COMPARATIVE HISTOMORPHOLOGICAL AND HISTOSTEREOLOGICAL TERATOGENIC EFFECTS OF *IN-UTERO* EXPOSURE TO LAMOTRIGINE AND LEVETIRACETAM ON THE FETAL KIDNEYS IN ALBINO RATS *(Rattus novegicus)*

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Comparative Histo-morphological and Histo-stereological Teratogenic Effects of *In-utero* Exposure to Lamotrigine and Levetiracetam on the Fetal Kidneys in Albino Rats *(Rattus novegicus)*

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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……...…….

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

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DEDICATION

I wish to dedicate this research thesis to my dear wife Lucy and my children, Ephraim, Joanne, and Nissi for their support and patience they have shown me in the entire process of my studies.

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ABBREVIATIONS AND ACRONYMS

AED	Antiepileptic Drug.
BS	Bowman Space
GT	Glomerular Tufts
JGA	Juxtaglomerular Apparatus
LTG	Lamotrigine
LVT	Levetiracetam.
MM	Molecular Mass/weight
SPSS	Social Package for Social Science.
TM1	Trimester One
TM2	Trimester Two
TM3	Trimester Three
US	Urinary Space

DEFINITION OF TERMS

Embryolithalities Number of dead fetuses that were recorded for each rat.

Gastric gavage procedure This is a procedure where the drug is introduced into the stomach of the experimental albino rats with the use of metallic tube.

Histo-stereology This is a three-dimensional measurement of microscopic structures to obtain reliable and important quantitative data that enables calculation of volumes and volume ratio, the area of samples, the number of particles per units' volume, particle size, unit volume, length and weight.

Litter sizes The number of life fetuses that were harvested from each rat

Morphometry This is the process of measuring the external shape and dimensions an organ.

Sickness This is the coordinated set of behavioral changes that develop in sick rats during the course of infection. They include; little interest in their surroundings, reduced feeding or complete refusal to feed, lethargic, isolation and being less playful, weight loss, abnormal fecal material in terms of color and appearance.

Teratogenic Ability of a substance to cause congenital abnormality to the developing fetal organs.

OPERATIONAL DEFINITIONS

Fetal pregnancy outcome parameters These were parameters that assessed the effects of the interventions to the fetuses. They included;

1.The intrauterine observations *(i.e the observations made when the fetuses were still attached to the uterine horns by their placenta*) including (i) the litter sizes, (ii) the resorbed glands/ devoured glands and (iii) the number of dead fetuses.

2.The individual fetal growth and development parameters that included; **(i)** the fetal weight (FT), **(ii)** head circumference (HC) and **(iii)** crown rump length (CRL).

Histo-morphological parameters These were parameters that assessed the histo-morphological effects of the intervention to the kidneys. They included, (**i**) the histo-morphological shapes of the glomeruli, (**ii**) the organization of the juxtaglomerular cells, (**iii**) the glomerular capillary tufts and associated cells, (**iv**) the bowman spaces and their sizes, (**v**) glomerular distribution of the per field of view, (**vi**) histological thicknesses of the fetal kidney cortex and medulla and (**vii**) histological organization of the medullary kidney tubules.

Histo-stereological parameters These were parameters that evaluated the gross morphometric and the histo-stereological organization of the fetal kidneys namely:

1. The gross morphometric parameters of the fetal kidneys including (i) the kidney weights, (ii) the kidney lengths, (iii) the kidney width and (iv) the initial total kidney volume.

2. The parameters that assessed the histo-quantitative components of the fetal kidneys (i) the calculated Cavalieri kidney volumes, (ii) the cortical and the medullary thicknesses (iii) the cortical and the medullary volume densities.

Maternal fetal outcome parameters These were parameters that assessed the effects of the intervention to the mothers. They included; daily maternal weight gain, terminal maternal weight and terminal placental weight.

ABSTRACT

Lamotrigine and levetiracetam are second-generation antiepileptic medicines that are currently being used widely in the management of maternal epilepsy and other neuralgic disorders during pregnancy. However, their comparative histo-morphological and histo-stereological teratogenic risks on the developing fetal kidneys remains unclear. The broad objective of this study therefore was to comparatively evaluate their histo-morphological and histo-stereological nephron-teratogenic effects on the developing fetal kidney when exposed in varied doses and at different gestational periods. In carrying out this study, a posttest experimental study design with a control group was adopted. All animal experimentation procedures that included breeding of the animals, feeding, drug administration up to harvesting of fetuses was done in Nairobi university (UON) while tissue processing for histology and stereological analysis was done in the department of Human anatomy in JKUAT. A sample size of 30 albino rat dams was used for each of lamotrigine and the levetiracetam treatment groups. These 30 albino rat dams for each of the two study categories were first randomly assigned into two broad study groups of 3 rats control and 27 rats experimental. The 27 rats in each of the experimental category were further randomly assigned into three subgroups of 9 rats each according to the three dose groups of low, medium and high dose as follows, 9 rats low dose lamotrigine/ levetiracetam treatment group, 9 rats for the medium dose lamotrigine/ levetiracetam treatment group and 9 rats for the high dose lamotrigine/ levetiracetam treatment groups. To further evaluate the effects of the time of exposure, the 9 rats in each of the three dose categories were further randomly sub-divided into three sub-groups of 3 rats each as follows; 3 rats for trimester I, the next 3rats for trimester II; and the last 3 rats for Trimester III. The study findings showed that in both lamotrigine and levetiracetam treatment groups, there was a statistical significance increase ($p<0.05$) in medullary thickness while the cortical thickness reduced in a proportionate manner. Stereologically there was marked reduction in glomerular numbers per field of view as well as in their volume densities resulting to their observed sparse distribution. There was an observed remarkable condensation of glomerular tufts capillaries with significantly hypertrophied bowman spaces. The critical nephron-teratogenic period was noted to be when both the drugs were administered in trimester one and trimester two with critical teratogenic doses being both the high dose of the two medicines. Lamotrigine was established to be more teratogenic compared to levetiracetam across all dose groups and across all the trimesters of exposure. In conclusion, both lamotrigine and levetiracetam are teratogenic to the developing fetal kidney and their teratogenic effects depicted patterns of both time-dose dependency. The study recommends caution when using high doses of both drugs by pregnant mothers particularly during trimester one and trimester two. It also recommends further studies on higher primates with close relations to humans.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Lamotrigine (LTG) has a molecular weight of 256.09 $g \cdot mol^{-1}$ and chemical a formula of $C_9H_7Cl_2N_5$ while Levetiracetam (LVT) has a molecular weight of 170.21 $g \cdot mol^{-1}$ and a chemical formula of $C_8H_{14}N_2O_5$, these are the new second-generation antiepileptic medicines (AEDs) which are gaining popular usage in the management of maternal neurological disorders during pregnancy (Bansal *et al.*, 2018).

The maternal neurological disorders that necessitate use of AEDs during pregnancy are usually of great concern as some anticonvulsant medicines are classified as class C medicines in the American food drug and substance administration (FDA)(Gupta *et al*., 2017), as they have been shown to have some degree of teratogenic risks to the developing fetal organ systems particularly during the period of organogenesis(Gupta *et al*., 2017; Meador & Loring, 2016).

Mothers with these neurological conditions would however continue to be managed with these medicines as their therapeutic benefits have also been shown to out-weigh the risks particularly with regards to the survival of the mother (Macfarlane & Greenhalgh, 2018). As such there is need to carry out more in-depth histo-stereological studies to establish their teratogenic risks to the developing fetal organs and in particular the fetal kidneys due to the rising cases of juvenile and adult renal failures of unknown causes(Schreuder *et al*., 2011), (Neild, 2009).

In particular, the histo-morphological and the histo-stereological teratogenic effects of these two commonly used anticonvulsants is generally lacking. Furthermore, whether or not their teratogenic effects are dose and time dependent is yet to be elucidated (Mohamed, 2021). AEDs for a long time have been found to be useful in the management of a wide range of conditions like neuropathic pain, migraine headaches, as well as in psychiatric disorders like insomnia, bipolar disorders, anxiety etc. (Borrelli *et al*., 2018),(Hill *et al.*, 2010) in females of reproductive age.

The expansion in the clinical application of AEDs has greatly increased the chances of exposure of potentially pregnant women to teratogenic effect of these compounds (Tomson & Battino, 2012). Though lamotrigine and levetiracetam have gained popular use in the recent past, their teratogenic effects on the developing fetal kidneys and other genital urinary structures have been a subject of disagreement (Elgndy *et al*., 2019), (Bromley *et al*., 2018).

Some studies have recommended the use of these two new 2nd generation antiepileptic medicines on the basis that they are relatively safer than the older generation AEDs and that they have good efficacy and high tolerability (Kim *et al*., 2020) (Elgndy *et al*., 2019) while other studies have reported that they bear relatively low risk in triggering congenital malformations(Meador & Loring, 2016).

It is important to note that even though the teratogenic risk of AEDs has been clearly established, it still remains unclear for newer second generation AEDs like lamotrigine and levetiracetam on the developing fetal kidneys(Elgndy *et al*., 2019). For example, in the USA alone, the risks linked with *in utero* antiepileptic drug (AED) exposure has been of critical importance because of the estimated 30,000 children born to epileptic mothers each year (Hill *et al*., 2010).

Previous studies have also shown that most women with epilepsy usually requires use of AEDs throughout their entire pregnancy period to control seizures (Bromley *et al*., 2018)*(*Tomson *et al*., 2011). Further to this, these studies have also shown that when AEDs are used either as monotherapy, doubles therapy, or as polytherapy, the risk for major congenital malformations doubles and triples with these combinations (Wlodarczyk *et al*., 2012).

Evidence is still building up where investigations have indicated that exposure to select AEDs leads to unexplained increase in renal failure among children born of mothers who had an in-utero exposure to AEDs (Schreuder *et al*., 2011). Because of this increase in cases of renal failure of unknown causes, kidney diseases have become a global public health problem where in a global scale over 750 million

people worldwide are suffering from kidney disease as reported by (Crews *et al*., 2019).

Even though anticonvulsant medicines are teratogenic and can affect the kidneys too, cases of uncontrolled epilepsy can lead to severe and critical consequences to both the mother and unborn child, therefore necessitating the use of at least one or two antiepileptic medicines as monotherapy, duo or poly-therapy in pregnancy (Falco-Walter and Bleck 2016). The *in-utero* exposure to AEDs is usually a subject of controversy as all AEDs have either been linked with some risks of major or minor congenital malformations (MCMs) (Wlodarczyk *et al*., 2012). In a separate study done by (Vajda et al., 2013), they reported association between fetal congenital malformations and antiepileptic drugs where they noted that all AEDs cause congenital malformations to the urinary system .

Lamotrigine and levetiracetam are being widely prescribed for use during pregnancy because of their perceived low risk for teratogenesis to the fetus as described in literature when compared with the other anticonvulsants (Abou-Khalil, 2008). Both of these two medicine like other AEDs are documented to cross the maternal blood placenta barrier as indicated by studies that calculated the Umbilical cord AEDs concentrations vis a vis maternal concentrations providing valuable indicators on the fetal AEDs exposure in-utero (Myllynen *et al.*, 2003).

The fetal tissue accumulation of lamotrigine and levetiracetam with their principal metabolites following in-utero exposure are thought to be influenced by drug transporting proteins in the placenta, including P-glycoprotein (P-gp), multidrug resistance protein (MRP) 1, and breast cancer resistance protein (BCRP) that are located in the syncytiotrophoblast plasma membrane that form the interface of the maternal and fetal circulations (Morrow *et al* 2006). Even though these proteins are supposed to offer protection to the fetus, the genetic variations in the expression and activity of these transport proteins are the ones indicated to influence fetal exposure to AEDs and thus increasing the risk of fetal viscera teratogenicity like the kidneys and the others (Atkinson *et al* 2006).

1.2 Problem Statement

While nephron-teratogenic effect of different AEDs has been explored, little attention has been paid on teratogenic effects of lamotrigine and levetiracetam on fetal kidneys development and in particular their teratogenic effects on histo-morphology and histo-stereology of the glomeruli and other kidneys histological apparatus. This is despite the fact that these two anticonvulsant medicines are currently gaining a lot of popular usage in management of maternal neurological disorders like epilepsy, bipolar disease, neuralgia among others during pregnancy in spite of the known fact that nephrogenesis continues up to around 36 weeks of intra uterine life (Schreuder *et al.,* 2011).

It is not clear what would be the effects on the developing fetal kidneys when the two medicines are exposed *in-utero* in varied doses and at different window periods. Health seeking behaviors and the affordability of health services in developing countries like Kenya as reported by Bigogo *et al*., 2010 in their study are influenced by the poverty levels among other factors. Kenya being a developing country is regarded as low-middle income country (World Bank list of economies 2020) which is transitioning from low-income country to a low-middle income country.

Majority of her population is poor and the economy is growing at a slow rate. This poverty level affects the lifestyle of her people including health seeking behaviors and the affordability of health services where this also influences the use of over-the-counter medications. Even in such situations, unfortunately, there could be no other medicines that can substitute the use of AEDs in management of maternal epilepsy in developed or in developing countries. The use of lamotrigine and levetiracetam in management of maternal neurological disorder like epilepsy during pregnancy is riddled with some degree of uncertainty when it comes to their teratogenic outcomes to the fetus (Elgndy *et al*., 2019). This has posed some degree of a challenge to the clinicians in making appropriate choices and rational applications of these two AEDs in management of maternal health issues requiring the use of an AED during pregnancy (Elgndy *et al*., 2019). This challenge on whether or not the *in-utero* exposure to lamotrigine and levetiracetam has any teratogenic effect on the

development of fetal viscera like the kidneys is compounded by lack of comparative histo-morphological and histo-stereological teratogenic data on the two medicines when exposed *in-utero* at varied doses and at different window periods. At the same time whether or not the teratogenic effects of these two anticonvulsant medicines on the development of fetal kidneys are dose and time dependent is yet to be established.

1.3 Justification of the Study

Insufficiency of a scientific data source on the histo-morphological and histo-stereological teratogenic effects of lamotrigine and levetiracetam to the developing fetal kidneys as well as other fetal viscera on these two commonly used second generation medicines will continue posing a challenge to the clinicians in making appropriate choices between these two medicines as well as in their rational application in management of maternal health issues like epilepsy requiring the use of AEDs during pregnancy. This will subsequently impact negatively on the patients and continue posing risks to the fetuses. The study findings seek to unravel the controversy surrounding the use of lamotrigine and levetiracetam during pregnancy.

1.4 Significance of the Study

The availability of the histo-morphological and histo-stereological nephron-teratogenic data that clearly indicates the comparative nephron-teratogenic risks between these two antiepileptic medicines on the developing fetal kidneys would serve as useful guide into the rational applications of these two antiepileptic medicines in management of maternal health issues like epilepsy requiring the use of an AEDs during pregnancy. This data will be useful to the clinicians when deciding on the appropriate dosages for mothers that have to use these two medicines during pregnancy.

These findings will also be useful in ensuring that the mothers derive optimal treatment benefits from the two medicines while protecting the fetus from the teratogenic risks that would arise from the maternal application of the two medicines.

The findings of this study will also serve as a guiding platform to anchor future research studies using the non-human primates that have close relation to humans.

1.5 Broad Objective

To comparatively evaluate the histo-morphological and histo-stereological nephron-teratogenic effects of prenatal exposure to varied doses of levetiracetam and lamotrigine on the developing fetal kidneys when exposed at different gestational periods in the albino rats. (*Rattus norvegicus*).

1.6 Specific Objectives

1. To evaluate the effects of prenatal exposure to varied doses of levetiracetam and lamotrigine on the fetal and maternal pregnancy outcomes when exposed at different gestational periods in albino rats.
2. To evaluate the histo-morphological teratogenic effects of prenatal exposure to varied doses of levetiracetam and lamotrigine on the developing fetal kidneys when exposed at different gestational periods in the albino rats.
3. To evaluate the histo-stereological teratogenic effects of prenatal exposure to varied doses of levetiracetam and lamotrigine on the developing fetal kidneys when exposed at different gestational periods in the albino rats.
4. To evaluate whether the observed histo-morphological and histo-stereological teratogenic effects of levetiracetam and lamotrigine on the developing fetal kidneys are time and dose dependent.

1.7 Null Hypothesis (Ho)

There are no significant differences in the histo-morphological and histo-stereological teratogenic effects on the developing fetal kidneys following prenatal exposure to levetiracetam and lamotrigine at varied doses and at varied window periods in albino rats.

1.8 Aim of the Study

The study aimed to comparatively evaluate histo-morphological and histo-stereological nephron-teratogenic effects of prenatal exposure to varied doses of levetiracetam and lamotrigine on the developing fetal kidneys when exposed at different gestational periods in the albino rats.

1.9 Assumption of the Study

In carrying out this study using the albino rat model, the study assumed that the teratogenic mechanisms in inducing injurious effects to the developing fetal kidneys in rats, would be the same teratogenic injurious mechanisms that would apply to both humans and non-human primates. The study further assumes that since rats are mammals just like the humans, the developmental process of the fetal kidneys would also be the same to the ones in humans. The study also assumes that the rat fetal kidney histo-morphological and histo-stereological features are the same to those in humans and therefore the injurious teratogenic effects of the two study medicines in rats would depict the same scenario as in humans.

1.10 Assumptions of the Study Model Used

Existing literature has shown that this breed of albino rats (*Rattus Novegicus*) has very close relationship to the human biological and functional characteristics. The study hence assumes that the results obtained in the process of carrying out these teratogenic experiments would mimic a similar histo-morphological and histo-stereological teratogenic outcomes in the development of fetal kidneys in humans as well as the non-human primates.

1.11 Study Limitations

Some study limitations included:

1. Unavailability of electron microscope which is very expensive. If it was available, it would have been possible to analyze finer and minute histological details of the kidneys.

2. Delayed pregnancy in some of the rat led to delays in completing the study.
3. Sickness of some of the treatment rats led to their discontinuation from the study.

1.12 Delimitations of the Study

1. The current study was carried out on the albino rats' species only. This was in cognizant of the fact that the non-human primates could have probably given more better findings that could simulate human findings.
2. Only two antiepileptic medicines in the second-generation antiepileptic class were studied even though there are other medicines in this class that were not studied. Furthermore, there are other classes of AEDs which were not studied in this current study.
3. The period of study was only limited to *in-utero* period only before the fetuses were born where postnatal effects of the drugs was not studied
4. The current study only concentrated on teratogenic effects of the ADEs on the fetal kidneys while these study medicines affected all the other fetal viscera.

1.13 The Conceptual Frame Work

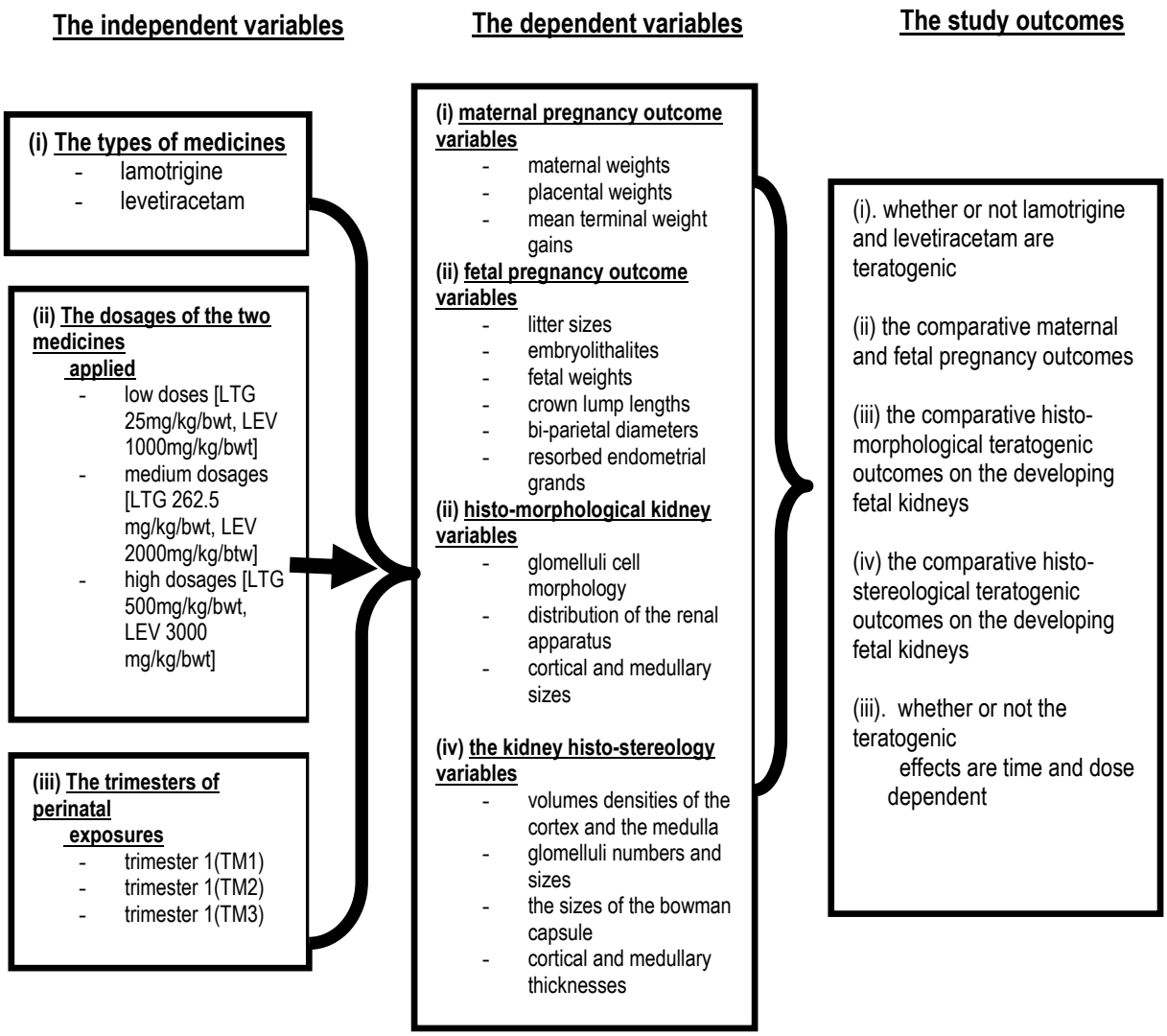

Figure 1.1: The Conceptual Frame Work

CHAPTER TWO

LITERATURE REVIEW

2.1 The General Teratogenic Background of Antiepileptic Drugs

Existing literature has shown that the *in-utero* exposure to almost all AEDs is associated with teratogenic risks of either a major or a minor congenital malformation (MCM) to the fetus (Wlodarczyk *et al*., 2012). As such, the American food and drugs administration (AFDA) classifies the AEDs as either class C medicines(Lee *et al*., 2013),(Mølgaard-Nielsen *et al.*, 2011) that should only be applied with caution during pregnancy or class D medicines (Bhakta *et al*., 2015)*(*Lee *et al.,* 2013) that should be used with great caution during pregnancy because they are known to be highly teratogenic. Some studies done previously have reported that AEDs are associated with some types of neonatal complications (Tomson *et al.,* Ba, 2012). Similarly, other studies have reported that, 2nd generation AEDs like levetiracetam and lamotrigine when used during the implantation or the organogenesis period can induce a wide range of both minor and major congenital malformations to the fetal viscera including the kidney too (Al-Ibrahimi *et al.,* 2015).

In some other studies done by Schreuder *et al*., (2011) and El Makawy *et al*., (2022), they pointed out that, any AED drug given during organogenesis and the implantation period is prone to affecting normal development of the fetal viscera including the kidneys. In line with this, (Carta *et al*., 2007) in their study indicated that prenatal use of AEDs is associated with one of the most severe multicystic dysplastic kidney (MCKD) disease expressed as the most common congenital anomalies of kidney and urinary tract (CAKUT). In a separate study done by (Schedl *et al.,* 2007), they reported developmental anomalies of the kidney which included renal agenesis, renal hypoplasia, dysplasia, malformation of ureters as well as CAKUT.

There is a substantive amount of evidence in the occurrence of congenital anomalies linked with the exposure to lamotrigine (LTG) during pregnancy as reported by International Lamotrigine Pregnancy Registry(Kong *et al.,* 2018). In a different

comparative study done by (Elgndy *et al.,* 2019) on lamotrigine and levetiracetam, they reported that both drugs are teratogenic to some degree with lamotrigine being more teratogenic than levetiracetam. As such, the *In-utero* exposure to AEDs is usually a subject of controversy as all AEDs have either been linked with some risks of major or minor congenital malformations (MCMs) (Wlodarczyk *et al*., 2012). The scarcity of histostereological and histomorphological scientific data on the *in-utero* exposure to lamotrigine and levetiracetam poses a challenge to the clinicians when dealing with a pregnant woman who has a health issue like epileptic in choosing the most appropriate antiepileptic medicine which is effective and less teratogenic. Furthermore, other than epilepsy, maternal health issues during pregnancy are at increased risk of antiepileptic drugs exposure since AEDs have gained a wide application in management of other health issues such as psychiatric disorders, migraine headaches as well as neuropathic pain (Hill *et al*., 2010).

2.2. The Pharmacological Description of Lamotrigine and Levetiracetam

Lamotrigine (LTG) is an organic compound with a chemical name 6- (2, 3-dichlorophenyl)-1, 2, 4-triazine-3, 5-diamine, and its molecular formula is C9H7Cl2N5 and a molecular weight of 263.09g/mol (Yasam *et al*., 2016). Lamotrigine is a 2,5-diamino-triazine which acts by blocking voltage-gated sodium channels (VGSCs) and voltage-gated calcium channels (VGCCs) where both of which contributes to the ant-seizure activity of this drug (Mitra-Ghosh *et al*., 2020). It is sold as oral tablets of different strengths (Mitra-Ghosh *et al.*, 2020). Lamotrigine (LTG) has a broad spectrum of activity with a great efficacy against focal or refractory partial epilepsy, bipolar disorder, absence seizures, generalized tonic-clonic seizures and Lennox-Gastaut syndrome (Mitra-Ghosh *et al*., 2020),(Yasam *et al*., 2016). It has a rapid and complete oral bioavailability reaching a peak concentration 1-3 hours after oral administration and it has no hepatic microsomal enzyme inducing activity and is metabolized by UDP- glucuronosyl transferase (UGT) enzymes mainly with glucuronidation conjugation (Carcak *et al.*, 2018). Lamotrigine (LTG) has a half-life of 24-34 hours in normal healthy individuals and it is excreted by kidneys (Yasam *et al.*, 2016). It was approved by

United State Food and Drug Administration and is categorized in class C as for drug to use in pregnancy (Lee *et al.,* 2013).

Levetiracetam is a (S)-α-ethyl-2-oxo-1-pyrrolidine acetamide with a chemical formula C8H14N2O2 and a Molar Mass (MM) of 170.209 g/mol (Kozłowski *et al.,* 2015). It is approved worldwide as an antiepileptic drug for adjunctive treatment of partial seizures (Isoherranen *et al.,* 2003). It binds selectively to the synaptic vesicles protein (SV2A) mainly in glutamatergic synapses in the brain and is an efficient well-tolerated agent used in treatment of focal and secondarily generalized tonic-clonic seizures and absence seizures (Carcak *et al.,* 2018). Selective inhibition of N-type calcium channels and blocking the inhibition of GABA- and glycine- gated currents by negative allosteric modulators is also thought to be another way through which LEV works (Kozłowski *et al*., 2015). It is rapidly and almost completely absorbed after oral administration reaching peak plasma concentration 1 hour after oral administration and its metabolism is not dependent on liver cytochrome P450 enzyme system (Abou-Khalil *et al.,* 2008).

Levetiracetam has a half-life of LEV is 7+/- 1 hour as indicated by (Kozłowski *et al*., 2015) in their study. Levetiracetam (LEV) is majorly excreted unchanged through the kidney with only around 27% metabolized and the main metabolic pathway is hydrolysis of acetamide group in the blood (Abou-Khalil *et al.,* 2008). The recommended initial dose in adults is 500 mg twice a day with dose increment based upon tolerance and effect to a maximum of 1500 mg twice a day or 3000 mg of extended release formulations once a day while dosing in children is based upon body weight (Overview *et al.,* 2020).

2.3 The Comparative Fetal Kidneys Morphogenetic Development Process Between the Rat and Humans

Existing literature has shown that the morphogenic process in the development of the fetal kidneys in both the rat and the human are closely related. Kidney development follows similar pathways in almost all mammals but the time frame in reference to the periods when the kidney morphogenesis begins up to the point of birth varies between on species to the other, the pattern of growth and morphological features of

every cell type, numbers present and division rate is similar for rats and human(Zoetis *et al.,* 2003). Existing literature has clarified that in normal state the gestation period in humans is around 40 weeks and in rats it's around 21 days (Zoetis *et al.,* 2003)

According to a study done by (Zoetis *et al.,* 2003), he explained that, in humans, nephrogenesis and kidney blood vessel development happens at similar time period and are completed at around 35 weeks of gestation while in the rat, nephrogenesis starts prenatally but was observed to occurs at a rapid rate between when the rats are born and the first eight days where it is completed by eleven days of age with tubular differentiation going on up to weaning time with functional maturity occurring even far much later.

In mammals like humans and rats, (Cullen-McEwen *et al*., 2015) in their study explained kidney development as involving development of three sets of primordial excretory structures namely pronephros, mesonephros and metanephros and these renal organs develop from intermediate mesoderm under strict control. Regression of the first two primordial excretory structures happens cranially to caudally as development progresses where later metanephros develop into mature kidneys. In another study done by (Michos *et al.,* 2009) also established that kidney development in human and rats happens in three stages but in different development time periods. The first stage involves pronephros together with its duct and these begins approximately around gestational day 22 in humans and gestational day 11 in the rats. The pronephros and mesonephros regress since they are transient structures, allowing the metanephros to differentiate into the adult kidney

Further explanation from (Cullen-McEwen *et al*., 2015) established that the pronephros are nonfunctional representing a transitory organ, with the anterior parts degenerating and the posterior parts develops. The nephric or Wolffian ducts remain and the induction of the nephric duct during pronephric development is important for development of the permanent kidneys, tubule formation in the adjacent mesonephric mesenchyme and giving rise to the ureteric bud in the metanephros. Ureteric bud continued contacts with the metanephric mesenchyme stimulates branching to continues with each branch representing what will develop into the future nephron,

also ureteric bud has controls over kidney morphogenesis, determining the future numbers of nephrons in the kidney and also contributes to the formation of the collecting ducts, renal pelvis, and ureters (Michos *et al.,* 2009).

2.4 The Comparative Teratogenic Mechanism of Both Lamotrigine and Levetiracetam

Physiological body changes during pregnancy influences dosing adjustment of AEDs. The distribution volume increases necessitating the need for increasing the maternal dose of the AEDs so as to meet the therapeutic benefits (Emanuela Voinescu *et al.,* 2018). As a result of this, there is an increased *in-utero* exposure of AEDs to the developing fetus (Emanuela Voinescu *et al.,* 2018). Subsequently, this would as well require an increased renal clearance of these AEDs from the maternal blood by her kidneys to reduce the AEDs plasma concentration as well as the exposure time of this drugs to the fetus.

Existing literature has established that drugs with molecular weight of less than 500 kilo Dalton and those that predominantly do not dissociate at physiologic PH completely pass through the placenta and though indirectly, protein binding and maternal variation in placental transporter protein expression also affect placental passage (Bank *et al.,* 2017). Further, existing literature indicates that lamotrigine has a molecular weight of 263.09g/mol thus it can diffuse freely through the placenta, it reaches a peak plasma concentration 1-3 hour thus reaching the fetus shortly after oral administration and it has a very long half-life of 24-34 hours leading to considerable *in-utero* exposure to the fetus (Yasam *et al*., 2016).

Trans-placental passage of lamotrigine through passive diffusion or non-saturable active transport are therefore suggested as the placental transfer mechanism of this medicine (Myllynen *et al.,* 2003). These properties are independent of maternal concentration of lamotrigine, whereas other physiochemical properties like lipid solubility, protein binding and degree of ionization also tend to affect placental transfer of lamotrigine by passive diffusion (Myllynen *et al.,* 2003). Teratogenicity caused by lamotrigine is due to its ability to rapidly and easily cross the placenta and as such maternal treatment leads to considerable exposure of lamotrigine to the

growing fetus (Selman *et al.,* 2016). Furthermore after lamotrigine crosses the placenta, it decreases fetal folate levels contributing to the development of congenital anomalies as reported by (Mobini *et al*., 2019). This agrees with a different study done by (Soni *et al.,* 2017) they reported that lamotrigine inhibits dihydrofolate reductase a critical enzyme in folate metabolism that catalyzes the reduction of dihydrofolate to tetrahydrofolate critical cofactor for biological processes.

Existing literature on teratogenicity of levetiracetam indicated that this drug crosses the placenta considerably because it has a molecular weight of 170.209g/mol (Kozłowski *et al.,* 2015) and it shows high levels of accumulations in the neonates with majority of it being eliminated by the kidney unchanged (Wlodarczyk *et al.,* 2012). Further studies have shown that levetiracetam reaches peak plasma concentration one hours after oral administration thus reaching the fetus after a very short period of oral ingestion of the drug and has a half-life of 6-8 hours after oral administration leading to a considerable *in-utero* exposure time to the developing fetus (Kozłowski *et al*., 2015). This therefore makes this medicine readily available to the developing fetus only one hour after administration. According to a study done by (Hernandez- Diaz *et al.,* 2014), they reported that after crossing the placenta, levetiracetam free radicals interferes with the endogenous bioelectrical mechanisms and voltage gradient that function as instructive cues guiding cell division, cell positioning and orientation, programmed cell death in the developing fetus.

2.5 The Teratogenic Effects of Prenatal Exposure to Antiepileptic Drugs on the Pregnancy Outcomes

The existing literature has reported that the prenatal exposure to AEDs is associated with a wide range of deleterious pregnancy outcomes that includes failure of the rats to become pregnant, poor maternal weight gain, increased fetal resorption, increased dead fetuses or decreased gross fetal parameters. This however is dependent on the category of anticonvulsant used where the first line anticonvulsants are reported to have more teratogenic effects on the pregnancy outcomes than the succeeding second or third generation anticonvulsants medicines(Singh *et al.,* 2019). Another recent study done on first generation antiepileptic medicines namely valproic acid,

carbamazepine and phenobarbital indicated that these drugs increase their teratogenicity in a dose dependent manner (Fujimura *et al.*, 2017).

In a separate study done by (Singh *et al.,* 2019), it indicated that first generation antiepileptic drugs are more teratogenic than the second generation antiepileptic drugs. It still went further and projected multifactorial inducing mechanisms like drug, dose and time of expose as being possible factor that may induce teratogenicity in humans and animals. Existing literature from a recent study done by (El-Gaafarawi *et al.,* 2015) on teratogenic effects of carbamazepine (CBZ) a first line anticonvulsant medicine at doses of 3.6mg and 10.6mg/100g body weight per day administered to pregnant rats for 15 days recorded abnormal fetal pregnancy outcomes as decreased fetal body weight, decreased crown-rump length and increased dead fetuses.

In a comparative study done by (Erisgin *et al.,* 2019) on teratogenic effects of second-generation antiepileptic drug oxcarbazepine (OXC) and gabapentin (GBP) it indicated that at a dosage of 100mg/kg/day of GBP, the rat dams failed to become pregnant and there was total fetal resorption or abortus. At 50mg/kg/day and 100mg/kg/day for OXC, the dams became pregnant but there was a preterm delivery observed for OXC treated group.

A similar study done on *in-utero* exposure to third generation antiepileptic drugs clobazam and eslicarbazepine acetate indicated that there was marked reduction in fetal weight where these effects were dose-dependent *(*Gupta *et al.,* 2019). Because of teratogenic effects of anticonvulsant medicines leading to poor pregnancy outcomes, continued use of AEDs throughout the pregnancy to control seizures is however risky since doses of these drugs are at time escalated so as to achieve their therapeutic benefit to the mother as reported by (Hill *et al*., 2010). Other studies have suggested that the risk of teratogenicity and poor pregnancy outcomes increases in a dose dependent manner (Wlodarczyk *et al*., 2012).

In a similar study done by (Tomson *et al.*, 2011) it agreed with these findings indicating that irrespective of the kind of anticonvulsant drug used by the pregnant woman, the dose and other variables influences the pregnancy outcomes in terms of

increased risk of major congenital malformations, therefore great care must be exercised to prevent or reduce this poor pregnancy outcomes to women potentially capable of bearing children and pregnant mothers.

2.6 The General Histo-Morphological Teratogenic Effects of Antiepileptic Drugs on the Developing Fetal Kidneys.

Existing literature on the histo-morphological teratogenic effects of AEDs indicate that anticonvulsant medicines do damage to renal tubules with increased lymphocytes deposition and varied damages to the glomeruli apparatus that tend to be atrophied and filled with hyaline material in the glomeruli pores (El-Gaafarawi *et al.,* 2015). A recent study done by (Hamdi *et al.,* 2017) on oxcarbazepine at a dose of 108mg/kg from gestation day 7 to gestation day 20 revealed histo-morphological teratogenic alternation of the renal corpuscles that included, a significantly atrophied glomeruli, widened capsular space in the bowman's capsule, hydrophobic degeneration of the tubules and cytoplasmic vacuole. A similar study done on effects of the valproic acid another (AED) at a dose of 400mg/kg and at gestation days 8^{th}, 9^{th} and 10^{th} days, indicated that there was degenerative changes in renal glomerular basal lamina and the primary and secondary foot processes of the podocytes (Erkan *et al.,* 2014).

2.7 The General Histo-Stereological Teratogenicity of Antiepileptic Drugs on the Developing Fetal Kidneys

Histo-stereological quantification of effects of AEDs and many other medicines given during nephrogenesis and their long term renal sequelae is yet to be established despite the fact that many drugs are known to affect kidney development (Schreuder *et al*., 2011). Though there is a comparative study that was done by (Elgndy *et al*., 2019)on effects of levetiracetam and lamotrigine on developing fetal albino rats, they only demonstrated the effects on the gross morphological features of the fetuses indicating that there was significant decrease in the bi-parietal diameter, crown-rump length and head length of the fetuses.

Previous studies however haven’t provided histo-stereological quantification of fetal viscera. As such, generally no data on the comparative histo-stereological quantification of viscera following *in-utero* exposure of the fetus to levetiracetam and lamotrigine is available. The actual histo-stereological and histo-morphological data on developing fetal kidneys is lacking and that is the data that this study is aiming to provide.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Setting

This study had two settings; the first study site was the university of Nairobi (OUN) department of Biological Sciences Chiromo Campus. In this first setting, all the procedures in animal handling and the animal experimentation that included breeding of the rats, randomization, feeding, weighing, drug administration, harvesting of the fetuses as well as harvesting of the fetal kidneys were done there. The second study setting was in the histology laboratory section in the Department of Human Anatomy of Jomo Kenyatta University of Agriculture and Technology –(JKUAT), where tissue processing for microscopy plus all the histo-morphological and histo-stereological analysis were carried out.

3.2 The Study Design

A Post-test only with a control experimental study design was adopted. This study design was adopted because apart from daily maternal weight, all the other measurements that included the fetal pregnancy outcome parameters, the histo-morphological and histo-stereological were done after the animal experimentation period was over making this study design the most suitable for this study.

3.3 The Study Sample

In carrying out the study, adult female albino rat dams *(Rattus norvegicus*) were used as the study models for levetiracetam and the lamotrigine treatment groups. All these rats used in the study were 9 weeks old and weighed between 220 ± 50 grams. This breed of Albino rats that were used as the study model were selected because of the following known scientific facts;

1. They have a big litter size of between 8-12 fetuses per rat.
2. They have low chances of spontaneous abortions as well as low incidence of spontaneous major and minor congenital defects.

3. They have a short breeding period of 21 days hence ease of obtaining a pure breed colony.
4. They are easily available in research animal houses.
5. They feed on a wide range of readily prepared rodent foods hence low cost of their maintenance during animal experimentation.
6. There is a huge amount of the scientific data on experimentation maneuvers with this breed of rats.
7. They are the breed of rats widely known to withstand a wide range of study medicines as they are quite resilient. (Bailey *et al.,* 2014).

3.3.1 The Description of the Albino Rats Breed (Rattus Novegicus) Used in this Study

The Abino rats breed (*Rattus Novegicus*) used in this study resemble the Japanese hooded rats in appearance where both male and female albino rats have red eyes and white fur. They are hence genetically identical from a common ancestor (Pritchett & Corning, 2016). For a long time in scientific research, these rats have been in use since they were the first mammalian species domesticated in research animal houses for research. Their life span is about 2-3.5 years (average 3 years), they develop rapidly during infancy period where they become sexually mature at about 4-5weeks in females and around 6-7 weeks in males.

They are confirmed to be sexually mature by vaginal opening in females and by balano-preputial separation in males(Yoshimura *et al*., 2005). Reproductive aging in the female rats occurs between 15-20 months of age (Pallav *et al.*, 2013) and their gestational period is generally estimated to range from 21-23 days. Their gestation period has 3 trimesters, with trimester one comprising the first 7 days after conception, second trimester begins from gestation day 8 to gestation day 14 and the third trimester starts from gestation day 15 to gestation days 21-23.

Palpating the bulging and enlarging abdomen, noticing weight gain, breast development and pregnant females making a nest are good indicators of pregnancy which on day 14 are noticeable. The usual number of fetuses born by a normal healthy pregnant rat range from 3 to 14 fetuses and these fetuses are usually born

deaf and blind and weaning usually occurs about 3 weeks later. Male rats usually have a larger body size than females and are about 9 to 11 inches long.

The 30 adult female rats used for each study medicine were a pure breed colony bred in the animal house of Nairobi university department of veterinary sciences Chiromo campus. Since one male was allocated two females for mating purposes in the current study, 15 sexually mature male albino rats of the same breed as the females were used for mating with sexually mature female albino for each study medicine in this study.

3.4 Sample Size Determination

The samples used for this study were in two levels as follows:

3.4.1 Sample Size Determination on the Number of Mature Female Rats Used for the Study

In determining the sample size of the 30 mature female albino rats per each of the study categories of either the lamotrigine or the levetiracetam treated groups, the resource equation formula used was n = DF/k + 1, with an acceptable range of the degrees of freedom (DF) on the error term taken to be between 10 and 20(Charan *et al.*, 2013) where;

n = number of subjects per group

DF =degree of freedom,

k = number of groups, and

Therefore, n=20/10+1 =3.

To get the total number of 30 mature female albino rats used for each of the two-anticonvulsant study medicine, the number of subjects per group were multiplied with the number of groups (3*10=30).

3.4.2 The Number of Fetuses Used for Histological Analysis

After sacrificing all the rats on the gestational day 20 (GD_{20}), three fetuses from each rat were used for histo-morphological and histo-stereological analysis. Simple random sampling method was used in selecting these three fetuses from the entire litter size harvested per rat where all the fetuses were assigned numbers then the selection of the three fetuses was random with replacement of the numbers to give all the fetuses equal chances of being selected. Three fetuses selected per rat multiplied with a total of thirty mother rats gave a total sample size of 90 fetuses. Therefore 90 fetuses were used for each study medicine and a total of 180 fetuses were used for the entire study (90*2=180). The remaining fetuses were fixed in 10% formaldehyde for future use.

3.5 The Criteria for Selecting the Study Subjects

3.5.1 Inclusion Criteria

- ✓ All the rats that were 9 weeks old and weighing between 220 ± 50 grams from a pure colony.
- ✓ All the rats that tested positive for pregnancy in the morning after being left to mate with a male of the same pure colony overnight.
- ✓ All healthy pregnant rats.

3.5.2 Exclusion Criteria

- Any pregnant rats that showed any signs of sickness before the administration of anticonvulsant medicines.
- Any animals that become sick in the course of the experiment either in the control group or in the treatment group following administration of any of the treatment medicines.
- All fetuses whose mothers became sick in the course of experimentation.

3.6 Breeding of Albino Rats

Breeding of the rats was done in Nairobi University animal house. This ensured that only pure breed male rat dams and female rat dams from the 3rd series breed of albino rats was used. They were serially in-bred until the 3rd series breed was obtained. It was from this third series pool that the sample size of 30 albino rats for each anticonvulsant medicine was obtained for the study.

3.7 Grouping of the Rats for Experimentation

The 30 rats in each of the study categories of either the lamotrigine or the levetiracetam were first randomly assigned into two broad study categories of 3 rats' control and 27 rats experimental. To evaluate the teratogenic effects of the two medicines based on dosages, the 27 rats in each of the experimental category was further subdivided into three study groups of 9 rats each as per the dosages as follows; the first 9 rats for the low dose lamotrigine (25mg/kg/bwt) or low dose levetiracetam treatment group (1000mg/kg/bwt), the second 9 rats for the medium dose lamotrigine (262.5mg/kg/bwt) or medium levetiracetam treatment group (2000mg/kg/bwt) and the last 9 rats for the high dose lamotrigine (500mg/kg/bwt) or high dose levetiracetam treatment groups (3000mg/kg/bwt).

To further evaluate the nephron-teratogenic effects of the two medicines based on the time of exposure, the 9 rats in each of the three dose groups of low, medium and high were further randomly subdivided into three sub-groups of 3 rats each based on the trimesters of exposure as follows; the first 3 rats for trimester one (TM_1), the second 3 rats for trimester II(TM_2) and the last 3 rats for trimester III(TM_3) as shown in **(Figure 3.6 and figure 3.5).** After randomly assigning the rats into the two study groups they were then put into their respective experimental polystyrene rat cages for 3 days to allow them acclimatize.

The Grouping of Animals in the Lamotrigine Treated Groups

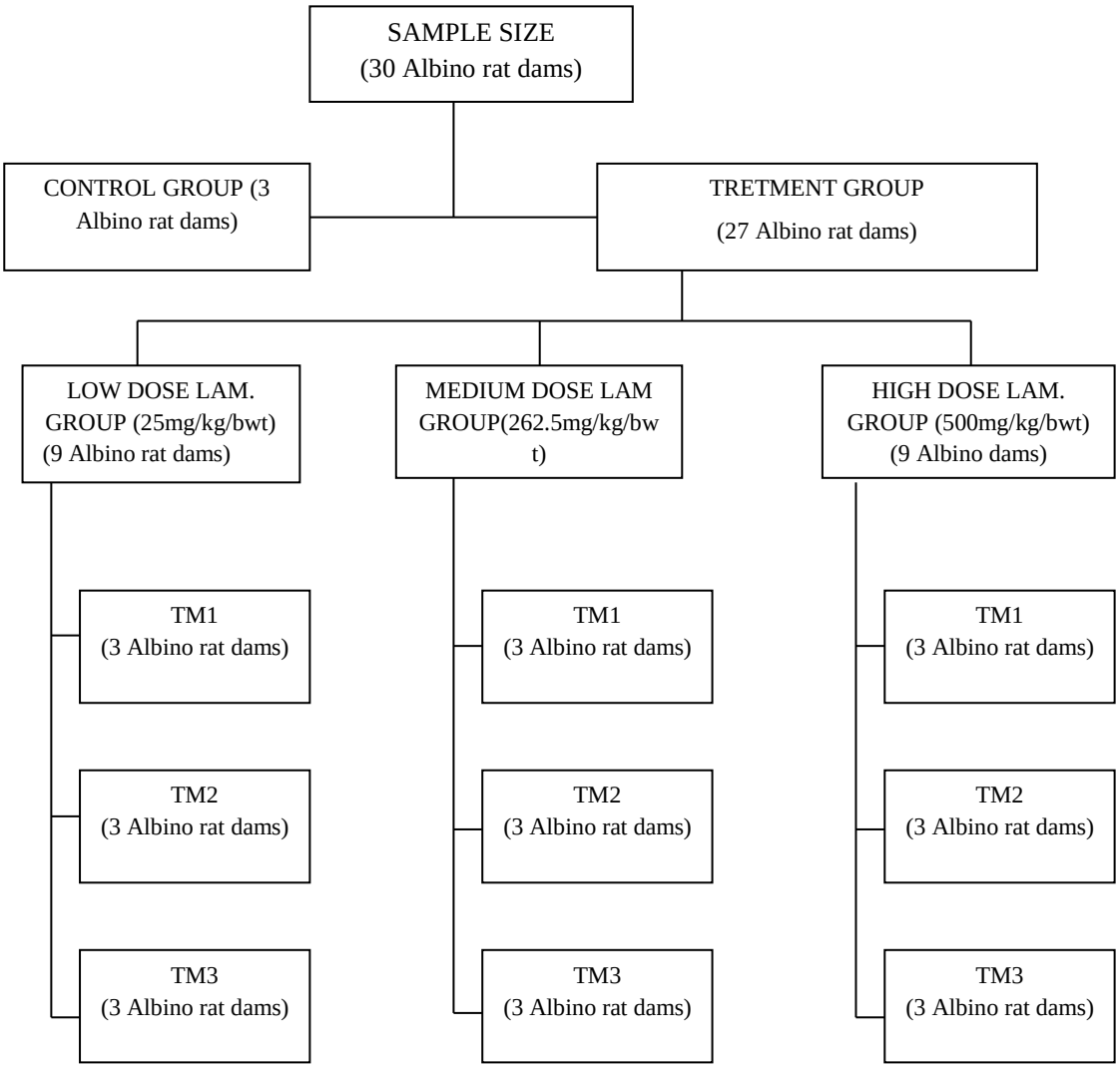

Figure 3:1: The Grouping of the 30 Albino Rat Dams for Lamotrigine Study Groups of 3 Rats Control and the Treatment Sub Groups of 3 Rats Each.

The animal grouping in the Levetiracetam treatment group

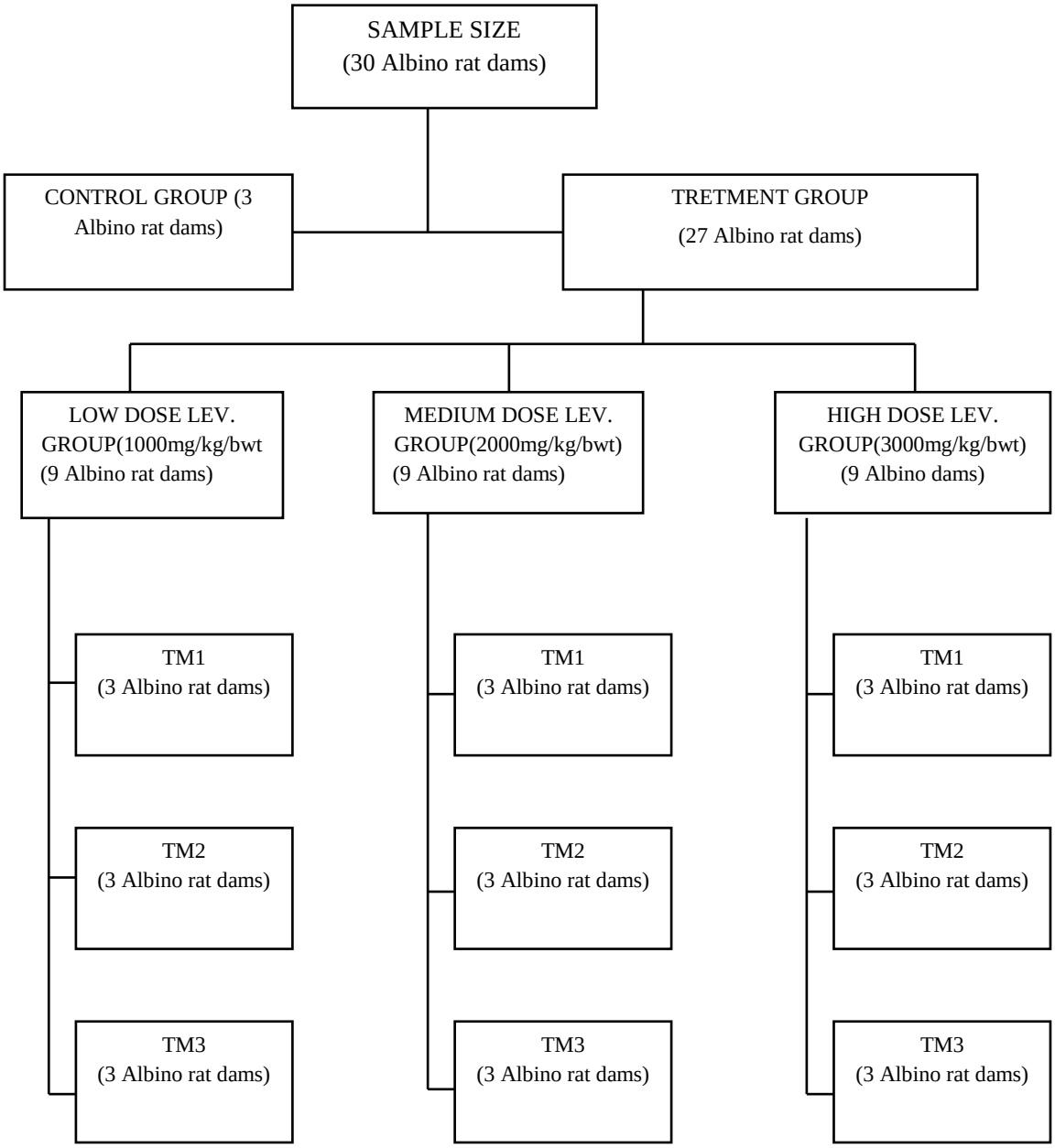

Figure 3:2: The Grouping of the 30 Albino Rat Dams for Levetiracetam Study Category Into the 3 Rats Control and Treatment Sub Groups of 3 Rats Each.

3.8 Feeding and Handling

(a) Feeding of Rats

All the 30 rats in both the two study categories of either lamotrigine or levetiracetam were fed on a standard rat pellets diet containing; 55% carbohydrates, 35% proteins, 5% fibres, 2% trace minerals, 2% vitamins and water *ad libitum* for the entire study period. This standard rat pellets were obtained from Unga Farm Care limited-located in Nairobi, Kenya, which is an authorized dealer in animal feeds production.

(b) Handling of Rats

Each morning the general health assessment and the feeding habits of the rats was done at 0900hrs, and any sick rat identified was isolated. Weight taking was done daily using high precision weighing scale **(figure 3.3(A))** and these weights recorded. Changing of soiled beddings, cleaning of cages, cleaning of the plastic drinking bottle and replacing the drinking water was done on a daily basis. The animal behaviors were assessed daily to ensure that they withstood the rigors of cleaning and regular handling.

To maintain the highest standards of hygiene and comfort to the animals, the cages used to house the rats had smooth impervious surfaces with minimal ledges, angles, corners and with no overlapping surfaces. This reduced accumulation of dirt, debris and moisture and didn't impair cleaning and disinfection process **(figure 3.3(B)).** Any cage that was broken or had sharp edges that could accidentally entrap the rats or their appendages or any cage that was considered unfit for the research work was changed.

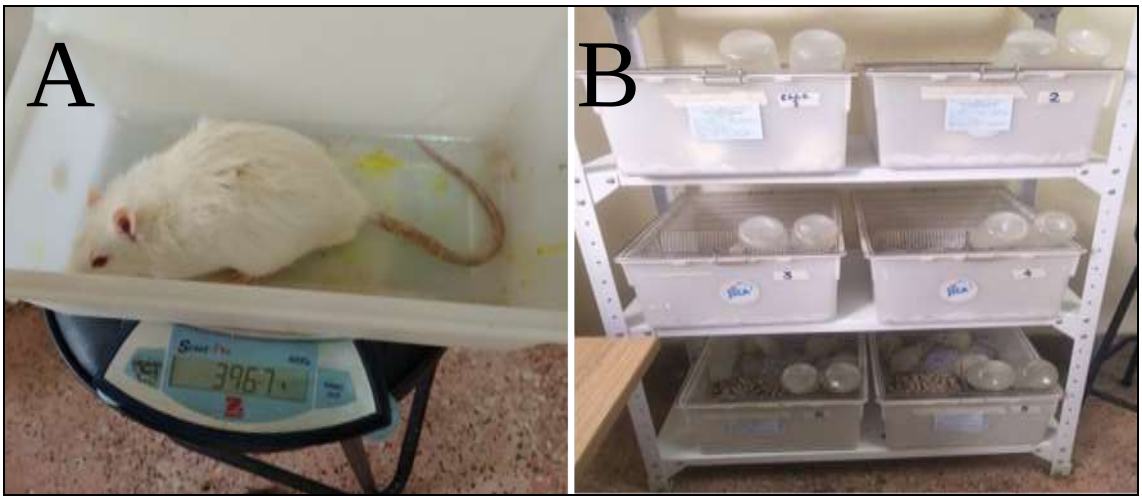

Figure 3.3: (A, B) Weight Taking and Rat Cages.

KEY: (a) daily weighing of the rats was done using high precision weighing scale.

(b)cages and bottles used for housing the rats.

(c) The Occupational Safety Measures

To avoid animal to human transmission of infections, protective gears were used every time when handling the animals. The safety measures included; a foot dip filled with a disinfectant was placed at the main entrance to the animal house to ensure anyone entering the animal house dipped their feet in it when entering or when leaving the animal house. The gate to the animal house was always kept under lock and key to avoid access by strangers to the animal house during the entire experimentation period.

Short-sleeved white laboratory coat and disposable gloves were always used to prevent any microbial transmission. Nose masks were used to prevent airborne aerosol spillages. Regular disinfection of the cages was done daily to prevent growth or spread of any form of microorganisms. Clear legible first aid steps to be followed were fixed on the walls inside the animal house.

3.9 Mating and Confirmation of the 1st Day of Pregnancy

For mating purposes, one male rat was introduced into a cage with 2 female rats overnight and then the males removed and returned to their separate cages the following morning. Pregnancy confirmation was done soon after the female rats spent one night with the males in the same cage. To confirm that mating had taken place, a wet cotton wool smear was gently inserted into the vagina rolled gently while inside the vagina, removed then swabbed on a glass slide and stained. The presence of sperms in the wet slide smear upon observing them under the microscope confirmed that coitus had taken place. To subsequently confirm fertilization and the first day of pregnancy, it was confirmed by estrous changes after taking a vaginal swab where the presence of huge polyhedral epithelial cells in the smear confirmed the estrous change indicating fertilization and the first day of pregnancy.

3.9.1The Procedure to be Followed in Determination of Pregnancy

After being left for 12 hours to mate, fertilization or pregnancy was tested through the following procedure;

1. Using a gauze holder, the rats were restrained and held by the back.
2. Using a micro syringe, a blunt tipped disposable pipette with a 1ml of saline was introduced into the rat's vaginal cavity.
3. Using a cotton tipped moist swab, a phosphate buffered saline was gently inserted into the rat's vaginal cavity.
4. Very gently a cotton swab was gently rolled in the vaginal canal before withdrawing to avoid injuring the animal vaginal walls.
5. Then the obtained moist swab was rolled onto a clean glass microscope slide after withdrawing from the rat's vaginal canal.
6. Before staining, 95% ethanol was sprayed on the smear on the microscope slide to fix the specimen on the slide.
7. One time dipping of the slides in 100% alcohol was done and they were left to air dry for 5 minutes.
8. The slides were then stained with Giemsa stain since it is the recommended stain for spermatozoa and polyhedral epithelial cells.

9. The stained slides were then observed under a microscope at magnification of X40 and X100 to denote presence of polyhedral epithelial cells that were scattered as well as many neutrophils on the smear.

Rats that were not pregnant were allowed for another 24hours after which pregnancy test was repeated.

3.10 Drug Administration

Drug administration was done at a consistent time daily at 0930hrs. Using a metallic gastric gavage gauge 16, the drug was introduced into the stomach of the experimental albino rats. To ensure there was no process error arising in the animal experimentation, the drug administration was done by the investigator only.

3.10.1 Calculating the Doses of the Two Drugs

In calculating the animal doses for the two drugs, a simplified guide for conversion of animal dosages from human dosages was applied as explained by Nair & Jacob (2016), they stated that dose is equally related to body weight although it is not the only factor which influences the scaling for dose calculation. The correction factor (Km) is estimated by dividing the average body weight (kg) of species to its body surface area (m^2). For example, the average human body weight is estimated to be 60 kg, and the body surface area is estimated to be 1.62 m^2. Therefore, the Km factor for human is calculated by dividing 60kgs by 1.62, which is 37. The Km factor values of various animal species is used to estimate the HED as:

HED mg / kg = Animal dose mg / kg Animal K /Human K Eq.

As the Km factor for each species is constant, the Km ratio is used to simplify calculations. Hence, Equation 2 is modified as:

HED mg / kg = Animal dose mg / kg K ratio Eq.

The Km ratio values are already provided and are obtained by dividing human Km factor by animal Km factor or vice versa. The adult LTG dosages in human ranges between 25mg-500mg per day while LVT ranges between 1000-3000mg in divided

dosages (Warshavsky *et al*., 2016),(Abou-Khalil, 2008). Both medicines were obtained from the government chemist in Nairobi considering their batch number and reconstituted using distilled water.

In calculating the lamotrigine and levetiracetam doses of either low, medium or high doses of the two study medicines, the conversion formula from human to animal dosages that entails multiplying the human equivalent dose in mg/kg/bwt by a constant ratio of 6.2 as determined by toxicological studies and lethal doses of levetiracetam and lamotrigine was applied as follows.

3.10.2 Calculation of Lamotrigine Doses for the Rats

The minimum lamotrigine dose in humans is 25mg, medium dose is 262.5mg and maximum dose is 500mg, while the average weight of an adult human is approximately 60kg.

Calculating the LTG dosages for the high dose groups;

Humans have an average weight of 60kg.

Determining the high dosage lamotrigine group:

The high dose lamotrigine =500mg in humans with an average weight of 60kg/bwt

500mg in 60kgs body weight

500mg = 60kg,

X mg =1 kg

The dose of lamotrigine in X mg=1x500/60 = 8.333mg/kg

Converting human equivalent dose to a rat equivalent dose;

AED = HED x Km factor.

Therefore, for a rat weighing 1kg = 8.3mg/kg x 6.2 =51.6667mg/kg.

1kg = 1000g.

1000g = 51.6667mg

1g =? mg.

= 51.6667mg/1000 =0.0517mg

Therefore, each rat's weight in grams was multiplied by 0.0517mg to obtain the amount of high dose drug each rat was supposed to take.

Calculating the LTG dosages for the medium dose groups;

The medium dose Lamotrigine 262.5mg in humans with an average weight of 60kg/bwt

262.5mg in 60kgs body weight

262.5mg =60kg,

X mg = 1kg

The dose of lamotrigine in X=1x262.5/60 = 4.375mg/kg

Converting human equivalent dose to a rat equivalent dose;

AED = HED X Km factor.

Therefore, for a rat with 1kg = 4.375mg/kg x 6.2 =27.125mg/kg

1kg = 1000g.

1000g = 27.125mg

1g =? mg.

= 27.125mg/1000 =0.0271mg

Therefore, each rat's weight in grams was multiplied by 0.0271mg to obtain the amount of medium dose drug each rat was supposed to take.

Calculating the LTG dosages for low dose groups;

Low dose lamotrigine in humans is 25mg/kg/ bwt

25mg in a 60kg/bwt

25mg = 60kg/bwt.

X mg = 1kg

X=1x25/60 = 0.4167mg/kg

Converting human equivalent dose to a rat equivalent dose;

AED = HED x Km factor-constant at 6.2

Therefore, in a rat of 1 kg = 0.4167mg/kg x 6.2 =2.5833mg/kg/bwt

1kg = 1000g.

1000g = 2.5833mg

1g =? mg.

= 2.5833mg/1000 =0.0026mg

Therefore, each rat's weight in grams was multiplied by 0.0026mg to obtain the amount of low dose drug each rat was supposed to take.

3.10.3 Calculation of Levetiracetam Doses for the Rats

The lowest dose for levetiracetam in humans is 1000mg, medium dose is 2000mg while the highest dose is 3000mg.

Calculating doses for the high dose levetiracetam groups;

The highest levetiracetam dose in humans of an average weight of 60kgs= 3000mg

3000mgs in 60kg/bwt

3000mg = 60kg

X mg = 1 kg

X = 1x 3000/60 = 50mg/kg

Converting the human equivalent dose to a rat equivalent dose;

AED = HED X Km factor (6.2)

Therefore, for a rat 1kg = 50mg/kg x 6.2 =<u>310mg/kg/bwt</u>

1kg = 1000g.

1000g = 310mg

1g =? mg.

= 310mg/1000 =0.310mg

Therefore, each rat's weight in grams was multiplied by 0.310mg to obtain the amount of high dose drug each rat was supposed to take.

Calculating doses for the medium levetiracetam groups;

The medium levetiracetam dose in humans with an average weight of 60kgs = 2000mg

2000mg =60kg

X mg = 1 kg

X = 1x 2000/60 = 33.333mg/kg

Converting the human equivalent dose to a rat equivalent dose;

AED = HED X Km factor (6.2)

Therefore, for a rat 1kg = 33.333mg/kg x 6.2 =206.6667mg/kg/bwt

1kg = 1000g.

1000g = 206.6667mg

1g =? mg.

= 206.6667mg/1000 =0.2067mg

Therefore, each rat's weight in grams was multiplied by 0.2067mg to obtain the amount of medium dose drug each rat was supposed to take.

Calculating dose for the low levetiracetam group;

The lowest human dose of levetiracetam is 1000mg/kg/bwt

1000mg will be equivalent to = 60kg average body weight

1000mg = 60mg

X mg = 1 kg

X = 1x 1000/60 = 16.6667mg/kg

Converting human equivalent dose to a rat equivalent dose;

AED = HED X Km factor (6.2)

Therefore, for a rat 1kg = 16.6667mg/kg x 6.2 =103.3333mg/kg/bwt

1kg = 1000g.

1000g = 103.3333mg

1g =? mg.

= 103.3333mg/1000 =0.1033mg

Therefore, each rat's weight in grams was multiplied by 0.1033mg to obtain the amount of low dose drug each rat was supposed to take.

The dosages for the low, medium and high treatment groups were as follows for both the two medicines: (i) the low dose doses for lamotrigine was 25 Mg/kg/bwt and levetiracetam 1000 Mg/kg/bwt. (ii) the medium dose was lamotrigine 262.5Mg/kg/bwt and levetiracetam medium dose 2000Mg/kg/bwt (iii) high doses lamotrigine was 500Mg/kg/bwt lamotrigine and 3000Mg/kg/bwt levetiracetam.

3.10.4 Administration of Lamotrigine and Levetiracetam to the Rats

Both lamotrigine and levetiracetam were administered on daily bases at 0930hrs using the following procedure:

1. The rats were held at the neck region with the left hand and they were wrapped with a piece of cloth to avoid them from soiling the investigators clothing's.
2. With the rat mouth facing the investigator, a gavage needle gauge 16 was gently introduced into the mouth of the rat turning it gently to pass the esophageal constrictions and the cardiac sphincter of the stomach.
3. The treatment drug bolus was put in the stomach of the rat.
4. Then the gavage needle was gently removed.

The periods for the drug exposures based on the trimesters for both lamotrigine and levetiracetam were hence as follows; (i) For the 3 rats for Trimester I (TM1) they started the treatment from gestation days one to gestation day 20; (ii) for the 3 rats for trimester II(TM2) started the treatment from gestation day 7 to gestation day 20 and; (iii) for the 3 rats for trimester III (TM3) started the treatment from gestation day 14 to gestation day 20 in each study group.

3.11 Humane Sacrificing of the Pregnant Rats, Harvesting of the Fetuses and Fetal Kidneys

The following procedures were applied in the humane sacrificing of the pregnant rats at GD_{20}:

1. The pregnant rat was humanely sacrificed by putting it into the bell jar with a tight-fitting lid which was connected with carbon dioxide source. The rat stayed in the jar for between 10-15 minutes until they died.
2. The rat was then removed from the bell jar and mounted onto a dissecting board fitted with a dissecting tray using mounting pins with dorsal side on the board and the ventral (abdomen) facing upward **(figure 3.4).**
3. Using a pair of scissors and forceps the abdomen was opened along the Linea alba in the ventral sagittal plane from the xiphoid process to the pubic symphysis.
4. 400mls of normal saline was introduced to the left ventricle of the heart using a perfusion needle that was inserted to the heart while connected to the perfusion set containing the normal saline.
5. Blood was cleared from the rat by introducing a physiological normal saline (200mls of 0.85mol/litre) through the left ventricle of the heart (saline flows by the force of gravity from one of the drips set hence it was able to clear the blood.)
6. When the blood was sufficiently cleared, the normal saline drip was removed (the needle in the left ventricle of the heart was left in situ (left in position of the heart) and then the fixative that is 10% formaldehyde was then introduced.

7. To confirm whether fixation had fully taken place the firmness of the tail was checked as a sign of effective fixation of the animal.
8. The drip set was disconnected and the perfusion needle removed from the heart.
9. The rat was later immersed in a container with 10% formalin to continue with fixation for 24 hours

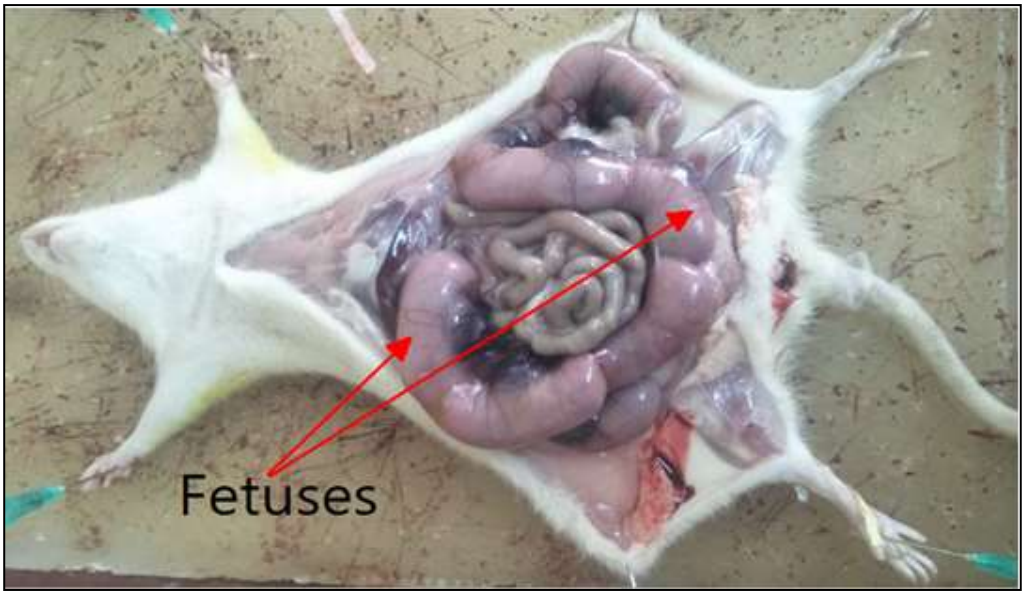

Figure 3.4: Opening of Anterior Abdominal Wall Along the Linea Alba for Fetal Harvesting.

3.11.1 The Harvesting of the Fetuses

The harvesting of the fetuses was done by opening the anterior abdominal wall of the mother along the linear alba from the symphysis pubis to the xiphisterna joint to expose the uterine horns. The fetal positions were observed within the horns, then the numbers of live and dead fetuses were determined by use of a gentle probe where movements of the fetuses inside the horns denoted the life fetuses while no movement denoted the dead fetuses.

The litter size was then noted and recorded. The numbers of devoured endometrial glands and resorbed fetus were also counted and recorded **(figure 3.4).** To expose the

fetuses, the uterine horns were excised along the anti-mesomentrial border using a pair of scissors. Utilizing the blunt end of a pair of forceps, fetuses and placentas were gently removed in totality from the uterus.

After harvesting the fetuses and before fixing them in formaldehyde fixative, the following fetal parameters were taken; **(i)**The general fetal morphology and abnormalities of the fetuses were examined and recorded. **(ii)** the placental weights, **(iii)** the fetal weights, **(iv)** the crown-lump length and **(v)** the head circumferences were taken and recorded**. (Figure 3.5 (A, B, C)**

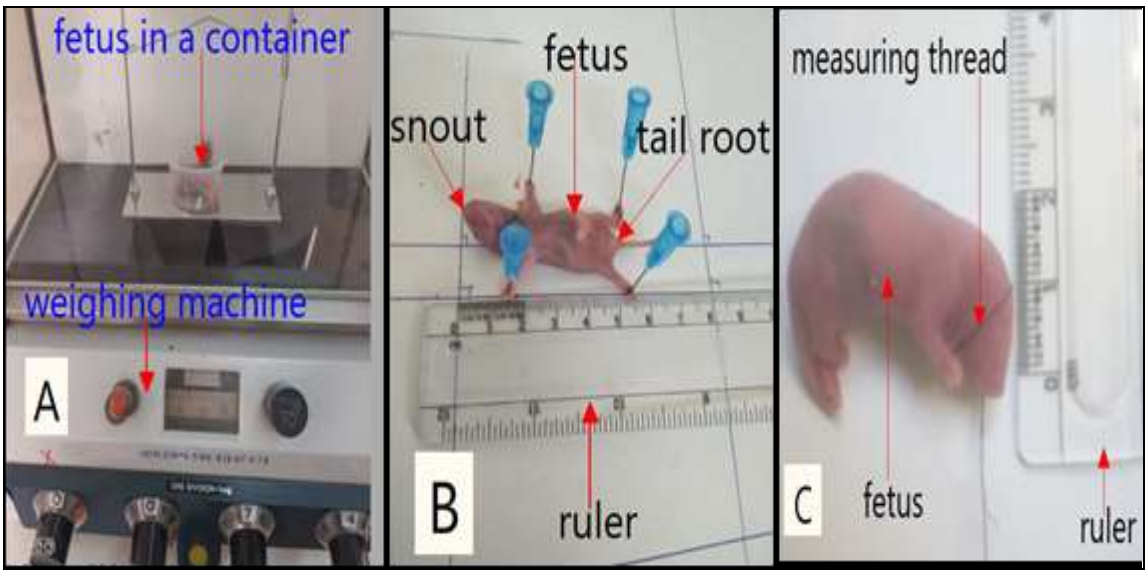

Figure 3.5: (A, B, C) Showing How the Gross Fetal Parameters Were Measured.

***Key**: **(A);** shows how the fetal weight using a high precision weighing scale was done.*

***(B);** Shows how the measuring of the crown-rump length in centimeters using a calibrated ruler was done.*

***(C);** shows how the measuring of the bi-parietal head circumference using a thread tied around the skull slightly above the ears and then straightening the thread alongside the ruler to get the actual measurement.*

3.11.2 The Harvesting of the Fetal Kidneys

The harvesting of the fetal kidneys was done as follows: -

A. Procedure for Harvesting Fetal Kidneys

Kidneys from the three fetuses that were selected per rat were harvested for both histo-morphological and histo-stereological analysis as per the following procedure;

1. The fetuses were put in a jar fitted with air tight lid and was connected to carbon dioxide source and they stayed inside this jar for 15 minutes and were removed after they died.
2. With the dorsal side facing the board, all the three fetuses were mounted on dissection board using pins.
3. With a pair of scissors and forceps the layers of the abdominal muscle were dissected along the midline to expose the abdominal viscera of the fetus.
4. Since the kidneys lie in the retroperitoneal space of the abdomen, the parietal peritoneum of the posterior abdominal was opened carefully in the center along the vertebral column, retracted carefully to avoid damaging the kidneys.
5. Using a magnifying glass, the whole fetal kidneys were identified.
6. Then the entire kidneys were excised at the level of the renal pelvis.
7. The kidney gross morphometric parameters were taken which included kidney weights which were carefully taken using high sensitivity measuring scale, the kidney lengths, the kidney widths and initial total kidney volumes **(Figure 3.6 A, B, C.)**
8. The kidneys were then immersed in 10% formalin solution to enable fixation to later proceed with processing for light microscopy and histo-stereology.

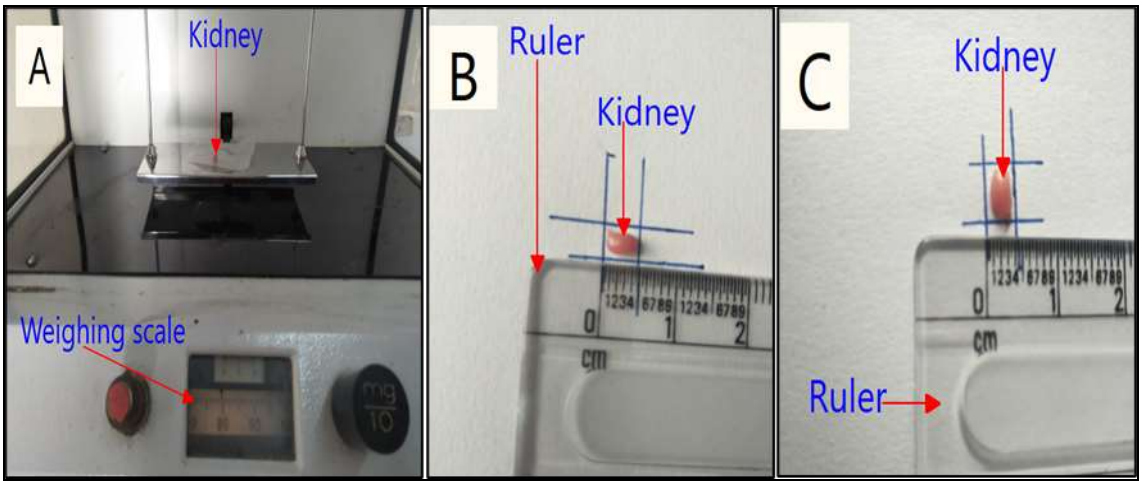

Figure 3.6: Showing How Gross Kidney Parameters Were Measured

Key: ***(A)*** *taking the kidney weight using high sensitivity measuring scale.*

(B) *Measuring the kidney lengths using a ruler in cm.*

(C) *Measuring the kidney widths using a ruler in cm.*

B. Processing Fetal Kidneys for Light Microscopy and Stereology

Procedure used;

1. Fixation of fetal kidneys was done with 10% formalin solution for 24 hours.
2. Dehydration was done in an ascending grade of alcohol (50%, 60%, 70%, 80%, 90%, 95% and 100% each for a period of one hour.
3. They were cleared by immersion with xylene for 12 hours.
4. They were infiltrated with paraffin wax for 12 hours at 56^0 Celsius
5. Orientation of fetal kidneys was done in longitudinal axis
6. They were embedded in paraffin wax on the wooden blocks
7. Trimming-off the excess wax was done to expose the entire length of the fetal kidney tissue.
8. Leitz sledge rotary microtome was used to cut 5µm thick longitudinal sections
9. To spread the tissue, they were floated in water at 37^0 Celsius.
10. A thin film of egg albumin was applied to the glass slides by use of a micro-dropper and cut sections stuck on them.

11. The stuck slides were then dried in an oven at 37^0 for 24 hours
12. Blinding by coding all the slides was done in the absence of the investigator.
13. Hematoxylin and Eosin were used for staining.

3.12 Quantitative Stereological Analysis

Quantitative stereological analysis of the kidney involved evaluation of **(i)** initial total kidney volumes, **(ii)** calculated Cavalieri volumes, **(iii)** stereological correction for kidney tissue shrinkage and **(iv)** medullary and cortical volume densities.

3.12.1 The Calculation of Initial Total Kidney Volume by Use of Archimedes Water Displacement Method

In calculating the initial kidney volumes, the Archimedes principle (water displacement method) was used. The total fetal kidney volumes were hence determined by immersing them in insulin syringe (which had its plunger removed) containing isotonic saline and the amount of fluid displaced was then measured by the amount of fluid displaced upward after immersing the fetal kidneys inside the syringe. The initial total fetal kidney volume was hence calculated as the difference between readings after immersion subtracting the initial readings before immersing the fetal kidneys in mm^3.

3.12.2 The Calculation of Total Kidney Volume by Use of Cavalieri Point Counting Method

The Cavalieri point counting method on the total kidney volume was applied in calculating the kidney volumes after fixation and processing for histology. The kidney was then fully sectioned into a series of slices where twenty sections of 5µm thickness from each kidney were selected using systematic uniform random sampling with a random start. These glass slides were mounted on microscope's stage and viewed at a magnification of X10 using 20megapixel high resolution digital swift camera where digital images were captured and uploaded into the computer swift software for analysis and labeling. These images were superimposed in a STEPanizer

tool for point counting to get the slice surface area. A consistent guard area was set to be used throughout the entire experiment.

The following are the steps that were followed in calculating the final total kidney volume using Cavalieri point counting method.

1. Prepared kidney sections of 5µ thickness were used.
2. Spacing for the point probe was determined.
3. In each section, the point probe was randomly tossed.
4. All points that were hitting the region of interest were counted keeping a tally of counts per section.
5. Cavalieri formula was used to calculate the volume.

The kidney volume was then calculated as the product of the sum of the cut slices surface areas multiplied with the slice thicknesses (starting with the first to the last section). The sum of points that hit the structure was estimated (the area of the structure 'i'). Point counting was done using the STEPanizer software as determined by Bolender and Weibel (1973). A test system that uses a transparent cast grid was superimposed on the computer screen projected images, whereby all points hitting the area of interest within the inclusion line were counted.

The following formula was then used to calculate the total kidney volume (**figure 3.7**);

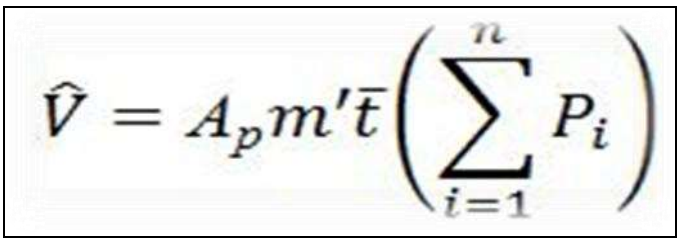

Figure 3.7: The Formula Used to Calculate the Cavalieri Kidney Volume (Welniak-Kaminska *et al.,* 2019).

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

3.12.3 The Stereological Correction for Kidney Tissue Shrinkage

In calculating the total percentage kidney tissue shrinkage due to histological procedures, shrinkage was calculated by subtracting Cavalieri kidney volume calculated after sectioning the kidney from the initial kidney volume calculated by use of Archimedes principal method of water displacement using the following formula;

Shrinkage = Volume before-Volume after

Where: Volume before is Archimedes volume while volume after is the Cavalieri volume.

3.12.4 Determination of Volume Densities of the Kidney Cortex and Medulla Using Cavalieri Method of Point Counting

In determining the volume densities of the kidney cortex and kidney medulla, Cavalieri method of point counting using the STEPanizer tool was used **(figure 3.8).** The number of points falling on the area of interest were counted and compared with the points falling on the entire kidney and the following formula was therefore used;

Est Vv = **P** (Part)/**P** (ref)

Where;

Est Vv -Estimated volume density

P (part) - All points falling in the area of interest (medulla structures / cortical structures).

P (ref) - All points falling on the entire kidney (reference space)

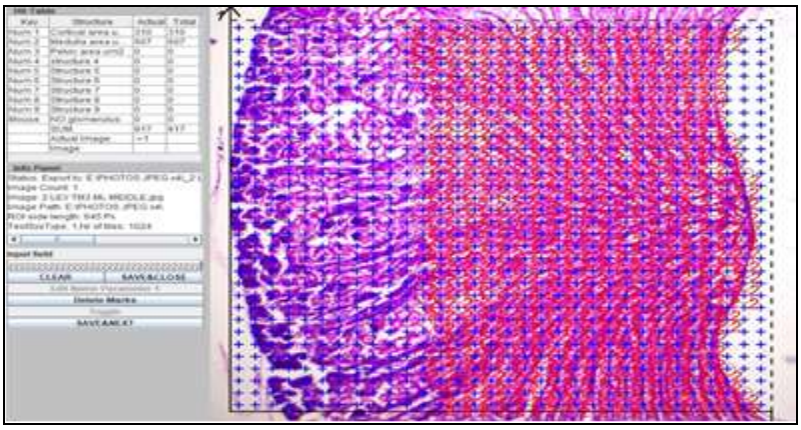

Figure 3.8: A Kidney Photomicrograph Superimposed on the Stepanizer Software Tool for Calculation of the Cortical and Medullary Volume Densities Using Cavalieri Point Counting Method.

3.13: Materials and Procedure of Taking Photographs

a. Materials Required

1. A swift digital camera
2. A microscope
3. External hard-drive
4. Glass slides

b. The Histology Photomicrographs Image Capturing and Labelling.

The following procedure was used in the histology photomicrograph images capturing and labelling.

1. Mounting of histological slides on the BH-Olympus light microscopic stage.
2. Adjustment of focus until the image came on focus.

3. Appropriate magnification field was selected.

4. The viewing of the Photomicrograph images done as section of the appropriate views for taking of photo-micrographic images done.

5. Capturing of the appropriate histological photomicrographic images was done using 20megapixel high resolution digital swift camera fitted on the BH-Olympus microscope. Images were taken from the lowest to the highest microscope magnifications of (X40, X100 and X400) as shown in ***figure 3.9 below.***

6. Then the transfer of the photo-microphotographs into the computer by use of a 32 GB-memory card.

7. Then this was followed with the uploading the captured photomicrographs in the computer swift software program for labelling as shown in ***figure 3.9 below*.**

Figure 3.9: A BH-Olympus Microscope Fitted with Swift Camera and a Computer with Installed Swift Imaging Software for Analysis of the Histo-Photomicrographic Images.

3.14 Data Management and Statistical Analysis

The quantitative data that was recorded in the structured data capture checklists that included; the dairy maternal weights, the litter sizes, the numbers of resorbed endometrial glands, devoured fetuses, embryolithalities, fetal weights, crown lump lengths, head circumferences and quantitative histo-stereological data was entered in the Microsoft excel spread sheets windows 2010 version 16 for data cleaning and running of descriptive statistics. The qualitative histo-morphological data that was collected in form of histo-photo-micrographs was entered into the computer using the swift image-J version 3.0 software Chicago Illinois.

Both the quantitative and qualitative histo-morphological data was kept safely under lock and key for further analysis. The quantitative data from the Microsoft excel spreadsheet was then transferred into the SPSS program version 25 for windows for statistical analysis. The descriptive statistics were expressed in form of means $\pm$ standard deviation (SD) of the means. The univariate and the bivariate regression analysis to comparatively evaluate how the three independent variables of **the drug, dose and time** influenced the dependent variables of the maternal and the fetal pregnancy out comes as well as the histo-quantitative parameters was analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison tests.

The multivariate regression 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 histo-quantitative parameters assessed was done by using Multiple Analysis of Variance (MANOVA). All results whose $P<0.05$ were considered statistically significant.

3.15 Ethical Approval

In carrying out the study the ethical approval was sought from the Animal research Ethics Committee at the University of Nairobi, Faculty of veterinary medicine, department of veterinary anatomy and physiology; certificate REF NO: FVM BAUEC/2021/323 (*as attached in appendices section*). This was done after

successful completion of a training on animal handling that was done in the Small Animal Facility for Research and Innovation (SAFARI) in Jomo Kenyatta University of Agriculture and Technology-JKUAT and thereafter being certified qualified to handle the experimental animals.

The specified animal ethical handling procedures were adhered to in all the processes of carrying out the research including; breeding of rats, handling of rats, feeding of rats, sacrificing and harvesting of fetal organs as they were stipulated in the protocols of animal ethics and as outlined by guidelines for care of laboratory animals.

CHAPTER FOUR

RESULTS

4.1 Introduction

The findings of this study are presented in line with the study objectives. However, the findings of objective 4 that was to evaluate whether or not the observed teratogenic effects of the two medicines were both dose and time dependent are integrated in the findings of the first three objectives. [*NB> some tables were big and are going beyond the margins and in some cases, they spill over from one page to the next*].

The Maternal and Fetal Pregnancy Outcomes

4.2. Objective 1: The Comparative Evaluation on How the Prenatal Exposure to Varied Doses of Lamotrigine and Levetiracetam Influenced the Maternal and the Fetal Pregnancy Outcomes.

In assessing how the two medicines influenced the maternal and fetal pregnancy outcomes, the findings are presented in two stages as follows:

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

Stage 2: The comparative findings on how the two medicines influenced the fetal pregnancy outcomes.

4.2.1: Stage 1: The Comparative Findings on the Maternal Pregnancy Outcomes

In determining how the two medicines influenced the maternal pregnancy outcomes, the following parameters were evaluated; **(i)** the mean maternal terminal weights, **(ii)** the total maternal weight gain, and (ii) the terminal placental weights. As such, a univariate and the bivariate regression analysis was subsequently done to establish

how the three independent variables namely **the drugs**, **the doses** and **the time of exposure** influenced the three maternal pregnancy outcome parameters within groups and between different study groups of both lamotrigine and levetiracetam treated groups. The intragroup and intergroup comparative findings were as follows.

(i) The means of the three maternal pregnancy outcome parameters including; the mean maternal terminal weight, the total maternal weight gain, and the terminal placental weights were significantly lower ($P<0.005$) in all the treatment groups of both the lamotrigine and levetiracetam treated groups when compared with the control. ***Table 4.1.***

(ii) Upon carrying out one way Analysis of Variance (ANOVA), to establish the differences in the observed significant levels between the treatment groups against the control, it was observed that, at the global level the treatment groups were remarkably different from the control as follows; [(i) mean maternal terminal weight =$F(18,38)=292.324$, $P=0.001$), (ii) total maternal weight gained during pregnancy=$F(18,38)=258.681$, $P=0.001$), and (iii) means terminal placental weights $F(18,38)=618.786$, $P=0.001$ respectively] ***table 4.1***.

(iii)Upon evaluating how the trimesters of exposure influenced the three maternal pregnancy outcome parameters, it was observed that, when the two medicines were exposed in trimester one (TM_1) and trimester two (TM_2) and particularly in the high and the medium doses the three maternal pregnancy outcome parameters were greatly reduced as compared to the means of the three maternal pregnancy parameters when the treatment was instituted at trimester three TM_3 (***table 4.1*** These finding hence points to the fact that the observed maternal pregnancy outcome parameters could have been due to perturbations of a prolonged nutritional disturbances to the mother, or due to GIT irritation or, due to prolonged disturbances in the nutritional assimilation process in the maternal GIT = (small intestines).

(iv). With regard to how the varied dosages of exposure influenced the three maternal pregnancy outcomes; it was observed that the medium and the high doses of both the lamotrigine and levetiracetam had the most deleterious influence to the three maternal pregnancy outcomes as compared to the low doses of both medicines. It was however notable that for all the lamotrigine treatment groups of low, medium and high they had more deleterious effects on the maternal pregnancy outcomes as compared to the effects of the same dose groups for the levetiracetam treatment groups. ***(Table 4.1)***

Table 4.1: The Comparative Means for The Mean Terminal Weight, Total Maternal Weight Gain and Then Terminal Placental Weight for the Treatment Groups Against the Control at TM1, TM2 &TM3.

Study groups	Dosage levels	Exposure to treatment	The comparative means for the terminal weight, total maternal weight gain and the terminal placental weights.		
			Mean terminal weight ± SD	Mean weight gain ± SD.	Mean terminal placental weight ± SD
Control	No treatment	-	388.33±.081	131.00±5.56	5.61±.03
	Low dose	TM1	334.33±6.02*	71.00±4.35*	4.95±039*
		TM2	351.33±1.53*	111.00±2.66*	5.29±.017*
		TM3	371.67±1.53	119.67±1.53	5.38±.039*
	Medium dose	TM1	274.33±2.31*	20.00±4.36*	4.66±.058*
Levetiracetam treated group		TM2	310.67±2.08*	67.00± 2.65*	5.10±.067*
		TM3	350.33± 4.51*	99.00±6.00*	5.37±.020*
	High dose	TM1	245.33± 3.79*	25.33±37.61*	4.27±.034*
		TM2	256.67± 2.87*	60.00±47.57*	4.73±.026*
		TM3	275.67± 2.52*	83.55±39.83*	5.24±.028*
	Low dose	TM1	296.33±1.15*	36.000±4.583*	3.54±.034*
		TM2	325.33±1.53*	75.000±11.00*	4.18±.012*
Lamotrigine treated group		TM3	333.33±10.59*	73.666±1.528*	4.45±.006*
	Medium dose	TM1	233.67±2.08*	21.666±4.163*	3.48±.057*
		TM2	266.66±2.52*	14.333±1.155*	4.03±.020*
		TM3	311.67±3.06*	56.000±3.464*	4.23±.067*
	High dose	TM1	245.33±3.79*	-55.000±1.732*	3.23±.020*
		TM2	256.67±2.89*	-25.666±2.887*	3.65±.006*
		TM3	275.67±2.52*	-24.000±18.35*	3.93±.058*
Comparison by ANOVA [F, P values]			F(18,38)=292.324 P=0.001	F(18,38)=258.681 P=0.001	F(18,38)=618.786 P=0.001

Key: values are expressed as means ± standard deviation of mean n=3 per group. The figures bearing the asterisk (*) means that they are significantly different with the control.

Upon carrying out a multivariate regression analysis between the two treatment groups by use of MANOVA to find out how the three independent variables [drug, dose and time of exposure] either acting alone, or when combined in two ways, or when combined in three ways influenced the overall/main global effects of all the three maternal pregnancy outcome variables either when combined together, or to a specific maternal pregnancy outcome parameter, the results are presented in three parts based on three stages on how the MANOVA analysis done, as follows:

Part 1: Are the findings on the overall global effects on how the three independents variables and their interactions (Acting alone, in two way or in three ways) influenced all the three maternal pregnancy outcomes parameters combined.

Part 2: Are the findings on the how each of the three independent variables and their interactions (alone, in two way or in three ways) influenced each of the three maternal pregnancy outcomes parameters.

Part 3: Are the pairwise comparative findings on how the two medicines influenced the maternal pregnancy outcome when exposed at the same dosage levels and in the same trimesters.

Part 1: The MANOVA Level 1 Findings on the Overall/ Global Effects on How the Three Independent Variables and Their Interaction Influenced on the Overall Maternal Pregnancy Outcomes Together

Upon completing the global stage 1 multivariate regression analysis using MANOVA and applying Wilks' Lambda statistical test to evaluate the overall/ global effects of the independent variables (drugs, trimesters and dosages) on the maternal pregnancy outcomes, the global main effects and their combined interaction effects (*), there was a notable significant interaction effects exacted on the three maternal dependent variables combined in different proportions of the independent variables of the drug, dose, and time of exposure as shown by the values of (partial Eta square (η^2) as shown in ***table*** 4.1 below as follows; -

(i) when each of the independent variables of the drug, dose and time of exposure were acting alone on the three maternal dependent variables

combined together, the overall/global main effects were as follows; (a) drugs, F(3, 36)=2421.21, P< .001 wilks ∧=.005, partial η^2 =.995. (b) doses; F(6,72)=130.73, P< .001 wilks ∧=.007 partial η^2 =.916. (c) trimesters; F(6, 72)=118.742 P< .001 wilks ∧=.008, partial η^2 .908. This meant that drug and dose acting alone had the greatest influence on all the three maternal pregnancy outcomes combined ***(Table 4.2)***

(ii) when combined in two ways that is any of two the independent variables [drug, dose and time of exposure] acting together on the three maternal dependent variables combined together, the overall/global main effects were as follows; Two-way combination interaction effects was noted between (a) drugs *trimesters; F(6, 72)= 9.004, P< .001 wilks ∧=.326 partial η^2 =.429, (b) dosages trimesters F(12, 95.539)= 23.461 P<0.0001 wilks ∧=.026 partial η^2 =.702, ***(Table 4.2)***

(iii)Three-way interaction effects among drugs*dosages*trimesters was noted as F(12, 95.539)=8.533, P<0.0001 wilks ∧=.146 partial η^2 =.474. ***(Table 4.2).***

Table 4.2: The MANOVA Level 1 Table on the Overall/ Global Effects of the Independent Variables and Their Interaction Effects on the Maternal Pregnancy Outcomes

Type of MANOVA evaluation at level 1	The comparative global effects assessed		MANOVA test statistic Wilks' Lambda	Statistics (F)	Hypothesis degree of freedom	Error degree of freedom	Sig.<0.05	Proportion of variance (Partial Eta Squared)
(i)test on whether the observed results were due to chance	Were the observed effects due to chance or the treatment	intercept	.000	211559.962[b]	3.000	36.000	.000	1.000
(ii)the individual main effects of the drugs, time of exposure and dosages on the maternal dependent variables	Were the observed effects due to drugs	Drugs	.005	2421.210[b]	3.000	36.000	.000	.995
	Were the observed effects due to varied drug doses	Doses	.007	130.725[b]	6.000	72.000	.000	.916
	Were the observed effects due to differing trimesters	Trimesters	.008	118.742[b]	6.000	72.000	.000	.908
	Were the observed overall effects due to interaction between varied doses and the drugs	Doses *Drugs	.874	.833[b]	6.000	72.000	.549	.065
(iii) Two-way interaction effects on the maternal dependent variables	Were the observed effects due to interaction between drug and differing trimesters	Drugs *Trimesters	.326	9.004[b]	6.000	72.000	.000	.429
	Were the observed effects due to interaction between varied doses and differing trimesters	Doses * Trimesters	.026	23.461	12.000	95.539	.000	.702
(iv)The three-way interaction effects.	Were the observed effects due to interaction between drugs, varied doses or differing trimesters.	Drugs *Doses *Trimesters	.146	8.533	12.000	95.539	.000	.474

a. Design: Intercept + Drugs + Dosages + TRIMESTERS + Drugs * Dosages + Drugs * TRIMESTERS + Dosages * TRIMESTERS + Drugs * Dosages * TRIMESTERS

b. Exact statistic

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

*Key, * means interaction effect*

Part 2: The MANOVA Level 2 Findings on How Each of the Independent Variable Plus Their Interaction Influenced the Individual Maternal Pregnancy Outcome Parameters

Upon performing **level 2 MANOVA analysis** on how each of the independent variables [**drug**, **dose** or **time of exposure**] plus their interactions influenced the individual dependent variable of the mean maternal terminal weight, the mean weight gain and the mean placental weights, there was marked significant differences on how each of the three independent variables either acting alone or when combined in two ways or when combined in three ways influenced the three maternal dependent variable in different proportions as indicated by the values of partial Eta (η^2) as follows:

(i) At the individual level, the global contribution for each independent variable to each individual maternal pregnancy outcome (maternal terminal weight, weight gain and placental weight) a statistically significant difference (P< .001) at different proportion of variance (partial Eta (η^2) was noted. From the three independent variables, the drug had the strongest contribution on placental weight at partial Eta (η^2) proportion of 99.4% but on maternal weight gain, still the drug was noted to have the weakest contribution at partial Eta (η^2) proportion of 94.7% **(*Table 4.3*).**

(ii) At two-way combinations, [i. e when any of the two independent variables were combined and their statistical significance contribution evaluated] as in between; **drug*dose**, **drugs*trimesters** as well as between **dosages*trimesters**, it was notable that at two-way interaction the severest effects was between **dosage*trimesters** on placental weight at partial eta (η^2) proportion of 86.5%, while the weakest effects was between **drug*dosage** on placental weights at partial eta proportion of (η^2) 3.6% **(*Table 4.3*).**

(iii) On further analysis, at three-way statistical significant interaction effects of **drugs*doses*trimesters** was noted influencing the individual maternal pregnancy outcomes in different proportions as listed below (a) maternal terminal weight F(4,38)=5.283, P<0.001, partial Eta (η^2)=.719, (b) weight gain F(4,38)=3.958, P<0.001, partial Eta (η^2)=.294 and (c) placental weight F(4,38)=20.696, P<0.001, partial Eta (η^2)=.685.

It was noted that each individual independent variables [drug, dosage or trimester] played a major role in determining the maternal pregnancy outcome rather than the observed combined effects at two-way or at three-way interaction effects. **(*Table 4.3*)**

Table 4.3: The MANOVA Level 2 Table Showing the Main Global Effects of Each Independent Variables and how their Interaction Effects Influenced the Maternal Pregnancy Outcome.

	The group being tested.	**Dependent Variable**	**Measurement of variability in dependent variables (Type III Sum of Squares)**	**Degree of freedom**	**The ratio type 111 sum of square to its correspondi ng degree of freedom (Mean Square)**	**The ratio of the mean square for the independent variable to the mean square for error (F statistics)**	**Sig.<. 05**	**Proportio n of variance (Partial Eta Squared)**
(i)the evaluation of the correctness of the model used for the study	Corrected Model	Terminal weight	155177.895[a]	18	8620.994	292.324	.000	.993
		Weight gain	162887.333[b]	18	9049.296	258.681	.000	.992
		Placental weight	29.048[c]	18	1.614	618.786	.000	.997
(ii) Test on whether the observed results were due to chance	Intercept	Terminal weight	3889515.825	1	3889515.825	131887.211	.000	1.000
		Weight gain	118147.666	1	118147.666	3377.341	.000	.989
		Placental weight	886.961	1	886.961	340090.485	.000	1.000
(iii)the individual independent variable and its effects on the maternal pregnancy outcome	Drugs	Terminal weight	19078.241	1	19078.241	646.912	.000	.945
		Weight gain	23814.000	1	23814.000	680.741	.000	**.947**
		Placental weight	17.771	1	17.771	6813.857	.000	**.994**
	Dosages	Terminal weight	81261.148	2	40630.574	1377.717	.000	.986
		Weight gain	82188.926	2	41094.463	1174.716	.000	.984
		Placental weight	1.861	2	.931	356.814	.000	.949
	Trimesters	Terminal weight	21370.815	2	10685.407	362.325	.000	.950
		Weight gain	26522.926	2	13261.463	379.089	.000	.952
		Placental weight	4.514	2	2.257	865.455	.000	.979
(iv)Two-way interaction effects on each of the maternal dependent variable	Drugs * Dosages	Terminal weight	110.259	2	55.130	1.869	.168	.090
		Weight gain	107.444	2	53.722	1.536	.228	.075
		Placental weight	.004	2	.002	.714	.496	.036
	Drugs * Trimesters	Terminal weight	581.481	2	290.741	9.859	.000	.342
		Weight gain	270.778	2	135.389	3.870	.030	.169
		Placental weight	.027	2	.014	5.212	.010	.215
	Dosages *	Terminal weight	4119.852	4	1029.963	34.924	.000	.786

	Trimesters	Weight gain	3398.074	4	849.519	24.284	.000	.719
		Placental weight	.637	4	.159	61.037	.000	**.865**
(v)Three-way interaction effects on each of the three maternal variables	Drugs * Dosages * Trimesters	Terminal weight	623.185	4	155.796	5.283	.002	.357
		Weight gain	553.778	4	138.444	3.958	.009	.294
		Placental weight	.216	4	.054	20.696	.000	.685
	Error	Terminal weight	1120.667	38	29.491			
		Weight gain	1329.333	38	34.982			
(vi)Overall inferential statistics on the model results		Placental weight	.099	38	.003			
	Total	Terminal weight	5091386.000	57				
		Weight gain	256943.000	57				
		Placental weight	1176.624	57				
	Corrected Total	Terminal weight	156298.561	56				
		Weight gain	164216.667	56				
		Placental weight	29.148	56				
	a. R Squared = .993 (Adjusted R Squared = .989)							
	b. R Squared = .992 (Adjusted R Squared = .988)							
	c. R Squared = .997 (Adjusted R Squared = .995)							

*Key; * means interaction effects*

Part 3: The MANOVA Level 3 Pairwise Comparison Effects on how the Two Medicines Influence the Maternal Pregnancy Outcome when Exposed at the Same Time and in the Same Trimester

Upon carrying out the MANOVA level 3 analysis on the pairwise comparison on how the two medicines influenced the three maternal pregnancy outcomes when the treatments were done at the same dosage levels and within the same trimesters of exposure, it was observed that in all doses and across all trimesters for both the treatment groups, there was a statistical significance difference ($P<0.05$) between the means of lamotrigine and the levetiracetam treated groups. In overall, the teratogenic effects of lamotrigine were seen to be more than those of the levetiracetam treated groups as shown in table ***(Table 4.4).***

Table 4.4: The MANOVA Level 3 Pairwise Comparison Effects on How the Two Medicines Influence the Maternal Pregnancy Outcome When Exposed at the Same Time and in the Same Trimester.

Dependent variable	Dose level	The time of exposure to treatment	Levetiracetam treatment	Lamotrigine treatment	Mean difference between LEV and LAM treatment	Std Error	Sig.< 0.05	95%confidence interval Lower bound	Upper bound
TERMINAL	LOW	TM1	LEV	LTG	38.000	4.43	**.034**	29.02	46.9
WEIGHT	DOSE	TM2	LEV	LTG	18.000	4.43	**.042**	9.024	26.9
		TM3	LEV	LTG	46.333	4.43	**.067**	37.35	55.3
	MEDIUM	TM1	LEV	LTG	40.667	4.43	**.025**	31.69	49.6
	DOSE	TM2	LEV	LTG	44.000	4.43	**.034**	35.02	52.9
		TM3	LEV	LTG	38.667	4.43	**.045**	29.69	47.6
	HIGH	TM1	LEV	LTG	49.667	4.43	**.003**	40.69	58.6
	DOSE	TM2	LEV	LTG	23.000	4.43	**.004**	.000	31.9
		TM3	LEV	LTG	40.000	4.43	**.004**	31.02	48.9
WEIGHT	LOW	TM1	LEV	LTG	35.000	4.82	**.002**	25.22	44.7
GAIN	DOSE	TM2	LEV	LTG	36.000	4.82	**.003**	26.22	45.7
		TM3	LEV	LTG	46.000	4.82	**.007**	36.22	55.7
	MEDIUM	TM1	LEV	LTG	41.667	4.82	**.002**	31.89	51.4
	DOSE	TM2	LEV	LTG	52.667	4.82	**.002**	42.89	62.4
		TM3	LEV	LTG	43.000	4.82	**.006**	33.22	52.7
	HIGH	TM1	LEV	LTG	40.000	4.82	**.001**	30.22	49.7
	DOSE	TM2	LEV	LTG	27.667	4.82	**.001**	17.89	37.4
		TM3	LEV	LTG	56.000	4.82	**.001**	46.22	65.7
PLACENTA	LOW	TM1	LEV	LTG	1.413	.042	**.009**	1.329	1.49
L WEIGHT	DOSE	TM2	LEV	LTG	1.161	.042	**.021**	1.077	1.24
		TM3	LEV	LTG	.935	.042	**.034**	.851	1.02
	MEDIUM	TM1	LEV	LTG	1.180	.042	**.050**	1.09	1.22
	DOSE	TM2	LEV	LTG	1.141	.042	**.048**	1.05	1.26
		TM3	LEV	LTG	1.071	.042	**.043**	.986	1.16
	HIGH	TM1	LEV	LTG	1.155	.042	**.009**	.950	1.11
	DOSE	TM2	LEV	LTG	1.085	.042	**.019**	1.001	1.17
		TM3	LEV	LTG	1.305	.042	**.006**	1.221	1.39

4.2.2: The Fetal Pregnancy Outcomes

The fetal pregnancy outcomes were evaluated at two levels:

Stage 1: The intrauterine observations *(i.e the observations made when the fetuses were still attached to the uterine horns by their placenta*) including (i) the litter sizes, (ii) the resorbed glands/ devoured glands and (iii) the number of dead fetuses.

Stage 11: The individual fetal growth and development parameters that included; **(i)** the fetal weight (FT), **(ii)** head circumference (HC) and **(iii)** crown rump length (CRL).

4.2.2.1: Stage 1: The Intrauterine Observation on Fetal Pregnancy Outcomes While the Fetuses Were Still Inside the Uterine Horns Between the Treatment Groups Against the Control

Upon opening the uterine horns, the intra-uterine fetal pregnancy outcomes evaluated comprised of the observations made while the fetuses were still attached to their placenta in the uterine horns which included **(i)** the litter sizes (life fetuses), **(ii)** the numbers of the dead fetuses and **(iii)** the numbers of the resorbed endometrial glands or devoured fetuses. Upon carrying out the inter and intra group comparisons, the number of counts done on these three parameters indicated that the control had the highest number of life fetuses ranging from 13-16 fetuses compared with 3-9 fetuses in the lamotrigine and 3-11 in the levetiracetam treated groups. **(*Figures 4.1 A, B and C*)**

On how the doses of exposure influenced the intrauterine fetal pregnancy outcomes, it was observed that the three fetal pregnancy outcomes parameters depicted an inverse dose-response relationship in that as the doses of the two medicines increased the counts of the life fetuses reduced appreciably and particularly when the treatments were done at TM1 and TM2 for both medicines. **(*Figures 4.1 A, B and C*)**. As such the rats that were treated at TM1 treated rats had the least number of life fetuses for both drugs while those treated at TM3 treated rats had the highest number of life fetuses. The highest numbers of dead fetuses as well as the numbers of

resorbed endometrial glands /devoured fetuses were observed to be in TM1 treated groups followed with those in TM2 then lastly the ones in TM3**(*figures 4.1 A, B and C*)**.

The comparative findings shown in ***figures 4.1 A, B and C*** clearly indicate that lamotrigine has more deleterious teratogenic effects on fetal pregnancy outcomes than it is to levetiracetam. It can further be observed that for the dosages they depict an inverse dose response relationship while for the time of exposure, they depicted a direct time responses relationship on the three fetal pregnancy outcome parameters evaluated.

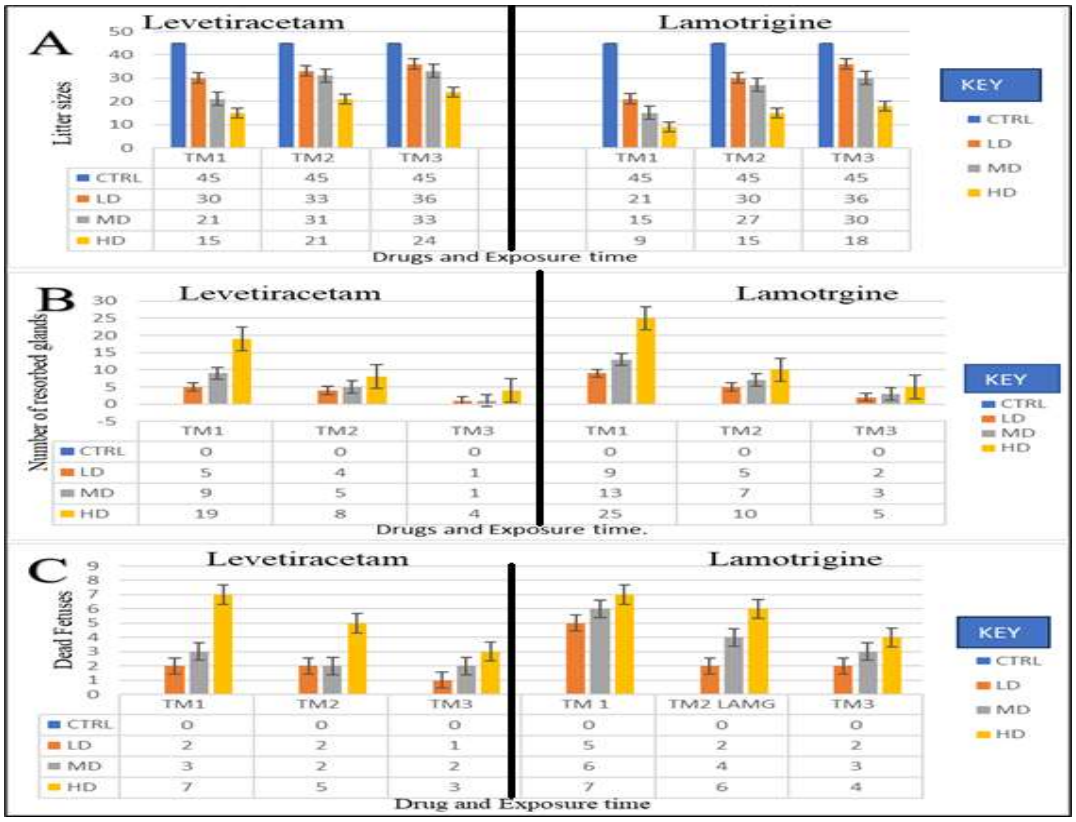

Figure 4.1: Comparative Fetal Pregnancy Outcomes on Litter Sizes, Number of Dead Fetuses and Resorbed Endometrial Glands

Key: (A): *Comparative litter size between control and the low dose, medium dose and high dose treatment groups (levetiracetam and lamotrigine).* ***(B):*** *Comparative resorbed glands between control and the low dose, medium dose and high dose treatment groups (levetiracetam and lamotrigine).* ***(C):*** *Comparative number of dead fetuses between control and the low dose, medium dose and high dose treatment groups (levetiracetam and lamotrigine).*

4.2.2.2 Stage 2: The Comparative Findings on the Individual Fetal Growth and Development Pregnancy Outcomes Between the Treatment Groups Compared with the Control

In evaluating how the two medicines influenced the individual fetal growth and development parameters *in-utero*, the following parameters were evaluated; (i) the mean fetal weights, (ii) the mean head circumferences, and (iii) the mean crown-rump lengths. A one-way Analysis of Variance (ANOVA) was first of all carried out to evaluate how the two treatment groups differed with the control. The study established that the two treatment groups of both the lamotrigine and the levetiracetam treatment groups were significantly different from the control as follows; **(a)** fetal weight $F(18,38) = 818.864$, $P < .001$), **(b)** head circumference $F(18,38) = 1083.820$, $P<0.001$), and **(c)** crown-rump length $F(18,38) = 224.211$, $P<0.001$**.(*Table 4.5*).**

When comparisons on how the different trimesters influenced the individual fetal growth and development parameters across the three trimesters, it was observed that rats exposed to treatment at TM_1 and TM_2 depicted a severe intra-uterine growth restriction as opposed to the ones exposed at TM_3. In terms of the dosages, the rats exposed to the low dosages for both medicines recorded minimal deleterious effects as compared to those that were exposed to medium and high dosages of both medicines. The study further established that the effects on the fetal growth and development in utero depicted an inverse-dose-response relationship and a direct time-response relationship for the two treatment medicines studied **(*Table 4.5*).**

Table 4.5: The ANOVA Table Showing the Comparative Means of the Individual Fetal Growth and Development Pregnancy Outcome Parameters In-Utero Between the Treatment Groups against the Control.

The study groups	Study group and dosage levels	Time of exposure to treatment	The comparative means for the fetal weight, crown lump length and head circumference.		
			Mean fetal weight ± SD	Mean crown lump length ± SD.	Mean head circumference ± SD
Control	No treatment	None.	7.74 ±.045	7.913 ±.059	4.200 ±.047
	Low dose	TM1	6.444 ±.010*	7.317 ±.300*	3.686 ±.092*
		TM2	6.510 ±.013*	7.454 ±.018*	3.834 ±.009*
		TM3	6.683 ±.024*	7.741 ±.010	4.040 ±.007*
Levetiracetam treated group	Medium dose	TM1	6.343 ±.083*	6.878 ±.066*	3.466 ± .046*
		TM2	6.438 ±.025*	7.131 ±.006*	3.710 ±.036*
		TM3	6.556 ±.017*	7.504 ±.078*	3.844 ±.026*
	High dose	TM1	5.443 ±.033*	5.445 ±.081*	3.003 ±.075*
		TM2	5.949 ±.011*	6.065 ±.010*	3.604 ±.071*
		TM3	6.237 ±.017*	6.437 ±.051*	3.540 ±.020*
	Low dose	TM1	7.012 ±.052*	4.132 ±.016*	3.263 ±.050*
		TM2	7.466 ±.067*	4.450 ±.014*	3.497 ±.050*
		TM3	7.637 ±.005*	4.545 ±.047*	3.609 ±.065*
	Medium dose	TM1	6.429 ±.006*	3.879 ±.068*	3.035 ±.056*
Lamotrigine treated group		TM2	6.837 ±.007*	4.155 ±.001*	3.249 ±.031*
		TM3	6.652 ±.007*	4.437 ±.048*	3.548 ±.025*
	High dose	TM1	5.571 ±.054*	3.376 ±.040*	2.397 ±.021*
		TM2	6.110 ±.059*	4.054 ±.003*	3.074 ±.039*
		TM3	6.330 ±.014*	4.310 ±.020*	3.450 ±.042*
Comparison by ANOVA [F, P values]			F(18,36)=818.864 P=0.0001	F(18,38)=1083.820 P=0.0001	F(18,38)=224.211 P=0.0001

Key: values are expressed as means ± standard deviation of mean n=3 per group. (*) the figures bearing the asterisk means that they are significantly different with the control.

Upon carrying out the multivariate analysis using MANOVA to establish how each of the three independent variables of **the drugs**, the **dose** and the **time of exposure** either acting alone or in combinations plus their interaction effects influenced the three fetal growth and development outcome of; (i) the fetal weight, (ii) crown lump length and (iii) the head circumference, the results are presented in three parts as per the three stages of MANOVA analysis as follows:

Part 1: The findings on the overall global effects of how the three independent variables namely the drug, dose and trimester influenced the three fetal pregnancy outcomes namely the fetal weight, the crown lump length and the head circumference combined.

Part 2: The main effects of each independent variable of either drug, dosage and trimesters and how its interaction effects influenced each of the three fetal pregnancy outcome namely fetal weight, the crown lump length and the head circumference.

Part 3: The pairwise comparison effects on how the two medicines influenced the fetal pregnancy outcome when exposed at the same dosage levels and within the same trimester.

Part 1: The MANOVA Level 1 Findings on the Global Teratogenic Effects on How the Three Independent Variables of the Drug, Dose and Trimester Influenced the Fetal Growth and Development Parameters Combined

Upon carrying out the MANOVA level 1 analysis on the global teratogenic effects on how the three independent variables namely **the drug**, **dose** and **trimester** of exposure influenced the three fetal growth and development parameters namely (i) the fetal weight (ii) the crown-rump length (iii) and head circumference, the analysis was done on the three independent variables when they were either acting alone, or when they acted in a two-way combination or at three-way combination. The Wilks' Lambda statistical test was applied during the MANOVA analysis to establish the global main effects or their interaction effects (*). Their proportionate contributions to the observed teratogenic effects were hence shown as significant interaction effects by the Partial Eta square (η^2) as shown below **(*Table 4.6*).**

(i) The overall main contributory effects of each of the individual independent variables on the three fetal growth and developmental outcomes when all were combined as follows:- **(a) drugs** F(3, 36)=6447.686, Wilks' Lambda (∧)=.002, P<0.001 partial Eta (η^2)=.998, **(b) dosage** F(6,72)=246.870, Wilks' Lambda (∧)=.002, P<0.001 partial Eta (η^2)=.954, **(c) trimesters** F(6,72)=144.384, Wilks' Lambda (∧)=.006 P<0.001 partial Eta (η^2)=.923. This shows that the drug had the strongest effects at 99.8%, followed by dose at 95.4% and lastly the weakest contributor being the trimester at 92.3% **(*Table 4.6*).**

(ii) At two-way combination interaction effects of (a) **doses*drugs** F(6,72)=166.393, Wilks' Lambda(∧)=.005 P<.001, partial η^2=.933, (b) **drugs*trimesters** F6,72)=20.203, Wilks Lambda (∧)=.139 P<.001 partial η^2 =.627, (c) **doses*trimesters** F(12, 95.539)= 31.623, Wilks' Lambda (∧)=.014, P<.001 partial η^2 =.757. The strongest interaction effect was noted between doses *drugs at partial Eta proportion of (η^2)93.3% while the weakest interaction effect was noted between drugs* trimesters at partial Eta (η^2) proportion of 62.7% **(*Table 4.6*).**

(iii) At three-way combination interaction effects of **drugs * doses * trimesters**, F(12,95.539)=9.856, Wilks' Lambda (∧) =.119 P<.001 partial η^2=.509.

It was evident that the individual independent variables had the major contribution to the observed fetal pregnancy outcomes with the drugs having the highest contribution with partial Eta proportion of 99.8% while trimesters had the lowest contribution to the observed fetal pregnancy outcomes at partial Eta proportion of 92.3%. **(*Table 4.6*).**

Table 4.6: The MANOVA Level 1 Findings on the Global Teratogenic Effects on How the Three Independent Variables of the Drug, Dose and Trimester Influenced the Fetal Growth and Development Parameters Combined

			Multivariate Tests					
Types of MANOVA evaluation at level 1	**The comparative analyzed**	**global effects**	**MANOVA test statistic Wilks' Lambda**	**Statistics (F)**	**Hypothesis degree of freedom**	**Error degree of freedom**	**Sig.<0.05**	**Proportion of variance (Partial Eta Squared)**
(ii) test on whether the observed results were due to chance	Were the observed effects due to chance or the treatment	The intercept parameter	.000	617079.527[b]	3.000	36.000	.000	1.000
(iii)the individual main effects of the drugs time of exposure and dosages on the fetal pregnancy	Were the observed effects due to LEV or LAM	Drugs	.002	6447.686[b]	3.000	36.000	.000	**.998**
	Were the observed effects due to variation in doses	Doses	.002	246.870[b]	6.000	72.000	.000	.954
	Were the observed effects due to differing trimesters	Trimesters	.006	144.304[b]	6.000	72.000	.000	**.923**
(iv)two-way interaction effects on the fetal dependent variable	Were the observed effects due to interaction between varied doses and the drugs	Doses *Drugs	.005	166.393[b]	6.000	72.000	.000	.933
	Were the observed effects due to interaction between drugs and differing trimesters	Drugs *Trimesters.	.139	20.203[b]	6.000	72.000	.000	.627
	Were the observed effects due to interaction between varied doses and differing trimesters	Doses * Trimesters.	.014	31.623	12.000	95.539	.000	.757
(v)three-way interaction effects on fetal pregnancy outcome	Were the observed effects due to interaction between drugs, varied doses or differing trimesters.	Drugs *Doses *Trimesters.	.119	9.856	12.000	95.539	.000	.509
	a. Design: Intercept + Drugs + Dosages + TRIMESTERS + Drugs * Dosages + Drugs * TRIMESTERS + Dosages * TRIMESTERS + Drugs * Dosages * TRIMESTERS							
	b. Exact statistic							
	c. The statistic is an upper bound on F that yields a lower bound on the significance level.							

*Key; * Means interaction effects*

Part 2: The Main and the Interaction Effects of Each Independent Variable and How Their Interaction Effects Individually Influenced Each of the Three Fetal Development Parameters *In-Utero*

Upon further analysis at MANOVA level 2 on how the main and the interaction effects of the three independent variables together with their interaction influenced each of the three individual fetal growth and development parameters during pregnancy, it was noted that;

(i) At a global level, contribution of each of the three independent variables to each of the three dependent fetal growth and development variables (fetal weight, head circumference and crown-rump length), showed that they were all statistically significant (P< .05). They influenced each of fetal growth and development parameters in different proportions (Partial Eta squared η^2) with each of the independent variable's proportion of variance being above 94% for all fetal dependent variables **(*Table 4.7:*).**

(ii) Two-way interaction effects of the three independent variables on the three fetal dependent variables was statistically significant (P< .05) between (a) **drug*dosages**, (b) **drug*trimester** and (c) **dosage*trimesters**. It was noted that, two-way interaction effects between **drugs * dosage** had the strongest effects on fetal weight at partial Eta (η^2) proportion of 95.9% while two-way interaction effect between **drug * dosages** had the weakest effects on fetal head circumference at partial Eta (η^2) proportion of 0.5% **(Table *4.7:*).**

(iii) At three-way interaction effects between **drugs * dosages * trimesters** on the three fetal growth and development parameters, a statistically significant interaction effect (P< .05) was noted. **Table *4.7***

The observed *in-utero* fetal pregnancy outcomes were majorly due to the individual independent variables (drug, dosage, or trimester) rather than due to their combinations at two-way or at three-way interaction levels. This suggested that all the three independent variables individually played a major role in determining the *in-utero* fetal pregnancy outcomes with the drugs having the strongest effects at partial Eta (proportion of variance being 99.7%) on fetal

crown-rump length and trimesters having the weakest effects at partial Eta (proportion of variance being 94%) on fetal crown rump length. **(Table *4.7*).**

Table 4.7: The MANOVA Level 3 Table the Findings on the Main and the Interaction Effects of Each Independent Variable Influenced Each of the Three Fetal Growth and Development Parameters In-Utero.

	The group being tested.	**Dependent Variable**	**Measurement of variability in dependent variables (Type III Sum of Squares)**	**Degree of freedom df**	**The ratio type 111 sum of square to its corresponding degree of freedom (Mean Square)**	**The ratio of the mean square for the independent variable to the mean square for error(F statistics)**	**The ratio of the mean square for the independent variable to the mean square for error (F statistics)**	**Proportion of variance (Partial Eta Squared)**
Evaluation on the correctness of the model used for the study	Corrected	FETAL WT	20.256[a]	18	1.125	818.864	.000	.997
	Model	FETAL CRL	133.862[b]	18	7.437	1083.820	.000	.998
		FETAL HC	9.276[c]	18	.515	224.211	.000	.991
Test on whether the observed results were due to chance	Intercept	FETAL WT	1854.635	1	1854.635	1349551.472	.000	1.000
		FETAL CRL	1466.216	1	1466.216	213683.031	.000	1.000
		FETAL HC	525.964	1	525.964	228833.129	.000	1.000
Individual independent variable and its effects on each of the three fetal dependent variables	Drugs	FETAL WT	1.880	1	1.880	1368.128	.000	.973
		FETAL CRL	100.467	1	100.467	14641.805	.000	**.997**
		FETAL HC	2.166	1	2.166	942.434	.000	.961
	Dosages	FETAL WT	9.675	2	4.837	3520.048	.000	.995
		FETAL CRL	9.158	2	4.579	667.326	.000	.972
		FETAL HC	2.090	2	1.045	454.573	.000	.960
	TRIMESTERS	FETAL WT	2.211	2	1.105	804.352	.000	.977
		FETAL CRL	4.115	2	2.057	299.852	.000	**.940**
		FETAL HC	2.624	2	1.312	570.887	.000	.968
	Drugs	FETAL WT	1.235	2	.617	449.182	.000	**.959**
	* Dosages	FETAL CRL	3.235	2	1.618	235.753	.000	.925
		FETAL HC	.000	2	.000	.088	.050	.005

	The group being tested.	Dependent Variable	Measurement of variability in dependent variables (Type III Sum of Squares)	Degree of freedom df	The ratio type 111 sum of square to its corresponding degree of freedom (Mean Square)	The ratio of the mean square for the independent variable to the mean square for error(F statistics)	The ratio of the mean square for the independent variable to the mean square for error (F statistics)	Proportion of variance (Partial Eta Squared)
Two-way interaction effects on each of the maternal dependent variables	Drugs	FETAL WT	.108	2	.054	39.278	.000	.674
	* TRIMESTERS	FETAL CRL	.029	2	.014	2.110	.005	.100
		FETAL HC	.116	2	.058	25.129	.000	.569
	Dosages	FETAL WT	.483	4	.121	87.839	.000	.902
	* TRIMESTERS	FETAL CRL	.609	4	.152	22.203	.000	.700
		FETAL HC	.471	4	.118	51.195	.000	.843
Three-interaction effects on each of the fetal dependent variable	Drugs	FETAL WT	.116	4	.029	21.119	.000	.690
	* Dosages	FETAL CRL	.017	4	.004	.621	.050	.061
	* TRIMESTERS	FETAL HC	.149	4	.037	16.223	.000	.631
	Error	FETAL WT	.052	38	.001			
Overall inferential statistical on the models results.		FETAL CRL	.261	38	.007			
		FETAL HC	.087	38	.002			
	Total	FETAL WT	2466.857	57				
		FETAL CRL	1952.735	57				
		FETAL HC	698.166	57				
	Corrected	FETAL WT	20.308	56				
	Total	FETAL CRL	134.123	56				
		FETAL HC	9.363	56				

a. R Squared = .997 (Adjusted R Squared = .996)

b. R Squared = .998 (Adjusted R Squared = .997)

c. R Squared = .991 (Adjusted R Squared = .986)

*Key; * means interaction effects.*

Part 3: The MANOVA Level 3 On Pairwise Comparison on how the Two Medicines Influence the Fetal Pregnancy Outcome When Exposed at the Same Dosage Levels and Within the Same Trimesters of Exposure

Upon carrying out the MANOVA level 3 analyses on the pairwise comparisons on how the two medicines influenced the individual fetal growth and development pregnancy outcome parameters in-utero when exposed at the same dosage levels and within the same trimesters. It was observed that in all dose levels of the two treatment medicines and across the three trimesters they were all statistically significant different (P< .001) between the lamotrigine and the levetiracetam treated groups. The overall finding on this pairwise comparison was that in all the dose groups of the low, medium and high dose lamotrigine treated groups, they had a more deleterious effects that the same dose groups of the levetiracetam treated groups on fetal weights, head circumference and crown-rump length when the exposed within the same trimesters **(*Table 4.8*).**

Table 4.8: MANOVA Stage III Table Showing the Pairwise Comparative Finding on How the Two Medicines Influenced the Three Fetal Growth and Developmental Parameters Within the Same Dosage Levels and Within the Same Trimesters of Exposures.

Dependent variable	**Dose level**	**Exposure time to treatment**	**Levetiracetam treatment**	**Lamotrigine treatment**	**Mean difference between LEV and LAM treatment**	**Std Error**	**Sig.<0.05**	**95%confidence interval**	
								Lower bound	Upper bound
FETAL WEIGHT	LOW DOSE	TM1	LEV	LTG	.567	.030	.000	.506	.629
		TM2	LEV	LTG	.876	.030	.000	.815	.938
		TM3	LEV	LTG	.954	.030	.000	.892	1.01
	MEDIUM	TM1	LEV	LTG	.085	.030	.008	.008	.147
	DOSE	TM2	LEV	LTG	.399	.030	.000	.338	.460
		TM3	LEV	LTG	.095	.030	.003	.034	.156
	HIGH	TM1	LEV	LTG	.127	.030	.000	.066	.189
	DOSE	TM2	LEV	LTG	.161	.030	.000	.100	.222
		TM3	LEV	LTG	.093	.030	.000	.032	.155
HEAD CIRCUMFERENCE	LOW DOSE	TM1	LEV	LTG	.423	.039	.000	.344	.502
		TM2	LEV	LTG	.337	.039	.000	.258	.416
		TM3	LEV	LTG	.431	.039	.000	.352	.510
	MEDIUM	TM1	LEV	LTG	.431	.039	.000	.352	.510
	DOSE	TM2	LEV	LTG	.462	.039	.000	.382	.541
		TM3	LEV	LTG	.296	.039	.000	.217	.376
	HIGH	TM1	LEV	LTG	.606	.039	.000	.527	.685
	DOSE	TM2	LEV	LTG	.529	.039	.000	.450	.609
		TM3	LEV	LTG	.089	.039	.000	.010	.169
CROWN-RUMP LENGTH	LOW DOSE	TM1	LEV	LTG	3.185	.068	.000	3.04	3.32
		TM2	LEV	LTG	3.004	.068	.000	2.86	3.14
		TM3	LEV	LTG	3.205	.068	.000	3.06	3.34
	MEDIUM	TM1	LEV	LTG	2.999	.068	.000	2.86	3.13
	DOSE	TM2	LEV	LTG	2.976	.068	.000	2.83	3.11
		TM3	LEV	LTG	3.067	.068	.000	2.93	3.20
	HIGH	TM1	LEV	LTG	2.069	.068	.000	1.93	2.20
	DOSE	TM2	LEV	LTG	2.010	.068	.000	1.87	2.14
		TM3	LEV	LTG	2.037	.068	.000	1.90	2.17

The Histo-morphological Findings.

4.3 Objective 2: The Comparative Histo-Morphological Evaluation on How the Two Medicines Influenced the Histological Organization of the Developing Fetal Kidneys.

In evaluating how the two medicines influenced the histological organization of the developing fetal kidneys, these results are presented in two stages as follows:

Stage 1: The comparative histo-morphological findings on how the two medicines influenced the histological organization of the renal capsule.

Stage 2: The comparative findings on how the two medicines influenced the histo-morphological structure and the distribution of the glomeruli.

Stage 3: The comparative findings on how the two medicines influenced the histo-morphological structure of the renal medullary tubules.

Stage 4: Comparative findings on the two medicines influenced the histo-morphological thickness of the kidney cortex and medulla.

4.3.1: Stage 1. The Comparative Findings on How the Two Medicines Influenced the Histological Organization of the Renal Capsule.

In evaluating how the two medicines influenced the histological organization of the renal capsule the following parameters were evaluated; (**i**) the histo-morphological shapes of the glomeruli, (**ii**) the organization of the juxtaglomerular cells, (**iii**) the glomerular capillary tufts and associated cells, (**iv**) the bowman spaces including the appearances of the simple squamous epithelial linings of both the visceral and the parietal layers of the bowman space. In this evaluation, the histological slides were analyzed at a magnification of (X1000) in order to clearly see how the glomerular cells were organized, establish the histo-morphological sizes of the glomeruli, the bowman's spaces and the glomerular capillary tufts.

This current study established that the histo-morphological shapes of the glomerular, the entire of the glomeruli appeared morphologically bigger in sizes in the treatment

groups as compared with the control ***(figures 4.2 A to figures 4.4 A).*** However, on further analysis on the internal histological differentiation of the glomerular structures that included the tufts of capillaries, juxtaglomerular cells and the bowman's space, it was observed that in the two treatment groups for the two treatment medicines, they both had smaller and compacted glomerular capillary tufts and the associated juxtaglomerular cells and they had a conspicuously enlarged bowman spaces as compared with the control ***(figures 4.2 B to G to figures 4.4 B to G).***

This phenomenon of smaller and compacted glomerular capillary tufts and the associated juxtaglomerular cells with enlarged bowman space suggested a possibility of excessive fluid accumulation in the bowman space either due to improper clearance of the renal fluid filtrates that could be due to maldevelopment of the renal tubules particularly when the treatments were instituted at TM_1 and TM_2 (***figure 4.1 B to G, and figure 4.4 B to G).*** This was not the case when the treatments were done at TM3 as shown in ***figure 4.4 B to G***. The compaction of the glomerular sizes was observed to be more intensified with increasing dosages of the two medicines in the medium and high doses and with the early periods of exposure where the greatest effects were when the medium and high doses were also instituted at TM_1 and TM_2 (***figure 4.2 B to G to figure 4.3 B to G***).

This phenomenon of alteration in the glomerular sizes following prenatal exposure to the two medicines was noted to depict a direct dose response relationship in that when the doses of the two medicines increased there was a corresponding proportionate increase in the renal capsule particularly for the treatment groups that received medium and high doses of the two treatment medicines in the first two trimesters as seen in ***figures 4.2 C, D, F, G to figure 4.4 C, D, F, G.*** On the juxtaglomerular apparatus, it was observed that the juxtaglomerular apparatus (*indicated by the red arrows*) for the two treatment groups were characterized by compacted cells that were also slightly hypertrophied in trimester 3 **(*figure 4.4 B to G*)**, moderately hypertrophied in trimester two **(*figure 4.3 B to G*)** and severely hypertrophied juxtaglomerular cells in trimester 1 ***(figure 4.2 B to G*).**

On the intra-glomerular mesangial cells (*marked with yellow arrows*) it was evident that they were many well developed cells that were evenly distributed in the control **(*figure 4.2 A, to figure 4.4 A*)** while in the two treatment groups, the cells condensed together, were few in numbers and at times they were seen to be sparsely distributed when the low dosages were applied **(*figures 4.2 B, E, figure 4.3 B, E, and figure 4.4 B, E*)**. In the medium and the high dose treatment groups, these cells were hypertrophied and densely packed together within glomerular capillary tufts a sign of impaired glomeruli capillaries tuft differentiation **(*figure 4.2 D, G figure 4.3 D to G and figure 4.4 D to G*).**

On the simple squamous epithelial lining of both the parietal layer and the visceral layers of the bowman spaces (*indicated by the blue arrows*) they depicted a smooth marginal outlines in the control group **(*figure 4.2 A, figure 4.3 A and figure 4.4 A*)**, while in the treatment groups the two layers shown some signs of serration probably showing the perturbations caused by the two medicines in the process of morphogenetic differentiation of the renal filtrate collecting apparatus that start from the bowman's capsule to the renal tubules a pointer as to the observed hypertrophied bowman's spaces **(*figure 4.2 B to G to figure 4.4 B to G*.).** This phenomenon of distortion of the simple squamous epithelial cells in the parietal layer of the bowman's capsule was seen to be more pronounced when the treatments were done in the first and the second trimester and less severe the third trimester **(*figure 4.2 B to G to figure 4.4 B to G*).**

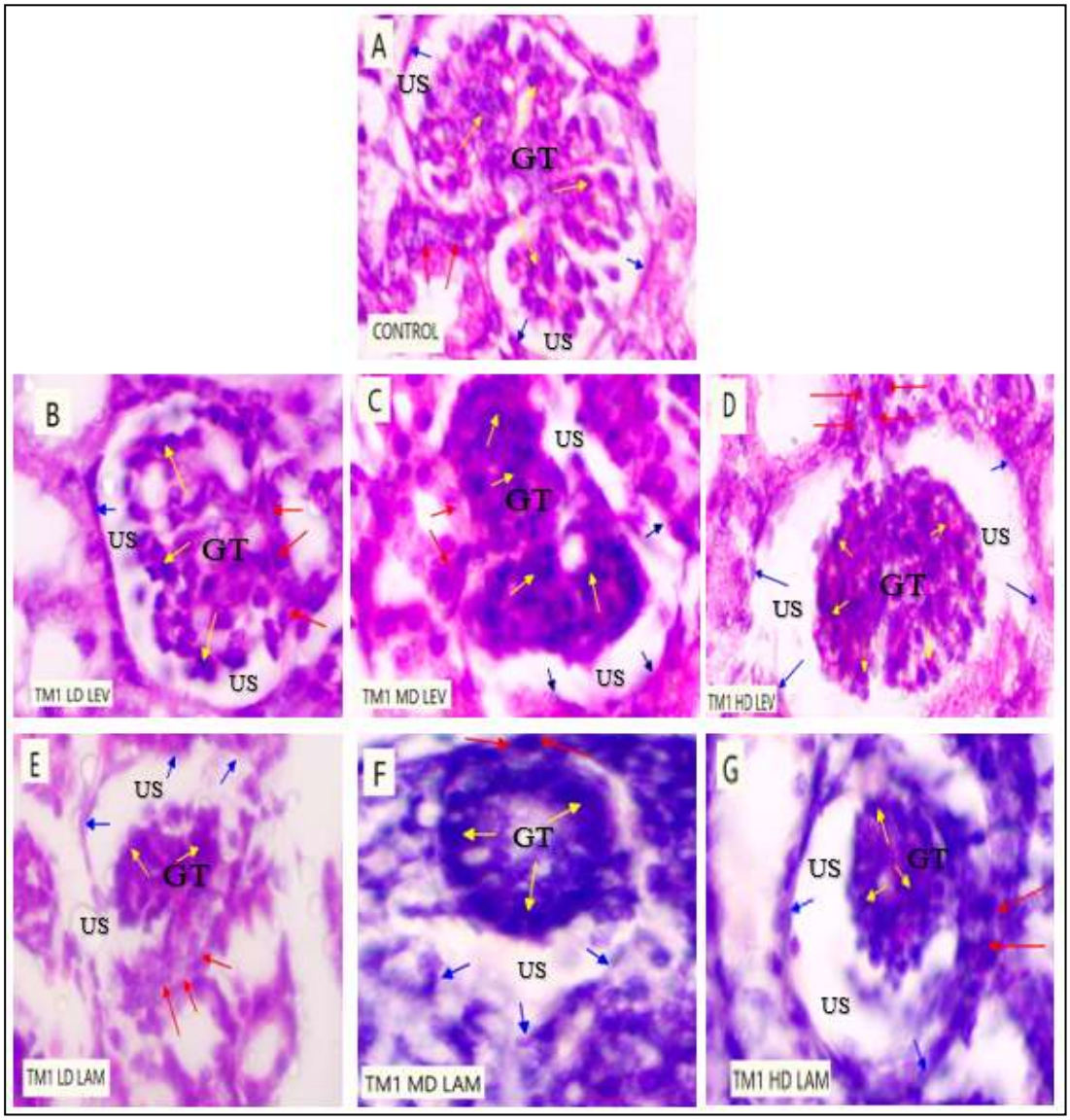

Figure 4.2. The Photomicrographs of the Comparative Histo-Morphological Features of The Fetal Kidney Showing Glomerular Sizes, Urinary Spaces and Juxtaglomerular Apparatus in TM1. Stained with H&E at Magnification of X1000

Key:

NB>: *The yellow arrows indicate the mesangial intra-glomerular cells; the blue arrow shows the parietal layer of the renal capsule and the red arrows indicate the juxtaglomerular apparatus.*

A: CONTROL; Normal glomerular sizes, normal urinary space, normal juxtaglomerular apparatus, normal intraglomerular mesangial cells and smooth outline of the parietal layer of squamous epithelial cells of the bowman space.

B:TM1 LD LEV; Increased glomerular sizes, increased urinary spaces without a notable change in the juxtaglomerular apparatus and intraglomerular mesangial cells.

C:TM1 MD LEV; Increased glomerular sizes with increased urinary spaces and juxtaglomerular apparatus and slightly increased intraglomerular mesangial cells.

D:TM1 HD LEV; Increased glomerular sizes, highly increased urinary space, hypertrophied juxtaglomerular apparatus, poor outline of parietal layer of squamous epithelial cells of the bowman space.

E:TM1 LD LTG; Increased glomerular sizes, increased urinary space, increased juxtaglomerular apparatus and intraglomerular mesangial cells and lost outline of the of the parietal layer of squamous epithelial cells of the bowman space.

F:TM1 MD LTG; Increased glomerular sizes, highly increased urinary space, hypertrophied juxtaglomerular apparatus and intraglomerular mesangial cells and lost outline of the of the parietal layer of squamous epithelial cells of the bowman space.

G:TM1 HD LTG; Highly increased glomerular sizes, highly increased urinary space, hypertrophied juxtaglomerular apparatus and intraglomerular mesangial cells and distorted outline of the parietal layer of squamous epithelial cells of the bowman space.

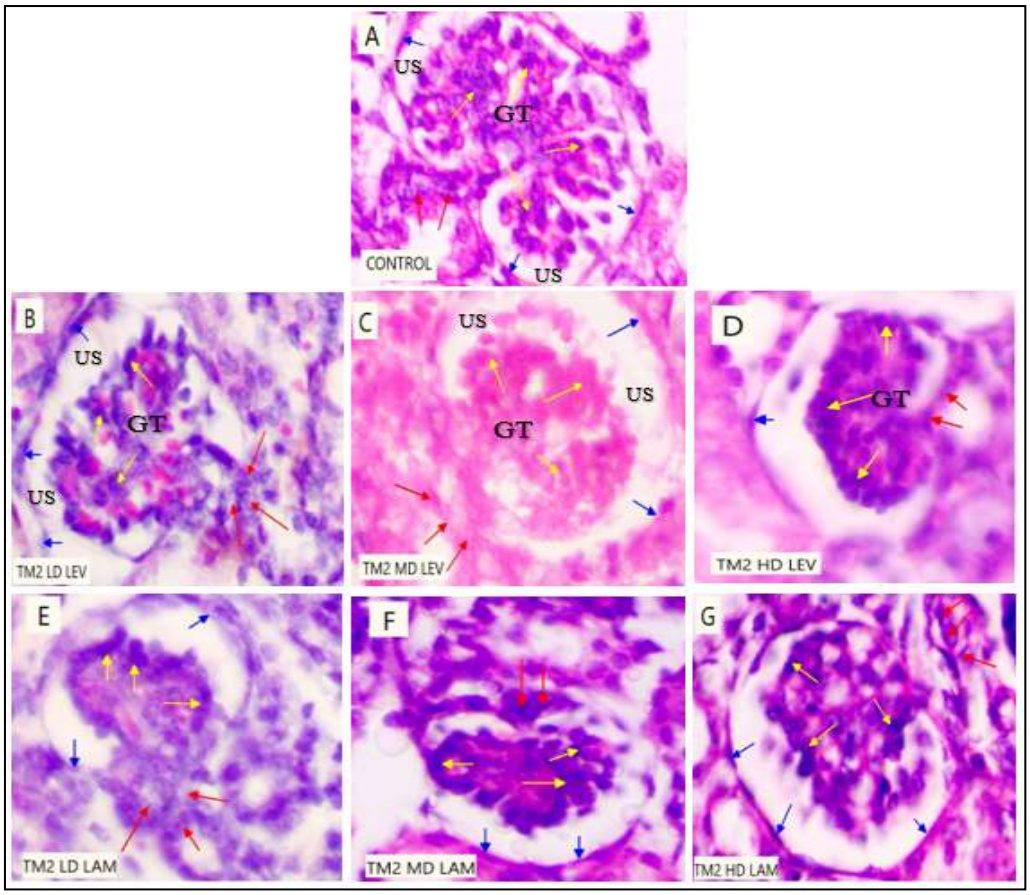

Figure 4.3. The Photomicrographs of the Comparative Histo-Morphological Features of the Fetal Kidney Showing Glomerular Sizes, Urinary Spaces and Juxtaglomerular Apparatus in TM2. Stained with H&E at Magnification of X1000.

Key:

(NB> The yellow arrows indicate the mesangial intraglomerular cells, the blue arrow shows the parietal layer of the renal capsule and the red arrows indicate the juxtaglomerular apparatus.)

A: CONTROL; Normal glomerular size, normal urinary space, normal juxtaglomerular apparatus, normal intraglomerular mesangial cells and smooth outline of the parietal layer of squamous epithelial cells of the bowman space.

B:TM2 LD LEV; Increased glomerular size, increased urinary spaces without a notable change in the juxtaglomerular apparatus and intraglomerular mesangial cells.

C:TM2 MD LEV; Increased glomerular size with increased urinary spaces and juxtaglomerular apparatus and slightly increased intraglomerular mesangial cells.

D:TM2 HD LEV; Increased glomerular size, increased urinary space, hypertrophied juxtaglomerular apparatus, poor outline of parietal layer of squamous epithelial cells of the bowman space.

E:TM2 LD LTG; Increased glomerular size, increased urinary space, slightly increased juxtaglomerular apparatus and intraglomerular mesangial cells.

F:TM2 MD LTG; Increased glomerular size, increased urinary space, densely populated intraglomerular

G:TM2 HD LTG; Increased glomerular size, increased urinary space, hypertrophied juxtaglomerular apparatus and intraglomerular mesangial cells and outline of the parietal layer of squamous epithelial cells of the bowman space.

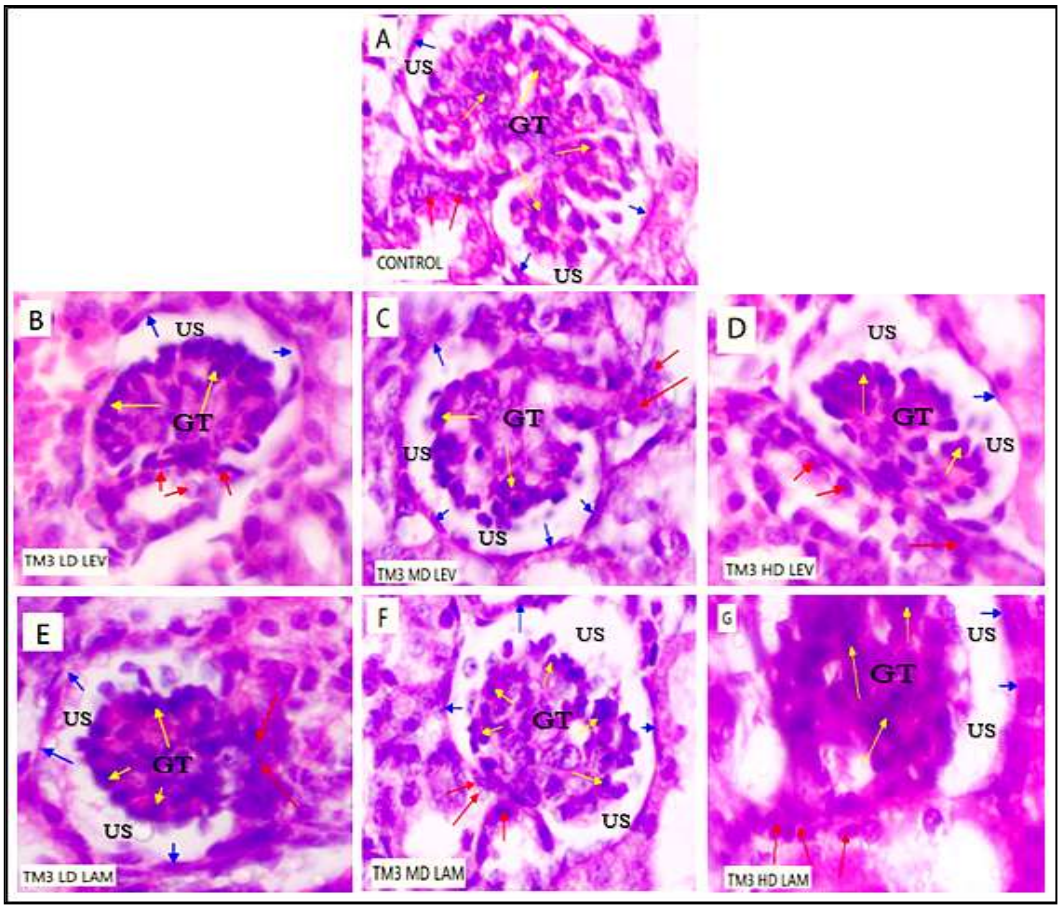

Figure 4.4. The Photomicrographs of the Comparative Histo-Morphological Features of The Fetal Kidney Showing Glomerular Capillary Sizes, Urinary Spaces and Juxtaglomerular Apparatus in TM3. Stained with H&E at Magnification of X1000.

Key:

NB> *The yellow arrows indicate the mesangial intraglomerular cells, the blue arrow shows the parietal layer of the renal capsule and the red arrows indicate the juxtaglomerular apparatus.*

A: CONTROL; Normal glomerular size, normal urinary space, normal juxtaglomerular apparatus, normal intraglomerular mesangial cells and smooth outline of the parietal layer of squamous epithelial cells of the bowman space.

B:TM3 LD LEV; Slightly increased glomerular size, increased urinary spaces without a big change to the juxtaglomerular apparatus and intraglomerular mesangial cells.

C:TM3 MD LEV; Slightly increased glomerular size with increased urinary spaces and juxtaglomerular apparatus and slightly increased intraglomerular mesangial cells.

D:TM3 HD LEV; Slightly increased glomerular size, increased urinary space, hypertrophied juxtaglomerular apparatus, poor outline of parietal layer of squamous epithelial cells of the bowman space.

E:TM3 LD LTG; Slightly increased glomerular size, increased urinary space, slightly increased juxtaglomerular apparatus and intraglomerular mesangial cells.

F:TM3 MD LTG; Slightly increased glomerular size, increased urinary space, poor outline of the parietal layer of squamous epithelial cells of the bowman space.

G:TM3 HD LTG; Slightly increase glomerular size, increased urinary space, hypertrophied juxtaglomerular apparatus and intraglomerular mesangial cells and distorted outline of the parietal layer of squamous epithelial cells of the bowman space.

4.3.2: Stage 2. The Comparative Findings on how the Two Medicines Influenced the Shapes and Distribution of Glomeruli.

In evaluating how the two medicines influenced the shapes and the distribution of the glomeruli in the developing fetal kidneys the following parameters were analyzed; **(i)** the glomerular sizes, **(ii)** the shapes and **(iii)** the glomerular distribution of the per field of view and **(iv)** the bowman space sizes. In comparing the four parameters between the treatment groups against the control, the study findings showed that the treatment groups were significantly different from the control in that both the treatments groups depicted the following features: (i) the glomerular sizes were enlarged due to bigger bowman's spaces, (ii) they had reduced glomeruli numbers per filed of views, the cells in the glomeruli appeared fewer and either condensed or sparsely distributed and (iv) they had enlarged bowman spaces between the visceral and the parietal layers when compared with those of the control **(*figures 4. 4 A to figure 4.4 A*)** and the treatment groups **(*figures 4. 4 B to G to figures 4.4 B to G*).**

With regards to the number of glomeruli per field of *Mag x 400,* it was observed that the control group had a significantly higher numbers of glomeruli per field that ranged between 4-7 and per field **(*figure 4.5 A to figure 4.7 A*)** while in the treatment groups they ranged between 3-5 per field **(*figure 4.5 B to G and figure 4.7 B to G*)**. The highest reduction in the number of glomeruli per field was noted in the medium and the high treatment groups and when the treatments were done at TM_1 and TM_2. It was also notable that between the two treatment medicines, lamotrigine had the worst deleterious effects as compared with the levetiracetam treatment groups **(*figure 4.5 C, F and figure 4.7 C, F*)**.

With regards to the glomerular shapes, the study established that most of the glomeruli in the treatment groups had irregular and distorted glomerular shapes while those in the control looked regular in shape **(*figures 4.5 A, to figure 4.7 A*).** Similarly, the bowman space in the two treatment groups was observed to be increasing with the upward variations in the doses applied **(*figures 4.5 B to G to figure 4.7 B to G*).**

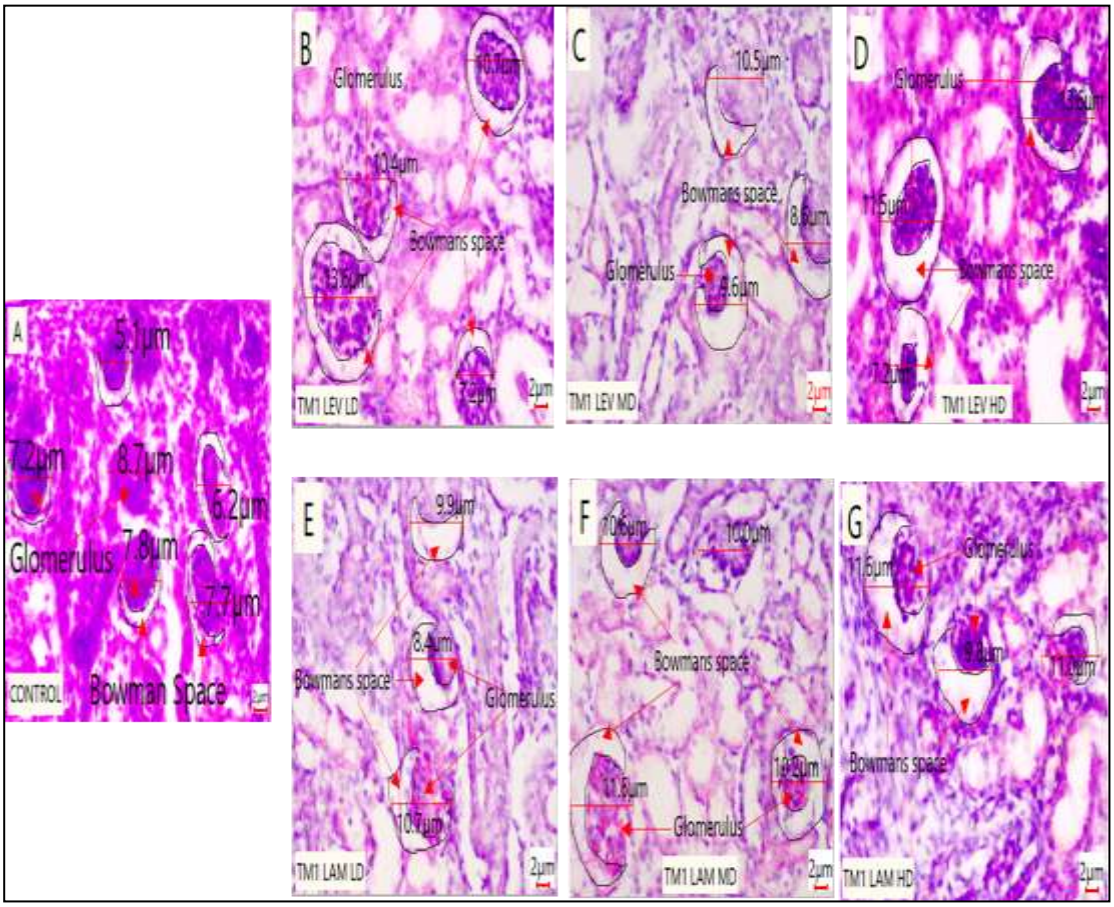

Figure 4.5. The Histo-Photomicrographs of Comparative Histo-Morphological Features of the Fetal Kidney Glomerular Sizes, Their Distribution and their Bowman Space in TM1. Stained with H&E at Magnification of X400.

KEY:

A: *CONTROL; Normal glomerular size and normal bowman's space.*

B: *TM1 LD LEV; Increased glomerular size and increased bowman's space.*

C*: TM1 MD LEV; Increased glomerular size and increased bowman's space.*

D*: TM1 HD LEV; Increased glomerular size and increased bowman's space.*

E*: TM1 LD LTG; Slightly increased glomerular space and bowman's space.*

F*: TM1 MD LTG; Increased glomerular space and increased bowman's space.*

G*:TM1 HD LTG; Highly increased glomerular space and increased bowman's space.*

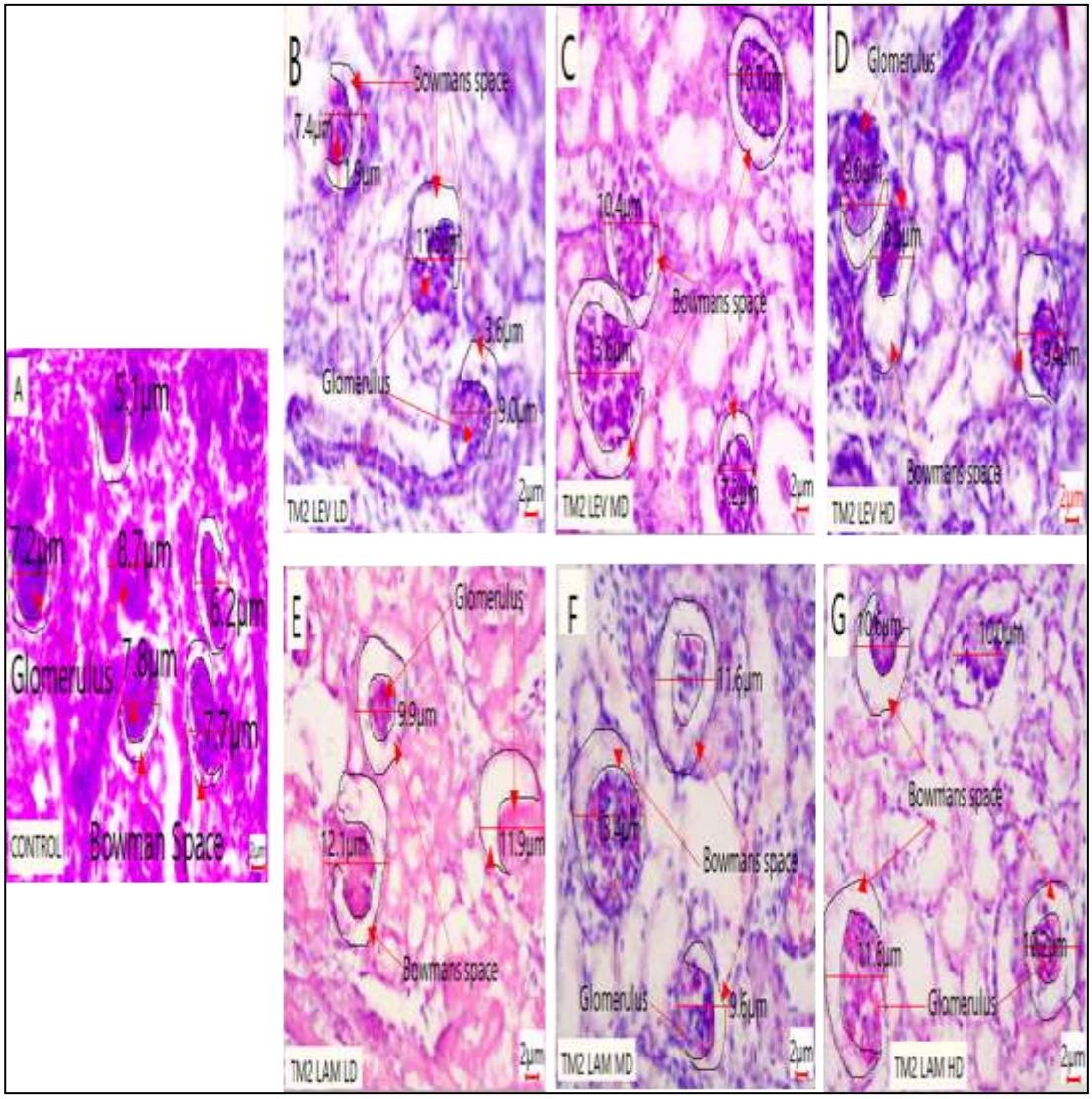

Figure 4.6. A Histo-Photomicrograph of Comparative Histo-Morphological Features of the Fetal Kidney Showing Glomerular Sizes, Their Distributions and Their Bowman Spaces in TM2. Stained with H&E at Magnification of X400.

KEY:

A: CONTROL; Normal glomerular size and bowman's space.
B: TM2 LEV LD; Increased glomerular size and bowman's space.
C: TM2 LEV MD; Increased glomerulus size and increased bowman's space.
D: TM2 LEV HD; Increased glomerular size and highly increased bowman's space.
E: TM2 LTG LD; Increased glomerular size with the increased bowman's space.
F: TM2 LTG MD; Increased glomerular size with the increased bowman's space.
G: TM2 LTG HD; Increased glomerular size with increased bowman's space.

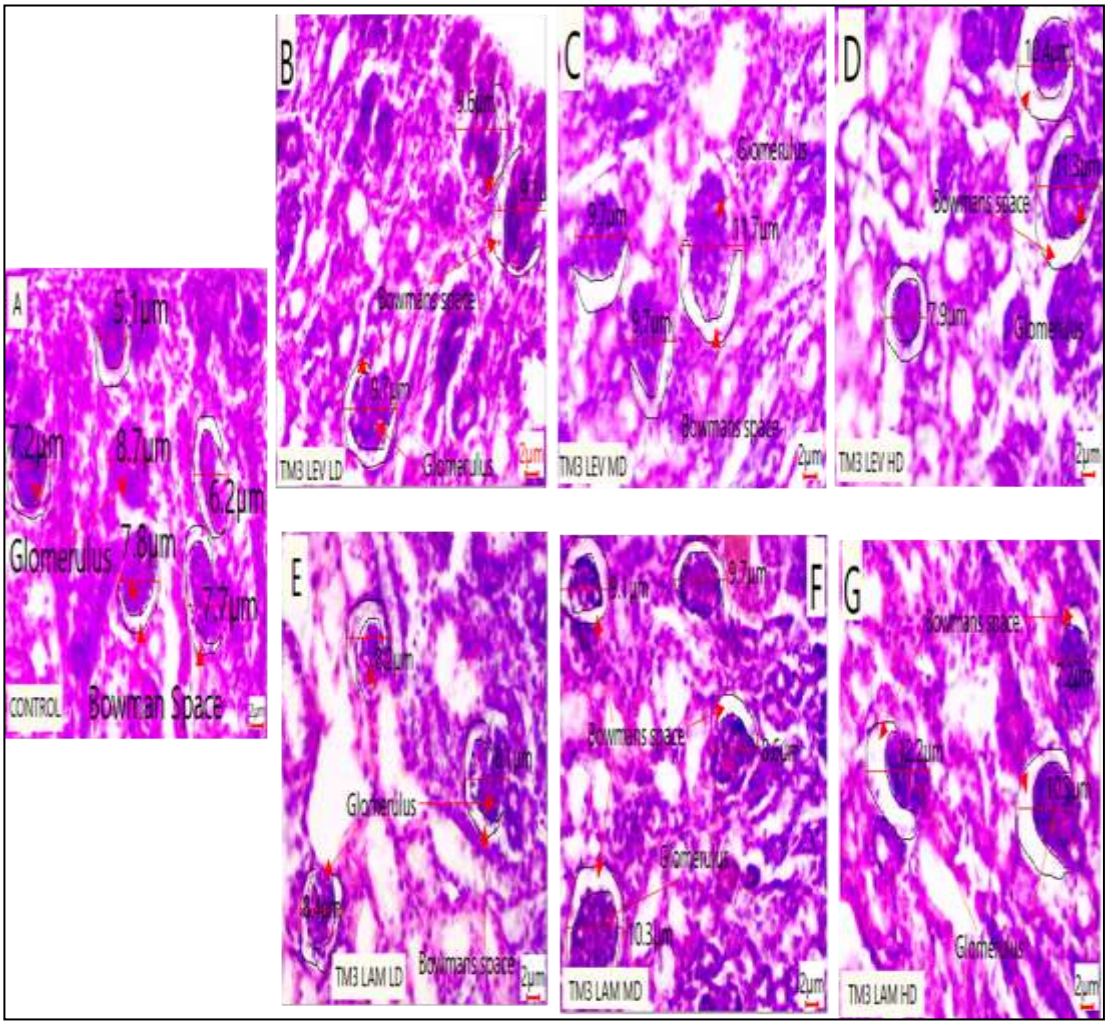

Figure 4.7. The Photomicrograph of Comparative Histo-Morphological Features of the Fetal Kidney Showing Glomerular Distribution, Their Sizes and Bowman Spaces TM3. Stained with H&E at Magnification of X400.

KEY:

A: CONTROL; Normal glomerulus size and normal bowman spaces, higher number of glomeruli per the field of view.
B: TM3 LEV LD; Slight increase in size in the glomerular size and bowman's space.
C: TM3 LEV MD; Slight change in the glomerular sizes and slightly increased bowman's space.
D: TM3 LEV HD; Slightly increased glomerulus sizes and bowman's space.
E: TM3 LTG LD; Slightly reduced glomerulus and reduced bowman's space.
F: TM3 LTG MD; Slightly increased glomerular size and bowman's space.
G: TM3 LTG HD; Increased glomerular size and increased bowman's space.

4.3.3: Stage 3. Comparative Findings on how the Two Medicines Influenced the Histological Structure of the Medullary Renal Tubules in the Developing Fetal Kidney.

Concerning how the two medicines influenced the histological organization of the medullary kidney tubules in the developing fetal kidneys, the study noted that the kidney tubules in the two treatment groups depicted some histomorphological alterations when compared with the control ***(figures 4.8 A to figures 4.10 A)***. In particular, it was observed that the kidney medullary tubules depicted some degree of tubular degenerative damage and interstitial edematous expansions or at times they depicted a combination of both tubulo-interstitial edema with some pockets of tubulo-interstitial adhesion. This phenomenon was particularly noted for the rats that were treated in the medium and high dose groups at TM_1 and TM_2 ***(figures 4.8 C, D, F, G and figure 4.9 C, D, F, G)***. Further in the fetal kidneys that were noted to depict tubulo-interstitail adhesion with enlarged bowmans capsule, the oveall sizes of the kidneys were also noted to be big because of enlarged bowmans spaces with smaller condensed sizes of glomeluli tuft of capillaries pushed in towards the vascular pole of the glomeluli as seen in photomicrographs figures ***figures 4.8 B to G to figure 4.10 B to G.***

The observed enlarged bownan spaces were thought to be a sign of fluid acculation and impaired renal fluid clearance that could have been ocassioned by the observed histomorphological alterations in the renal tubles that either made them to be malfunctional in allowing the flow of urine in the urinary pole of the nephron in the medulla. These tubular changes in the kidney tubules were also noted to depict a dose response relationship in that when the doses of the two medicines increased there was a correponding remakable increase in the degenerative and interstiatil edema or adhesions with the highest changes being in TM_1 and TM_2 as shown in ***figures 4.8 B to G to figure 4.9 B to G.***

The lumen of these kidney tubules were also noted to be disproportinately enlarging with the increasing dosage of the two medicines as shown in ***figures 4.8 B, to G to figure 4.10 B, to G.*** Inaddition, the study noted that the collagen fiber deposition was

increasing with increase in the dosage of the tretment medicines particularly in high dose treated groups for the two treatment medicines in trimester one and trimaster two ***(figures 4.8 D to G to figure 4.9 D to G.).*** In particular for the rats that were treated in TM_1 and TM_2 in both the two treatment groups the fetal kidneys were noted to have islands of undifferentiated renal tissues particularly in the lamotrigen treated rats that were exposed to medium and high doses as shown in ***figure 4.9 F, G and figure 4.10 F, G.*** In summary the comparative anlysis on how the two medicines differed in influenceing the difrentiation and the morphogenesis of the renal tubules in the fetal kidney, it was notable that lamotrigine has more deleterious effects as compared with the levetiracetum ***(figures 4.8 E, F, G to figure 4.10 B, C, D)***.

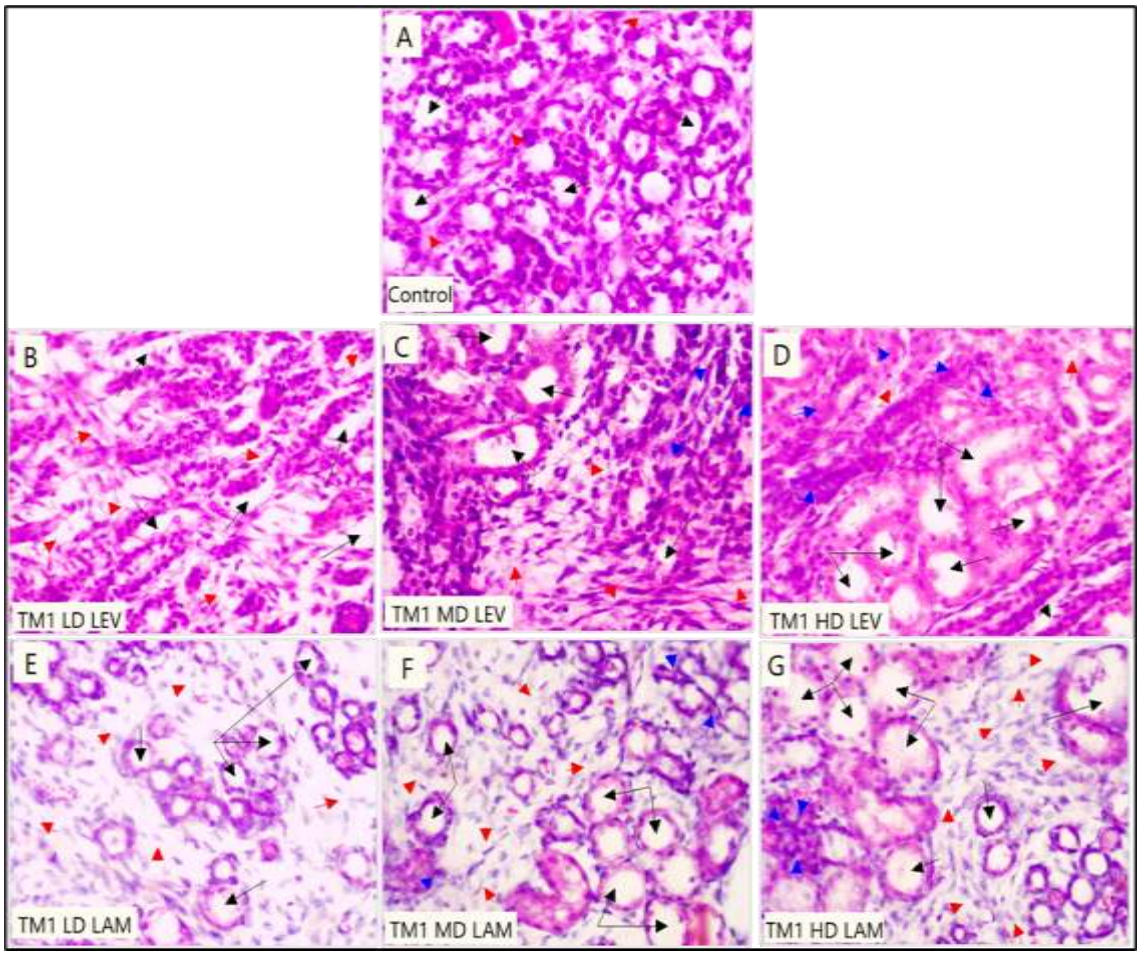

Figure 4.8. A Histo-Photomicrograph of Comparative Histo-Morphological Features of the Fetal Kidney Showing Renal Tubule and Other Medullary Changes in TM1. Stained with H&E at Magnification of X400.

KEY:

NB> The black arrow shows the kidney tubules, the red arrow heads show the collagen fibres and the blue arrow heads show the un-differentiated kidney tissue masses.

***A: CONTROL**; Normal kidney tubules with normally distributed collagen fibres in-between the kidney tubules. **B: TM1 LEV LD**: Enlarged kidney tubules with increased collagen fibres deposition in-between the kidney tubules. **C: TM1 LEV MD**; Moderately enlarged kidney tubules with increased collagen fibres deposition in-between the kidney tubules with un-differentiated kidney tissue mass. **D: TM1 LEV HD**; Highly enlarged kidney tubules with increased collagen fibres deposition in-between the kidney tubules and poorly differentiated kidney tissue masses. **E: TM1 LTG LD**; Slight increased kidney tubules with increased amount of collagen fibres in-between the tubules. **F: TM1 LTG MD**; Increased kidney tubules with increased collagen fibres deposition in-between the kidney tubules and un-differentiated kidney tissue mass. **G: TM1 LTG HD**; Highly increased kidney tubules with increased collagen fibres in-between the kidney tubules with un-differentiated kidney tissue mass.*

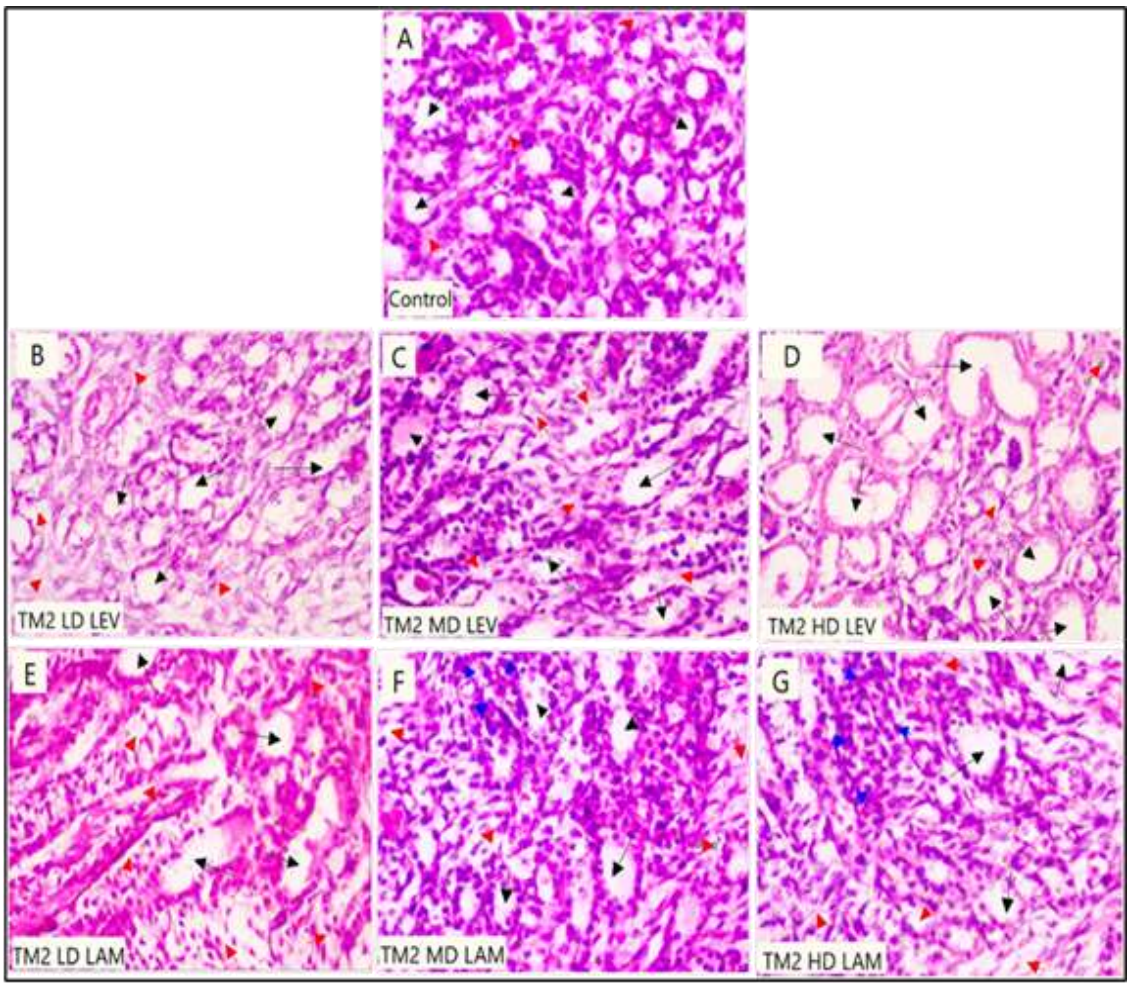

Figure 4.9. A Histo-Photomicrograph of Comparative Histo-Morphological Features of the Fetal Kidney Showing Renal Tubules and Other Medullary Changes in TM2. Stained with H&E at Magnification of X400.

KEY:

A: CONTROL; *Normal kidney tubules with normally distributed collagen fibres in-between the kidey tubules.* ***B: TM2 LEV LD****: Enlarged kidney tubules with increased collagen fibres deposition in-between the tubules;* ***C: TM2 LEV MD****; Moderately enlarged kidney tubules with increased collagen fibres deposition in-between the kidney tubules.* ***D: TM2 LEV HD;*** *Enlarged kidney tubules with increased collagen fibres deposition in-between the kidney tubules.* ***E: TM2 LTG LD;*** *Slight increased kidney tubules with increased amount of collagen fibres in-between the kidney tubules.* ***F: TM2 LTG MD;*** *Enlarged kidney tubules with highly increased collagen fibres deposition in-between the kidney tubules with some islands of undifferentiated kidney tissues.* ***G: TM2 LTG HD;*** *Highly enlarged kidney tubules, increased collagen fibres in-between the kidney tubules and large islands of un-differentiated kidney tissue mass.* ***NB>*** *The black arrow shows the kidney tubules, the red arrow heads show the collagen fibres and the blue arrow heads show the un-differentiated kidney tissue masses.*

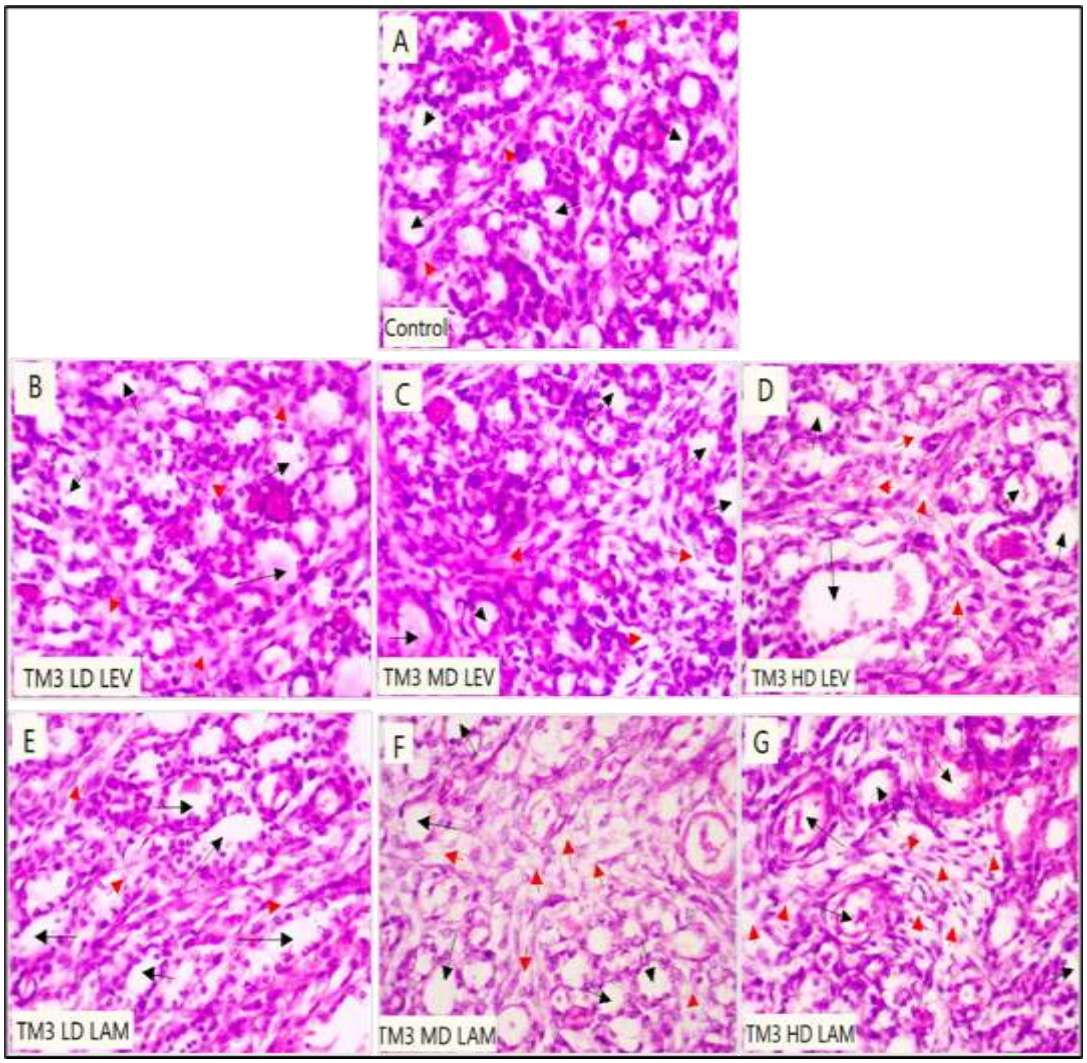

Figure 4.10. A Histo-Photomicrograph of Comparative Histo-Morphological Features of the Fetal Kidney Showing Renal Tubules and Other Medullary Changes in TM3 Stained with H&E at Magnification of X400.

KEY:

A: CONTROL*; Normal kidney tubules with normally distributed collagen in-between the kidney tubules fibres.* ***B: TM3 LEV LD****: slightly enlarged kidney tubules with slight increase in collagen fibres deposition in-between the tubules.* ***C: TM3 LEV MD****; Moderately enlarged kidney tubules with increased collagen fibres deposition in-between the tubules.* ***D: TM3 LEV HD****; Enlarged kidney tubules with increased collagen fibres deposition in-between the tubules.* ***E: TM3 LTG LD****; Slight increased kidney tubules with increased amount of collagen fibres in-between the tubules.* ***F: TM3 LTG MD****; Increased kidney tubules with increased collagen fibres deposition in-between the kidney tubules.* ***G: TM3 LTG HD****; Moderately increased kidney tubules with increased collagen fibres in-between the kidney tubules.*

NB> *The black arrow shows the kidney tubules and the red arrow heads show the collagen fibres.*

4.3.4: Stage 4. Comparative Findings on How the Two Medicines Influenced the Histological Thicknesses of the Cortex and The Medulla.

Concerning how the two medicines influenced the histological thicknesses of the fetal kidney cortex and medulla, this study established that in the two treatment groups the kidney medulla was remarkably hypertrophied in sizes with large tubulo-interstitial spaces and thickened collagen fibre depositions in the interstitial tubular spaces while kidney cortices were reduced as compared with the control ***(figure 4.11 A to figure 4.13 A)***. The kidney medullary thicknesses from the two treatment groups were increasing with increase in the dosage administered as shown in ***figure 4.11 B to G to figure 4.13 B to G.*** The kidney cortical thickness was however noted to be reducing in the two treatment medicines with increase in the dose administered as shown in the ***figure 4.11 B to G to figure 4.13 B to G.***

Across the trimesters of exposure for two treatment medicines, the medullary thicknesses were highly increased particularly in the treatment groups which were exposed to treatment from trimester one for the two treatment medicines ***(figure 4.11 B to G)***, for those exposed to treatment in trimester two for the two treatment medicines, they depicted moderate increment of their medullary thickness ***(figure 4.12 B, to G)*** while for those exposed to treatment in trimester three those kidneys were found to depict a slight increase of their medullary thickness ***(figure 4.13 B to G).***

Comparative analysis between the two study medicines indicated that, lamotrigine treated groups were severely affected and had the largest medullary thickness ***(figure 4.11 E, F, G to figure 4.13 E, F, G)*** as opposed to the medullary thicknesses that were observed in the levetiracetam treated groups ***(figures 4.11 B, C, D to 4.13 B, C, D)***.

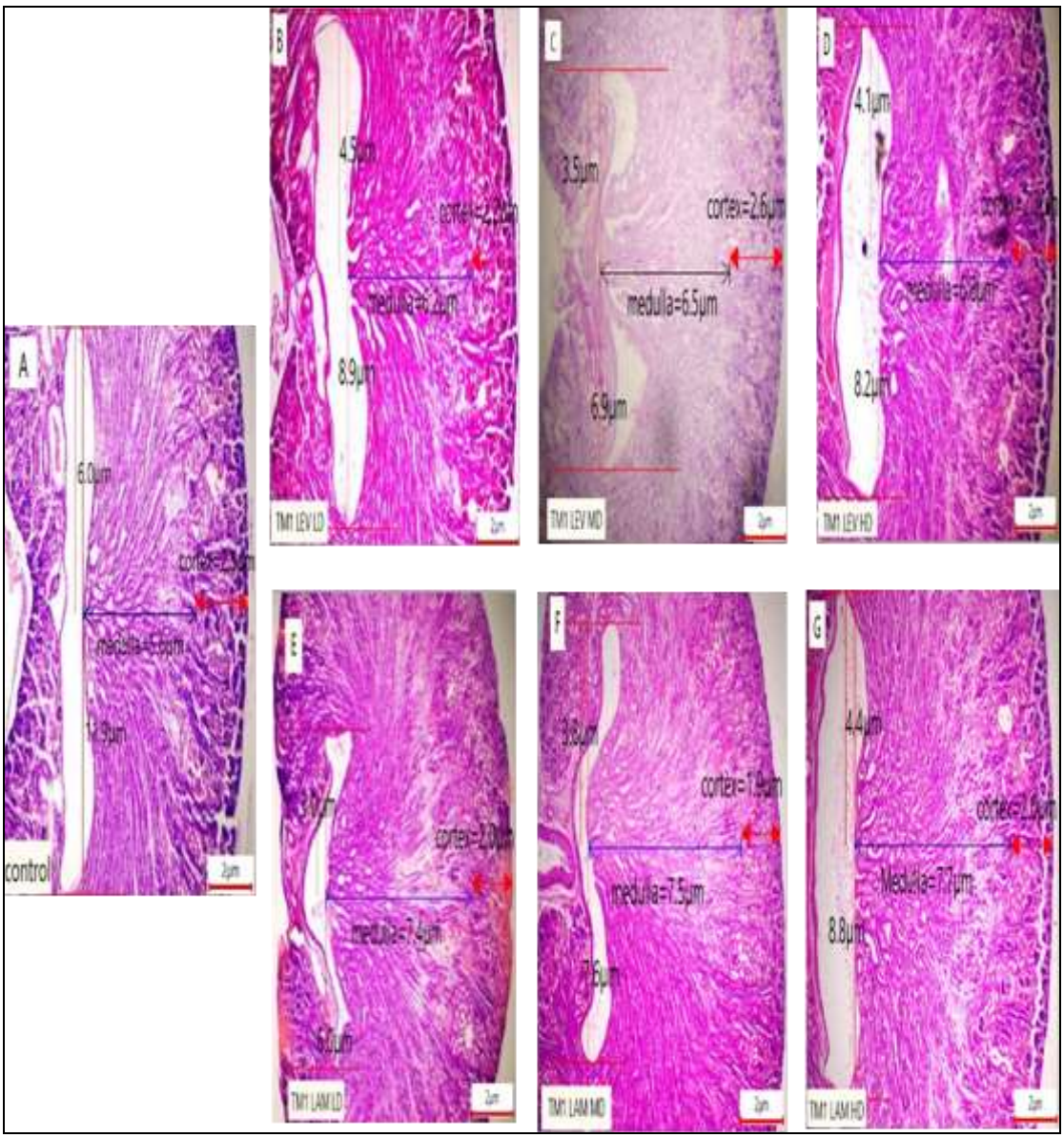

Figure 4.11 The Histo- Photomicrograph of Comparative Histo-Morphological Features of the Fetal Kidney Showing Medullary and Cortical Thicknesses in TM1. Stained with H&E at Magnification of X40.

KEY

: The blue arrow shows the medulla thickness. The red arrow shows the cortical thickness.
A: CONTROL; Normal medullary thickness and cortical thickness/lengths
B: TM1 LD LEV; Increased medullary thickness and slightly reduced cortical thickness
C: TM1 LEV MD; Increased medullary thickness and slightly increased cortical thickness
D: TM1 LEV HD; Increase in both medullary thickness and cortical thickness
E: TM1 LTG LD; Increase of both medullary thickness and cortical thickness.
F: TM1 LTG MD; High increase in the medullary thickness and reduced cortical thickness
G: TM1 LTG HD; Highly increased medullary thickness and reduced cortical thickness.

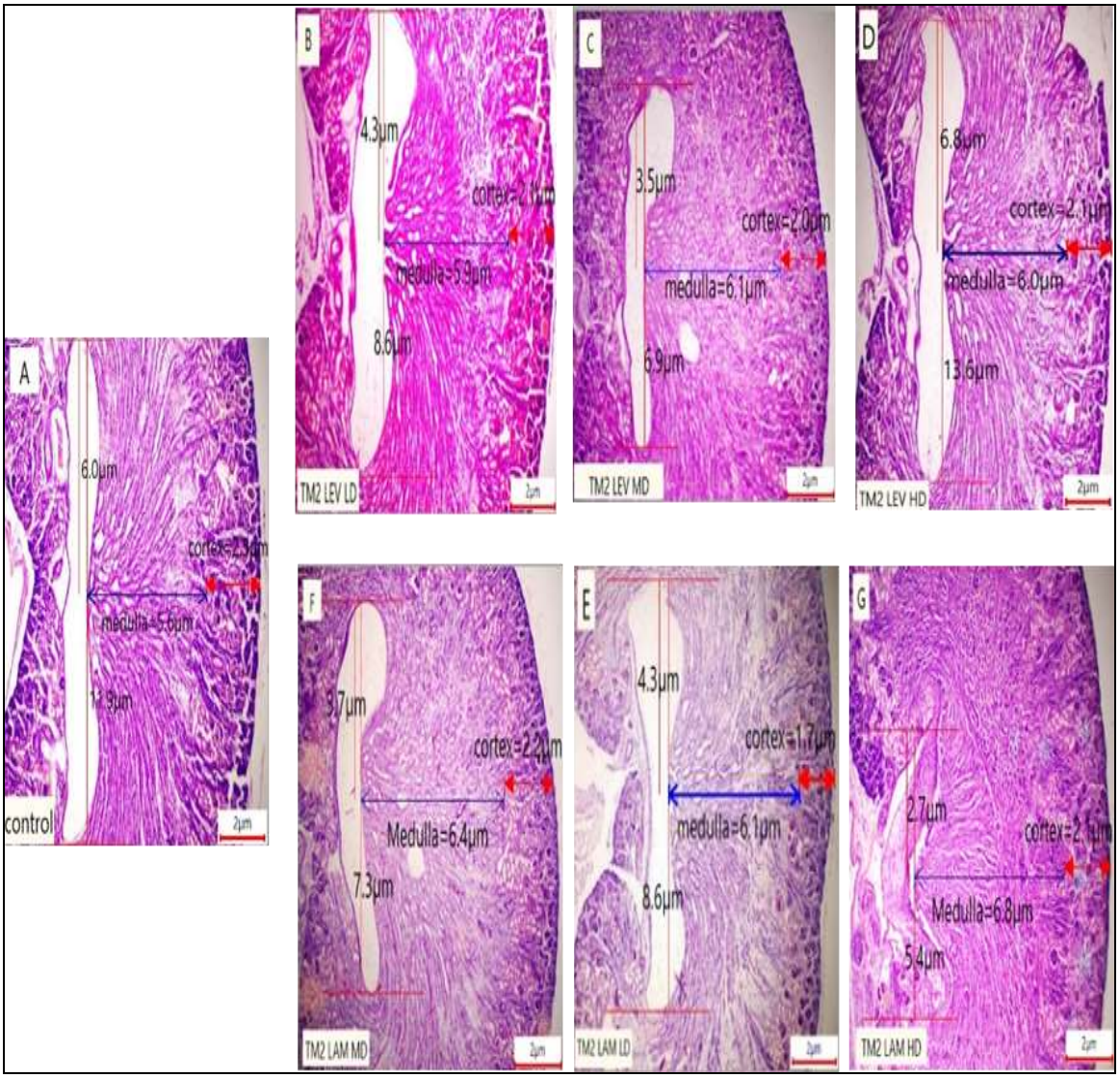

Figure 4.12. A Histo-Photomicrograph of Comparative Histo-Morphological Features of the Fetal Kidney Showing Medullary and Cortical Thicknesses in TM2. Stained H&E at Magnification of X40.

KEY:
The blue arrow shows the medulla thickness. The red arrow shows the cortical thickness.
A: CONTROL; Normal medullary and cortical thickness/ lengths
B: TM2 LEV LD; Increased medullary thickness and slightly reduced cortical thickness.
C: TM2 LEV MD; Increased medullary thickness and reduced cortical thickness.
D: TM2 LEV HD; Increased medullary thickness and reduced cortical thickness.
E: TM2 LTG LD; Increased medullary thickness and reduced cortical thickness
F: TM2 LTG MD; Increased medullary thickness and slightly reduced cortical thickness.
G: TM2 LTG HD; Highly increased medullary thickness and slightly reduced cortical thickness

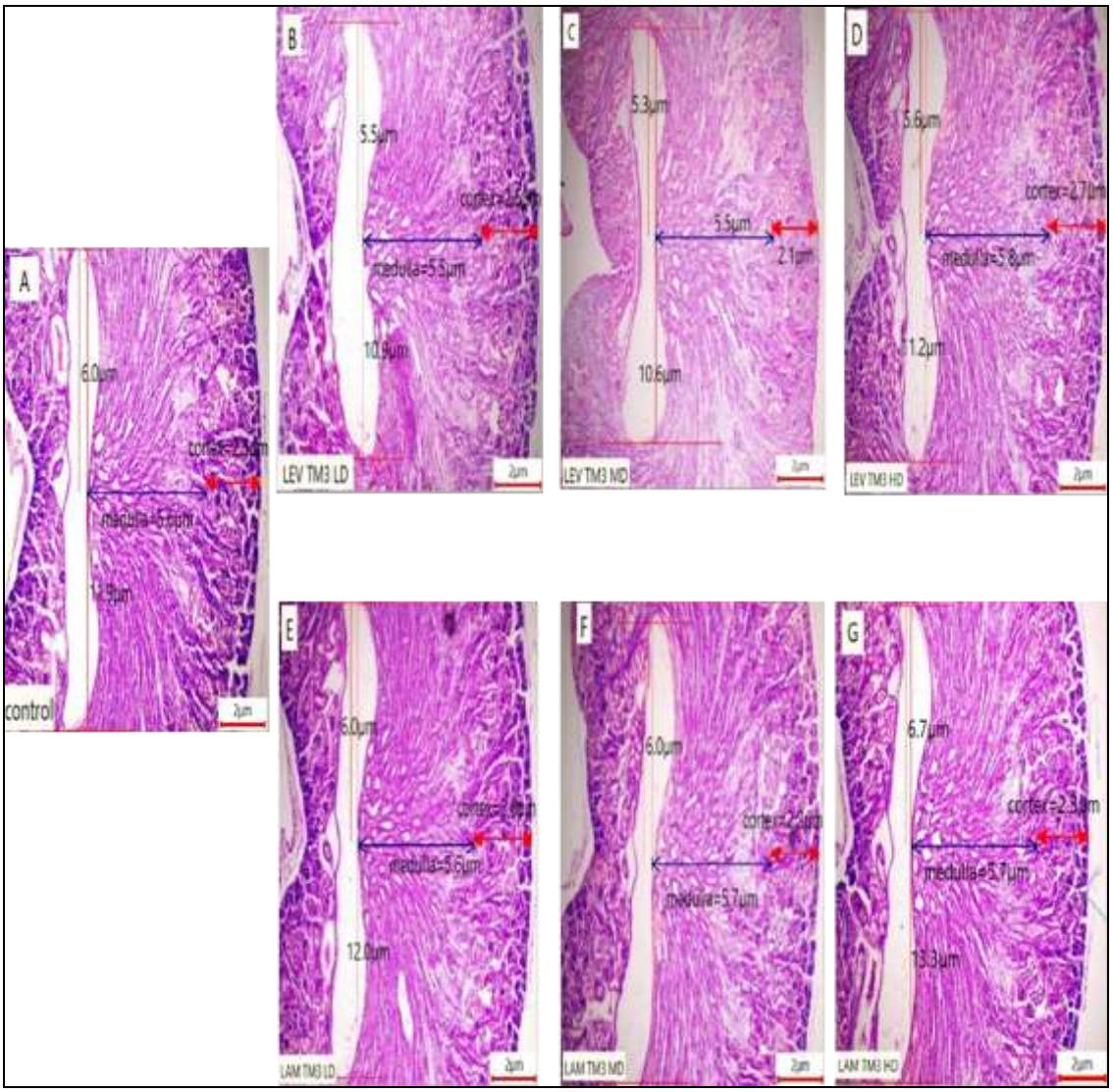

Figure 4.13. A Histo-Photomicrograph of Comparative Histo-Morphological Features of the Fetal Kidney Showing Medullary and Cortical Thicknesses in TM3 Stained with H&E at Magnification of X40.

KEY:
The blue arrow shows the medulla thickness. The red arrow shows the cortical thickness.
A: CONTROL; Normal cortical and medullary thickness.
B: TM3 LEV LD: Slightly reduced medullary thickness and slightly reduced cortical thickness
C: TM3 LEV MD; Slightly reduced medullary thickness and reduced cortical thickness
D: TM3 LEV HD; Increased medullary thickness and cortical thickness
E: TM3 LTG LD; Slight increase in both medullary and cortical thickness.
F: TM3 LTG MD; Slight increase in the medullary thickness and cortical thickness
G: TM3 LTG HD; Slight increase in both medullary thickness and cortical thickness.

The Histo-stereological Findings

4.4 Objective 3: The Comparative Evaluation on How the Two Medicines Influenced the Morphometric and the Histo-Stereological Organization of the Developing Fetal Kidney.

In evaluating how the two medicines influenced the gross morphometric and the histo-stereological organization of the fetal kidneys. The findings are presented in two steps as follows:

Step 1: The comparative results on how the two medicines influenced the gross morphometric parameters of the fetal kidneys including (i) the kidney weights, (ii) the kidney lengths, (iii) the kidney width and (iv) the initial total kidney volume.

Step 2: The comparative histo-stereological findings on how the two medicines influenced the histo-quantitative components of the fetal kidneys including (i) the calculated Cavalieri kidney volumes, (ii) the cortical and the medullary thicknesses (iii) the cortical and the medullary volume densities.

4.4.1: Step 1: The Comparative Findings on How the Two Medicines Influenced the Gross Morphometric Parameters of the Developing Fetal Kidneys

In evaluating how the two medicines influenced the gross morphometric parameters of the developing fetal kidneys, that included; (i) The mean kidney weights, (ii) the mean kidney lengths, (iii) the mean kidney widths and (iv) the mean initial total kidney volumes. The first level analysis was the one-way Analysis of Variance (ANOVA) that was meant to establish how the means of the four fetal kidney morphometric parameters differed with those of the control. The study established that, there was a remarkable significant difference($P<0.005$) in the means of the four gross morphometric parameters evaluated in the treatment groups when compared with the control as follows; **(a) kidney weights** $F(18,38) = 50.48$, **P=.001**), (b) **kidney lengths** $F(18,38) = 21.14$, **P=.001**), (c) **kidney widths** $F(18,38) = 14.82$, $P=.001$ and (d) **the initial total kidney volume** $F(18.38) = 17.03$, **P= .001**. ***(Table 4.9)***

On further comparative analysis on the teratogenic effects of the various doses (low dose, medium dose or the high doses) on the two treatment groups for the two treatment medicines, this study established that low dose treated groups for the two treatment medicines slightly affected the kidney gross morphometric parameters, while high doses for the two treatment medicines severely affected the gross morphometric kidney parameters. It was noted that these parameters depicted an inverse dose response relationship. ***(Table 4.9).*** With regards to the trimesters of exposure [TM_1, TM_2 or TM_3] between the two treatment groups, the comparative analysis between the two medicines established that the teratogenic effects of the two medicines under the study depicted a direct response relationship with the time of exposure where those rats that were exposed in trimester one (TM_1) treated had the highest teratogenic effects, followed by those in trimester two (TM_2) and those in trimester three (TM_3) ***(Table 4.9).***

Table 4.9: The ANOVA Table Showing the Comparative Findings on How the Two Medicines Influenced Gross Morphometric Parameters of Developing Fetus as Compared with the Control:

The study groups	Dosage levels	Treatment exposure time	Mean kidney weight ± SD	Mean kidney length ± SD.	Mean kidney width ± SD	Initial Total Kidney volume ± SD
Control	No treatment	-	.052 ± .01	.541 ±.02	.355± .010	.024 ±.001
	Low dose	TM1	.054 ±.01*	.563 ±.05*	.399 ±.001*	.020 ±.001
		TM2	.053 ±.01*	.558 ±.02*	.388 ±.001*	.021 ±.001
		TM3	.051 ±.04	.554 ±.03	.372 ±.004	.023 ±.001
	Medium dose	TM1	.055 +.02*	.579±.00*	.400 ±.001*	.018 ±.001*
Levetiracetam treated group		TM2	.054 ±.06*	.570 ±.02*	.390 ±.001*	.018 ±.001*
		TM3	.053 ±.05	.564 ±.06	.372 ±.004	.021 ±.001
	High dose	TM1	.056 ±.05*	.584 ±.04*	.407 ±.006*	.014 ±.003*
		TM2	.054 ±.04*	.576 ±.07*	.394 ±.003*	.017 ±.002*
		TM3	.054 ±.04*	.569 ±.01*	.386 ±.007*	.021 ±.001
	Low dose	TM1	.058± .06*	.576 ±.04*	.407 ±.003*	.019 ±.001*
		TM2	.057 ±.07*	.564 ±.08*	.402 ±.006*	.021 ±.001
		TM3	.054 ± .04	.599 ±.00	.389 ±.001*	.023 ±.001
Lamotrigine treated groups	Medium dose	TM1	.062 ±.05*	.583 ±.03*	.426 ±.006*	.014 ±.002*
		TM2	.058 ±.01*	.575 ±.05*	.407 ±.008*	.016 ±.002*
		TM3	.054 ± 04*	.561 ±.01*	.393 ±.002*	.021 ±.001
	High dose	TM1	.068 ±.00*	.646 ±.04*	.430 ±.002*	.011 ±.002*
		TM2	.061 ±.01*	.580 ±.02*	.421 +.004*	.014 ±.003*
		TM3	.055 ±.01*	.570 ±.03*	.405 ±.010*	.020 ±.001
Comparison by (ANOVA) [F,P values]			F(18,38)=50.478 P=.001	F(18,38)=21.135 P=.001	F(18,38)=14.822 P=.001	F(18,38)=17.03 P=.001

Key: values are expressed as means ± standard deviation of mean n=3 per group. (*) the figures bearing the asterisk means that they are significantly different with the control.

The second level entailed the multivariate analysis of variance (MANOVA) which was conducted in three levels to establish how the three independent variable of; (i) **the drug**, (ii) **the dose** and (iii) **the trimester of exposure** globally and at individual influenced the fetal kidneys gross morphometric dependent variables of the; **(i)** the kidney weight (KDY WT), **(ii)** the kidney length (KDY LGHT), **(iii)** the kidney width (KDY WDTH) and **(iv)** the initial/reference total kidney volume (I. KDY. VOL.) when either acting individually, or when combined in two-way or when combined in three-ways, the findings on the global/main effects and their interaction effects are shown by the significant proportions of the partial Eta (η^2) as shown below by the MANOVA level one analysis as follows;

(i) At individual level when each of the three independent variables was acting alone, the total contributory influence of each of the three independent variables on the gross morphometric kidney parameters were as follows; (a) **drugs** F (4,35) = 126.867, P<.001, Wilks' Lambda (∧) =.065, partial Eta (η^2) = .938. (b) **dosage** F (8,70) = 14.049, P< .001, Wilks' Lambda (∧) =.147, partial Eta (η^2) =.616. (c) **trimesters** F (8,70) = 24.057, P< .001, Wilks' Lambda (∧) = .071, partial Eta (η^2) = .733. It was established that each of the three independent variables influenced the fetal kidney parameters in different proportions with the drug having the strongest influence at partial Eta (η^2) of 93.8% while trimesters had a weak influence at partial Eta (η^2) of 73.3% and dosage had the weakest influence at partial Eta (η^2) of 61.6% ***(Table 4.10)*.**

(ii) At two-way combined interaction effects of either of the two independent variable (drug, dosage or trimester) they had their influence as follows; (a) **drugs * dosage** F(8,70) =2.4, P<.024 Wilks' Lambda (∧)= .616, partial Eta (η^2) = .215, (b) **drugs*trimesters** F(8,70) =3.231, P< .003 Wilks' Lambda (∧)= .533, partial Eta (η^2) = .270, (c) **trimester*dosages** F(16,107.564) =3.941, P<.001, Wilks' Lambda (∧) = .244, partial Eta (η^2) = .297 ***(Table 4.10).***

It was observed that the interaction effects at two-way level was weak ranging between partial Eta (η^2) 21.5% for the **drug*dosage** to partial Eta (η^2) 29.7% for the **dosage*trimesters**. It was therefore established that the observed influence on the

fetal kidney outcomes at the global scale was majorly because of the individual independent variable but not because of their interaction effects at two-way interaction level.

Table 4.10: The MANOVA Level 1 Table Showing the Overall/Global Effects of The Three Independent Variables and Their Interaction Effects on the Gross Morphometric Fetal Kidney Parameters.

Types of MANOVA evaluation at level 1	Comparative global effects assessed	Multivariate Tests						
		Parameters used	Wilks' Lambda Value	Statistics (F)	Hypothesis degree of freedom	Error degree of freedom	Sig.<.05	Proportion of variance Partial Eta Squared
Test on whether the observed results were due to chance	Overall evaluation of whether or not the observed effects were due to chance or treatment	Intercept	.000	71561.725[b]	4.000	35.000	.000	1.000
The individual main effects of the drugs, dosages and time of exposure on fetal kidneys	Evaluation of whether or not the observed effects were due to drugs	DRUG	.065	126.867[b]	4.000	35.000	.000	.935
	Evaluation of whether or not observed effects were due to dosages	DOSAGE	.147	14.049[b]	8.000	70.000	.000	.616
	Evaluation of whether or not the observed effects were due to time of exposure	TRIMESTERS	.071	24.057[b]	8.000	70.000	.000	.733
Two-way interaction effects on the fetal kidney parameters	Evaluation of whether or not the observed effects were due to interaction between dugs and dosages.	DRUG * DOSAGE	.616	2.400[b]	8.000	70.000	.024	.215
	Evaluation of whether or not the observed effects were due to drugs interaction between drugs and trimesters	DRUG * TRIMESTERS	.533	3.231[b]	8.000	70.000	.003	.270
	Evaluation of whether or not the observed effects were due to interaction between dosage and trimesters	DOSAGE * TRIMESTERS	.244	3.941	16.000	107.564	.000	.297
Three-way interaction effects one the fetal parameters.	Evaluation of whether or not the observed effects were due to interaction between drug, dosage and trimester.	DRUG * DOSAGE * TRIMESTERS	.674	.927	16.000	107.564	.542	.094

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

b. Exact statistic

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

Upon carrying **out the MANOVA level 2 analysis** to evaluate how each of the three independent variable of (i) the drug, (ii) the dosage and (iii) the trimester together with their interaction effects influenced the four gross morphometric fetal parameters of [kidney weight, kidney length, kidney width and initial total kidney volumes], it was established that there was significant main effects and significant interaction effects of the drugs, dosages and trimesters influencing each of the four fetal parameters *in-utero* as shown below:

(i) At individual level, the global contribution of either the drug used, the dosages administered or the trimesters of exposure was observed to statistically significantly (P<.001) influence each of the four kidney parameters (kidney weights, kidney lengths, kidney widths and initial total kidney volumes) at varying proportions (partial Eta η^2). This suggested that all the three independent variables individually played a role in determining the individual kidney outcomes with the drugs having the strongest influence on kidney weight at partial Eta (η^2) (proportion of variance being) of 99.9% while the weakest influence was from dosages on kidney weight at partial Eta (η^2) (proportion of variance being) of 49.7%. ***(Table 4.11).***

(ii) Combined two-way interaction effects of the independent variables on the four dependent fetal parameters showed that between **drug*dosages** only kidney weights and kidney widths had statistically significant interaction effects (P< .05). Between **drug*trimesters** only kidney weights and initial/reference total kidney volumes had statistically significant interaction effects (P< .05). Between **dosage*trimesters**, statistically significant effects were noted on kidney lengths and initial/reference total kidney volumes (P< .05).

It was noted that the observed kidney gross morphometric outcomes were majorly due to the influence of the individual independent variables other than the influence of the combined interaction effects at two-way or at three-way interaction effects levels of the independent variables. Drugs had the strongest influence on kidney weight at partial eta (η^2) of 81.5% while doses had the weakest influence again on kidney weight at partial eta (η^2) of 49.7%. ***(Table 4.11).***

Table 4.11: The MANOVA Level II Table Showing How Each of the Three Independents Variables of the Drug, Dose and Time of Exposure Influenced Each of the Four Fetal Kidneys Gross Morphometric Parameters.

	Groups being tested	Dependent Variables	Measurements of the variability in the dependent variable. (Type III Sum of Squares)	Degree of freedom	The ratio type 111 sum of square to its corresponding degree of freedom (Mean Square)	The ratio of the mean square for the independent variable to the mean square for error (F statistics)	Sig. <.05	Proportion of variance (Partial Eta Squared)
Evaluation on the correctness of the model used for the study	Corrected Model	KDY WGT	.001[a]	18	5.296E-5	21.668	.000	.911
		KDY LGTH	.050[b]	18	.003	17.527	.000	.893
		KDY WIDTH	.014[c]	18	.001	22.596	.000	.915
		I. KDY. VOL.	.001[d]	18	3.559E-5	11.641	.000	.846
Test on whether the observed results are due to chance	Intercept	KDY WGT	.125	1	.125	51034.067	.000	.999
		KDY LGTH	12.622	1	12.622	79782.901	.000	1.000
		KDY WIDTH	6.419	1	6.419	188849.795	.000	1.000
		I. KDY. VOL.	.032	1	.032	10516.073	.000	.996
The individual independent variable and its effects on each of the three fetal dependent variables.	DRUG	KDY WGT	.000	1	.000	167.541	.000	**.815**
		KDY LGTH	.025	1	.025	156.563	.000	.805
		KDY WIDTH	.005	1	.005	141.987	.000	.789
		I. KDY. VOL.	.000	1	.000	52.616	.000	.581
	DOSAGE	KDY WGT	9.186E-5	2	4.593E-5	18.793	.000	**.497**
		KDY LGTH	.007	2	.003	21.573	.000	.532
		KDY WIDTH	.002	2	.001	26.835	.000	.585
		I. KDY. VOL..	.000	2	8.349E-5	27.308	.000	.590
	TRIMESTERS	KDY WGT	.000	2	.000	60.253	.000	.760
		KDY LGTH	.010	2	.005	30.027	.000	.612
		KDY WIDTH	.006	2	.003	83.114	.000	.814
		I. KDY. VOL.	.000	2	8.904E-5	29.124	.000	.605
Two-way interaction effects on each of the three fetal dependent variables	DRUG * DOSAGE	KDY WGT	2.572E-5	2	1.286E-5	5.261	.010	.217
		KDY LGTH	.000	2	.000	.806	.454	.041
		KDY WIDTH	.000	2	.000	3.673	.035	.162
		I. KDY. VOL.	7.148E-6	2	3.574E-6	1.169	.322	.058
	DRUG * TRIMESTERS	KDY WGT	3.680E-5	2	1.840E-5	7.530	.002	.284
		KDY LGTH	.000	2	.000	1.358	.269	.067
		KDY WIDTH	2.070E-7	2	1.035E-7	.003	.997	.000
		I. KDY. VOL.	2.013E-5	2	1.007E-5	3.293	.048	.148
	DOSAGE * TRIMESTERS	KDY WGT	3.102E-5	4	7.756E-6	3.173	.024	.250
		KDY LGTH	.007	4	.002	11.126	.000	.539
		KDY WIDTH	.000	4	4.044E-5	1.190	.331	.111
		I. KDY. VOL.	3.750E-5	4	9.375E-6	3.066	.028	.244
Three-way interaction effects on the three fetal dependent variables	DRUG * DOSAGE * TRIMESTERS	KDY WGT	2.169E-5	4	5.421E-6	2.218	.085	.189
		KDY LGTH	.000	4	4.441E-5	.281	.889	.029
		KDY WIDTH	.000	4	3.668E-5	1.079	.380	.102
		I. KDY. VOL.	3.235E-6	4	8.088E-7	.265	.899	.027
Overall inferential statistics on the model results.	Error	KDY WGT	9.287E-5	38	2.444E-6			
		KDY LGTH	.006	38	.000			
		KDY WIDTH	.001	38	3.399E-5			
		I. KDY. VOL.	.000	38	3.057E-6			
	Total	KDY WGT	.178	57				
		KDY LGTH	17.775	57				
		KDY WIDTH	9.062	57				
		I. KDY. VOL.	.048	57				
	Corrected Total	KDY WGT	.001	56				
		KDY LGTH	.056	56				
		KDY WIDTH	.015	56				
		I. KDY. VOL.	.001	56				

a. R Squared = .911 (Adjusted R Squared = .869)
b. R Squared = .893 (Adjusted R Squared = .842)
c. R Squared = .915 (Adjusted R Squared = .874)
d. R Squared = .846 (Adjusted R Squared = .774)

Further, upon carrying out the **MANOVA level III pairwise comparative analysis** on how the two medicines influenced the kidney gross morphometric parameters of the kidney weights, kidney widths, kidney lengths, and initial total kidney volumes in the same dosage groups and in the same trimester, a MANOVA pairwise comparisons test was conducted. It was observed that the gross morphometric kidney parameters in all doses and across all trimesters were statistically significantly different (P< .05) between the two treatment medicines. The overall finding from this pairwise comparisons on the gross morphometric kidney parameters was that lamotrigine had severest teratogenic effects than levetiracetam to the growing and developing kidney parameters. **(Table 4.12)**

Table 4.12: The MANOVA Level 111 Table Showing the Pairwise Comparative Findings on How the Two Medicines Influenced the Four Gross Morphometric Parameters of the Fetal Kidneys.

Dependent variable	Dose level	The time of exposure to treatment	Levetiracetam treatment	Lamotrigine treatment	Mean difference between LEV and LAM treatment	Std Error	Sig.<0.05	95%confidence interval	
								Lower bound	Upper bound
	LOW	TM1	LEV	LTG	-.004	.001	.001	-.007	-.002
	DOSE	TM2	LEV	LTG	-.004	.001	.002	-.007	-.002
		TM3	LEV	LTG	-.003	.001	.009	-.006	-.001
KIDNEY	MEDIUM	TM1	LEV	LTG	-.007	.001	.000	-.010	-.004
WEIGHT	DOSE	TM2	LEV	LTG	-.004	.001	.002	-.007	-.002
		TM3	LEV	LTG	-.004	.001	.003	-.007	-.002
	HIGH	TM1	LEV	LTG	-.012	.001	.000	-.014	-.009
	DOSE	TM2	LEV	LTG	-.006	.001	.000	-.009	-.004
		TM3	LEV	LTG	-.004	.001	.003	-.007	-.001
	LOW	TM1	LEV	LTG	-.046	.010	.000	-.067	-.025
	DOSE	TM2	LEV	LTG	-.039	.010	.000	-.060	-.019
		TM3	LEV	LTG	-.032	.010	.004	-.052	-.011
KIDNEY	MEDIUM	TM1	LEV	LTG	-.042	.010	.000	-.063	-.021
LENGTH	DOSE	TM2	LEV	LTG	-.044	.010	.000	-.065	-.023
		TM3	LEV	LTG	-.036	.010	.001	-.057	-.015
	HIGH	TM1	LEV	LTG	-.062	.010	.000	-.083	-.041
	DOSE	TM2	LEV	LTG	-.043	.010	.000	-.064	-.023
		TM3	LEV	LTG	-.041	.010	.000	-.062	-.021
	LOW	TM1	LEV	LTG	-.015	.005	.003	-.018	.001
	DOSE	TM2	LEV	LTG	-.013	.005	.008	-.023	-.004
		TM3	LEV	LTG	-.017	.005	.001	-.026	-.007
KIDNEY	MEDIUM	TM1	LEV	LTG	-.025	.005	.001	-.035	-.016
WIDTH	DOSE	TM2	LEV	LTG	-.017	.005	.001	-.026	-.007
		TM3	LEV	LTG	-.020	.005	.000	-.030	-.011
	HIGH	TM1	LEV	LTG	-.023	.005	.000	-.033	-.013
	DOSE	TM2	LEV	LTG	-.027	.005	.000	-.036	-.017
		TM3	LEV	LTG	-.019	.005	.000	-.029	-.010
		TM1	LEV	LTG	.004	.001	.015	.001	.007
INITIAL	LOW	TM2	LEV	LTG	.004	.001	.019	.001	.006
TOTAL	DOSE	TM3	LEV	LTG	-.001	.001	.043	-.002	.004
KIDNEY	MEDIUM	TM1	LEV	LTG	-.005	.001	.002	-.008	-.002
VOLUME	DOSE	TM2	LEV	LTG	-.003	.001	.050	-.006	0.00
		TM3	LEV	LTG	.002	.001	.048	.000	.005
	HIGH	TM1	LEV	LTG	-.006	.001	.000	-.009	-.003
	DOSE	TM2	LEV	LTG	-.004	.001	.005	-.007	-.001
		TM3	LEV	LTG	-.003	.001	.046	-.005	.000

4.4.2: Step 2: The Histo-Stereological Findings on How the Two Medicines Influenced the Histo-Quantitative Components of the Fetal Kidneys.

In evaluating how the two medicines influenced the histo-stereological parameters of the developing fetal kidneys, the following parameters were evaluated; - **(i)** the calculated Cavalieri kidney volumes, **(ii)** the histological thicknesses of the cortex (iii) the histological thicknesses of the medulla **(iv)** the volume densities of the cortex in relation to the entire kidneys, **(v)** the volume densities of the medulla in relation to the entire kidney. The analysis was done at two levels the ANOVA descriptive analysis and the second level was the MANOVA multivariate analysis to establish how multiple independent variables influenced the dependent variables either acting alone or in combination or by their interaction effects.

The findings of level one evaluation by use of ANOVA, the study established that all the five histo-stereological parameters of the fetal kidneys were statistically different from the controls as follows; **(a) the calculated Cavalieri kidney volumes**,= F (18,38) =17.54, **P=.001**), **(b) the medullary volume densities** F (18,38) =32.968, **P=.001**), (c) **the cortical volume densities** F (18,38) =7.228, **P=.001) (d) the medullary thickness** F(18,38)=4.1 P=.001), and **(e) the cortical thickness** F(18,38)=14.2 **P=.001**).

On further comparison on how the different dosages of the two medicines influenced the four histo-quantitative parameters evaluated [i.e the low, medium and high doses] it was established that the fetal kidneys of the rats treated with the high doses of both the two medicines were severely affected followed by the medium dose treatment groups **table 4.13.**

With regards to how the trimesters of exposure influenced the four stereological outcomes, this study established that the worst teratogenic effects were when the treatments were instituted in TM_1 and TM_2 denoting that the teratogenic perturbations were basically due to disruptions resulting during the process of cell differentiation, cell programming and kidney tissues maturation process during the prenatal period. This is confirmed by the observed less effects in the fetal kidneys of the rats that were exposed in trimester three (TM_3). **(Table 4.13)**

Table 4.13: An ANOVA Table Showing Histo-Stereological Findings on How the Two Medicines Influenced the Histo-Quantitative Components of the Fetal Kidneys.

Study groups	Dosage levels	Treatment exposure time	Cavalieri volume ± SD	Shrinkage ± SD	Medullary Volume Density± SD	Cortical Volume Density ±SD	Mean medullary thickness ±SD	Mean cortical thickness ± SD
Control	No treatment	None.	.023 ±.001	.001± .000	.015 ±.000	.008± .001	5.83± .17	2.73 ±.05
	Low dose	TM1	.019 ±.001	.001 ±.000	.013 ±.007	.006 ±.001	6.30 ±.26	2.46 ±.15
		TM2	.020 ±.001	.001 ±.000	.013 ±.000	.007 ±.001	5.83 ±.50	2.63 ±.21
		TM3	.022 ±.001	.001 ±.000	.014 ±.000	.008 ±.001	5.70 ±.10	2.70 ±.10
	Medium dose	TM1	.016 ±.001*	.001 ±.000	.011 ±.001*	.005 ±.001*	6.63 ±.50*	2.23±.21
Levetiracetam		TM2	.017 ±.001*	.001 ±.000	.012 ±.001*	.005 ±.001*	5.83 ±.30	2.60±.10
		TM3	.020 ±.001	.001 ±.000	.014 ±.001	.007 ±.001	5.76 ±.15	2.60±.10
	High dose	TM1	.013 ±.003*	.001 ±.000	.011 ±.007*	.004 ±.001*	6.90 ±.45*	2.27 ±.15
		TM2	.016 ±.002*	.001 ±.000	.010 ±.001*	.005 ±.010*	5.96 ±.37	2.47 ±.32
		TM3	.020 ±.001	.001 ±.000	.013 ±.000	.006 ±.000	5.96 ±.41	2.46 ±.15
	Low dose	TM1	.018 ±.001*	.001 ±.000	.012 ±.000*	.006 ±.001*	7.40 ±.26*	2.43 ±.21
		TM2	.020 ±.001	.001 ±.000	.013 ±.000	.007 ±.010	5.93 ±.41	2.63 ±.15
		TM3	.022 ±.001	.001 ±.000	.015 ±.001	.007 ±.001	5.67 ±.15	2.70 ±.10
	Medium dose	TM1	.013 ±.002*	.001 ±.000	.007 ±.001*	.004 ±.001*	7.50 ±.10*	2.20
Lamotrigine		TM2	.015 ±.002*	.001 ±.000	.010 ±.001*	.005 ±.001*	6.26 ±.15	±.20*
		TM3	.021 ±.001	.001 ±.000	.014 ±.001	.007 ±.000	5.66 ±.15	2.53 ±.21
								2.70 ±.10
	High dose	TM1	.012 ±.002*	.001 ±.000	.007 ±.001*	.003 ±.001*	7.60 ±.20*	2.00
		TM2	.013 ±.002*	.001 ±.001	.008 ±.000*	.004 ±.002*	6.24 ±.25	±.10*
		TM3	.019 ±.002	.001 ±.001	.012 ±.001*	.007 ±.001	5.72 ±.15	2.33 ±.25
								2.43 ±.15
			F(18,38)=17.54 P=.001	F(18,38)=1.567	F(18,38)=32.968 P=.001	F(18,38)=7.228 P=.001	F(18,38)=14.2 P=.001	F(18,32)=4.1 P=.001

Key: values are expressed as means ± standard deviation of mean n=3 per group. (*) the figures bearing the asterisk means that they are significantly different with the control.

The second level was a multivariate analysis of variance **(MANOVA)** which was conducted in three levels. The MANOVA analysis was meant to analyze the individual main effects, the interaction effects and the pairwise comparison between the two treatment medicines. Upon conducting a **MANOVA level I analysis** on how the three independent variables of **(i)** drug used, **(ii)** the dosage administered and **(iii)** the trimester of exposure influenced the histo-quantitative histo-stereological kidney parameters which included;- **(i)** the calculated Cavalieri kidney volumes, **(ii)** the histological thicknesses of the cortex **(iii)** the histological thicknesses of the medulla

(iv) the volume densities of the cortex in relation to the entire kidneys, **(v)** the volume densities of the medulla in relation to the entire kidney, a multivariate analysis of variance (MANOVA) and applying Wilks' Lambda statistical test was used. It was observed that there were statistically significant main effects together with significant interaction effects (*) which influenced the histo-quantitative histo-stereological fetal kidney parameters in different proportions (partial Eta (η^2) as listed below;

(i) The global main influence of each of the independent variable together with their interaction effects on the global histo-stereological fetal kidney the findings were as follows (a) **drugs** F(5,34) = 17.859, P< .001, Wilks' Lambda (∧) = .276, Partial Eta (η^2)= .724. (b) **dosages** F (10,68) = 13.451, P< .001, Wilks' Lambda (∧) = .113, Partial Eta (η^2) = .664. (c) **trimesters** F(10,68) = 16.434, P< .001, Wilks' Lambda (∧) = .086, Partial Eta (η^2) =.707. It was observed that the drugs had the strongest influence on these fetal parameters with a proportion of variance (Partial Eta (η^2)) of 72.4% followed by trimesters with a proportion of variance (Partial Eta (η^2)) of 70.7% and dosages had the weakest influence with a proportion of variance (Partial Eta (η^2)) of 66.4%.

(ii) At two-way combined interaction effects the findings were as follows (a) dosage * drugs F(10,68) = 5.397, P< .001, Wilks' Lambda (∧) = .311, Partial Eta (η^2) = .442. (b) drugs * trimesters F(10,68) = 5.687, P< .001, Wilks' Lambda (∧) =.297, Partial Eta (η^2) =.455. (c) dosage * trimesters F(20,113.715)= 3.173, P< .001, Wilks' Lambda (∧) = .230, Partial Eta (η^2) =.308. The strength of the interaction effects moderate to weak ranging between (Partial Eta (η^2)) 45.5% for the drugs * trimesters and (Partial Eta (η^2)) 30.8% between dosage * trimesters.

(iii) At three-way combined interaction effects of drugs * dosages * trimesters, the findings were as follows; F(20,113.715)= 2.974, P< .001, Wilks' Lambda (∧) = .248, Partial Eta (η^2) =.295. It was noted that at three-way interaction effects, the combined influence was weak at (Partial Eta (η^2)) 29.5%.

In summary, it can be observed that at the global level of MANOVA level one analysis, the overall histo-quantitative teratogenic outcomes observed were majorly

determined by the drugs having the strongest influence (proportionate variance of the drug being 72.4%) while the dosages had the weakest influence at 66.4% while the time of exposure contributed to 71%. The combined interaction effects at either two-way interaction level or at three-way interaction level played a lesser role in determining the fetal kidney stereological outcomes in the parameters evaluated. ***(Table 4.14).***

Table 4.14: The MANOVA Level 1 Table Showing the Overall/ Global Effects of the Independent Variables and Their Interaction Effects on the Histo-Quantitative Kidney Parameters.

Multivariate Tests

Types of MANOVA evaluation at level 1	Comparative global effect evaluated		MANOVA test statistic Wilks' Lambda	Statistics (F)	Hypothesis degree of freedom	Error degree of freedom	Sig. <0.05	Proportion of variance (Partial Eta Squared)
Test on whether the observed results were due to chance	Were the observed effects due to chance or the treatment	Intercept	.002	3563.228[b]	5.000	34.000	.000	.998
	Were the observed effects due to LEV or LAM	Drugs	.276	17.859[b]	5.000	34.000	.000	.724
The individual main effects of the drug, dosage and time of exposure on the fetal dependent variables.	Were the observed effects due to varied doses	Dosages	.113	13.451[b]	10.000	68.000	.000	.664
	Were the observed effects due to differing trimesters	Trimesters	.086	16.434[b]	10.000	68.000	.000	.707
Two-way interaction effects on the fetal dependent variables	Were the observed effects due to interaction between varied doses and the drugs	Doses *Drugs	.311	5.397[b]	10.000	68.000	.000	.442
	Were the observed effects due to interaction between drugs and differing trimesters	Drugs *Trimesters	.297	5.687[b]	10.000	68.000	.000	.455
	Were the observed effects due to interaction between drugs and differing trimesters	Doses * Trimesters.	.230	3.173	20.000	113.715	.000	.308
Three-way interaction effects	Were the observed effects due to interaction between drugs, varied doses or differing trimesters.	Drugs *Doses *Trimesters.	.248	2.974	20.000	113.715	.000	.295

a. Design: Intercept + Drugs + Dosages + TRIMESTERS + Drugs * Dosages + Drugs * TRIMESTERS + Dosages * TRIMESTERS + Drugs * Dosages * TRIMESTERS

b. Exact statistic

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

*Key; * means interaction effects*

Upon carrying out further **MANOVA level 11 analysis** on how the three independent variables of **(i)** drugs, **(ii)** the dosages and **(iii)** the trimester of exposure influenced the five kidney parameters which included;- of **(i)** drug used, **(ii)** the dosage administered and **(iii)** the trimester of exposure influenced the histo-quantitative histo-stereological kidney parameters which included;- **(i)** the calculated Cavalieri kidney volumes, **(ii)** the histological thicknesses of the cortex **(iii)** the histological thicknesses of the medulla **(iv)** the volume densities of the cortex in relation to the entire kidneys, **(v)** the volume densities of the medulla in relation to the entire, a MANOVA specific statistical test was applied which specified that there was individual main effects and two-way or three-way interaction effects influencing each of the five fetal parameters in-utero as shown below.

(i) At individual level, contribution of either the drugs, the dosages or the trimesters was observed to statistically significantly (**P<.001**) influence each of the five kidney parameters (Cavalieri volume, cortical volume densities, medullary volume densities, cortical thicknesses and medullary thicknesses) at varying proportions (partial Eta η^2). This suggested that all the three independent variables individually played a role in determining the individual histo-quantitative histo-stereological kidney parameters with the trimesters having the strongest influence (proportion of variance being 89.7%) on mean Cavalieri volumes and drugs having the weakest influence (proportion of variance being 3.1%) on mean cortical thicknesses ***(Table 4.15).***

(ii) At two-way interaction effects of the three independent variables on the five histo-quantitative histo-stereological kidney fetal parameters a statistically significant (P<.05) influence was observed between (a) **drug*dosages**, (b) **drug*trimester** and (c) **dosage*trimesters**. It was noted that at this level, the interaction effects between dosage * trimesters had the biggest proportion of variance on medullary volumes density at partial Eta (η2) at 58.2%. ***(Table 4.15).***

(iii) At three-way interaction effects between drugs * dosages * trimesters on the five histo-quantitative histo-stereological kidney parameters, a statistically significant influence was only observed on medullary volume densities and Cavalieri volumes (***Table 4.15).***

The overall observed main effects and both two-way or three-way interaction effects were majorly due to individual independent variables (drug, dosage, or trimester) rather than due to the combinations between these three independent variables. This suggested that all the three independent variables individually played a major role in determining the outcome with the trimesters contributing greatest (proportion of variance being 89.7%) on Cavalieri volume means while drugs had the weakest influence (proportion of variance being 3.1%) on mean cortical thicknesses ***(Table 4. 15).***

Table 4.15: The MANOVA Level 11 Table Showing How Each of the Three Independents Variables Influenced Each of the Five Kidney Parameters.

	Groups being tested	Dependent Variable	Measurements of the variability in the dependent variables. (Type III Sum of Squares)	Degrees of freedom	The ratio type 111 sum of square of its corresponding degree of freedom (Mean Square)	The ratio of the mean square for the independent variable to the mean square for error (F statistics)	Sig.<.05	Proportion of variance (Partial Eta Squared)
Evaluation the correctness of the model used for the study	Corrected Model	CAV VOL.	.000[a]	18	2.455E-5	41.295	.000	.951
		VOL shrink	3.616E-6[b]	18	2.009E-7	1.567	.120	.426
		MED. VOL.DENSITY	.000[c]	18	2.397E-5	16.844	.000	.889
		COR. VOL. DENSITY	9.873E-5[d]	18	5.485E-6	7.761	.000	.786
		MEDULLARY THICKNESS	22.573[e]	18	1.254	13.988	.000	.869
		CORTICAL THICKNESS	2.189[f]	18	.122	4.126	.000	.662
Test on whether the observed results were due to chance	Intercept	CAV. VOL.	.029	1	.029	48914.496	.000	.999
		VOL shrink	3.965E-5	1	3.965E-5	309.171	.000	.891
		MED VOL.DENSITY	.014	1	.014	9650.583	.000	.996
		COR VOL. DENSITY	.003	1	.003	4204.153	.000	.991
		MEDULLARY THICKNESS	1577.807	1	1577.807	17599.809	.000	.998
		CORTICAL THICKNESS	259.751	1	259.751	8812.966	.000	.996
Individual independent variable and its effects on fetal dependent variable	DRUG	CAV VOL.	9.335E-5	1	9.335E-5	157.010	.000	.805
		VOL shrink	1.707E-6	1	1.707E-6	13.308	.001	.259
		MED VOL.DENSITY	8.538E-5	1	8.538E-5	60.007	.000	.612
		COR VOL. DENSITY	5.143E-5	1	5.143E-5	72.780	.000	.657
		MEDULLARYTHICKNESS	2.042	1	2.042	22.774	.000	.375
		CORTICALTHICKNESS	.036	1	.036	1.231	.274	.031
	DOSAGE	CAV VOL.	4.714E-5	2	2.357E-5	39.639	.000	.676
		VOL shrink	1.893E-7	2	9.463E-8	.738	.485	.037
		MED VOL.DENSITY	5.474E-5	2	2.737E-5	19.237	.000	.503
		COR VOL. DENSITY	9.571E-6	2	4.786E-6	6.772	.003	.263
		MEDULLARY THICKNESS	.974	2	.487	5.435	.008	.222
		CORTICAL THICKNESS	.643	2	.322	10.914	.000	.365
	TRIMESTERS	CAV VOL.	.000	2	9.810E-5	165.000	.000	**.897**
		VOL shrink	1.593E-8	2	7.963E-9	.062	.940	.003
		MED VOL.DENSITY	.000	2	5.868E-5	41.245	.000	.685
		COR VOL. DENSITY	1.864E-5	2	9.319E-6	13.187	.000	.410
		MEDULLARY THICKNESS	16.421	2	8.211	91.585	.000	.828
		CORTICAL THICKNESS	1.120	2	.560	19.000	.000	.500
Two-way interaction effects on each of the independent variable on the fetal dependent variable	DRUG * DOSAGE	CAV VOL.	7.034E-6	2	3.517E-6	5.915	.006	.237
		VOL shrink	1.444E-8	2	7.222E-9	.056	.945	.003
		MED VOL.DENSITY	1.549E-5	2	7.746E-6	5.444	.008	.223
		COR VOL. DENSITY	3.938E-6	2	1.969E-6	2.786	.074	.128
		MEDULLARY THICKNESS	.001	2	.001	.006	.994	.000
		CORTICAL THICKNESS	.058	2	.029	.986	.382	.049
	DRUG * TRIMESTERS	CAV VOL.	5.174E-6	2	2.587E-6	4.351	.020	.186
		VOL shrink	1.900E-7	2	9.500E-8	.741	.484	.038
		MED VOL.DENSITY	1.680E-6	2	8.402E-7	.591	.559	.030
		COR VOL. DENSITY	4.125E-6	2	2.062E-6	2.919	.066	.133

	Groups being tested	Dependent Variable	Measurements of the variability in the dependent variables. (Type III Sum of Squares)	Degrees of freedom	The ratio type 111 sum of square of its corresponding degree of freedom (Mean Square)	The ratio of the mean square for the independent variable to the mean square for error (F statistics)	Sig.<.05	Proportion of variance (Partial Eta Squared)
		MEDULLARY THICKNESS	2.154	2	1.077	12.016	.000	.387
		CORTICAL THICKNESS	.041	2	.021	.704	.501	.036
	DOSAGE * TRIMESTERS	CAV VOL.	2.947E-5	4	7.368E-6	12.392	.000	.566
		VOL shrink	1.007E-6	4	2.519E-7	1.964	.120	.171
		MED VOL.DENSITY	7.528E-5	4	1.882E-5	13.227	.000	.582
		COR VOL. DENSITY	3.234E-6	4	8.085E-7	1.144	.351	.107
		MEDULLARY THICKNESS	.074	4	.019	.208	.933	.021
		CORTICAL THICKNESS	.063	4	.016	.537	.709	.054
Three-way interaction effects on the of the independent variables on each of the dependent variable.	DRUG * DOSAGE * TRIMESTERS	CAV VOL.	1.192E-5	4	2.980E-6	5.012	.002	.345
		VOL shrink	4.622E-7	4	1.156E-7	.901	.473	.087
		MED VOL.DENSITY	3.381E-5	4	8.451E-6	5.940	.001	.385
		COR VOL. DENSITY	7.354E-6	4	1.839E-6	2.602	.051	.215
		MEDULLARY THICKNESS	.301	4	.075	.840	.509	.081
		CORTICAL THICKNESS	.024	4	.006	.204	.935	.021
Overall inferential statistics on the model results.	Error	CAV VOL.	2.259E-5	38	5.946E-7			
		VOL shrink	4.873E-6	38	1.282E-7			
		MED VOL.DENSITY	5.407E-5	38	1.423E-6			
		COR VOL. DENSITY	2.685E-5	38	7.067E-7			
		MEDULLARY THICKNESS	3.407	38	.090			
		CORTICAL THICKNESS	1.120	38	.029			
	Total	CAV VOL.	.043	57				
		VOL shrink	6.529E-5	57				
		MED VOL.DENSITY	.021	57				
		COR VOL. DENSITY	.004	57				
		MEDULLARY THICKNESS	2266.940	57				
		CORTICAL THICKNESS	354.080	57				
	Corrected Total	CAV VOL.	.000	56				
		VOL shrink	8.490E-6	56				
		MED VOL.DENSITY	.000	56				
		COR VOL. DENSITY	.000	56				
		MEDULLARY THICKNESS	25.979	56				
		CORTICAL THICKNESS	3.309	56				

a. R Squared = .951 (Adjusted R Squared = .928)
b. R Squared = .426 (Adjusted R Squared = .154)
c. R Squared = .889 (Adjusted R Squared = .836)
d. R Squared = .786 (Adjusted R Squared = .685)
e. R Squared = .869 (Adjusted R Squared = .807)
f. R Squared = .662 (Adjusted R Squared = .501)

*Key; * means interaction effects*

Upon carrying out a further **MANOVA level 111 analysis** on how the two treatment medicines namely levetiracetam and lamotrigine influenced the five the histo-quantitative histo-stereological kidney parameters which included;- **(i)** the calculated Cavalieri kidney volumes, **(ii)** the histological thicknesses of the cortex **(iii)** the histological thicknesses of the medulla **(iv)** the volume densities of the cortex in relation to the entire kidneys, **(v)** the volume densities of the medulla in relation to the entire, a MANOVA pairwise comparisons test was conducted at the same dosage groups and at the same trimester of exposure. The overall finding from this pairwise comparisons test on the fetal kidney parameters was that lamotrigine had more deleterious teratogenic effects than levetiracetam on growing and developing fetal kidney parameters ***(Table 4.16).***

Table 4.16: A MANOVA Level 111 Pairwise Comparison Table Showing How the Two Medicines Influenced the Kidney Parameters When Administered at the Same Dosage and at the Same Trimester of Exposure.

Dependent variables	Dosage levels	Exposure time	Levetiracetam treated group	Lamotrigine treated group	LEV and LAM mean difference	Std Error	Sig.<0.05	95%confidence interval	
								Lower bound	Upper bound
		TM1	LEV	LTG	-.002	.001	.002	-.003	-.001
		TM2	LEV	LTG	-.006	.001	.000	-.009	-.003
		TM3	LEV	LTG	-.003	.001	.000	-.004	-.002
CAVALIERI	LOW DOSE								
VOLUME	MEDIUM	TM1	LEV	LTG	.003	.001	.000	.001	.004
	DOSE	TM2	LEV	LTG	-.003	.001	.000	-.004	-.001
		TM3	LEV	LTG	-.002	.001	.000	-.004	-.001
		TM1	LEV	LTG	-.006	.001	.000	-.007	-.004
		TM2	LEV	LTG	-.003	.001	.000	-.004	-.002
		TM3	LEV	LTG	-.002	.001	.005	-.003	-.001
	HIGH DOSE								
		TM1	LEV	LTG	.000	.000	.498	.001	.001
		TM2	LEV	LTG	.000	.000	.368	.001	.001
		TM3	LEV	LTG	.001	.000	.052	.001	.001
SHRINKAGE	LOW DOSE								
	MEDIUM	TM1	LEV	LTG	.001	.000	.060	.001	.001
	DOSE	TM2	LEV	LTG	.001	.000	.910	-.001	.001
		TM3	LEV	LTG	.000	.000	.179	.001	.001
		TM1	LEV	LTG	.001	.000	.057	.001	.001
		TM2	LEV	LTG	.000	.000	.261	.001	.001
		TM3	LEV	LTG	.000	.000	.572	.001	.001
	HIGH DOSE								
		TM1	LEV	LTG	-.002	.001	.017	-.004	.000
		TM2	LEV	LTG	-.002	.001	.027	-.004	.000
		TM3	LEV	LTG	.002	.001	.045	.001	.004
MEDULLARY	LOW DOSE								
VOLUME	MEDIUM	TM1	LEV	LTG	-.002	.001	.027	-.004	.000
DENSITIES	DOSE	TM2	LEV	LTG	-.002	.001	.035	-.004	.000
		TM3	LEV	LTG	-.005	.001	.000	-.007	-.003
		TM1	LEV	LTG	-.002	.001	.025	-.004	.000
		TM2	LEV	LTG	-.002	.001	.023	-.004	.000
		TM3	LEV	LTG	-.005	.001	.000	-.007	-.003
	HIGH DOSE								
		TM1	LEV	LTG	-.002	.001	.027	-.004	.000
		TM2	LEV	LTG	-.003	.001	.000	-.004	-.001
		TM3	LEV	LTG	-.002	.001	.007	-.003	-.001
CORTICAL	LOW DOSE								
VOLUME	MEDIUM	TM1	LEV	LTG	-.002	.001	.017	-.002	.001
DENSITIES	DOSE	TM2	LEV	LTG	-.002	.001	.002	-.004	-.001
		TM3	LEV	LTG	-.002	.001	.005	-.003	-.001
	HIGH DOSE	TM1	LEV	LTG	-.003	.001	.000	-.005	-.002
		TM2	LEV	LTG	-.003	.001	.000	-.004	-.001
		TM3	LEV	LTG	-.002	.001	.004	-.003	-.001
MEDULLARY	LOW DOSE	TM1	LEV	LTG	-1.10	.244	.000	-1.59	-.605
LENGTH		TM2	LEV	LTG	-.100	.244	.002	-.595	.395
		TM3	LEV	LTG	.033	.244	.053	-.462	.528
	MEDIUM	TM1	LEV	LTG	-.867	.244	.001	-1.36	-.372
	DOSE	TM2	LEV	LTG	-.433	.244	.044	-.928	.062
		TM3	LEV	LTG	.100	.244	.050	-.395	.595
	HIGH DOSE	TM1	LEV	LTG	-.700	.244	.007	-1.19	-.205
		TM2	LEV	LTG	-.567	.244	.026	-1.06	-.072
		TM3	LEV	LTG	-.453	.244	.050	-.362	.628
CORTICAL	LOW DOSE	TM1	LEV	LTG	.033	.140	.084	-.250	.317
LENGTH		TM2	LEV	LTG	.001	.140	.078	-.284	.284
		TM3	LEV	LTG	.001	.140	.063	-.284	.284
	MEDIUM	TM1	LEV	LTG	.033	.140	.043	-.250	.317
	DOSE	TM2	LEV	LTG	.067	.140	.067	-.217	.350
		TM3	LEV	LTG	-.100	.140	.057	-.384	.184
	HIGH DOSE	TM1	LEV	LTG	.267	.140	.065	-.017	.550
		TM2	LEV	LTG	.133	.140	.047	-.150	.417
		TM3	LEV	LTG	.033	.140	.003	-.250	.317

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECCOMENDATION

The aim of this study was to comparatively evaluate the histo-morphological and histo-stereological teratogenic effects of prenatal exposure to varied doses of lamotrigine and levetiracetam on the developing fetal kidneys in albino rats (*Rattus norvegicus*). The discussion on this study findings is presented along the study objectives as follows:

5.1 Objective One: To Comparatively Evaluate How the Prenatal Exposure to Varied Doses of Levetiracetam and Lamotrigine Influenced the Maternal and Fetal Pregnancy Outcome in Albino Rats.

With concerns as to how the prenatal exposure to the two medicines influenced the maternal pregnancy outcomes, this study established that the three maternal pregnancy outcome parameters including; the mean maternal terminal weight, the total maternal weight gain, and the terminal placental weights were statistically significant lower ($P<0.05$) in all the treatment groups of both the lamotrigine and levetiracetam treated groups when compared with the control ***(Table 4.1)***.These findings are in line with some others previous study by El-Qaafarawi *et al.,* 2015, Ali *et al.,* 2020, Mwangi *et al.,* (2018) and Sigei *et al.,* (2018) who reported that the prenatal exposure to carbamazepine and phenytoin resulted with reduction in all the maternal and fetal pregnancy outcome when exposed prenatally. On further analysis to establish the teratogenic relationships between the trimesters of exposures and the dosages of exposure on the three maternal pregnancy outcome parameters, it was observed that, when the two medicines were exposed in trimester one (TM_1) and trimester two (TM_2) and at both medium and high doses the means of the three maternal pregnancy outcome parameters were greatly reduced as compared to the means of the three maternal pregnancy parameters when the treatment was instituted at TM_3 ***(Table 4.1)***

These observed adverse effects on the maternal pregnancy outcomes following the prenatal exposure to the two medicines are in line with some other study findings by Mitra-Ghosh *et al*., (2020),(Yasam *et al.,* 2016) who observed that the overall effects of prenatal exposure to any of the anticonvulsant medicines have negative deleterious impacts on both the mother and the fetuses. On further analysis on how the observed deleterious effects on the three maternal pregnancy outcome parameters differed between the lamotrigine and the levetiracetam treated groups, the study established that in all the lamotrigine treatment groups of low, medium and high, the deleterious effects were more pronounced than it was the case for the levetiracetam treated groups ***(Table 4.4).***

In a nutshell, these finding on the overall reduction in all the three maternal pregnancy outcome parameters points out to the fact that the observed maternal pregnancy outcome parameters could have been due to either the perturbations of a prolonged nutritional disturbances to the mother, or due to GIT irritation or, due to prolonged disturbances in the nutritional assimilation process in the maternal GIT = (small intestines). This finding agrees with the study findings by Isoherranen *et al.,* 2003, Hill *et al.*, 2010, who have reported of the association between prenatal exposure to anticonvulsants with maternal nutritional perturbations.

Concerning the terminal placental weights, the study observed that the terminal placental weights in the two the treatment groups of lamotrigine and levetiracetam were remarkably lower than those of the control groups (***Table 4.1***). This reduction in the terminal placental weights could have been associated with poor maternal nutrition status. This could have been occasioned by poor gastrointestinal activities leading to decreased nutrients available in the maternal blood for the development of this vital exchange organ between the mother and the fetus. The placenta sizes and weights are an important parameter in maternal pregnancy outcomes. The placenta permits the exchange of nutrients and metabolic products between the mother and its fetus hence it's an indicator of appropriate fetal growth and development. These finding on the decreased placental weights are in line with a previous study by Erisgin *et al.*, 2019, Sigei *et al.,* 2017 and Mwangi *et al.,* 2017 who noted apparent

reductions in terminal placental weights in albino rats exposed to both phenytoin and carbamazepine.

On the fetal pregnancy outcomes parameters that included; the litter sizes, resorbed glands/ devoured fetuses and dead fetuses, the current study established that there was a statistically significant reduction (P< .05) in the numbers of live fetuses, with significantly increased numbers of resorbed endometrial glands and dead fetuses in both the treatment groups as compared with the control **(f*igure 4.1 A, B, C*)**. These increased numbers of the resorbed/ devoured glands as well as the increased numbers of dead fetuses in the treatment groups could be attributed to the nutritional perturbations because of gastrointestinal irritation and placental functional suppression. This observation was similar to what was reported by a study by Omar *et al.*, (2016) who reported that, anticonvulsant medicines induced placental functional suppression and impairment subsequently leading to interrupted fetal embryogenesis, organogenesis and impairment to fetal growth and development.

On further comparisons of how the three independent variables of the drug, their dosages, the trimester of exposure plus their interaction influenced the dependent variables of the fetal growth and developmental parameters that included the mean fetal weights, mean crown-rump length and mean head circumference, this current study, established that these parameters decreased substantially(P=0.001) comparing with the control ***(Tables 4.6)***. In comparing the contributory roles of each of the three independent variables of the drug, dose and the trimester of exposures by use of MANOVA, the study established that these four parameters that determine the fetal growth and development in utero had differing proportionate contributions on the observed fetal growth and development outcomes and it was evident that each individual independent variable had a major contribution to the observed fetal pregnancy outcomes with the drugs having the highest contribution with partial Eta proportion of 99.8% while trimesters had the lowest contribution to the observed fetal pregnancy outcomes at partial Eta proportion of 92.3%. **(*Table 4.5 to table 4.7*).**

These findings are in line with the studies done previously and tried to elucidate the mechanisms and principles of teratogenesis of anticonvulsant medicines by Myllynen *et al*., 2003, Ali *et al.,* 2020. They observed that teratogens influence teratogenesis

based on the teratogen itself, the mode in which it interacts with developing fetal tissues as well as their dosages. The findings of the current study further presumes that the fetal tissue accumulation of lamotrigine and levetiracetam with their principal metabolites following in-utero exposure are thought to be influenced by drug transporting proteins in the placenta, including P-glycoprotein (P-gp), multidrug resistance protein (MRP) 1, and breast cancer resistance protein (BCRP) that are located in the syncytiotrophoblast plasma membrane that form the interface of the maternal and fetal circulations. This concurs with a study done by (Morrow *et al.,* 2006) which had the same observations. Though these proteins are supposed to offer protection to the fetus the genetic variations in the expression and activity of these transport proteins are the ones indicated to influence fetal exposure to AEDs and thus increasing the risk of fetal viscera teratogenicity like the kidneys and the others. This is in agreement with the study done by (Atkinson *et al.,* 2006) who made the similar observations in their study.

5.2 Objective 2: The Comparative Evaluation on How the Prenatal Exposure to Varied Doses of Levetiracetam and Lamotrigine Influenced the Histo-Morphological Organization of the Developing Fetal Kidney in Albino Rats.

In evaluating how the prenatal exposure to the varied doses of lamotrigine and levetiracetam influenced the histological organization of the developing fetal kidneys glomeruli apparatus the following parameters were evaluated; (**i**) the glomeruli histo-morphological shapes (**ii**) the organization of the juxtaglomerular cells in the glomeruli, (**iii**) the glomerular tufts of capillaries and associated cells, (**iv**) the bowman spaces including the appearances of the simple squamous epithelial linings of both the visceral and the parietal layers of the bowman space. The current study established that, the entire of the glomeruli sizes appeared enlarged in their morphological sizes in the treatment groups as compared with the control. On closer examination of the internal histological structure of the renal nephrons, it was noted that there was an abnormal differentiation of the glomerular structures that included the tufts of capillaries, juxtaglomerular cells and the bowman's space. These findings are in line with some other study findings by Cullen-Mcewen *et al.,* 2015;

Addul-Rhahman *et al.,* 2011; Albrahimini *et al*., (2015) who observed similar effects in the developing fetal kidneys exposed to different types of medical substances.

Still on the glomeruli, it was further noted that in both the treatment groups, the glomeruli were quite big in size but with compacted glomerular tufts of capillaries plus fewer juxtaglomerular cells, and they depicted a conspicuously enlarged bowman spaces. Then between the renal tubules were clear empty spaces that marked possibilities of edema due to increased hydronephrosis or the renal filtrate leakages from the tubes that could also explain the observed excessive fluid accumulation in the bowman space either due to improper clearance of the renal fluid filtrates that could be due to maldevelopment of the renal tubules particularly when the treatments were instituted at TM_1 and TM_2 (***figure 4.2 B to G and figure B to G 4.3).*** These findings are in line with some other study findings by Addul-Rhahman *et al.,* 2011; Albrahimini *et al.,* (2015) who observed that fetal kidneys are in high risk of histological disorganization following the prenatal exposures to anti-epileptic medicines and other medical related teratogens as they may cause alteration to the histological organization of the Malpighian bodies of the developing fetal kidneys a risk factor to kidney failures in adult hood.

Further, the observed hypertrophication of the glomerular sizes in the treatment groups were noted to be more intensified when the dosages of the two medicines, were increased and when they were particularly exposed to the medium and high doses. This situation was worsened by the early exposures to the two medicines prenatally where the worst deleterious effects were noted when these medium and high doses were instituted at TM_1 and TM_2 (***figure 4.2 C, D, F, G to figure 4.4 C, D, F, G***). This phenomenal hypertrophic alteration on the glomerular sizes following prenatal exposure to the two medicines was hence noted to depict a direct dose response relationship in that when the doses of the two medicines increased there was a corresponding proportionate increase in the glomeruli sizes (***figures 4.2 B to G to figure 4.4 B to G).*** These findings are in line with the study findings by Elmakaway *et al.,* 2022, Elgndy *et al.,* 2019, Erisgin *et al.,* 2019, who reported that teratogens to developing fetal kidneys affect a wide range of structures including nephrons, and surrounding tissues to the kidney's tubules.

On the intra-glomerular mesangial cells, the study established that in the two treatment groups, the intra-glomerular mesangial cells appeared condensed or more clustered together and poorly developed particularly in the high and the medium dose groups, while in the low dose groups the cells appeared to be fewer in number as compared with the control but instead of them being condensed together on one pole they looked sparsely distributed within the tuft of the capillaries **(*figures 4.2 D,C,B,G,F,E, figure 4.3 D,C,B,G,F,E, and figure 4.4 D,C,B,G,F,E,*).** This was however not the case with the control in that the intra-glomerular mesangial cells appeared many in numbers, very well-developed cells and evenly distributed in the glomeruli of the control groups **(*figure 4.2 A, to figure 4.4 A*).** These findings are in concurrence with the study finding of Abdul-Rhaman *et al* 2011, Cullen-Mcewen *et al* 2015, Carta *et al* 2007 and Algndy *et al* 2019 who made similar observation in the developing fetal kidneys.

Concerning the histological organization in the bowman's capsule, the simple squamous epithelial lining to both the parietal layer and the visceral layers of the bowman's spaces that are meant to have smooth marginal outlines were noted to be serrated and at times having discontinuous outlines in the treatment groups unlike the smooth outline observed in the control group **(*figure 4.2 A, figure 4.3 A and figure 4.4 A*)**, in other words in the treatment groups the two layers shown some signs of serration probably showing the perturbations caused by the two medicines in the process of morphogenetic differentiation of the renal filtrate collecting apparatus that start from the bowman's capsule to the renal tubules a pointer as to the observed hypertrophied bowman's spaces (***figure 4.2 B to G and figure 4.4 B to G*).** This phenomenon of distortion of the simple squamous epithelial cells in the parietal layer of the bowman's capsule was seen to be more pronounced when the treatments were done in the first and the second trimester and less severe in the third trimester **(*figure 4.2 B to G to figure 4.4 B to G*).** These findings concur with the findings by Addul-Rhahman *et al* (2011) and Albrahimin and Al-bakri (2015) who also observed similar changes in bowman's capsule following prenatal exposure to anticonvulsant teratogenic agents.

Concerning the histological organization of the renal tubules the study noted that the kidney tubules in the two treatment groups depicted some histomorphological alterations when compared with the control. In particular, it was observed that the kidney medullary tubules depicted some degree of tubular degenerative damage and interstitial edematous expansions or at times they depicted a combination of both tubulo-interstitial edema with some pockets of tubulo-interstitial adhesion. This phenomenon was particularly noted for the rats that were treated in the medium and high dose groups at TM1 and TM2 ***(figures 4.8 B to G and figure 4.9 B to G)***. This is in concurence with what was was priorly observed on the renal tubules alterations by Addul-Rhahman *et al* (2011) and Albrahimin and Al-bakri (2015), carta eta al 2007, El-Makaway *et al* 2022, Elgndy *et al* 2019. Further in those fetal kidneys that were also noted to depict tubulo-interstitail edema with enlarged bowmans capsule, the oveall sizes of the kidneys were also noted to have a big size because of edematous bowmans spaces with smaller condensed sizes of glomeluli tuft of capillaries pushed in towards the afferent pole of the glomeluli as seen in photomicrographs figures ***figures 4.2 B to G to figure 4.4 B to G.*** The observed edematous bownan spaces were note to be a sign of fluid accummulation and impaired renal fluid clearance that could have been ocassioned by the observed histomorphological alterations in the renal tubles that either made them to be malfunctional in allowing the flow of urine in the urinary pole of the nephron in the medulla. These tubular changes in the kidney tubules were also noted to depict a dose response relationship in that when the doses of the two medicines increased there was a correponding remakable increase in the degenerative and interstiatil edema or adhesions with the highest changes being in TM1 and TM2 as shown in ***figures 4.8 B to G and figure 4.9 B to G.***

With regard to kidney medullary thickness and kidney cortical thicknesses this current study observed increased medullary thicknesses and reduction of cortical thickness from the treatment groups for the two treatment medicines ***(figures 4.8 B to G to figures 4.10 B to G).*** This current study presumes that the increase in medullary thicknesses was orchestrated by swelling of the kidneys since they were observed to have been edematous when they were subjected to the treatment medicines. The current study also presumes that the cortical thickness was also

reduced due to reduced number of glomeruli and immature condensed, shrunken and atrophied glomeruli in the treatment groups while the medullary thickness is supposed to have been increased due to increased deposition of collagen fibres particularly in the high dose medium dose treated groups ***(figures 4.8 C,D,F,G and figures 4.9 C,D,F,G).*** This study agrees with a study that was done by (Ismail *et al*., 2022) on pregabalin and they noted similar observation.

5.3 Objective 3: The Comparative Evaluation on How the Prenatal Exposure to Varied Doses of Levetiracetam and Lamotrigine Influenced the Gross Morphometric and Histo-Stereological Parameters of the Developing Fetal in Albino Rats.

In assessing how the two medicines influenced the gross morphometric development of the fetal kidneys, the following parameters were evaluated; (i) The mean kidney weights, (ii) the mean kidney lengths, (iii) the mean kidney widths and (iv) the mean initial total kidney volumes. The comparative gross morphometric analysis was done by applying both ANOVA and MANOVA regression analysis to establish how the three independent variables of the drug, dose and time of exposure influenced the four dependent morphometric variables either acting individually, or in two way or in three-way combinations. This was meant to establish how the means of the four fetal kidney morphometric parameters differed with those of the control. The study established that, there was a remarkable significant increase ($P<0.005$) in the means of the four gross morphometric parameters evaluated in the treatment groups when compared with the control as follows; **(a)** kidney weights $F(18,38) = 50.48$, $P=.001$), **(b)** kidney lengths $F(18,38) = 21.14$, $P=.001$), **(c)** kidney widths $F(18,38) = 14.82$, $P=.001$ and **(d)** the initial total kidney volume $F(18.38) = 17.03$, $P= .001$ ***(Table 4.9)***. These finding are in concurrence with the findings by Addul-Rhahman *et al* (2011) and Albrahimin and Al-bakri (2015) who noted that with prenatal exposures to some medical teratogens they resulted in hypertrophy and increase in interstitial edema in the fetal tissues increasing the gross morphometric measurements of the developing fetal kidneys.

On further comparative analysis on the teratogenic effects of the various doses (low dose, medium dose or the high doses) on the two treatment groups for the two treatment medicines, this study established that low dose treated groups for the two treatment medicines slightly affected the kidney gross morphometric parameters, while high doses for the two treatment medicines severely affected the gross morphometric kidney parameters. These findings could be attributed to the perturbations the two medicines had in the process of differentiation of the kidney tissues during the early periods of organogenesis. It was noted that these parameters depicted an inverse dose response relationship ***(Table 4.9).*** With regards to the trimesters of exposure [TM_1, TM_2 or TM_3] between the two treatment groups, the comparative analysis between the two medicines established that the teratogenic effects of the two medicines under the study depicted a direct response relationship with the time of exposure where those rats that were exposed to treatment in trimester one (TM_1) had the highest teratogenic effects, followed by those in trimester two (TM_2) and those in trimester three (TM_3) ***(Table 4.9)***. These findings are in line with the observations by Addul-Rhahman *et al* (2011) and Albrahimin and Al-bakri (2015), carta eta al 2007, El-Makaway *et al* 2022, Elgndy *et al* 2019.

This current work further agrees with a study done by (El Makawy *et al.*, 2022) on topiramate a drug in the same class with lamotrigine and levetiracetam where they similarly noted that there was increase in mice kidney weight when high doses of topiramate were used. The current study however disagrees with a study done on carbamazepine by (Al-bakri *et al*., 2016) which observed that there was no change in kidney weights after treatment. This is probably because the fetal kidneys were harvested seven days later after delivery and this could have given them time to resolve the effects caused by the drug.

On the comparative histo-quantitative evaluation of calculated Cavalieri kidney volumes, medullary volume densities, cortical volume densities, medullary thickness and cortical thickness noted that there were significant differences with the control and between the two treatment groups **(*tables 4.13*).** Increase in calculated Cavalieri volumes, medullary and cortical volume densities, medullary thickness and cortical thickness was directly related to the time of contact with the two study medicines, the

type of medicine administered while at the same time it was inversely related to the dosages **(*table 4.14 to table 4.16*).** These findings are also in concurrence with the stereological observation by Albrahimin and Al-bakri (2015), carta eta al 2007, El-Makaway *et al* 2022, Elgndy *et al* 2019. who noted that with increasing dosages of anticonvulsant medicine there was a probable increase in volumes and volume densities in the kidney structures following their fluid accumulations.

The changes in volumes and the volume densities of the different kidney components could also be attributed to the increase in the deposition of collagen fibers between the kidney tubules in the medulla and around the glomerulus in the cortex ***(figures 4.8 B to G to figure 4.10 B to G)*.** This can as well be presumed to be the reason why there was increased medullary thickness in the treatment groups for the two treatment medicines. This concurs with a study done by (Ismail *et al*., 2022) on pregabalin where they noted similar observation. The observation made could have been as a result of the ability of the two treatment drugs to cross the blood placenta barrier more readily because they have a low molecular weight of less than 500 kilodaltons and lamotrigine has a longer half-life that levetiracetam leading to longer fetal exposure time to this antiepileptic medicine. This is in agreement with a study done by (Bank *et al.*, 2017), (Selman *et al*., 2016). It was also noted that kidney nephrotoxicity teratogenic effects for lamotrigine were higher than the ones for the levetiracetam treated groups. This agrees with another study done by (Myllynen *et al*., 2003) (Ali & El, 2020) where they noted that lamotrigine crosses the placenta easily and readily leading to considerable fetal visceral and nephron-teratogenic effects.

5.4. The Study Conclusions

The conclusions made from this current work are in line with the study objectives as follows;

(i) the two medicines when prenatally exposed caused deleterious effects to all the maternal and fetal pregnancy outcome parameters by causing in-utero toxicity a risk factor to the fetal growth environment that predisposes them to congenital anomalies.

(ii) the two medicines caused histological derangement of the bowman's' space, caused glomerular histological disorganization as well as the histological alterations to the renal tubules in a dose and time dependent manner.

(iii)the two medicines caused hypotrophy of the gross morphometric parameters, while in stereology it caused derangements in volume densities of all the histo-stereological parameters evaluated in the fetal kidneys in a dose and time dependent manner.

(iv) the Comparative analysis established that levetiracetam has less teratogenic effects to the developing fetal kidneys as compared to lamotrigine.

(v) the prenatal exposure to either levetiracetam or lamotrigine has a dose and time dependent influence on the maternal and kidney parameters that substantiate a direct-linear relationship.

5.5 The Study Recommendations

The study recommends that:

(i) If possible, the use of levetiracetam and lamotrigine during pregnancy should be avoided during TM1 and TM2 since they have been shown to negatively effects the maternal and fetal pregnancy outcome as this is the period of cellular differentiation and organogenesis while administration of the drug in TM3 is less deleterious.

(ii) If these medicines have to be used during pregnancy, high doses need to be avoided particularly during early periods of pregnancy in the organogenesis period to minimize chances of causing fetal congenital perturbations to the developing fetal viscera that includes the developing fetal kidneys.

(iii)Between these two anticonvulsant drugs, levetiracetam has been shown to have less teratogenic effects than lamotrigine and it is better to use it when it is required during pregnancy. Use of other lesser teratogenic drugs is highly recommended.

(iv) Further studies in non-human primates are recommended as they would give more accurate result that are close to what would be observed in humans

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APPENDICES

Appendix I: Data Capture Sheets

Data capture sheet for expectant albino rats (Lamotrigine drug)

Albino Rat Identity……

Initial weight……Dose administered……..

DATE	WEIGHT IN GRAMS	LAMOTRIGINE DOSE (g\kg)	GENERAL CONDITION OF RAT

Data capture sheet for expectant albino rats (Levetiracetam drug)

Albino Rat Identity……

Initial weight……Dose administered……

DATE	WEIGHT IN GRAMS	LEVETIRACETAM DOSE (g\kg)	GENERAL CONDITION OF RAT

Appendix II: Fetal Details

Data capture sheet for the albino fetuses

Albino rat identity (mother)…….

Date of harvesting……. Fixative used…….

Total no. of fetuses…….

Total no. of resorptions……

Total number of fetuses with congenital malformations….

No. of dead fetuses…….

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
GROSS APPEARANCE												
FETAL WT(g)												
FETAL CROWN RUMP LENGTH(CM)												
OBVIOUS CONGENITAL ABNORMALITIES OF THE FETUS												
HEAD CIRCUMFERENCE												
BI-PARIETAL												

DIAMETER												
KIDNEYS												
GROSS APPEARANCE												
KIDNEY WT(g)Rt Kidney												
Left kidney												
TOTAL KIDNEY VOLUME(WIM) (Right)												
TOTAL KIDNEY VOLUME(WIM) Left Kidney												

Appendix III: Work Plan

Events	Aug to Sep 2020		Sep-Oct 2020	Oct 2020	Jan-Au2021	Oct 2021	Nov 2021	Dec 2021
Introduction of research project (theory)								
Developing research topic and objectives								
Developing data collection tool								
Introduction of the area and pre-testing of data collection tool								
Collecting data								
Data analysis								
Typing and data documentation								
Presentation of research project								
Printing and binding the final copy								
Submission of the research project for marking								

Appendix 1V: Budget

No items	Budget items	Cost per unit	Number /items	Total cost
1.	Albino rats	300	60	18,000
2.	Gastric gavage tubes	100	120	12,000
3.	Feed rodent pellets	4000	20	80,000
4.	Transport	50,000	1	50,000
5.	Stationary	50,000	1	50,000
6	lamotrigine	40	75	3000
	Levetiracetam	55	91	5225
7	Labour	3500	30	105,000
8	Histological and stereological reagents and tissue processing	450,000	1	450,000
9	Equipment (weighing machine, printer)	50,000	1	50,000
10.	Photography materials	45,000	1	45,000
11.	Miscellaneous	50,000	1	50,000
	Total			**915,000**

Appendix V: Publication

Journal of Agriculture Science & Technology JAGST 22 (2) 2023, 72-82

Embryonic teratogenic effect of levetiracetam on a developing fetus

ORIGINAL RESEARCH ARTICLE

Evaluation of embryonic teratogenic effects on fetal growth and development following prenatal exposure to different doses of levetiracetam in albino rats *(rattus norvegicus)*

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ABSTRACT

The teratogenic effects of embryonic contact with different doses of levetiracetam, a second-line anticonvulsant medicine, on the growth and development of the foetus remain unclear. Previous research has advocated for more research on levetiracetam's safety index on the growth and development of foetuses following prenatal exposure at various gestational periods and dosages. In this study, a post-test-only control experimental design was used. Animal experiments and measurements of foetal growth variables were carried out at the University of Nairobi's animal facility. The sample size used was 30 albino dam rats (*Rattus norvegicus*). Two clusters, each comprising three control rats and 27 investigational rats, were obtained by dividing these 30 rats into two broad study groups. To investigate the effects of different doses on foetal pregnancy outcomes, the 27 experimental rats were divided into three major groups of nine rats: low dose, medium dose, and high dose assemblies. Furthermore, to assess the comparative effects on different pregnancy periods, each set of the three-dose assemblies was split into three groups of three rats as per the trimesters (Trimester I, Trimester II, and Trimester III). Foetal weight, head circumference, bi-parietal diameter, head length, and crown-rump length were all collected and coded in Excel spreadsheets for Windows 10 edition 2013.Later, they were transferred to SPSS (Windows Edition 25) for evaluation. Subsequently, a one-way analysis of variance (ANOVA) together with Tukey's post hoc multiple comparison tests were used for analysis. The results of all values were presented as mean $\pm$ standard error of the mean (SEM). Levetiracetam was found to have a time- and dose-dependent effect on foetal growth parameters. Findings with a $P < 0.05$ level of significance were considered significant.

Keywords: Anticonvulsants, teratogenic, levetiracetam.

Appendix VI: Animal Ethics

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REF: FVM BAUEC/2021/323

Mr. Cyrus Kamau Kweri.
Dept. Human Anatomy,
JKUA & Technology.
10/11/2021

Dear Cyrus,

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

Comparative histomorphological and histostereological Teratogenic effects of in-utero exposure to Lamotrigen and Levetiracetam on the fetal kidneys in Albino rats.
Cyrus Kamau Kweri. HSM301-1194/2020.

We refer to your MSc. proposal submitted to our committee for review and your application letter dated 5th November 2021. We have reviewed your application for ethical clearance for the study. The number of albino rats and protocols used to assess how histomorphological and histostereological nephroteratogenic effects of in-utero exposure to Lamotrigen and Levetiracetam on 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