.1636
	Low dose treatment group (LPB)-[3.1 mg/kg/bw)	TM_1	4.6519±.1503	.7950±.0564	3.620±.0816	3.492±.1863
		TM_2	5.6130±.0697	1.0870±.0405	3.798±.0854	3.607±.0584
		TM_3	6.0445±.0415	1.442±.0186	4.248±.0373	4.187±.0827
	Medium dose treatment group(MPB)-[19.2mg/kg/bw)	TM_1	4.0304±.0351*	.679±.0262	3.270±.0151*	3.212±.1515*
		TM_2	5.0954±.0408	.933±.0283	3.584±.0599	3.453±.0953
		TM_3	5.9133±.0155	1.325±.0065	4.116±.0120	3.994±.0264
The phenobarbital treatment groups	High dose treatment group	TM_1	3.3102±.1635*	.526±.068*	3.0200±.0603*	2.9420±.2095*
		TM_2	4.6653±.0627*	.850±.0515*	3.4627±.0934*	3.2828±.0827*
	(HPB) (41.5 mg/kg/bw)	TM_3	5.6468±.0535	1.276±.0565	3.8471±.0436	3.8405±.0326;
	Low dose treatment group (LPT)-(31 mg/kg/bw).	TM_1	4.7701±.1547	.916±.06407	3.728±.0865	3.571±.1668
		TM_2	5.7201±.0769	1.193±.04945	3.911±.0842	3.696±.0390
		TM_3	6.1393±.0500	1.548±.01347	4.331±.0373	4.285±.0701
	Medium dose treatment group (MPT)-[62 mg/kg/bw).	TM_1	3.9706±.1543	.791±.0073	3.390±.0292	3.325±.0981
		TM_2	5.1604±.0761	1.055±.0306	3.699±.0611	3.520±.1063
		TM_3	6.0381±.0504	1.433±.0037	4.228±.0206	4.109±.0340
The phenytoin treatment groups	High dose treatment group(HPT) (124 mg/kg/bw).	TM_1	3.432±.157*	.671±.034*	3.116±.076*	3.082±.135*
		TM_2	4.788±.073*	.961±.048*	3.585±.099*	3.355±.126*
		TM_3	5.767±.046	1.353±.012	4.068±.139	3.958±.063
			F(18,38)= 332.303 P= 0.00	**F(18,38)= 178.154 P= 0.03**	**F(18,38)= 95.816 P= 0.01**	**F(18,38)= 37.390 P= 0.00**

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

Upon carrying out the MANOVA level II analysis to find out how the three independent variables of the **individual drug**, **dose** and **the time of exposure** plus

their interactions influenced each of the four fetal outcome parameters this study found out that: -

At individual level, this study found out that all the three independent variables had a significant role to play in influencing The four fetal pregnancy outcome parameters but in varying proportions as indicated by the values of wilk's labda Partial Eta squared (η^2) (***Table 4.5).*** It was observed that at individual level the type of drug, the doses and the time of administration of medicines had a statistical significant contribution ($P<.05$) with the type of drug contributing (Partial Eta squared (η^2) between 91-99% followed by the dosage between 71-96% and the time had (the least) contribution ranging between 49-90% (***Table 4.5).***

a) At two-way level interaction effects it was observed the element of the types of time of exposure in combination with dosages had the worst effects followed by the combination of drug and dosages then lastly the combinations of time of exposure and drug as shown in the four variables of the mean fetal weight (FW), crown rump length (CRL), head circumference (HC) with (Partial Eta squared (η^2) of 92.5%, 92.7% and 88.2% on the mean fetal weights respectively (***Table 4.5).***
b) At three -way level interaction effects of doses, drugs and the trimesters, the foetal weight had the highest contribution with Partial Eta squared (η^2) of 52.13% (***Table 4.5).***

Table 4.5: The Level 2 MANOVA Results on how the Individual Drug, Dose and the Time of Exposure plus Their Interactions Influenced Each of the Four Foetal Growth and Development Outcome Parameters Prenatally.

Types of MANOVA evaluation at level 2	The groups being tested	The three dependent variables.	Measurements of the variability in the depended variables (Type III Sum of square)	Degree of freedom df	The ratio Type III Sum of square to its corresponding degree of freedom. (Mean Square)	The ration of the mean square for the independent variable to the mean square for error (F Statistics)	Sig.	Proportion of variance (Partial Eta Squared)
(i) The evaluation on the correctness of the model used for the study.	Corrected Model	HC	48.061[a]	18	2.670	346.690	<.001	.994
		FW	6.009[b]	18	.334	197.581	<.001	.989
		CRL	9.815[c]	18	.545	102.948	<.001	.980
		BPD	9.807[d]	18	.545	37.603	<.001	.947
(ii) Test on whether the observed results were due to chance	Intercept	HC	1127.171	1	1127.171	146355.462	<.001	1.000
	(grand total)	FW	50.027	1	50.027	29611.205	<.001	.999
		CRL	599.679	1	599.679	113222.703	<.001	1.000
		BPD	558.351	1	558.351	38534.789	<.001	.999
	DOSES (Low, medium, high dose)	FW	7.113	2	3.556	461.786	<.001	.960
		HC	.455	2	.228	134.734	<.001	.876
		CRL	1.613	2	.807	152.306	<.001	.889
		BPD	1.380	2	.690	47.635	<.001	.715
(iii) The Individual independent variable and its effects on	DRUGS (PB,PT)	FW	31.719	2	15.860	2059.278	<.001	.991
		CRL	4.028	2	2.014	1192.040	<.001	.984
each of the three fetal dependent variables	TRIMESTER (TM_1,TM_2,TM_3)	HC	5.573	2	2.786	526.092	<.001	.965
		BPD	6.207	2	3.103	214.187	<.001	.919
		CRL	1.346	1	1.346	174.755	<.001	.821
		FW	.608	1	.608	359.637	<.001	.904
		HC	.661	1	.661	124.810	<.001	.767
		BPD	.531	1	.531	36.679	<.001	.491
(iii)Two-way interaction effects on each of the fetal dependent variables	DOSES (Low, medium, high dose) * DRUGS (PB,PT)	FW	6.273	4	3.490	442.381	<.001	.927
		CRL	1.234	4	.801	132.265	<.001	.874
		HC	.699	4	.705	42.734	<.001	.548
		BPD	.397	4	.158	29.005		.031.196
	DOSES (Low, medium, high dose) * TRIMESTER (TM_1,TM_2,TM_3)	FW	6.273	4	3.490	442.381	<.001	.927
		CRL	1.234	4	.801	132.265	<.001	.874
		HC	.699	4	.705	42.734	<.001	.548
		BPD	.397	4	.158	29.005		.031.196
	DRUGS (PB,PT) * TRIMESTER (TM_1,TM_2,TM_3)	FW	3.208	4	3.573	388.123	.000	.882
		BPD	.155	4	.98	57.130	.097	.039
		CRL	.913	4	.567	71.323	<.001	.526
		HC	.816	4	.428	18.895	<.001	.656
(iv)Three-way interaction effects on each of the fetal dependent variables	DOSES (Low, medium, high dose) * DRUGS (PB,PT) * TRIMESTER (TM_1,TM_2,TM_3)	FW	.535	2	.321	40.323	<.001	.523
		BPD	.255	2	.102	11.090	.148	.081
		HC	.381	2	.523	20.378	.051	.125
		CRL	.472	2	.281	32.936	<.001	.401
	Error	HC	.293	38	.008			
		FW	.064	38	.002			
		CRL	.201	38	.005			
		BPD	.551	38	.014			
	Total	HC	1493.043	57				
		FW	66.117	57				
		CRL	796.883	57				
		BPD	744.127	57				
(iv) Overall inferential	Corrected Total	HC	48.354	56				
		FW	6.073	56				

statistics on the model results.	CRL	10.016	56	
	BPD	10.358	56	

*Key:- *Indicates interaction effects among the independent variables.*

Upon carrying out the MANOVA pairwise comparison to find out how the two medicines differed in influencing the four foetal pregnancy outcomes in the same dosage levels this study found out that phenobarbital had more effects to the fetal pregnancy outcome as compared to phenytoin (***Table 4.6).***

Table 4.6: The Level Three MANOVA Pairwise Comparison Table on How the Two Medicines Influenced Four Foetal Growth and Development Outcome Parameters when Exposed Within the Same Dosage Level and Duration of Treatment.

Dependent variable	Dosage level	The time of exposure to treatment	Phenobarbital treatment.	Phenytoin treatment	Mean difference between phenobarbital and phenytoin treatment groups (PB-PT)	Std. Error	Sig.[d] <0.05	95% Confidence Interval for Difference[d]	
								Lower Bound	Upper Bound
HC	Low	TM_1	PB	PT	-.319*	.072	<.001	-.464	-.174
		TM_2	PB	PT	-.307*	.072	<.001	-.452	-.162
		TM3	PB	PT	-.173	.098	.087	-.372	.026
	medium	TM_1	PB	PT	-.324*	.072	<.001	-.469	-.179
		TM_2	PB	PT	-.312*	.072	<.001	-.457	-.167
		TM_3	PB	PT	-.318*	.072	<.001	-.463	-.172
	High	TM_1	PB	PT	-.323*	.072	<.001	-.468	-.178
		TM_2	PB	PT	-.322*	.072	<.001	-.467	-.177
		TM_3	PB	PT	-.322*	.072	<.001	-.467	-.177
FW	Low	TM_1	PB	PT	-.221*	.034	<.001	-.289	-.154
		TM_2	PB	PT	-.207*	.034	<.001	-.275	-.139
		TM_3	PB	PT	-.206*	.034	<.001	-.274	-.138
	medium	TM_1	PB	PT	-.222*	.034	<.001	-.290	-.155
		TM_2	PB	PT	-.212*	.034	<.001	-.280	-.144
		TM_3	PB	PT	-.208*	.034	<.001	-.276	-.140
	High.	TM_1	PB	PT	-.244*	.034	<.001	-.312	-.177
		TM_2	PB	PT	-.211*	.034	<.001	-.279	-.143
		TM_3	PB	PT	-.177*	.034	<.001	-.245	-.109
CRL	Low	TM_1	PB	PT	-.213*	.059	.001	-.333	-.093
		TM_2	PB	PT	-.208*	.059	.001	-.328	-.088
		TM3	PB	PT	-.168	.098	.051	-.367	.031
	Medium	TM_1	P B	PT	-.220*	.059	.001	-.340	-.099
		TM_2	PB	PT	-.215*	.059	.001	-.335	-.095
		TM_3	PB	PT	-.212*	.059	.001	-.332	-.092
	High	TM1	PB	PT	-.321*	.059	<.001	-.442	-.201
		TM2	PB	PT	-.223*	.059	.001	-.343	-.103
		TM3	PB	PT	-.197*	.059	.002	-.317	-.076
Bi-parietal diameter (BPD)	Low	TM_1	PB	PT	-.203*	.098	.045	-.402	-.005
		TM_2	PB	PT	-.198	.098	.051	-.397	.001
		TM_3	PB	PT	-.179	.098	.077	-.377	.020
	medium	TM_1	PB	PT	-.215*	.098	.035	-.414	-.016
		TM_2	PB	PT	-.213*	.098	.037	-.412	-.014
		TM3	PB	PT	-.183*	.059	.004	-.303	-.063
	High.	TM_1	PB	PT	-.219*	.098	.032	-.418	-.020
		TM_2	PB	PT	-.218*	.098	.033	-.417	-.019

Dependent variable	Dosage level	The time of exposure to treatment	Phenobarbital treatment.	Phenytoin treatment	Mean difference between phenobarbital and phenytoin treatment groups (PB-PT)	Std. Error	Sig.[d] <0.05	95% Confidence Interval for Difference[d] Lower Bound	Upper Bound
		TM_3	PB	PT	-.294*	.072	<.001	-.440	-.149

Key: - The mean difference of less than 0.05 is significant and is denoted by asterisk (*).

The Histomorphological Findings

4.2 Objective Two: The Comparative Histo-Morphological Evaluation on How the Medicines Phenobarbital and Phenytoin] Influenced the Histological Organization of the Developing Fetal Kidneys.

In evaluating the histo-morphological organization of the developing fetal kidneys, the two parenchymal layers that included the renal cortex and the medulla were assessed. this was done to establish the extent of the histological alteration of these two zones of the developing fetal kidneys as their alterations would serve as a predictor to an early onset of congenital renal disease that can lead to obstructive uropathy, renal dysplasia (Potter type IV) or multicystic dysplastic kidney (MDK) disease (Potter type II). In evaluating how the two medicines influenced the histological organization of the fetal kidneys, a step wise approach was used where; the first step was to establish how the two medicines influenced the histomorphological thicknesses, then followed by the histomorphological organization of the glomeruli, the histological organization of the bowman's capsule and lastly the histological organization of the collecting tubules in the medulla as follows: -

(i) Comparative histomorphological thicknesses of the two parenchymal layers [cortex and the medulla].
(ii) Comparative histomorphological organization of the glomeruli structures.
(iii) Comparative histomorphological findings on the bowman's space.
(iv) Comparative histomorphological appearances on the renal tubules.

4.2.1 The Comparative Evaluation on How the Two Medicines Influenced the Histo-Morphological Thicknesses of the Two Parenchymal Layers [Cortex and the Medulla].

Clinically the thicknesses of the renal cortex, the medulla plus the sizes of the glomeruli are key indicators in estimating how effective is the kidney glomerular filtration rate (GFR). As such, histo-morphological thicknesses were measured in the longitudinal histological sections of the fetal kidneys and the results presented in line with the comparative doses of exposure between the two medicines.

It was observed that the medullary thicknesses, looked hypertrophied or enlarged in sizes for both the treatment groups as compared with the controls. The cortical thicknesses on the other hand was slightly reduced. This enlargement of the medullary thickness was noted to be occasioned by the fluid accumulation in the interstitial spaces between the collecting tubules there were signs of edematous fluid while in some areas there was marked deposition of fibrous connective tissue within the interstitial spaces of the collecting tubules. On the other hand, the slight reduction of the cortical thickness was occasioned by the degeneration of the glomeruli as follows:

(i) **For the rats that received the low dose phenobarbital and phenytoin at TM_1, TM_2 and TM_3 the cortical and the medullary thicknesses changes were as follows:** among the phenobarbital treatment group the medullary thicknesses were 4.2 µm, 3.8 µm, 3.7 µm, for TM_1, TM_2 and TM_3 respectively while phenytoin treatment group on the other hand were the 4.1 µm, 3.7 µm, 3.5 µm for TM_1, TM_2 and TM_3 respectively,

The cortical thicknesses were 2.5 µm, 2.5 µm, 2.6 µm. for TM_1, TM_2 and TM_3 respectively for phenobarbital treatment group while phenytoin treatment group on the other hand were the cortical thickness 2.4 µm, $_{2.4}$µm, ***2.5*** µm for TM_1, TM_2 and TM_3 respectively ***(Figure 4.2).***

(ii) **For the rats that received the medium dose phenobarbital and phenytoin at TM_1, TM_2 and TM_3 the cortical and the medullary thicknesses**

changes were as follows: among the phenobarbital treatment group the medullary thicknesses were 4.4 µm, 4.4 µm, 4.5 µm for $TM_{1,}$ TM_2 and TM_3 respectively while phenytoin treatment group on the other hand were the .2 µm, 4.1 µm, 3.8 µm, for $TM_{1,}$ TM_2 and TM_3 respectively,

The cortical thicknesses were 2.4 µm, 2.5 µm, 2.5 µm. for $TM_{1,}$ TM_2 and TM_3 respectively for phenobarbital treatment group while phenytoin treatment group on the other hand were the cortical thickness 2.3 µm, 2.4 µm, - 2.5 µm for $TM_{1,}$ TM_2 and TM_3 respectively ***(Figure 4.3).***

(iii) For the rats that received the high dose phenobarbital and phenytoin at TM_1, TM_2 and TM_3 the cortical and the medullary thicknesses changes were as follows: - among the phenobarbital treatment group the medullary thicknesses were 6.5µm, 5.7 µm, 5.3 µm, for $TM_{1,}$ TM_2 and TM_3 respectively while phenytoin treatment group on the other hand were the 5.9µm, 5.5 µm, 5.1 µm, for $TM_{1,}$ TM_2 and TM_3 respectively,

the cortical thicknesses were 2.3 µm, 2.5 µm, *2.5* µm. for $TM_{1,}$ TM_2 and TM_3 respectively for phenobarbital treatment group while phenytoin treatment group on the other hand were the cortical thickness 2.3 µm, $_{2.4}$µm, 2.5 µm for $TM_{1,}$ TM_2 and TM_3 respectively ***(Figure 4.4).***

In summary, it can be noted that the kidney medullary thicknesses in the phenobarbital treated groups were relatively thicker than those in the phenytoin treated groups and in comparison with the control the medullary thicknesses were thicker in both treatment groups ***(Figure 4.2. 4.3, 4.4).***

The comparative medullary and cortical thickness at low dose at TM_1, TM_2 and TM_3.

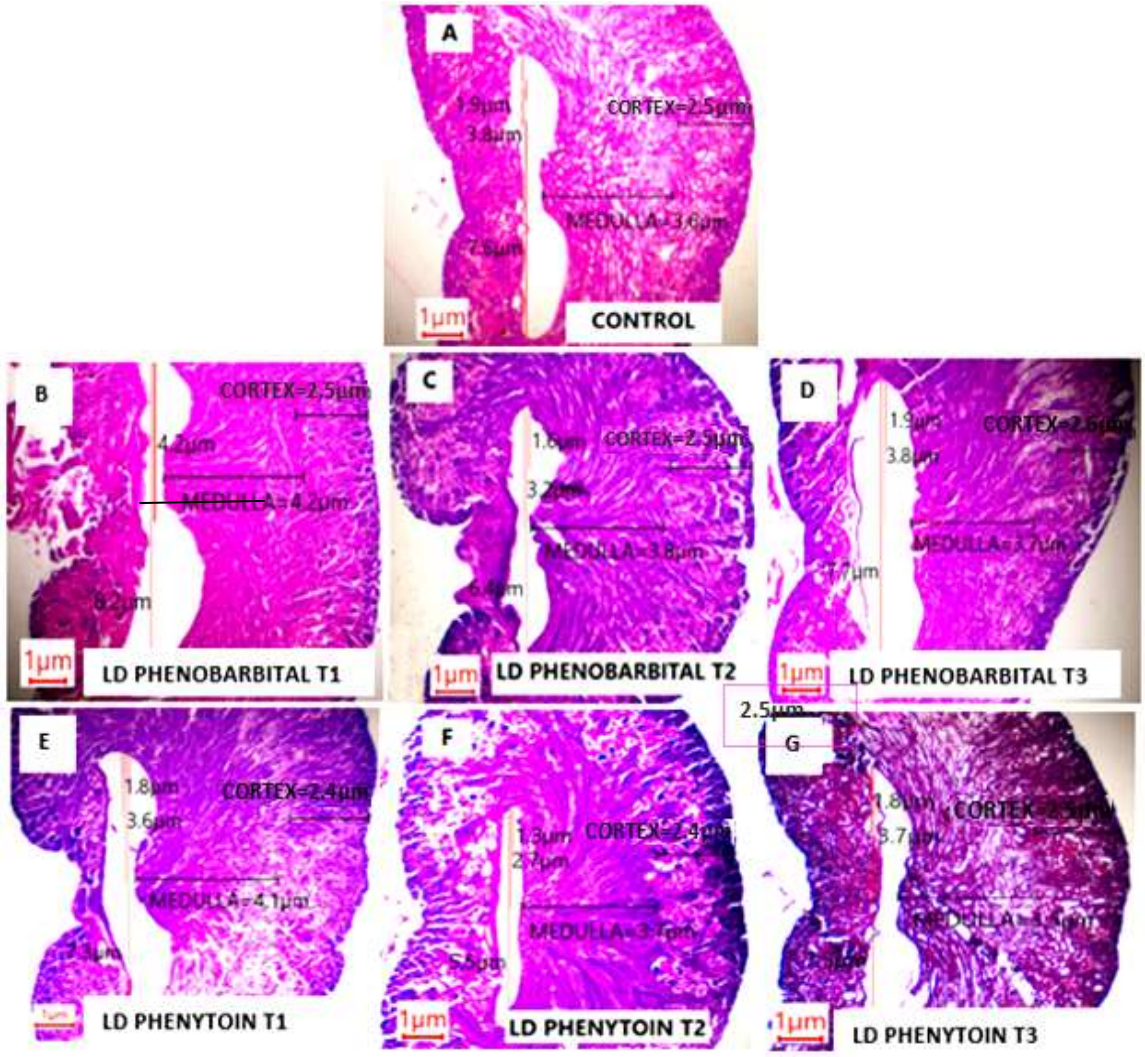


Figure 4.2: The Photomicrographs of the Longitudinal Sections of the Fetal Kidneys Showing the Medulla and the Cortical Thicknesses of the Phenobarbital and Phenytoin Low Dose Treated Groups against the Control Treated at TM1, TM2, TM3 (H&E Mag X4).

KEY: -
A: CONTROL,
B: - (LD PHENOBARBITAL T_1) = Low dose phenobarbital trimester one,
C: - (LD PHENOBARBITAL T_2) = Low dose phenobarbital trimester two,
D: - (LD PHENOBARBITAL T_3) = Low dose phenobarbital trimester three,
E: - (LD PHENYTOIN T1) = Low dose phenytoin trimester one,
F: - (LD PHENYTOIN T2) = Low dose phenytoin trimester two

G: - (LD PHENYTOIN T3) = Low dose phenytoin trimester three.

The comparative medullary and cortical thickness at medium dose.

Figure 4.3: The Photomicrographs of the Longitudinal Sections of the Fetal Kidneys Showing the Medulla and the Cortical Thicknesses of the Phenobarbital and Phenytoin Medium Dose Treated Groups against the Control Treated at TM_1, TM_2, TM_3 (H&E Mag X4).

KEY: -
A: CONTROL,
B: - (LD PHENOBARBITAL T1) = Low dose phenobarbital trimester one,
C: - (LD PHENOBARBITAL T2) = Low dose phenobarbital trimester two,
D: - (LD PHENOBARBITAL T3) = Low dose phenobarbital trimester three,
E: - (LD PHENYTOIN T1) = Low dose phenytoin trimester one,

F: - (LD PHENYTOIN T2) = Low dose phenytoin trimester two
G: - (LD PHENYTOIN T3) = Low dose phenytoin trimester three.

The comparative medullary and cortical thickness at high dose.

Figure 4.4: The Photomicrographs of the Longitudinal Sections of the Fetal Kidneys Showing the Medulla and the Cortical Thicknesses of the Phenobarbital and Phenytoin High Dose Treated Groups Against the Control Treated at TM_1, TM_2, TM_3 (H&E Mag X4).

KEY: -
A: CONTROL,
B: - (LD PHENOBARBITAL T_1) = Low dose phenobarbital trimester one,
C: - (LD PHENOBARBITAL T_2) = Low dose phenobarbital trimester two,
D: - (LD PHENOBARBITAL T_3) = Low dose phenobarbital trimester three,
E: - (LD PHENYTOIN T1) = Low dose phenytoin trimester one,
F: - (LD PHENYTOIN T2) = Low dose phenytoin trimester two
G: - (LD PHENYTOIN T3) = Low dose phenytoin trimester three.

4.2.2 The Comparative Effects of Phenobarbital and Phenytoin on the Number of the Glomeruli per Field.

Upon carrying out a comparative histo-morphological analysis on the numbers of the glomeruli per a field, it was observed that the numbers of the glomeruli were seen to be varying with the time and the dose of exposure as follows:

(i) For the rats that received the low dose phenobarbital and phenytoin at TM_1, TM_2 and TM_3 the numbers of the glomeruli per a field were as follows:-, for phenobarbital treatment group the numbers of the glomeruli per a field ranged between 9-14 while the phenytoin treatment group, the numbers of the glomeruli per a field ranged between 14-15 with trimester one having the least number of glomeruli per field followed by trimester two then trimester three with highest among the low dose treatment groups. However, the numbers were noted to be reduced as compared to the control which had 17 glomeruli per field and phenobarbital treated group had more reduced number of glomeruli per field as compared to phenytoin treated group shown in ***(Figure 4.5).***

(ii) For the rats that received the medium dose phenobarbital and phenytoin at TM_1, TM_2 and TM_3 the numbers of glomeruli per field were also noted to be reducing as compared with the control. The phenobarbital treatment group had glomeruli ranging from 4-13 while the phenytoin treatment group ranges between 8-14. It was evident that the phenobarbital treated group had more reduced number of glomeruli per field as compared to phenytoin treated group. It was also notable that trimester one had the least number of glomeruli per field followed by trimester two then trimester three with highest among the medium dose treatment groups. The numbers were also noted to be reduced as compared to the control which had 17 glomeruli per field shown in ***(Figure 4.6).***

(iii) For the rats that received the high dose phenobarbital and phenytoin at TM_1, TM_2 and TM_3 the numbers of the glomeruli per a field were as follows:-, for phenobarbital treatment group the numbers of the glomeruli per a field ranged between 2-5 while the phenytoin treatment group, the numbers of

the glomeruli per a field ranged between 7-14 with trimester one having the least number of glomeruli per field followed by trimester two then trimester three with highest among the low dose treatment groups. However, the numbers were also noted to be reduced as compared to the control which had 17 glomeruli per field and phenobarbital treated group had more reduced number of glomeruli per field as compared to phenytoin treated group (***Figure 4.7).***

In summary, the numbers of the glomeruli reduced per field with increasing doses of the two medicines and across all the dose groups of low, medium and high phenobarbital and phenytoin treated groups ***(Figure 4.5, Figure 4.6*** and ***Figure 4.7)***. Concerning the time of administration, it was observed that when the treatments were instituted at trimester one (TM_1), the glomeruli distribution were markedly reduced, followed by trimester two (TM_2) while during trimester three (TM_3) there was no marked significance reduction in the glomeruli distribution for both phenobarbital and phenytoin treatment groups especially when they were administered at low dose ***(Figure 4.5, Figure 4.6*** and ***Figure 4.7)***. Upon comparing the distribution of the glomeruli in the fetal kidneys exposed it was noted that there was remarkably reduced glomerular distribution in the phenobarbital treated groups as compared to phenytoin treated groups ***(Figure 4.5, Figure 4.6*** and ***Figure 4.7).***

The comparative number of the glomeruli per field at low dose.

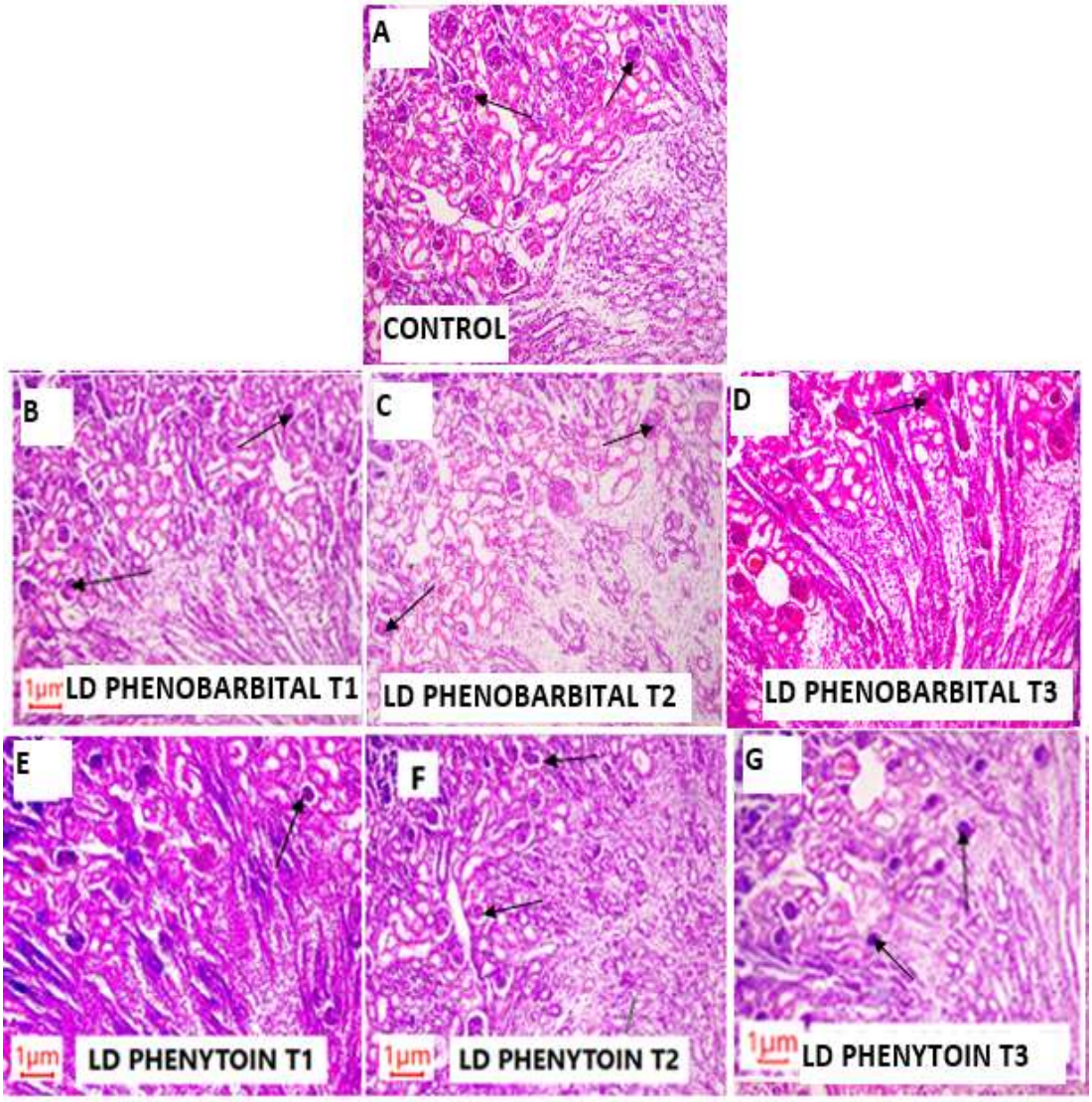


Figure 4.5: The Photomicrographs of the Fetal Kidneys Showing the Number of Glomeruli Per Field (Arrows) of View for the Phenobarbital and Phenytoin Low Dose Treated Groups Against the Control Treated at TM1, TM2, TM3 (H & E Mag X10).

KEY: -
A: CONTROL,
B: - (LD PHENOBARBITAL T_1) = Low dose phenobarbital trimester one,
C: - (LD PHENOBARBITAL T_2) = Low dose phenobarbital trimester two,
D: - (LD PHENOBARBITAL T_3) = Low dose phenobarbital trimester three,
E: - (LD PHENYTOIN T1) = Low dose phenytoin trimester one,
F: - (LD PHENYTOIN T2) = Low dose phenytoin trimester two
G: - (LD PHENYTOIN T3) = Low dose phenytoin trimester three.

The comparative number of the glomeruli per field at medium dose.

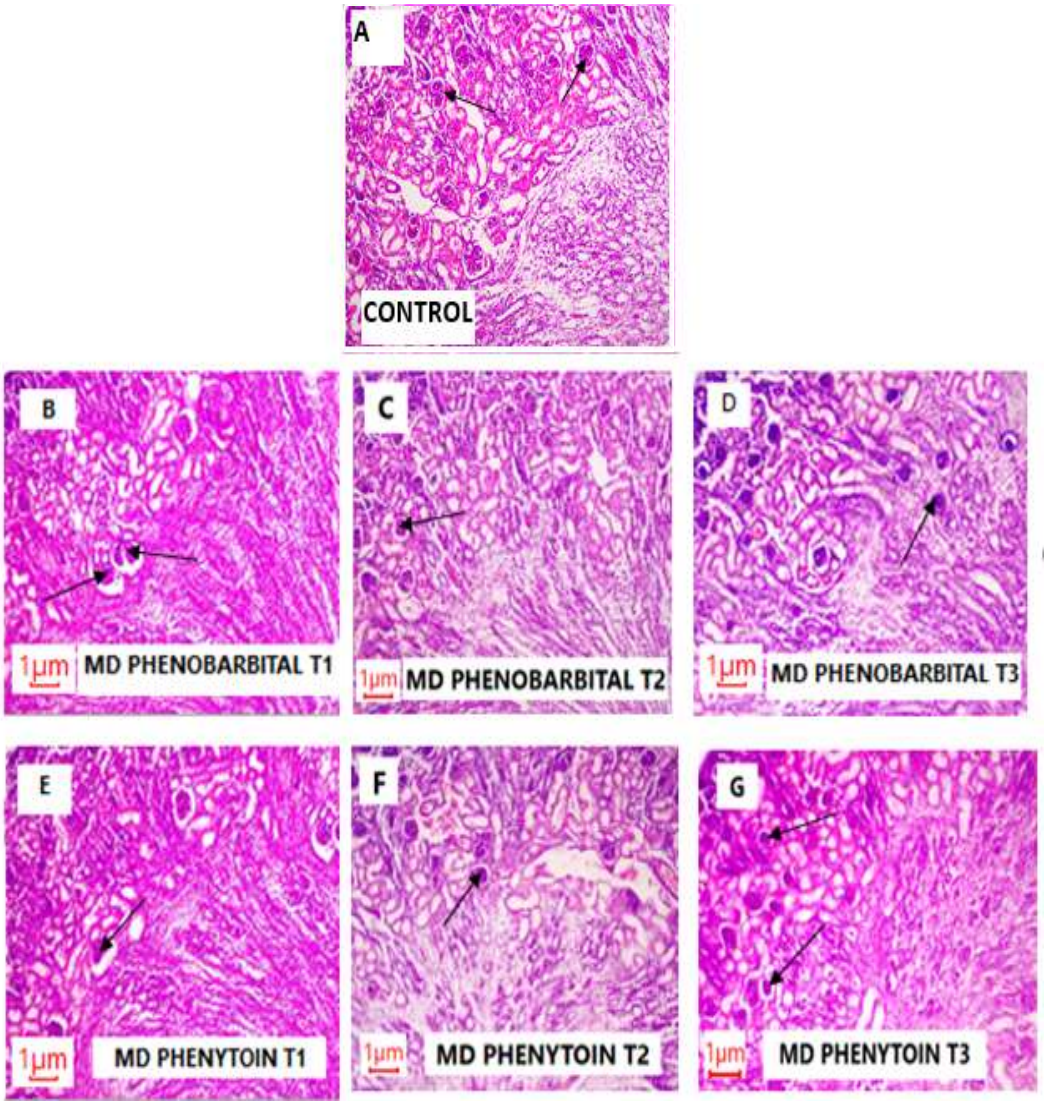


Figure 4.6: The Photomicrographs of the Fetal Kidneys Showing the Number of Glomeruli Per Field (Arrows) of View for the Phenobarbital and Phenytoin Medium Dose Treated Groups against the Control Treated at TM1, TM2, TM3 (H & E Mag X10).

KEY: -
A: CONTROL,
B: - (LD PHENOBARBITAL T_1) = Low dose phenobarbital trimester one,
C: - (LD PHENOBARBITAL T_2) = Low dose phenobarbital trimester two,
D: - (LD PHENOBARBITAL T_3) = Low dose phenobarbital trimester three,
E: - (LD PHENYTOIN T1) = Low dose phenytoin trimester one,

F: - (LD PHENYTOIN T2) = Low dose phenytoin trimester two
G: - (LD PHENYTOIN T3) = Low dose phenytoin trimester three.

The comparative number of the glomeruli per field at medium dose

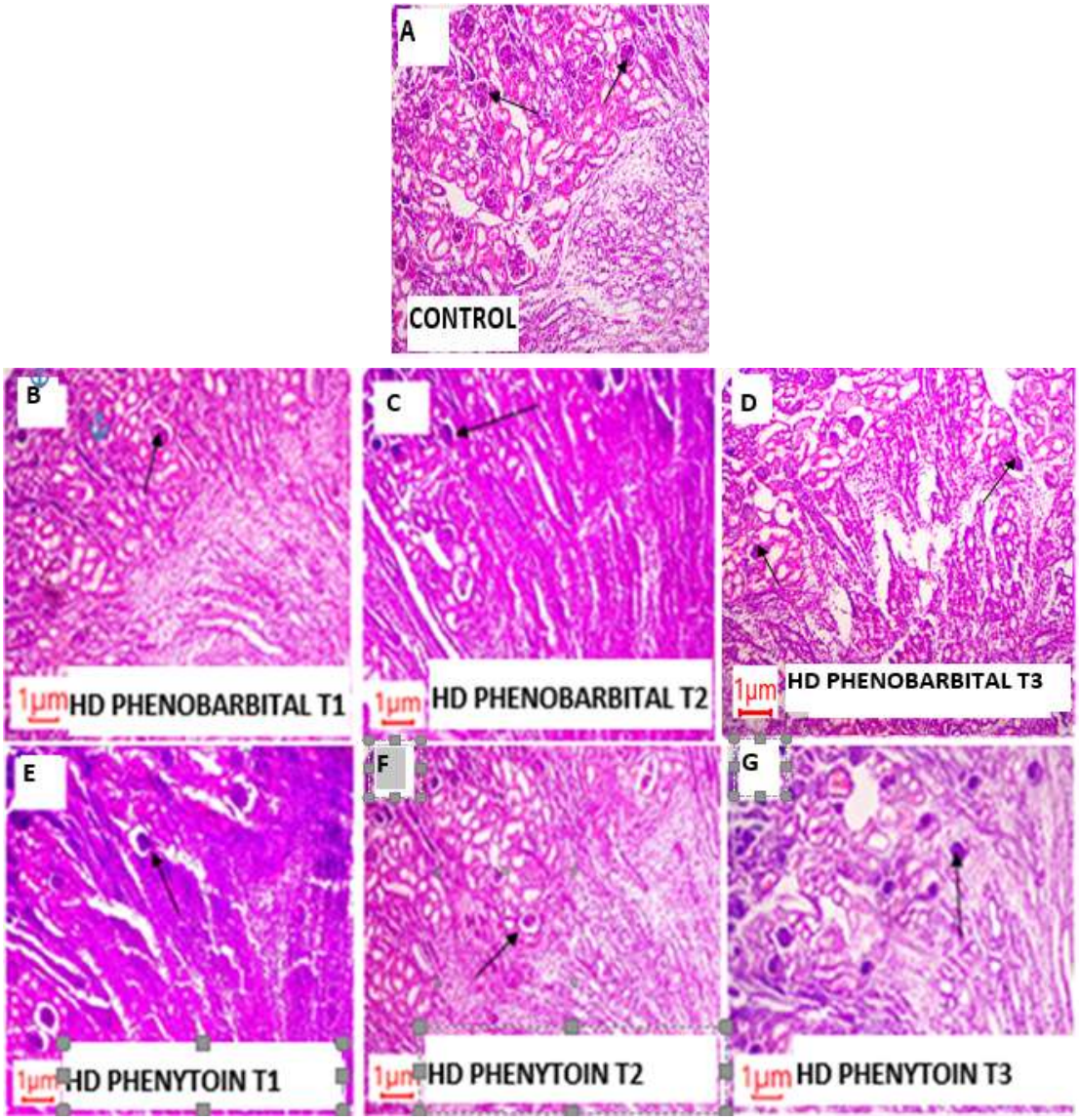


Figure 4.7: The Photomicrographs of the Fetal Kidneys Showing the Number of Glomeruli Per Field (Arrows) of View for the Phenobarbital and Phenytoin High Dose Treated Groups Against the Control Treated at TM_1, TM_2, TM_3 (H & E Mag X10).

KEY: -
A: CONTROL,
B: - (LD PHENOBARBITAL T_1) = Low dose phenobarbital trimester one,
C: - (LD PHENOBARBITAL T_2) = Low dose phenobarbital trimester two,

D: - (LD PHENOBARBITAL T_3) = Low dose phenobarbital trimester three,
E: - (LD PHENYTOIN T1) = Low dose phenytoin trimester one,
F: - (LD PHENYTOIN T2) = Low dose phenytoin trimester two
G: - (LD PHENYTOIN T3) = Low dose phenytoin trimester three.

4.2.3 The Comparative Effects of Phenobarbital and Phenytoin on the Bowman's Space, Cellular Distribution.

Upon carrying out comparative histo-morpological analysis on the size of the bowman's space and the distribution of the cells, this study found out that when the dose of both phenobarbital and phenytoin were increased, the bowman's spaces were noted to be increasing and also the distribution of the cells were also noted to be increasing directly with the dose of administration as follows: -

(i) For the rats that received the low dose phenobarbital and phenytoin at TM_1, TM_2 and TM_3 the bowman's size changes were as follows: - among the phenobarbital treatment group the bowman's sizes were 0.012 µm, 0.011 µm, 0.011 µm, for TM_1, TM_2 and TM_3 respectively while phenytoin treatment group on the other hand were 0.011µm, 0.010µm, 0.010 µm for TM_1, TM_2 and TM_3 respectively ***(Figure 4.8).***

(ii) For the rats that received the medium dose phenobarbital and phenytoin at TM_1, TM_2 and TM_3 the bowman's size changes were as follows: - among the phenobarbital treatment group the bowman's sizes were 0.017 µm, 0.014 µm, 0.014 µm for TM_1, TM_2 and TM_3 respectively while phenytoin treatment group on the other hand were 0.016 µm, 0.013 µm, 0.012 µm, for TM_1, TM_2 and TM_3 respectively ***(Figure 4.9).***

(iii)For the rats that received the high dose phenobarbital and phenytoin at TM_1, TM_2 and TM_3 the cortical and the bowman's size changes were as follows: - among the phenobarbital treatment group the **bowman's size** were 0.020µm, 0.020 µm, 0.019 µm, for TM_1, TM_2 and TM_3 respectively while phenytoin treatment group on the other hand were. 0.019 µm, 0.019 µm, 0.017 µm, for TM_1, TM_2 and TM_3 respectively ***(Figure 4.10).***

In summary, the bowman's spaces were noted to increasing directly with the dose of administration. For example, low dose treatment groups had the least widened

bowman's space, followed by medium dose treatment groups then high dose treatment groups had the most widened bowman's space. Concerning the duration of administration, the bowman' s spaces were noted to be more increased when the treatments were administered during the first trimester as compared to the second and the third trimester. It was notable that the widening of the bowman's space was marked with the high dose when the medicines were administered at trimester one, two and three and also medium doses when the medicines were administered at trimester one and two ***(Figure 4.8, Figure 4.9*** *and* ***Figure 4.10).***

In assessing the comparative cellular distribution at the glomeruli and the jugta-glomeruli apparatus (JGA), the cells specifically mesangial cells (MC) were seen to vary in their number and shapes based on the time of exposure as well as the dose of exposure. The treatment groups were seen to have hyper cellular glomeruli especially with medium and high dose treatment groups especially with the high dose treatment groups for both drugs particularly when the treatments were done during trimester one (TM_1) when it was compared with the control ***(Figure 4.10).*** The treatment groups (low dose group, medium dose groups and high dose groups) were noted to have varying cellular distribution in dose dependent manner in that high dose treatment groups had hyper cellular glomeruli followed by medium dose then the low dose had the least cellular distribution (almost the same with that of the control) among the treatment groups and the effects were seen to be more with the phenobarbital treatment group as compared with phenytoin especially with the high dose group (When comparison was made between the two medicines within the same duration of administration and the same dosage level, phenobarbital was noted to have more cells at the glomeruli as compared to phenytoin especially with the high dose treatment group when the medicines were administered during trimester one and two *(****Figure 4.8, Figure 4.9*** *and* ***Figure 4.10).***

The comparative cellular distribution at the glomeruli and the bowman's space at low dose.

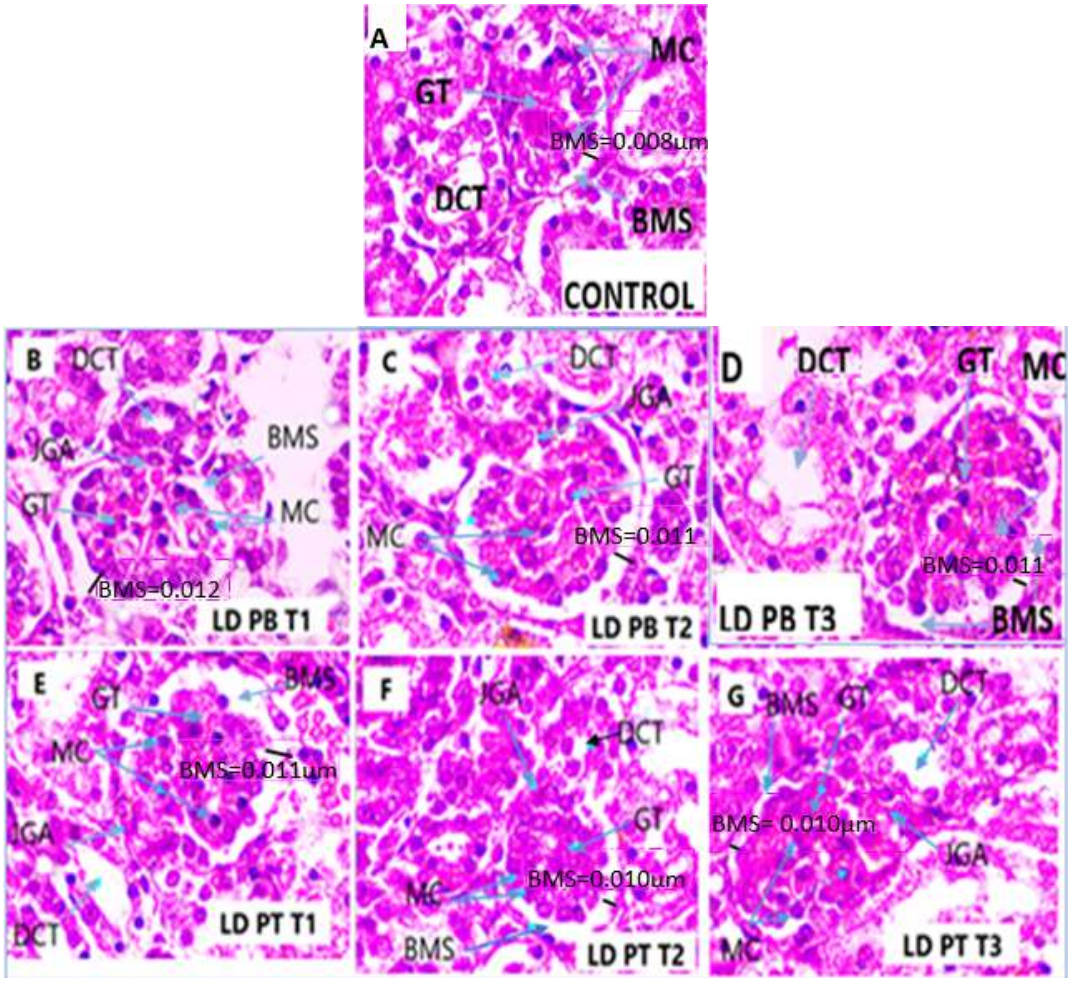


Figure 4.8: The Photomicrographs of the Fetal Kidneys Showing Bowman's Space and Distribution of Cells Per Field of View for Phenobarbital and Phenytoin Low Dose Treated Groups against the Control Treated at TM_1, TM_2, TM_3(H & E Mag X40).

KEY: -
A: CONTROL,
B: - (LD PHENOBARBITAL T1) = Low dose phenobarbital trimester one,
C: - (LD PHENOBARBITAL T2) = Low dose phenobarbital trimester two,
D: - (LD PHENOBARBITAL T3) = Low dose phenobarbital trimester three,
E: - (LD PHENYTOIN T1) = Low dose phenytoin trimester one,
F: - (LD PHENYTOIN T2) = Low dose phenytoin trimester two
G: - (LD PHENYTOIN T3) = Low dose phenytoin trimester three.

MC: - mesangial cells, BMS: - Bowman's space, GT: - Glomeruli tuft, DCT: - Distal convoluted tube, JGA: - Juxta-glomeruli apparatus,

The comparative cellular distribution at the glomeruli and the bowman's space at medium dose.

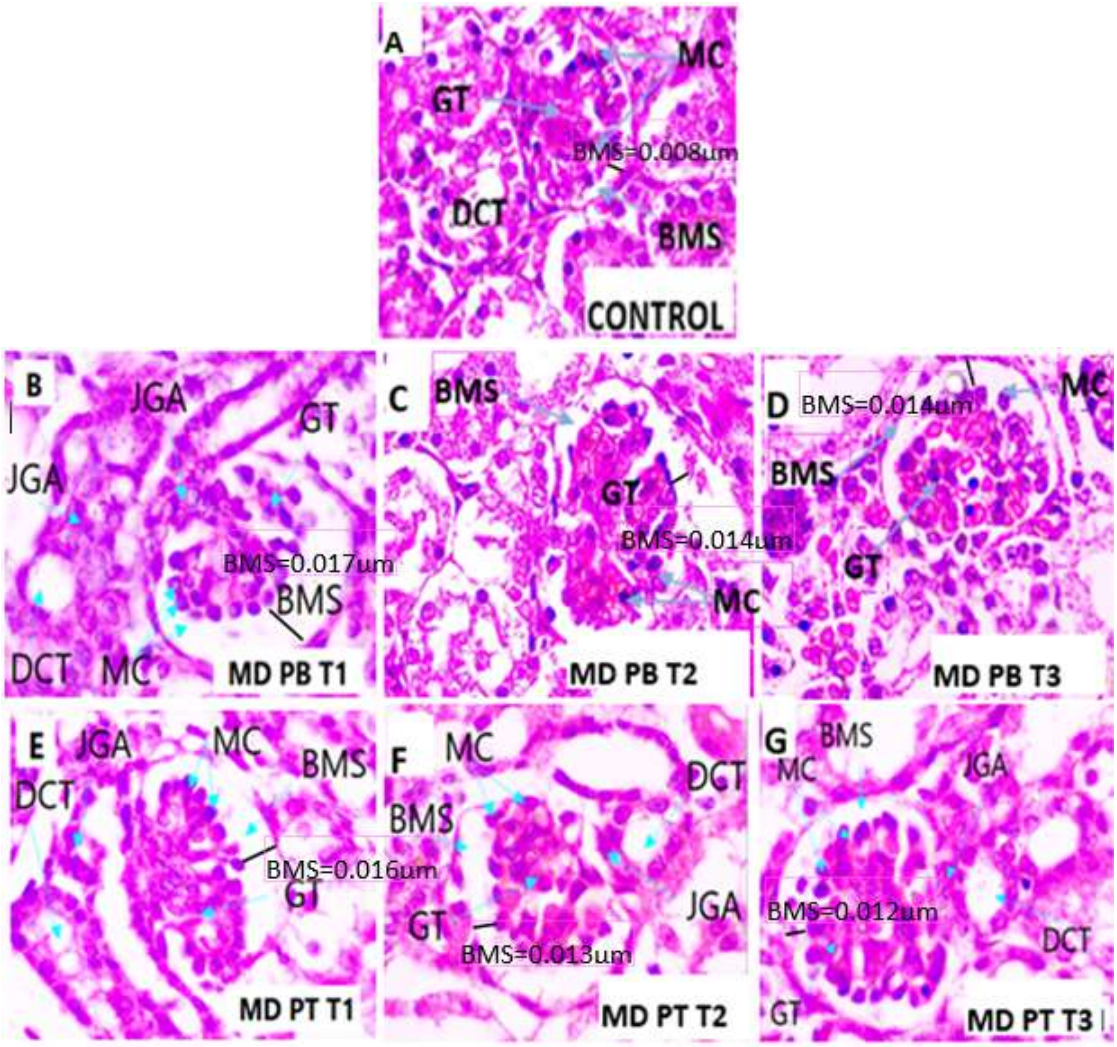


Figure 4.9: The Photomicrographs of the Fetal Kidneys Showing Bowman's Space and Distribution of Cells Per Field of View for Phenobarbital and Phenytoin Medium Dose Treated Groups against the Control Treated at TM_1, TM_2, TM_3 (H & E Mag X40).

KEY: -
A: CONTROL,
B: - (LD PHENOBARBITAL T_1) = Low dose phenobarbital trimester one,
C: - (LD PHENOBARBITAL T_2) = Low dose phenobarbital trimester two,
D: - (LD PHENOBARBITAL T_3) = Low dose phenobarbital trimester three,
E: - (LD PHENYTOIN T1) = Low dose phenytoin trimester one,
F: - (LD PHENYTOIN T2) = Low dose phenytoin trimester two

G: - (LD PHENYTOIN T3) = Low dose phenytoin trimester three.
MC: - mesangial cells, BMS: - Bowman's space, GT:- Glomeruli tuft, DCT:- Distal convoluted tube, JGA:- Juxta-glomeruli apparatus,

The comparative cellular distribution at the glomeruli and the bowman's space at high dose.

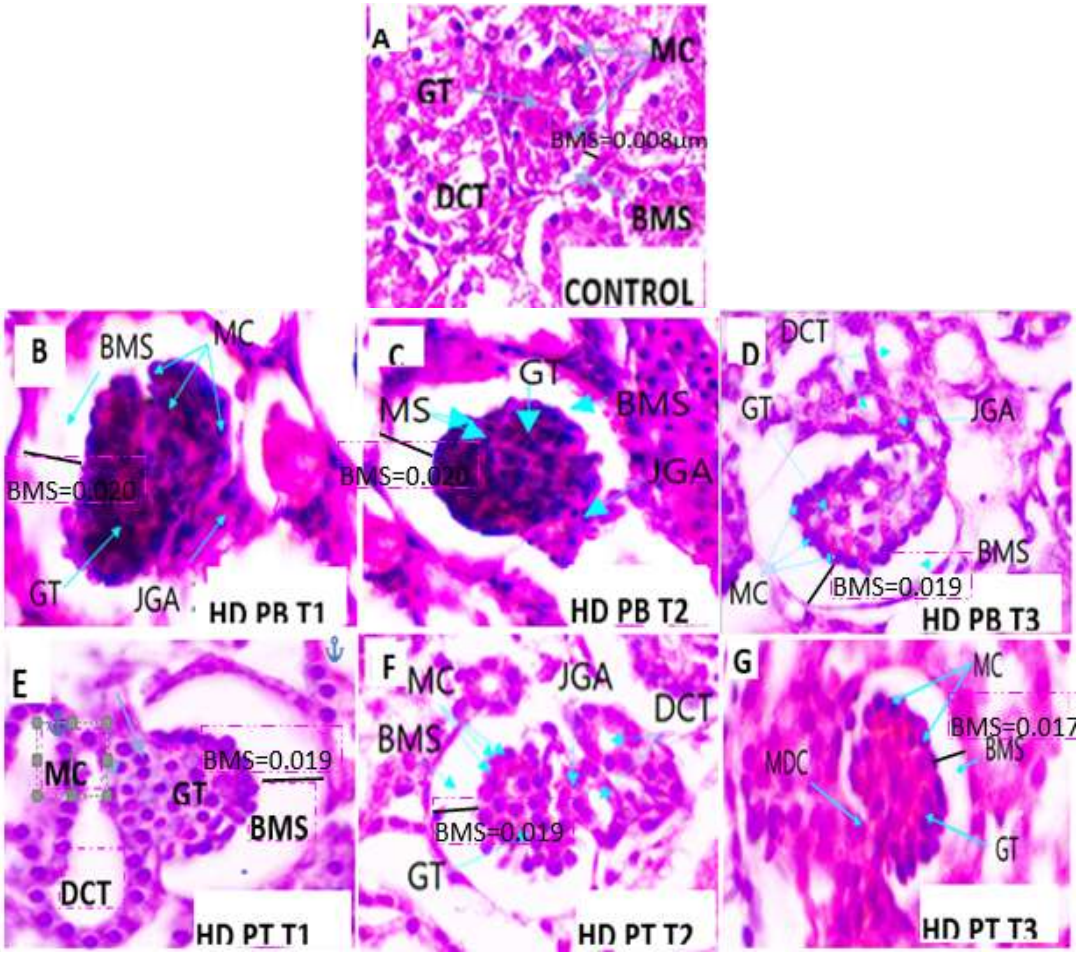


Figure 4.10: The Photomicrographs of the Fetal Kidneys Showing Bowman's Space and Distribution of Cells Per Field of View for Phenobarbital and Phenytoin High Dose Treated Groups against the Control Treated at TM_1, TM_2, TM_3 (H & E Mag X100).

KEY: -
A: CONTROL,
B: - (LD PHENOBARBITAL T_1) = Low dose phenobarbital trimester one,
C: - (LD PHENOBARBITAL T_2) = Low dose phenobarbital trimester two,
D: - (LD PHENOBARBITAL T_3) = Low dose phenobarbital trimester three,
E: - (LD PHENYTOIN T1) = Low dose phenytoin trimester one,
F: - (LD PHENYTOIN T2) = Low dose phenytoin trimester two

G: - (LD PHENYTOIN T3) = Low dose phenytoin trimester three.
MC: - mesangial cells, BMS: - Bowman's space, GT: - Glomeruli tuft, DCT: - Distal convoluted tube, JGA: - Juxta-glomeruli apparatus.

4.2.4 The Comparative Evaluation on How the Two Medicines Influenced the Histo-Morphological Differentiation of the Renal Tubules.

The comparative renal tubular appearance is presented as per dose as follows:

Upon doing comparative histo-morpological analysis on the appearance of the renal tubules, their appearances were noted to differ with the dose of administration and the time of exposure. Some of these changes noted were swelling of the renal tubules with the enlargement in the lumen of the renal tubules, and these changes were noted to be increasing with increasing doses across all the dose groups of low, medium and high phenobarbital and phenytoin treated groups ***(Figure 4.11, Figure 4.12*** and ***Figure 4.13).***

Concerning the time of administration, it was noted that when the treatments were instituted at trimester one (TM_1), the tubules were more swollen and also the tubular lumens were more enlarged, followed by trimester two (TM_2) while during trimester three (TM_3) there was no marked tubular changes for both phenobarbital and phenytoin treatment groups especially when they were administered at low dose ***(Figure 4.11, Figure 4.12*** *and* ***Figure 4.13).*** On comparing between the two medicines within the same duration of administration and the same dosage level to see how they contributed on the distribution of glomeruli, phenobarbital treated groups were noted to have markedly enlargement of the renal tubules as compared to phenytoin treated groups ***(Figure 4.11, Figure 4.12*** *and* ***Figure 4.13).***

The comparative appearance of the renal tubules at low dose.

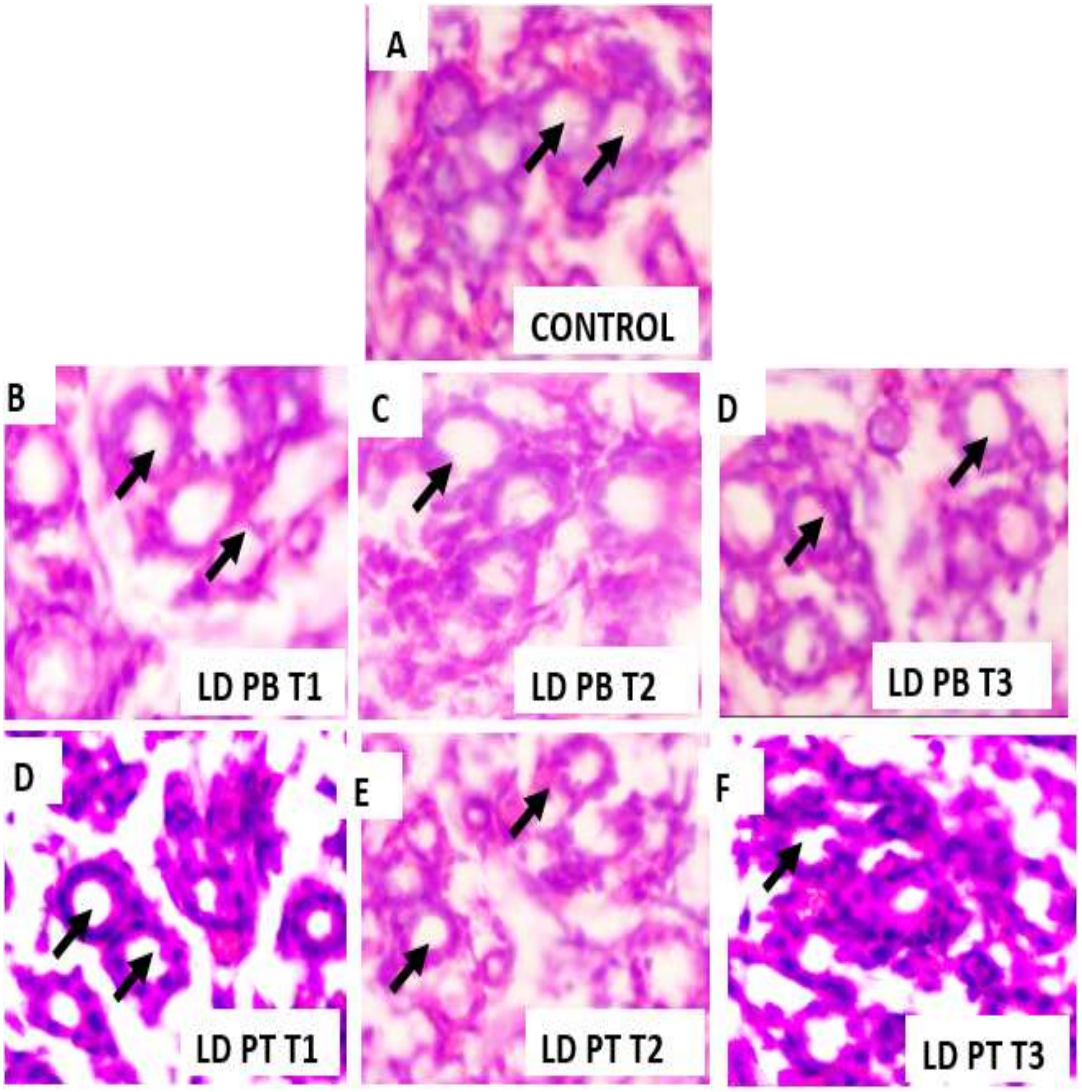


Figure 4.11: The Photomicrographs of the Transverse Sections of the Fetal Kidneys Showing Appearance of the Renal Tubules for Phenobarbital and Phenytoin Low Dose Treated Groups against Control Treated at TM1, TM2, TM3 (H & E Mag X100).

KEY: -
A: CONTROL,
B: - (LD PHENOBARBITAL T_1) = Low dose phenobarbital trimester one,
C: - (LD PHENOBARBITAL T_2) = Low dose phenobarbital trimester two,
D: - (LD PHENOBARBITAL T_3) = Low dose phenobarbital trimester three,
E: - (LD PHENYTOIN T1) = Low dose phenytoin trimester one,
F: - (LD PHENYTOIN T2) = Low dose phenytoin trimester two
G: - (LD PHENYTOIN T3) = Low dose phenytoin trimester three.

The comparative appearance of the renal tubules at medium dose.

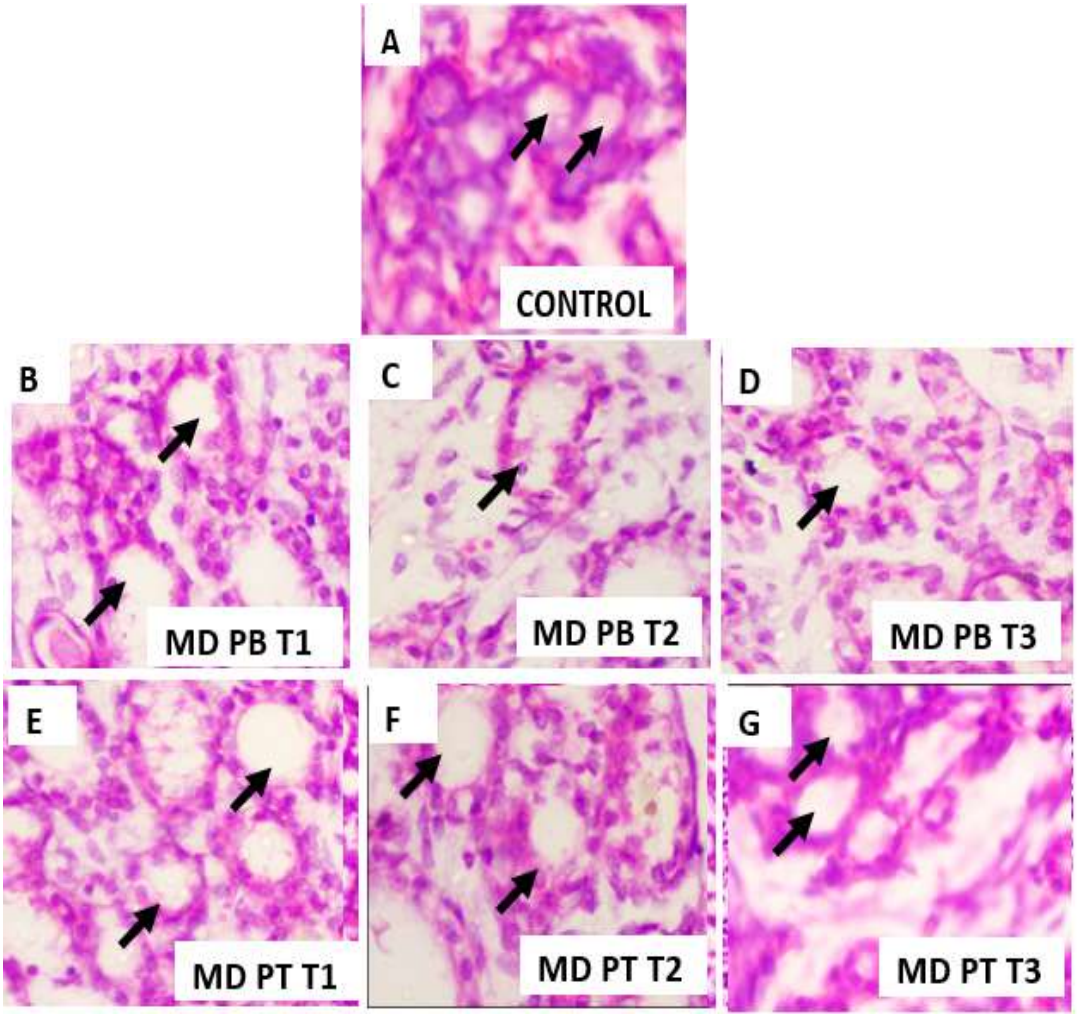


Figure 4.12: The Photomicrographs of the Transverse Sections of the Fetal Kidneys Showing Appearance of the Renal Tubules for Phenobarbital and Phenytoin Medium Dose Treated Groups against Control Treated at TM_1, TM_2, TM_3 (H & E Mag X100).

KEY: -
A: CONTROL,
B: - (LD PHENOBARBITAL T_1) = Low dose phenobarbital trimester one,
C: - (LD PHENOBARBITAL T_2) = Low dose phenobarbital trimester two,
D: - (LD PHENOBARBITAL T_3) = Low dose phenobarbital trimester three,
E: - (LD PHENYTOIN T1) = Low dose phenytoin trimester one,
F: - (LD PHENYTOIN T2) = Low dose phenytoin trimester two
G: - (LD PHENYTOIN T3) = Low dose phenytoin trimester three.

The comparative appearance of the renal tubules at low dose.

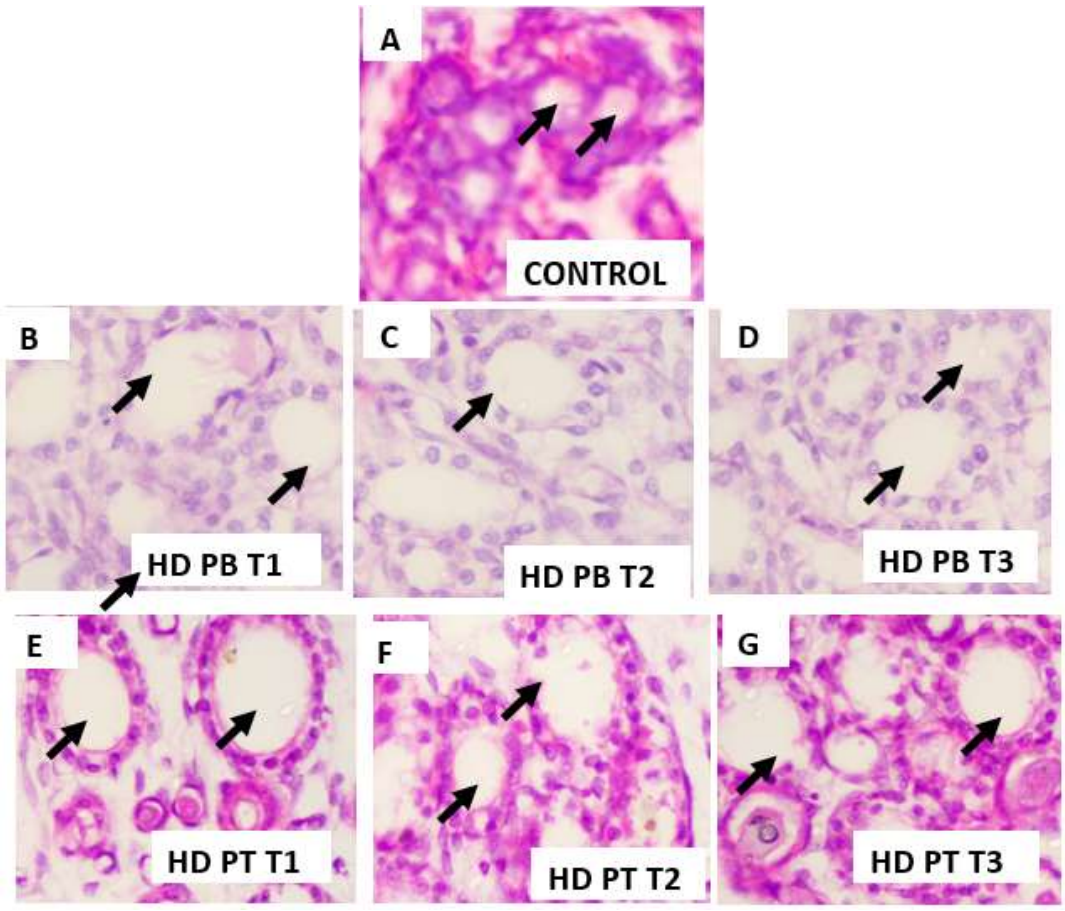


Figure 4.13: The Photomicrographs of the Transverse Sections of the Fetal Kidneys Showing Appearance of the Renal Tubules for Phenobarbital and Phenytoin High Dose Treated Groups Against Control Treated at TM_1, TM_2, TM_3 (H & E Mag X100).

KEY: -
A: CONTROL,
B: - (LD PHENOBARBITAL T_1) = Low dose phenobarbital trimester one,
C: - (LD PHENOBARBITAL T_2) = Low dose phenobarbital trimester two,
D: - (LD PHENOBARBITAL T_3) = Low dose phenobarbital trimester three,
E: - (LD PHENYTOIN T1) = Low dose phenytoin trimester one,
F: - (LD PHENYTOIN T2) = Low dose phenytoin trimester two
G: - (LD PHENYTOIN T3) = Low dose phenytoin trimester three.

The Histostereological Findings

4.3 Objective Three: The Comparative Evaluation on How the Two Medicines Influenced the Histo-Stereological Organization of the Fetal Kidneys

In evaluating how the two medicine influenced the histo-stereological differentiation of the developing fetal kidneys, the following parameters were evaluated; (a) morphometric parameters: - kidney length, kidney weight, kidney width and mean total kidney volume while (b) the stereological parameters included histological thicknesses of the medulla and the cortex and the medulla, the volume densities of the medulla and the cortex, the glomerular numbers per filed, the results are hence presented in three stages as follows: -

Stage 1: The comparative evaluation on how the two medicines influenced the gross morphometric parameters of the kidney length, kidney weight, kidney width and mean total kidney volume (Archimedes volumes).

Stage 2: The comparative evaluation on how the two medicines influenced the medullary and cortical thicknesses.

Stage 3: The comparative evaluation on how the two medicines influenced the total kidney volume and volume densities of the medulla and the cortex.

4.3.1 Stage 1: The Comparative Evaluation on How the Two Medicines Influenced the Gross Morphometric Parameters of the Kidney Length, Kidney Weight, Kidney Width and Mean Total Kidney Volume (Archimedes Volumes).

In evaluating how the two medicines influenced the fetal morphometric parameters of the developing fetal kidneys, the following parameters were evaluated; (i) Mean kidney length, (ii) Mean kidney weight (iii) Mean kidney width (iv) Mean total kidney volume (Archimedes volumes). This was done by doing the univariate, bivariate and multivariate regression analysis by use of both (ANOVA) and MANOVA: - The overall global effects of both phenobarbital and phenytoin on

kidney length, kidney weight, width, total kidney volume was assessed by the univariate and bivariate analysis using ANOVA. At the global level, the study established that the administration of varied doses of both phenobarbital and phenytoin during pregnancy caused a significant ($P<0.05$) detrimental increment to all the four dependent variables assessed when compared with the control (***Table 0.7)***. It was further observed that phenobarbital has more harmful detrimental effect on all the four dependent variables as compared to phenytoin as shown in the ANOVA results of (***Table 0.7)*** as follows: - (a) fetal kidneys weights $F(18, 38)= 381.437$ $P= 0.002$, (b) total kidney lengths $F(18,38)= 97.655$ $P= 0.039$ (c) kidney widths $F(18,38)= 39.003$ $P= 0.049$ (***Table 4.7)***

Concerning the time of exposure and the dosages, it was further noted that in both the two treatment categories of phenobarbital and phenytoin, the mean kidney length, kidney weight, kidney width and the mean total kidney volume (Archimedes volumes were highly affected when the treatments were initiated at TM_1 and TM_2 with the worst detrimental outcomes when high treatment dose were administered (***Table 4.7.)***

Table 4.7: Comparative Findings on Fetal Kidney Length, Kidney Weight, Kidney Width and the Mean Total Kidney Volume (Archimedes Volumes for Phenobarbital and Phenytoin at Different Doses Administered at Different Trimesters against the Control using ANOVA.

The study groups	**Study groups and dosage levels.**	**The time of exposure to treatment**	**The comparative mean kidney lengths, weights and widths for various study groups.** **Mean kidney weight (kwg) (g) ± SD)**	**Mean kidney length (kl) (mm) (cm) ± SD)**	**Mean kidney width (kw) (mm) ± SD)**	**Mean total kidney volume (KV)(mm^3) ± SD)**
Control.	Control (C)					.2627±.0031
The phenobarbital treatment groups.	No treatment	None.	.0259±.0006	.2611.0007	.1029±.0003	
	Low dose treatment group (LPB)- [3.1 mg/kg/bw)	TM_1	.0303±.0003	.2820±.0001	.1350±.0011	.2795±.0033
		TM_2	.0286±.0001	.2795±.0018	.1262±.0005	.2830±.0041
		TM_3	.0276±.0009	.2672±.0033	.1142±.0019	.2745±.0042
	Medium dose treatment group(MPB)- [19.2mg/kg/bw)	TM_1	.0339±0019*	.3179±.0068	.1513±.0047	.3253±.0032*
		TM_2	.0318±.0005	.2925±.0017	.1202±.0047	.3203±.0043
		TM_3	.0317±.0001	.2882±.0024	.1116±.0085	.3160±.0049
	High dose treatment group	TM_1	.0414±.0043*	.3666±.0025*	.1666±.0025	.3563±.0112*
	(HPB) (41.5 mg/kg/bw)	TM_2	.0383±.0009*	.3553±.0033*	.1553±.0033	.3441±.0064*
		TM_3	.0373±.0009	.3446±.0038	.1446±.0038	.3455±.0046
	Low dose treatment group (LPT)-(31 mg/kg/bw).	TM_1	.0286±.0001	.2717±.0033	.1204±.0058	.3097±.0030
		TM_2	.0265±.0006	.2645±.0048	.1092±.0054	.3085±.0074
		TM_3	.0261±.0008	.2643±.0019	.1052±.0036	.3040±.0048
The phenytoin treatment groups	Medium dose treatment group (MPT)-[62 mg/kg/bw).	TM_1	.0318±.0005*	.3050±.0032	.1095±.0013	.3022±.0032
		TM_2	.0310±.0009	.2890±.0012	.1085±.0003	.2972±.0041
		TM_3	.0302±.0002	.2835±.0016	.1050±.0032	.2880±.0036
	High dose treatment group (HPT) (124 mg/kg/bw).	TM_1	.0368±.0001*	.3350±.0011*	.1019±.0034	.3329±.0084*
		TM_2	.0355±.0001*	.3262±.0005*	.0964±.0040	.3349±.0029*
		TM_3	.0344±.0001 **F(18,38)= 381.437 P= 0.002**	.3126±.0008 **F(18,38)= 97.655 P= 0.039**	.0858±.0012 **F(18,38)= 39.003 P= 0.049**	.3315±.0009 **F(18,38)= 79.487 P= 0.034**

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

Upon carrying out the MANOVA level II analysis to find out how the three independent variables of the individual drug, dose and the time of exposure plus their interactions influenced fetal kidney length, kidney weight, kidney width and the total kidney volume (Archimedes volumes) individually, this study found out that: -

a) At individual level, this study found out that all the three independent variables had a significant role to play in influencing fetal kidney length, kidney weight, kidney width and the total kidney volume (Archimedes volumes) but in varying proportions as indicated by the values of wilk's labda Partial Eta squared (η^2) in (***Table 4.8).*** It was observed that at individual level the type of drug, the doses and the time of administration of medicines had a statistical significant contribution ($P<.05$) with the dosage contributed more between 52-99% followed by the type of drug between 53-96% and time had the least contribution ranging between 35-91% as shown in (***Table 4.8).***
b) At two-way level interaction effects it was observed the element of the types of time of exposure in combination with dosages had the worst effects followed by the combination of drug and dosages then lastly the combinations of time of exposure and drug as shown in the three variables of the mean fetal kidney length, kidney weight, kidney width and the total kidney volume (Archimedes volumes) with Partial Eta squared (η^2) of 91.7%, 91.5% and 51.3% on the mean kidney weight respectively as shown in ***(Table 4.8).***
d) At three -way level interaction effects of doses, drugs and the trimesters, the kidney weights had the highest contribution with Partial Eta squared (η^2) of 51.6 % as shown in ***(Table 4.8).***

Table 4.8: The MANOVA Findings on How the Individual Drug, Dose and the Time of Exposure plus Their Interactions Influenced Each of the Foetal Kidney Length, Kidney Weight, Width and Mean Total Kidney Volume (Archimedes Volumes) Prenatally.

Types of MANOVA evaluation At level 2.	The groups being tested.	The three dependent variables.	Measurements of the variability in the depended variables (Type III Sum of square).	Degree of freedom	The ratio Type III Sum of square to its corresponding degree of freedom. (Mean Square).	The ration of the mean square for the independent variable to the mean square for error (F Statistics).	Sig.	Proportion of variance (Partial Eta Squared).
(i) The evaluation on the correctness of the model used for the study	Corrected Model	kidney width.	.001[a]	18	0.000	39.002	<.001	.949
		Kidney length	.057[b]	18	.003	381.447	<.001	.994
		kidney weights	.026[c]	18	.001	97.655	<.001	.979
		kidney vol.	.037[a]	18	.002	79.486	<.001	.974
(ii) Test on whether the observed results were due to chance	Intercept (grand total)	kidney width.	.040	1	.040	26258.425	<.001	.999
		kidney length	3.555	1	3.555	424574.820	<.001	1.000
		kidney weights	.561	1	.561	38604.236	<.001	.999
		kidney vol.	3.791	1	3.791	145567.403	<.001	1.000
(iii) The Individual independent variable and its effects on each of the three kidney dependent variables	DOSES (Low, medium, high dose)	kidney width.	.001	2	.000	20.739	<.001	.522
		kidney length	.001	2	.000	262.693	<.001	.933
		kidney weights	.043	2	.022	2592.800	<.001	.993
		kidney vol.	.026	2	.013	494.178	<.001	.963
	DRUGS (PB,PT)	kidney width.	.000	1	0.00	44.199	<.001	.538
		kidney length	.003	1	.003	401.063	<.001	.913
		kidney weights	.013	1	.013	920.086	<.001	.960
		kidney vol.	.003	1	.003	118.884	<.001	.758
	TRIMESTER (TM_1,TM_2,TM_3)	kidney width.	.000	2	0.00	21.215	<.001	.528
		kidney weights	.004	2	.002	210.649	<.001	.917
		kidney length	.004	2	.002	121.188	<.001	.864
		kidney vol.	.001	2	.000	10.459	<.001	.355
(iv) Two-way	DOSES (Low, medium, high dose) * DRUGS	kidney width.	.000	2	0.000	3.578	.038	.158
		kidney length	.002	2	.001	92.653	<.001	.830

Types of MANOAVA evaluation At level 2.	The groups being tested.	The three dependent variables.	Measurements of the variability in the depended variables (Type III Sum of square).	Degree of freedom	The ratio Type III Sum of square to its corresponding degree of freedom. (Mean Square).	The ration of the mean square for the independent variable to the mean square for error (F Statistics).	Sig.	Proportion of variance (Partial Eta Squared).
interaction effects	(PB,PT)	kidney weights	.006	2	.003	203.339	<.001	.915
		kidney vol.	.001	2	.000	28.399	<.001	.599
on each of the kidney dependent variables	DOSES (Low, medium, high dose) * TRIMESTER	kidney width.	.000	2	0.00	21.215	<.001	.528
		kidney length	.004	2	.002	121.188	<.001	.864
		kidney weights	.004	2	.002	210.649	<.001	.917
	(TM_1,TM_2,TM_3)	kidney vol.	.000	2	.000	3.848	.030	.168
	DRUGS (PB,PT) * TRIMESTER	kidney width.	.000	2	10.000	.820	.448	.041
	(TM_1,TM_2,TM_3)	kidney length	.000	2	0.000	3.497	.040	.155
		kidney weights	.001	2	.000	20.028	<.001	.513
		kidney vol.	.000	2	.000	1.989	.116	.173
(v)	DOSES (Low, medium, high dose) * DRUGS (PB,PT) * TRIMESTER (TM_1,TM_2,TM_3)	kidney width.	0.000	4	0.000	.385	.818	.039
Three-way interaction effects		kidney length	.000	4	0.000	4.205	.006	.307
		kidney weights	.001	4	.000	10.145	<.001	.516
on each of the kidney dependent variables		kidney vol.	.000	4	.000	.442	.778	.044
	Error	kidney width.	.000	38	0.000			
		kidney length	.000	38	0.000			
		kidney weights	.001	38	0.000			
		kidney vol.	.001	38	.000			
(vi) Overall inferential statistics on the model results	Total	kidney width.	.059	57				
		kidney length	5.200	57				
		kidney weights	.840	57				
		kidney vol.	5.564	57				
	Corrected Total	kidney width.	.001	56				
		kidney length	.058	56				
		kidney weights	.026	56				
		kidney vol.	.038	56				

*Key:- *Indicates interaction effects among the independent variable*

Upon carrying out the MANOVA level III pairwise comparison to establish how the two medicines differed in influencing the fetal kidney length, kidney weight, kidney width and the total kidney volume (Archimedes volumes) when administered within the same dosage levels and in the same trimesters, the study established that that phenobarbital had more deleterious effects on the kidney morphometric parameters as shown in (***Table 4.9).***

Table 4.9: The MANOVA Pairwise Comparison Table on How the Two Medicines Influenced Fetal Kidney Length, Kidney Weight, Kidney Width and the Kidney Volumes When Exposed Within the Same Dosage Level and Duration of Treatment.

The comparative mean kidney lengths, weights and widths for various study groups	Study groups and dosage levels.	The time of exposure to treatment	Phenobarbital treatment (PB)	Phenytoin treatment (PT)	Mean difference (PB-PT)	Std. Error	Sig.[d]	95% Confidence Interval for difference[d] Lower Bound	Upper Bound
Kidney width	Low	TM_1	PB	PT	.002*	.001	.044	.000	.004
		TM_2	PB	PT	.002	.001	.087	.000	.004
		TM_3	PB	PT	.001	.001	.169	-.001	.003
	medium	TM_1	PB	PT	.003*	.001	.048	0.00	.005
		TM_2	PB	PT	.002*	.001	.044	-.001	.003
		TM_3	PB	PT	.002	.001	.145	-.001	.004
	High.	TM_1	PB	PT	.005*	.001	<.001	.003	.007
		TM_2	PB	PT	. 003*	.001	.005	.001	.005
		TM_3	PB	PT	.003*	.001	.008	.001	.005
Kidney. L	Low	TM_1	PB	PT	.010*	.002	<.001	.006	.015
		TM_2	PB	PT	.015*	.002	<.001	.010	.020
		TM_3	PB	PT	.003	.002	.240	-.002	.008
	medium	TM_1	PB	PT	.013*	.002	<.001	.008	.018
		TM_2	PB	PT	.005*	.002	.050	0.00	.009
		TM_3	PB	PT	.003	.002	.147	-.001	.008
	High.	TM_1	PB	PT	.032*	.002	<.001	.027	.036
		TM_2	PB	PT	.029*	.002	<.001	.024	.034
		TM_3	PB	PT	.032*	.002	<.001	.027	.037
Kidney. weight..	Low	TM_1	PB	PT	.017*	.003	<.001	.011	.023
		TM_2	PB	PT	.015*	.003	<.001	.008	.021
		TM_3	PB	PT	.009	.003	.007	.003	.015
	medium	TM_1	PB	PT	.042*	.003	<.001	.036	.048
		TM_2	PB	PT	.012*	.003	.001	.005	.018
		TM_3	PB	PT	.007*	.003	.041	.000	.013
	High.	TM_1	PB	PT	.065*	.003	<.001	.058	.071
		TM_2	PB	PT	.059*	.003	<.001	.053	.065
		TM_3	PB	PT	059*	.003	<.001	.053	.065

*Key:- * The mean difference of less than 0.05 is significant and was denoted by asterisk (*)*

4.3.2 Stage 2: The Comparative Evaluation on How the Two Medicines Influenced the Medullary and Cortical Thicknesses Using ANOVA

In assessing carrying out the comparative morphometric evaluation on how the two medicines influenced the gross morphometric outcomes of the fetal kidneys, the following parameters were evaluated; (i) kidney medullary thickness (ii) the kidney cortical thickness**.** The inferential statistical analysis was subsequently done by use of the univariate, bivariate and multivariate regression analysis by use of both (ANOVA) and MANOVA:- At the global level the study established that in both the phenobarbital and the phenytoin treated groups there was a significant ($P<0.05$) increment in the kidney medullary thickness with slight reduction in the kidney cortical thickness was observed on the administration of varied doses of both phenobarbital and phenytoin during pregnancy when comparison was done with the control ***(Table 4.10.)*****.** Upon comparing the two medicines, it was established that phenobarbital has more injurious teratogenic effect to both the kidney medulla and the cortex as compared to phenytoin as shown in the ANOVA results in ***(Table 4.10.)*** as follows: - (a) kidney medullary thickness $F(18,38) = 43.851$ $P= 0.036$ and (b) the kidney cortical thickness $F(18,38)= 205.096$ $P= 0.048$ ***(Table 4.10.)*****.**

It was further noted that in both the two treatment categories of phenobarbital and phenytoin, the kidney medullary thickness and the kidney cortical thickness were therefore highly reduced when the treatments were initiated at TM_1 and TM_2 with the worst detrimental outcomes when high treatment dose were administered ***(Table 4.10.)*****.**

Table 4.10*:* Comparative Findings on Kidney Medullary Thickness and the Kidney Cortical Thickness for Phenobarbital and Phenytoin at Different Doses Administered at Different Trimesters against the Control using ANOVA.

The study groups	Study groups and dosage levels.	The time of exposure to treatment	The comparative mean Kidney medullary thickness and cortical thickness (for various study groups)	
			Mean kidney medullary thickness (µm) ± SD)	Mean kidney cortical thickness (µm) ± SD)
Control.	Control (C) No treatment	None.	3.5288±0.0140	2.5951 ±0.0333
The phenobarbital treatment groups	Low dose treatment group (LPB)-[3.1 mg/kg/bw)	TM_1	4.8717±0.0214	2.5834±0.0459
		TM_2	4.7302±0.0410	2.5797±0.0285
		TM_3	4.6452±0.0712	2.5538±0.0090
	Medium dose treatment group(MPB)-[19.2mg/kg/bw)	TM_1	4.9876±0.0435	2.3511±0.0456
		TM_2	4.8901±0.0339	2.2586±0.0236
		TM_3	4.7981±0.0163	2.1951±0.0286
	High dose treatment group	TM_1	5.3549±0.0459*	2.1202±0.0337*
	(HPB) (41.5 mg/kg/bw)	TM_2	5.2612±0.0285*	1.9786±0.0287*
		TM_3	5.2153±0.0090	1.8936±0.0822
	Low dose treatment group (LPT)-(31 mg/kg/bw).	TM_1	4.6217±0.0231	2.5778±0.0842
		TM_2	4.5951±0.0538	2.5678±0.0842
		TM_3	4.58016±0.032	2.5463±0.4759
	Medium dose treatment group (MPT)-[62 mg/kg/bw).	TM_1	4.8526±0.0129	2.3135±0.0502
		TM_2	4.7601±0.0738	2.3087±0.0311
The phenytoin treatment groups		TM_3	4.6966±0.0133	2.1452±0.0034
	High dose treatment group(HPT) (124 mg/kg/bw).	TM_1	5.0791±0.3253*	1.7702±0.0318
		TM_2	4.9565±0.2863*	1.9287±0.0514
		TM_3	4.8689±0.2770	1.8437±0.0213
			F(18,38)= 43.851 P= 0.036	**F(18,38)= 205.096 P= 0.048**

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

Upon carrying out the MANOVA level II analysis to find out how the three independent variables of the individual drug, dose and the time of exposure plus their

interactions influenced kidney medullary thickness and kidney cortical thickness, this study found out that: -

a) At individual level, this study found out that all the three independent variables had a significant role to play in influencing kidney medullary thickness and kidney cortical thickness but in varying proportions as indicated by the values of wilk's labda Partial Eta squared (η^2) ***(Table 4.11).*** It was observed that at individual level the type of drug, the doses and the time of administration of medicines had a statistical significant contribution ($P<.05$) with the dosage contributed more between 97-99% followed by the type of drug between 45-91% and time had the least contribution ranging between 11-92% ***(Table 4.11).***
b) At two-way level interaction effects it was observed the element of the types of the type of drug in combination with dosages had the worst effects followed by the combination of time of exposure and dosages then lastly the combinations of time of exposure and drug with Partial Eta squared (η^2) of 89.5%, 75.6% and 66.3% on the mean kidney medullary thickness respectively ***(Table 4.11).***
c) At three-way interaction effect, it was observed that the dosages, the type of drug and the time of exposure all combined had a contribution with partial eta squared 67.0% on the kidney medullary thickness ***(Table 4.11).***

Table 4.11: The MANOVA Findings on How the Individual Drug, Dose and the Time of Exposure plus Their Interactions Influenced Each of the Kidney Medullary and Kidney Cortical Thickness Prenatally.

Types of MANOVA evaluation at level 2	The groups being tested	The two dependent variables.	Measurements of the variability in the depended variables (Type III Sum of square)	Degree of freedom	Mean square The ratio Type III Sum of square to its corresponding degree of freedom. (Mean Square)re	The ration of the mean square for the independent variable to the mean square for error (F Statistics)	Sig.	Proportion of variance (Partial Eta Squared)
(i) The evaluation on the correctness of the model used for the study	Corrected Model	medullary thickness	6.220a	18	.346	372.464	<.001	.994
		cortical thickness	4.652b	18	.258	105.778	<.001	.980
(ii) Test on whether the observed results were due to chance	Intercept (grand total)	medullary .length	33.567	1	33.567	36180.578	<.001	.999
		cortical thickness	786.858	1	786.858	322045.711	<.001	1.000
(iii) The Individual independent variable and its effects on each of the three maternal dependent variables	DOSES (Low, medium, high dose)	medullary thickness	4.776	2	2.388	2573.851	<.001	.993
		cortical thickness	4.185	2	2.092	856.320	<.001	.978
	DRUGS (PB,PT)	medullary thickness	.385	1	.385	414.733	<.001	.916
		cortical thickness	.076	1	.076	31.141	<.001	.450
	TRIMESTER (TM_1,TM_2,TM_3)	medullary thickness	.442	2	.221	237.948	<.001	.926
		cortical thickness	.012	2	.006	2.541	<.001	.118
(iv) Two-way interaction effects On each of the maternal dependent variables	DOSES (Low, medium, high dose)* DRUGS (PB,PT)	medullary thickness	.300	2	.150	161.409	<.001	.895
		cortical thickness	.044	2	.022	8.978	<.001	.321
	DOSES (Low, medium, high dose) * TRIMESTER (TM_1,TM_2,TM_3)	medullary thickness	.109	4	.027	29.460	<.001	.756
		cortical thickness	.035	4	.009	3.612	<.001	.275
	DRUGS (PB,PT) * TRIMESTER (TM_1,TM_2,TM_3)	medullary thickness	.001	2	.001	.606	.551	.031
		cortical thickness	.183	2	.092	37.462	<.001	.663
(iv) Three-way		medullary thickness	.072	4	.018	19.331	<.001	.670

Types of MANOVA evaluation at level 2	The groups being tested	The two dependent variables.	Measurements of the variability in the depended variables (Type III Sum of square)	Degree of freedom	Mean square The ratio Type III Sum of square to its corresponding degree of freedom. (Mean Square)re	The ration of the mean square for the independent variable to the mean square for error (F Statistics)	Sig.	Proportion of variance (Partial Eta Squared)
interaction effects	DOSES (Low, medium, high							
On each of the maternal dependent variables	dose) * DRUGS (PB,PT) * TRIMESTER (TM_1,TM_2,TM_3)	cortical thickness	.011	4	.003	1.133	.356	.107
	Error	medullary thickness	.035	38	.001			
(v)		cortical thickness	.093	38	.002			
Overall inferential statistics on the model results	Total	medullary thickness	56.166	57				
		cortical thickness	1112.980	57				
	Corrected Total	medullary thickness	6.255	56				
		medullary thickness	56.166	56				
	a. r squared = .993 (adjusted r squared = .990)							
	b. r squared = .975 (adjusted r squared = .963)							

*Key:- *Indicates interaction effects among the independent variables.*

Upon carrying out the MANOVA level III analysis on the pairwise comparison to establish how the two medicines differed in influencing the kidney medullary and kidney cortical thickness in the same dosage levels and within the same trimesters, the study established that the phenobarbital had more effects on both dependent variables as compared to phenytoin ***(Table 4.12).***

Table 4.12: The MANOVA Pairwise Comparison Table on How the Two Medicines Influenced the Kidney Medullary and Kidney Cortical Thickness When Exposed Within the Same Dosage Level and Duration of Treatment.

The comparative mean medullary and cortical thickness for various study groups	Study groups and dosage levels.	The time of exposure to treatment	Phenobarbital treatment (PB)	Phenytoin treatment (PT)	Mean Difference (PB-PT)	Std. Error	Sig.[d]	95% Confidence Interval for Difference[d] Lower Bound	Upper Bound
		TM_2	PB	PT	.078*	.025	.795	-.039	.117
		TM_3	PB	PT	.013	.025	.610	-.006	.019
	Medium	TM_1	PB	PT	.172*	.025	p<0.001	.122	.222
		TM_2	PB	PT	.104*	.025	p<0.001	.053	.154
		TM_3	PB	PT	.028	.025	.264	-.022	.079
	High.	TM_1	PB	PT	.406*	.025	P<0.001	.342	.469
		TM_2	PB	PT	.291*	.025	P<0.001	.241	.341
		TM_3	PB	PT	.315*	.025	P<0.001	.265	.366
Cortical. L	Low	TM_1	PB	PT	.120*	.040	.005	.039	.202
		TM_2	PB	PT	.106*	.040	.012	.024	.188
		TM_3	PB	PT	.050	.040	.223	.025	.075
	Medium	TM_1	PB	PT	.247*	.040	p<0.001	.166	.329
		TM_2	PB	PT	.126*	.040	p<0.001	.004	.207
		TM_3	PB	PT	.068	.040	.100	.150	.014
	High .	TM_1	PB	PT	.277*	.040	p<0.001	.195	.358
		TM_2	PB	PT	.150*	.040	p<0.001	.068	.232
		TM_3	PB	PT	.023	.040	.564	.012	.035

*Key:- * The mean difference of less than 0.05 is significant and was denoted by asterisk (*)*

4.3.3 The Comparative Evaluation on How the Two Medicines Influenced the Total Kidney Volumes and the Volume Densities of the Medulla and the Cortex Using ANOVA.

Upon carrying out a comparative evaluation on how the two medicines influenced the total kidney volumes as well as the volume densities of the cortex and the medulla, the following parameters were evaluated; (i) Calculation of mean total fetal kidney volumes by cavalieri method (cavalieri volume) (ii) Mean kidney medullary density (iii) Mean cortical density. the inferential statistical analysis was hence done by use of univariate, bivariate and multivariate regression analysis by use of both (ANOVA) and MANOVA: - The overall global effects of both phenobarbital and phenytoin on cavalieri volume, kidney medullary density and cortical density were assessed by the univariate and bivariate analysis using ANOVA. At the global level, the study established that the administration of varied doses of both phenobarbital and phenytoin during pregnancy caused a significant ($P<0.05$) detrimental increment to all the three dependent variables assessed when compared with the control (***Table 4.13.)*** It was further observed that phenobarbital has more harmful detrimental effect on all the three dependent variables as compared to phenytoin as shown in the ANOVA results (***Table 4.13)*** as follows: - (a) Cavalieri volume $F(18,38)= 495.683$ $P= 0.035$ (b) kidney medullary density $F(18,38)= 43.851$ $P= 0.038$, (c) cortical density $F(18,38)= 205.096$ $P= 0.042$ (***Table 4.13.)***

Concerning the time of exposure and the dosages, it was further noted that in both the two treatment categories of phenobarbital and phenytoin, the Cavalieri volume, mean kidney medullary density and the mean cortical density were highly affected when the treatments were initiated at TM_1 and TM_2 with the worst detrimental outcomes observed when the medium and the high dose levels were administered (***Table 4.13.)***

Table 4.13: Comparative Findings on Kidney Volume and Volume Densities for Phenobarbital and Phenytoin at Different Doses Administered at Different Trimesters Against the Control Using ANOVA.

The study groups	The study groups and dosage level	The time of exposure to treatment	The comparative Mean total kidney volume, medullary density, cortical density and carvalieli volume for various study groups. Mean medullary density (MD) (mm^3)± SD)	Mean Cortical density (CD) (mm^3) ± SD)	Mean carvalieli volume (CV) (mm^3) (cm) ± SD
Control	Control (C) No treatment		.1591±.0026	0.0794±0.0014	.2383±.0036
The phenobarbital treatment groups	Low phenobarbital group (PB)- [3.1 mg/kg/bw)	TM_1	.2086±.0140	.0792±.0012	.2958±0032
		TM_2	.1949±.0020	.0732±.0015	.2808±.0033
		TM_3	.1844±.0079	.0658±.0014	.2716±.0034
	Medium phenobarbital group (PB)- [19.2mg/kg/bw)	TM_1	.2221±.0037	.0576±.0016	.3181±.0041*
		TM_2	.2011±.0033	.0545±.0019	.3089±.0044
		TM_3	.1939±.0012	.0519±.001	.3010±.0041
	High phenobarbital group (PB) (41.5 mg/kg/bw)	TM_1	.2444±.0035*	.0486±.0012	.3589±.0007*
		TM_2	.2364±.0039*	.0436±.0017	.3461±.0004*
		TM_3	.2217±.0016	.0405±.0018	.3314±.0009
The phenytoin treatment groups	Low phenytoin treatment group (PT)-(31 mg/kg/bw)	TM_1	.1923±.0135	.0677±.0010	.2910±.0041
		TM_2	.1792±.0031	.0639±.0008	.2764±.0041
		TM_3	.1654±.0079	.0604±.0003	.2672±.0041
	Medium phenytoin treatment group (PT)-[62 mg/kg/bw)	TM_1	.2005±.0023	.0560±.0018	.2928±.0033
		TM_2	.1921±.0027	.0530±.0014	.2734±.0036
		TM_3	.1852±.0026	.0503±.0010	.2758±.0034
	High phenytoin treatment group(PT) (124 mg/kg/bw)	TM_1	.2146±.0003*	.0570±.0018	.3356±.0037*
		TM_2	.2093±.0010*	.0521±.0016	.3242±.0033*
		TM_3	.2033±.0017	.0491±.0012	.3175±.0034
			F(18,38)= 43.851 P= 0.038	F(18,38)= 205.096 P= 0.042	F(18,38)= 495.683 P= 0.035

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

Upon carrying out the MANOVA level II analysis to find out how the three independent variables of the individual drug, dose and the time of exposure plus their

interactions influenced kidney volume (KV), carvalieli volume (CV), cortical density(CD) and kidney medullary density (MD)**,** this study found out that: -

a) At individual level, this study found out that all the three independent variables had a significant role to play in influencing kidney volume (KV), carvalieli volume (CV), cortical density(CD) and kidney medullary density (MD) but in varying proportions as indicated by the values of wilk's labda Partial Eta squared (η^2) (***Table 4.14).*** It was observed that at individual level the type of drug, the doses and the time of administration of medicines had a statistical significant contribution ($P<.05$) with the type of drug contributed more between 89-98% followed by the dosage between 72-90% and the time had the least contribution ranging between 70-81% ***(Table 4.14).***
b) At two-way level interaction effects it was observed that the element of the types of the type of drug in combination with dosages had the worst effects followed by the combination of time of exposure and dosages then lastly the combinations of time of exposure and drug as shown in the two variables of the mean kidney medullary density and kidney cortical density that is 63.8%, 28.4% on the mean kidney volume and 10.3% on the mean *cortical density* respectively ***(Table 4.14).***
c) At three-way interaction effect, it was observed that the dosages, the type of drug and the time of exposure all combined had a contribution with partial eta squared 10.8% on the cavalieli volume ***(Table 4.14).***

Table 4.14: The MANOVA Findings on how the Individual Drug, Dose and the Time of Exposure Plus their Interactions Influenced Each of the Kidney Volume (KV), Carvalieli Volume (CV), Cortical Density(CD) and Kidney Medullary Density (MD) Prenatally.

Types of MANOVA evaluation at level 2	The groups being tested	The four dependent variables.	Measurements of the variability in the depended variables (Type III Sum of square)	Degree of freedom	The ratio Type III Sum of square to its corresponding degree of freedom. (Mean Square)	The ration of the mean square for the independent variable to the mean square for error (F Statistics)	Sig.	Proportion of variance (Partial Eta Squared)
(i) The evaluation on the correctness of the model used for the study	Corrected Model	medullary density	.025[b]	18	.001	43.851	<.001	.954
		cortical density	.009[c]	18	.000	205.101	<.001	.990
		cavalieli volume	.048[d]	18	.003	223.269	<.001	.991
(ii) Test on whether the observed results were due to chance	Intercept	medullary density	1.556	1	1.556	48773.080	<.001	.999
		cortical density	.412	1	.412	178535.861	<.001	1.000
		cavalieli volume	3.580	1	3.580	299473.313	<.001	1.000
(iii) The Individual independent variable and its effects on	DOSES (Low, medium, high dose)	medullary density	.003	2	.002	49.006	<.001	.721
		cortical density	.001	2	.000	124.885	<.001	.868
		cavalieli volume	.004	2	.002	171.971	<.001	.901
Each of the three kidney dependent variables	DRUGS (PB,PT)	medullary density	.011	2	.005	166.946	<.001	.898
		cortical density	.006	2	.003	1218.504	<.001	.985
		cavalieli volume	.028	2	.014	1151.918	<.001	.986
	TRIMESTER (TM_1,TM_2,TM_3)	medullary density	.005	1	.005	164.155	<.001	.812
		cortical density	.000	1	.000	90.630	<.001	.705
		cavalieli volume	.001	1	.001	104.982	<.001	.734
(iv) Two-way interaction effects On each of the kidney dependent variables	DOSES (Low, medium, high dose)* DRUGS (PB,PT)	cortical density.	.000	2	.000	.49	.564	.030
		medullary density	.000	2	.000	3.072	.058	.139
		cavalieli volume	.000	2	.000	33.521	<.001	.638
	DOSES (Low, medium, high dose) * TRIMESTER (TM_1, TM_2, TM_3)	medullary density	.000	2	.000	.097	.908	.005
		cortical density	.000	2	.000	2.184	.126	.103
		cavalieli volume	.000	2	.000	1.118	.337	.056
	DRUGS	cortical density	.000	4	.000	.889	.480	.086

Types of MANOVA evaluation at level 2	The groups being tested	The four dependent variables.	Measurements of the variability in the depended variables (Type III Sum of square)	Degree of freedom	The ratio Type III Sum of square to its corresponding degree of freedom. (Mean Square)	The ration of the mean square for the independent variable to the mean square for error (F Statistics)	Sig.	Proportion of variance (Partial Eta Squared)
	(PB,PT) * TRIMESTER (TM_1,TM_2,TM_3)	medullary density	.000	4	.000	1.969	.119	.172
		kidney vol.	.000	4	.000	3.775	.011	.284
Three-way interaction effects	DOSES (Low, medium, high dose)* DRUGS	medullary density	.000	4	.000	.966	.437	.092
		cortical density	.000	4	.000	2.080	.103	.101
On each of the maternal dependent variables	(PB,PT) * TRIMESTER (TM_1,TM_2,TM_3	cavalieli volume	.000	4	.000	1.068	.386	.180
(v)Overall inferential statistics on the model results.	Error	medullary density	.001	38	.000			
		cortical density	.000	38	.000			
		cavalieli volume	.000	38	.000			
	Total	medullary density	2.331	57				
		cortical density	.624	57				
		cavalieli volume	5.369	57				
	Corrected Total	medullary density	.026	56				
		cortical density	.009	56				
		cavalieli volume	.048	56				

R Squared = .974 (Adjusted R Squared = .962)$_a$
R Squared = .954 (Adjusted R Squared = .932)$_b$
R Squared = .990 (Adjusted R Squared = .985)$_c$
R Squared = .991 (Adjusted R Squared = .986)$_d$

Upon carrying out the MANOVA level III on the pairwise comparison to establish how the two medicines differed in influencing the kidney volume (KV), carvalieli volume (CV), cortical density(CD) and medullary density (MD) in the same dosage levels this study found out that phenobarbital contributed more to increment in those parameters as compared to phenytoin shown in ***(Table 4.15).***

Table 4.15: The MANOVA Pairwise Comparison Table on How the Two Medicines Influenced the Kidney Volume (KV), Carvalieli Volume (CV), Cortical Density (CD) and Medullary Density (MD) when Exposed Within the Same Dosage Level and Duration of Treatment.

The comparative mean kidney volume (KV), carvalieli volume (CV), cortical density(CD) and medullary density (MD) for various study groups	Study groups and dosage levels.	The time of exposure to treatment	Phenobarbital treatment (PB)	Phenytoin treatment (PT)	Mean Difference (PB-PT)	Std. Error	Sig.[d]	95% Confidence Interval for Difference[d] Lower Bound	Upper Bound
Medullar	Low	TM_1	PB	PT	.019*	.005	.000	.010	.028
		TM_2	PB	PT	.016*	.005	.002	.006	.025
		TM_3	PB	PT	.016*	.005	.001	.007	.026
y. d	medium	TM_1	PB	PT	.019*	.005	.000	.010	.028
		TM_2	PB	PT	.017*	.005	.001	.008	.026
		TM_3	PB	PT	.015*	.005	.002	.006	.024
	High.	TM_1	PB	PT	.030*	.005	.000	.020	.039
		TM_2	PB	PT	.027*	.005	.000	.018	.036
		TM_3	PB	PT	.018*	.005	.000	.009	.028
Cortical. d	Low	TM_1	PB	PT	.007*	.001	..000	.003	.011
		TM_2	PB	PT	.004*	.001	.000	.002	.006
		TM_3	PB	PT	.001	.001	.058	-.001	.004
	medium	TM_1	PB	PT	.008*	.001	.000	.004	.012
		TM_2	PB	PT	.006*	.001	.000	.003	.009
		TM_3	PB	PT	.002	.001	.082	-.001	.004
	High.	TM_1	PB	PT	.012*	.001	.000	.009	.014
		TM_2	PB	PT	.009*	.001	.000	.007	.012
		TM_3	PB	PT	.005*	.001	.000	.003	.008
	Low	TM_1	PB	PT	.020*	.003	.000	.010	.030
		TM_2	PB	PT	.012*	.003	.000	.006	.018
		TM_3	PB	PT	.010*	.003	.001	.005	.015
		TM_1	PB	PT	.014*	.003	.001	.007	.021
Cavalieli vol.	medium	TM_2	PB	PT	.011*	.003	.004	.005	.017
		TM_3	PB	PT	.010*	.003	.005	.005	.015
		TM_1	PB	PT	.022*	.003	.000	.011	.033
	High	TM_2	PB	PT	.013*	.003	.001	.006	.019
		TM_3	PB	PT	.010*	.003	.005	.005	.015

*Key:- * The mean difference of less than 0.05 is significant.*

CHAPTER FIVE

DISCUSSIONS, CONCLUSIONS AND RECOMMEDATIONS

5.1 Discussion

The study discussion is aligned to the study objectives.

5.1.1 Objective 1: The Comparative Evaluation on How the Prenatal Exposure to Varied Doses of Phenobarbital and Phenytoin Influenced the Maternal and Fetal Pregnancy Outcomes When Exposed In Different Gestational Periods.

In abeyance to the principles of teratogenesis where the *in-utero* environmental toxicity is a predictive pointer to the fetal teratogenic perturbations that can interfere with the normal fetal growth and development *in-utero,* an evaluation of the maternal and fetal pregnancy outcomes was carried out. The maternal pregnancy outcome parameters evaluated included; (i) the maternal weights gains trends, (ii) resorbed endometrial glands, (iii) placental weights, (iv) litter size, (v) number of dead fetuses. On the other hand, the fetal pregnancy outcomes parameters evaluated included; (i) the mean fetal weights, (ii) head circumferences, (iii) crown rump length, and the (iv) bi- parietal diameters. On the maternal pregnancy outcomes, the study established that all the five parameters evaluated on the maternal pregnancy outcome parameters were significantly worse ($P<0.05$) as compared with those of the control (***Table 4.1)***. The observed reduction in maternal weight gain treads and placental weights could be attributed to the interference of the two medicines to the maternal nutritional status that could be occasioned by the gastrointestinal irritations, or Nutrients mal-absorptions in the intestines while the reduction in litter sizes, increased number of dead fetuses, as well as the increase in the number of the resorbed endometrial glands could be attributed to inhibitory effects of the two medicines in the closure of the two medicines plus their metabolites in the blood placental barriers. These findings are in line with (Ritchie *et al*., 2019) who made similar observations. These findings could as well be due to several side effects of the two medicines like the maternal

catalepsy or anorexia that are some of the most commonly reported side-effects of anticonvulsant therapy as reported by (Venâncio *et al*., 2014; Kaplan & Demir, 2021). Similarly, a study by (Gupta, 2016) observed that maternal food intake was reduced up to 14.61 % in gabapentin and 22.29 % in valproic acid treatment while maternal body weight deficit was 34.55 % in gabapentin and 38.73 % in valproic acid exposed subjects (both drugs with the same mode of action with phenobarbital) when administered at higher doses respectively.

With regards to the fetal pregnancy outcomes that included, (i) the mean fetal weights, (ii) head circumferences, (iii) crown rump length, and the (iv) bi- parietal diameters, this study established that, in both the treatment groups of phenobarbital and phenytoin they were significantly lower ($P<0.05$) when compared with the control. It was further noted that in the phenobarbital treatment groups were significantly lower ($p<0.05$) than those of the phenytoin treated groups (***Table 4.2).*** In carrying out MANOVA analysis to establish the contributory effects of the drug, dose and the time of exposure; either acting alone, or in two way combinations or in three way combinations, it was observed the greatest contributor was the type of drug contributing more (Partial Eta squared (η^2) between 91-99%; followed by the dosage at between 71-96% while the time of exposure had the least contribution of between 49-90% (***Table 4.2).***

These finding are in concurrence with some previous findings by (Ritchie *et al*., 2019) of reduced birth weight, head circumference, crown rump length and bi-parietal diameters in rats administered with phenobarbital. They are also in agreement with another report by (Hamdi *et al*., 2016b) that reported that valproic acid that is an anticonvulsant medicine with the same mode of action as phenobarbital, caused a significant reduction in crown rump length and fetal weight in experimental rats. similarly, another study done by (El-Gaafarawi & Abouel-Magd, 2015) showed that upon administration of anticonvulsants like carbamazepine, that is in the same generation with phenobarbital, there was decreased crown-rump length and fetal body weights.

The above findings on the fetal and maternal pregnancy outcomes could be attributed to the fact that phenobarbital up regulates cytochrome P450s of the 2B family and produces oxidative stress through the generation of superoxide radicals, leading to the production of hydroxyl radicals, resulting in the formation of 8-oxodeoxyguanine that results in guanine, cytosine to thymine, adenine transversions (Baldelomar *et al*., 2018; *Margulis et al.*, 2019). These findings therefore demonstrate that phenobarbital-induced oxidative stress may be the one that causes the observed developmental defects of the fetal viscera in-utero (Tomson & Battino, 2012). The other possible mechanisms that are thought to induce fetal growth restriction, are due to involvement of estrogen biosynthesis, leptin expression, placental dysfunction, increased oxidative stress and poor antioxidant mechanism poor mitochondrial function and enhanced apoptosis (Baldelomar *et al*., 2018,). This findings are also in agreement with results of previous study done by (Czeizel *et al*., 2011) who demonstrated that microcephaly has been associated with in utero AED exposure. Another multicenter prospective study reported that there is increased risk for small head circumference was increased use of phenobarbital during pregnancy. Margulis *et al*.,(2019) also found out that there was a reduction in head circumference when valproic acid whose mode of action is similar to that of phenobarbital was applied in-utero. A study by Gibson and Patel, (2008) reported that apoptotic neuro-degeneration triggered by AEDs during the critical stages of development are at least partly the contributors of reduced head circumference and this was due to the fact that experimental data provided evidence that barbiturates like phenobarbital induced massive apoptotic response in immature rodents.

5.1.2 Objective 2: To Comparatively Evaluate the Histo-Morphological Teratogenic Effects of the Prenatal Exposure o Varied Doses of Phenobarbital and Phenytoin on the Development of the Fetal Kidneys

In evaluating how the two medicines influenced the histological differentiation of the developing fetal kidneys the following parameters were evaluated (i) the histomorphological thicknesses of the cortex and the medulla, (ii) the distribution of the glomeruli per field of views, (iii) the distribution of the juxtaglomerular mesangial cells, (iv) the bowman's space, (v) the histo-morphological appearances

on the renal tubules. The histo-photomicrographs were analyzed at different magnifications of X10, X40, and X100. Concerning the overall sizes of the fetal kidneys, the fetal kidneys from the treatment groups were noted to be overlay bigger in size than those of the control. However, upon analyzing their longitudinal histological sections, the medulla was the one that was seen to be greatly hypertrophied as compared with slight hypertrophy of the cortex. However, in comparing the levels of the fetal kidney histological development and histological differentiation of the nephrogenic structures of both the glomeruli and the renal tubules, the feta kidneys of the control depicted clear differentiated renal structures with reduced no cases of excessive deposition of stromal tissues in the tubule-interstitial spaces, very well developed proximal and distal convoluted tubules, well differentiated glomeruli and no cases of hypertrophied glomerular spaces. On the other hand, the fetal kidneys of both the treatment groups of both the phenobarbital and phenytoin they showed variances in the levels of differentiation of the renal structures depending on the dosages of exposure and the time of exposures with the medium and high doses recording the worst teratogenic effects in altering the differentiation of the fetal kidneys ***(Figures 4.9, figure 4.10, figure 4.12*** and ***figure 4.13)***. These findings are in concurrence with findings by Baldelomar *et al* (2018) who reported reduction in glomeruli sizes and in their distributions.

In particular, the fetal kidneys of both the phenobarbital and phenytoin that were exposed at TM_1 and TM_2 and in the medium and the high dosages, they were noted to have excessive deposition of stromal tissues in the tubule-interstitial spaces with pockets of edematous fluid accumulations in these spaces, then poorly developed distal convoluted tubules that had clogged masses of luminal undifferentiated cells. This clogging of the luminal spaces with masses of undifferentiated cell masses seemed to block the urine flow in the renal tubules hence causing back flow that caused hypertrophication of the bowman's spaces in the treatment groups and eventual enlargement of the thicknesses of both the cortical and medullary layers **(*Figures 4.3, figure 4.4, figures 4.9, figure 4.10, figure 4.12 and figure 4.13)*.** This study also found out that phenobarbital had more deleterious effects to the cortical thickness and the medullary thickness as compared to phenytoin ***(Figure 4.2, Figure 4.3 and Figure 4.4)*.** These observations could be as a result of the effects on the

mode of action of phenobarbital where it acts by production of superoxide that generates of oxidative stress to the developing fetal nephrogenic tissues leading to injurious effects on the differentiating embryonic tissues. The main cause in cortical thickness was noted to be due to hypertrophy of the bowman's spaces that looked dilated due to clogging of the distal convoluted tubules that seemed to have masses of undifferentiated tissues that could have been blocking the urine flow in the renal tubules either causing a back-flow and dilatation of the bowman's space and the increased pressure was transmitted proximally leading to proximal tubular edema. This findings are in concurrence with some previous studies done on sodium valproate that reported interstitial hemorrhage, cloudy swelling of renal tubules with enlargement of the lumen, proliferation of mesangial cells, hypercellular glomeruli, and blood vessels congestion, hydropic changes on the proximal and distal convoluted tubules (El-Shenawy & Hamza, 2016). This also agrees with previous studies done by (Jassim, 2013) that reported changes of kidney tissue treated with valproate which has the same mode of action as phenobarbital revealed proximal and the distal convoluted tubules showed hydropic changes and there was increased number of cells in the glomeruli.

The changes in the cortical thickness could be as a result of the reduction in the size of the glomerular tuft of capillaries and the reduction in the number of glomeruli due to the toxicity of the drugs hence poorly developed and some degenerated. This concurs with the previous studies done on the fetuses of mothers injected with gabapentin whose mode of cation is similar to that of phenobarbital that exhibited degeneration of renal corpuscles in the form of shrunken or absent glomeruli along with increased peri-glomerular space (Badawy *et al*., 2019). However on the other hand it disagreed with other studies done by (Alsereah, 2018) which showed that there was hypertrophy of renal cortical secondary to phenobarbital exposure. From light microscopy micrograph, the experimental epileptic mother rats showed that the glomeruli had a marked increase of cellularity, lobulated and filling almost of the bowman's capsule space El-Gaafarawi and Abouel-Magd, (2015).

With regards to the juxta-glomeruli Mesangial cells, it was noted that in both the treatment groups they depicted hyper-cellularity and clumping together of the cells

that corresponded to those exposures to medium and high doses of both phenobarbital and phenytoin particularly when exposed during TM_1 and TM_2 as compared with the control whose features were more or less similar to those fetuses of mother exposed at TM_3 and in low doses. It further established that the glomeruli cellular distribution had an inverse-time-dependent relationship in that when phenobarbital and phenytoin treatment was administered early at TM_1, TM_2, the cellular distribution decreased directly with time of exposure. When the pairwise histo-morphological comparisons were done between phenobarbital and phenytoin treatment groups at the same time of exposure and within the same dosage levels, the study further established that phenobarbital treated groups had their cells reduce in sizes and became clumped together in the glomerular capillary tuft pole as compared to phenytoin ***(Figure 4.8, figure 4.9*** and ***figure 4.10).***

This could be due to the fact that phenobarbital induces excessive oxidative stress leading to kidney injury as a result of free radicle damage and poor differentiation of the cellular components of the fetal kidneys. Mesangial cells act as macrophages in the intra-glomerular, therefore these cells proliferate specifically at the glomeruli in response to kidney injury. This results resonates well reports with some previous studies done by El-Shenawy and Hamza, (2016) who reported that sodium valproate which is also a first-generation anticonvulsant medicine with the same mode of action as phenobarbital showed some changes in the kidney for example proliferation of mesangial cell, hyper cellular glomeruli. It was further noted that in phenobarbital treatment groups the bowman's spaces were highly increased when the treatments were administered in high doses and at TM_1 with 0.020µm, compared to control which had 0.008 µm. In similar fashion, phenytoin depicted the same trend but to a lesser effect in that bowman's spaces were highly increased when the treatments were prescribed in high doses and when the treatments were initiated at TM_1 with 0.019µm, compared to control which had 0.008µm. It was further noted that phenobarbital had more deleterious effects to the appearance of the bowman's space as compared to phenytoin ***(Figure 4.8, figure 4.9*** and ***figure 4.10).***

This results also concurs with previous which showed that first-generation antiepileptic for example carbamazepine which are in the same generation with

phenobarbital, the rats which were in treatment group kidneys showed histological changes like, atrophy of glomeruli, expansion of bowman's space *(*Al-bakri *et al.,* 2016*).*

Concerning the appearance of the renal tubules, this study noted that they were seen to differ in their histological appearance based on the doses of phenobarbital and phenytoin administered. the histological changes observed included cloudy swelling of renal tubules, and hydropic changes on the proximal and distal convoluted tubules with enlargement of the lumen in the proximal convoluted tubules with clogging of undifferentiated tissues in the lumen of the distal convoluted tubules (***Figure 4.11, figure 4.12*** and ***figure 4.13).*** These results could have been due to interference of renal development by phenobarbital due to production of superoxide radicals causing oxidative stress leading to tubular injury. These findings are in tandem with previous studies done on sodium valproate which has the same mode of action as phenobarbital and also a first-generation anticonvulsant medicine, this study also noted some changes in the kidney for example:-, cloudy swelling of renal tubules, and hydropic changes on the proximal and distal convoluted tubules with enlargement of the lumen (El-Shenawy & Hamza, 2016). Other studies also done previously showed alterations in the histological structures that included damage of renal tubules for the fetal kidneys born of mothers who had been treated with AEDs in the management of epilepsy.

The glomeruli become swollen, lobulated and occluded almost of the bowman's capsule space. Peri-glomerular renal cell infiltration was also detected (El-Gaafarawi & Abouel-Magd, 2015).

5.1.3 Objective 3: To Comparatively Evaluate the Histo-Stereological Teratogenic Effects of the Prenatal Exposure to Varied Doses of Phenobarbital and Phenytoin on the Development of the Fetal Kidneys

The histo-stereological teratogenic effects of phenobarbital and phenytoin was done to quantitatively evaluate the histological changes that occurred on the different kidney structures of the fetal kidneys following the prenatal exposure to varied doses of phenobarbital and phenytoin *in-utero*.

The following parameters were subsequently evaluated; (i) Kidney length, (ii) Kidney weight, (iii) kidney width and (iv) total Kidney volume (Archimedes volumes), (v) the medullary and cortical thicknesses carvalieri volume, (vii) the volume densities of both cortex and medulla, and the (viii) number of glomeruli per kidney for both phenobarbital and phenytoin treatment groups and also the control group. The study established that upon administration of the various doses of both phenobarbital and phenytoin during pregnancy there was a marked significant ($P<0.05$) increase in the fetal kidneys weights, kidney lengths and kidney widths when compared with the control. For the phenobarbital treated groups these parameters were as follows when the treatment was done at TM_1 :- [0.0414±.0043, .3666±.0025, and .1666±.0025 respectively and TM_2 with mean .0383±.0009, .3553±.0033, and .3441±.0064] respectively compared to control whose values were=.0259±.0006, .2611.0007 and .1029±.0003 while for the phenytoin the parameters were as follows when the treatments were initiated at TM_1 with mean as follows:- .0368±.0001, .3350±.0011 and 1019±.0034 respectively and TM_2 with mean .0355±.0001, .3262±.0005, and .3349±.0029 respectively compared to control .0259±.0006, .2611.0007 and .1029±.0003 depicted (***Table 4.7).***

These stereological findings could have been due to interference with the fetal kidney teratogenicity that interfered with the normal morphogenesis of the entire kidneys structures as was also reported by Wlodarczyk *et al*., (2012) who indicated that phenobarbital up regulates cytochrome P450s of the 2B family and produces oxidative stress through the generation of superoxide radicals. Phenobarbital induces oxidative stress leading to kidney injury as a result of free radicle damage resulting edema and eventual swelling of the kidney. This could then explain the observed overall increase in the total sizes of the prenatally exposed fetal kidney as a result of edema and associated kidney swelling. The resultant Glomeruli hyper filtration is associated with early phases of acute kidney injury hence increased glomerular size and tearing of the linings of the renal tubules. However, the increase in the size of the glomeruli was mostly accounted for by an increase in bowman's space size and not by an increase in glomerular tuft size. The fact that the tubular lumen size was also increased suggests that Bowman's space dilation was insufficient to normalize bowman's space pressure and that the increased pressure was transmitted distally.

The observed injurious effects on the renal tubules and the glomeruli resulted in the overall increase in the size of the kidney as well as the increase in the size of the urinary space (bowman's space and the tubular size). These observations are in concurrence with a study done by Elshama *et al*., n.d. (2015) who also found out that when carbamazepine, (anticonvulsant in the same generation with phenobarbital) was administered to pregnant mice, it showed that weight of fetal kidneys had a significant increase. Another study done on sodium valproate that has the same mode of action with phenobarbital showed changes in the kidney for example:- interstitial hemorrhage, cloudy swelling of renal tubules, proliferation of mesangial cells, hypercellular glomeruli, and blood vessels congestion, hydropic changes on the proximal and distal convoluted tubules (El-Shenawy & Hamza, 2016) and this leads to swelling of the kidneys therefore increase in weight and size of the kidneys. These results would help explain the increase in the total kidney weight due to edema. Previous studies done on the rabbits with administration of high dose of gabapentin 1500mg/kg which has the same mode of action as phenobarbital showed that there was enlargement of renal pelvis in rabbits (Petrere & Anderson, 2014) and this can lead to enlargement of the kidney size observed in this study.

In assessing how the phenobarbital and phenytoin influenced the volume densities of the medulla and the cortex including the carvalieli volume, this current study established a significant increase ($P<0.05$) in the medullary and the cortical volume densities including the carvalieli volumes when compared with the control. It was further noted that the in phenobarbital treatment groups the medullary density, cortical density and carvalieli volume were highly affected when the treatments were initiated at TM_1 at high dose with mean as follows .2444±.0035, .0486±.0012 and .3589±.0007 respectively and TM_2 with mean (.2364±.0039, .0436±.0017, 3461±.0004 0383±.0009, ·3553±.0033, .3441±.0064) respectively compared to control (.0259±.0006), (.2611.0007) (.1029±.0003. similarly, phenytoin depicted the same trend in the medullary volume densities, cortical density as well as the carvalieli volumes when treatment was done at TM_1 as follows (.2146±.0003, .0570±.0018, .3242±.0033 respectively and TM_2 with mean (.2093±.0010, .0521±.0016, .3242±.0033) respectively compared to control (.1591±.0026, 0.0794±0.0014, 2383±.0036 (***Table 4.7).*** Upon applying the MANOVA analysis to

find out contributory role of each of the three independent variables of the drug, dose and time of exposure the study established that the type of drug contributed 89-98% followed by the dosage at 72-90% and the time of exposure 70-81% shown in the ***(Table 4.11).*** It was further noted that phenobarbital had severe effects to the medullary density, cortical density and carvalieri volume as compared to phenytoin shown in the ***(Table 4.12).*** These findings are similar to what was observed in previous studies by Mifsud, (2010), El-Shenawy & Hamza, (2016).

5.2 Conclusions and Recommendations

The conclusions of the study are in line with the study objectives as follows: -

(i) On the first objective that was to comparatively evaluate the prenatal effects of prenatal exposure to the varied doses of phenobarbital and phenytoin on the maternal and fetal pregnancy outcomes, it can be concluded that the prenatal exposure to phenobarbital and phenytoin causes teratogenic perturbations to the fetal growth and development environment *in-utero* that is first occasioned by maternal nutrition disturbances plus the inhibitory mechanisms of the two medicines plus their metabolites that perturb the functions of the maternal blood placental barriers. As such the phenobarbital and phenytoin perturb maternal and fetal pregnancy outcome parameters across all the stages with phenobarbital having more deleterious effects as compared to phenytoin

(ii) Concerning the comparative histo-morphogical teratogenic effects of prenatal exposure phenobarbital of both and phenytoin on the developing fetal kidneys, both medicines were also seen to perturb the fetal kidney histological differentiation in utero. The two were noted to affect the functional structures of the kidney- nephrons with phenobarbital having more detrimental effects on the fetal histological organization as compared to phenytoin

(iii) Regarding the comparative histo-stereological teratogenic effects of prenatal exposure phenobarbital of both phenobarbital and phenytoin on the developing fetal kidneys, both medicines were also seen to perturb the

fetal kidney structures for instance the kidney volume and volume densities were noted to increase, the number of glomeruli per field were noted to decrease.

(iv) In terms of whether or not the teratogenic effects of the two medicines are dose and time dependent, it can be concluded that the teratogenicity of the two medicines on the maternal and fetal pregnancy outcomes outcome, histo-morphological teratogenic effects and the histo-stereological teratogenic are all doses and time dependent with exposures at TM_1 and TM_2 giving the worst outcomes and when administered in medium and high doses.

5.3 Recommendations

The study recommends that: -

- If a mother gets pregnant while on medium and high doses of phenobarbital or phenytoin, the doses should be adjusted to low dose as other safer drugs are introduced.
- If a mothers get pregnant while on any of these two medicines, the medicine should be down regulated as a safer drug is introduced.
- More studies also need to be done on the higher primates to ascertain its safety in pregnancy in order to curb the rising cases kidney diseases and also congenital anomalies which may be associated with it.

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APPENDICES

AppendixI:Publication

IOSR Journal Of Humanities And Social Science (IOSR-JHSS)
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The Intra-Uterine Stereological Tratogenic Effects of Phenytoin on Fetal Kidneys in Albino Rats (*Rattus Norvegicus*)

Jennifer Segut[1], Joseph Kweri[2], Ann Mwangi[3], Caroline Sigei[4], James Kanyoni[5], Peris Macharia[6], Walter Rono[7], Cyrus Kamau[8], Anne Njoki[9], Cynthia Chebii[10], Jane Kuria[11], Joseph Wachira[12], Christopher Mramba[13], Jane Karanja[14]

[1-13]*(Department of Human Anatomy, School of Medicine (SOMED), College of Health Sciences (COHES) Jomo Kenyatta University of Agriculture and Technology (JKUAT) Kenya.*
[14]*(School of Nursing (SON), College of Health Sciences (COHES) Jomo Kenyatta University of Agriculture and Technology (JKUAT) Kenya.*

Abstract:
Background: *The teratogenic stereological effects on the fetal kidney development after exposing to differing doses of phenytoin remain poorly elucidated. This study, therefore, set to evaluate the intrauterine stereological teratogenic effects of varied doses of phenytoin on fetal kidneys in albino rats when prescribed at different gestational period in albino rats as the experimental model.*
Materials and methods: *In conducting this study, a post-test only control experimental study design was used. A resource equation for One-way Analysis of Variance (ANOVA) was applied to determine the sample size and therefore a sample size of thirty Albino rats (Rattus norvegicus) weights ranging from 150-250 mg were used in this study. These 30 albino rat were hence obtained from the school of biomedical sciences of Jomo Kenyatta University of Agriculture and Technology (JKUAT) at the Small Animal Facility for Research and Innovation (SAFARI). This sample size of 30 albino rats were randomly allocated into two wide study categories of 27 rats experimental and the 3 rats control group. To assess the intrauterine stereological teratogenic effect of phenytoin when administered in differing doses, in experimental group, the twenty seven rats were still split into 3 study categories consisting of 9 rats each depending on the three study doses of low, medium, and high phenytoin doses applied in the study hence: 9 rats for the high phenytoin group -that received 124 mg/kg/BW; 9 rats for the medium phenytoin group whereby 62 mg/kg/BW was prescribed and lastly 9 rats for the low phenytoin group that received 31mg/kg/BW. To gauge the intrauterine teratogenic stereological end results of phenytoin when administered on varying incubation periods, the nine rats in each of the 3 study dose categories were still split into 3 small groups of 3 rats depending on the trimester when they received treatment as follows; 3 rats that received the treatment from Trimester I; 3rats that received treatment from trimester II and 3 rats that received treatment from trimester III respectively. At gestation day 20, all the rats were humanely sacrificed and 3 fetuses from each rat were selected based on their weights as follows; the first one with the highest weight, another one with the median weight, and the last one with the lowest weight. Their kidneys were then harvested for stereological analysis. The stereological parametric data that included fetal kidneys weights, total kidney volume, medullary volume and corical volume density of the fetal kidney structure which was obtained by using carvalieri technique of point counting and water immersion method (WIM). The data was collected using a structured a check list, then entered into the computer using an excel spreadsheet for windows version 10, the data in the excel spreadsheets was then exported to the Statistical Package for the Social Scientist (SPSS) to analyze. To determine the causal effects and interaction effects the statistical significance was determined by use of Turkey's post hoc multiple comparison tests and all values whose P<0.05 were considered to be significant.*
Results: *The results of this research has shown that there was statistical significant increase (P<0.05) in fetal kidney stereological parameters especially during the first trimester. Phenytoin administered prenatally had a time and dose dependent impact on fetal parameters in that effects were more with (HPTG)-124 mg/kg, and during the first trimester (TM_1) when compared with control.*
Conclusion: *Therefore more studies needs to be done on higher primates to ascertain its teratogenicity prenatally.*
Key words: *stereological, teratogenic, albino rats, phenytoin.*

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Appendix II: Letter of Ethical Approval

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

P.O. Box 30197,
00100 Nairobi,
Kenya.

Tel: 4449004/4442014/ 6
Ext. 2300
Direct Line: 4448648

REF: FVM BAUEC/2021/327

Ms. Jennifer Segut.
Dept. Human Anatomy,
JKUA & Technology.
10/11/2021

Dear Jennifer,

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

Comparative histomorphological and histostereological teratogenic effects of phenobarbital and Phenytoin on the fetal kidneys in Albino rats.

Jennifer Segut. HSM301-1488/2020.

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 the proposed protocol that will be used to compare histomorphological and histostereological teratogenic effects of phenobarbital and Phenytoin on the fetal kidneys 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,

Kaluwa

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

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