COMPARATIVE HISTOMORPHOLOGICAL AND HISTOSTEREOLOGICAL TERATOGENIC EFFECTS OF PRENATAL EXPOSURE TO LAMOTRIGINE AND LEVETIRACETAM ON FETAL MEMORY CIRCUITRY STRUCTURES IN ALBINO RATS (*Rattus novegicus*)

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Comparative Histomorphological and Histostereological Teratogenic Effects of Prenatal Exposure to Lamotrigine and Levetiracetam on Fetal Memory Circuitry Structures 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

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DEDICATION

I dedicate this thesis to my daughters Gael Courtney and Victorin Loverna, who provided me with moral support throughout this study period.

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ABBREVIATIONS AND ACRONYMS

AED	Antiepileptic drug
AED	Animal equivalent dose
ANOVA	Analysis of variance
AN	Amygdaloid Nucleus
C	Control
CNS	Central nervous system
COHES	College of Health Sciences
CRL	Crown rump length
DG	Dentate Gyrus
ETC	Entorhinal Cortex
GD	Gestational period by Dates
GP	Gestation Period
HC	Hippocampus
JKUAT	Jomo Kenyatta University of Agriculture and Technology
Keq	Constant equilibrium -is a characteristic numerical value
Km	Is a constant factor used to convert the mg/kg in to mg/m^2 (body weight in to surface area).
Kg	Kilogram
LAM	Lamotrigine
LAMTG	Lamotrigine Group
LEV	Levetiracetam
LEVG	Levetiracetam Group

Mg	Milligram
Mm	Millimetre
MRI	Magnetic Resonance Imaging
MTL	Medial temporal lobe
NIH	National Institute of Health
PC	Perirhinal Cortex
PFC	Prefrontal Cortex
SUB	Subiculum
PrSUB	Presubiculum
PaSUB	Parasabiculum
SEM	Standard Error of the mean
SPSS	Statistical Package of Social Sciences
TM_1	Trimester one
TM_2	Trimester two
TM_3	Trimester three
PHC	Para hippocampus
μ_m	Micrometre
WHO	World health organization
WIM	Water immersion method

DEFINITION OF TERMS

Anticonvulsants (ACs) Are a wide range of medicines used in management of various conditions like seizures associated with epilepsy, mood stabilizers in bipolar disorders, mania, neuropathic pains among others.

Lamotrigine (LAM) Brand name; (lamictal) -is a 2nd generation anticonvulsant medicine used as first line in management of various conditions like bi- polar disorders, epileptic seizures, among other uses.

Levetiracetam (LEV) Brand name; (Keppra) -is a 2nd generation anticonvulsant medicine used as first line in control certain types of seizures (e.g., partial seizures myoclonic seizures, or tonic-clonic seizures) in the treatment of epilepsy

Histosmophology This is the use of histology to study the morphology of cells i.e. (their size, shape, structure and their arrangement)

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

In-Utero Is a latin word meaning ‘in the womb’

Adlibitum Is a latin word meaning "in accordance with one's wishes”

ABSTRACT

Though lamotrigine and levetiracetam are the most commonly used first line anticonvulsant medicines in the management of epileptic seizures and other convulsion disorders during pregnancy, the *in-utero*-teratogenic effects in the development of fetal memory circuitry structures remain equivocal. Furthermore, whether or not their teratogenic effects are both dose and time-dependent also remains unclear and hence the basis of this study. In carrying out this study, a post-test-only experimental study design was adopted. The animal experimentation was done in the animal research facility in the University of Nairobi (UON), while tissue processing for histology and stereological analysis was done at JKUAT. A Sample size of 30 albino rat dams weighing between 220 to 250 grams for each of the study medicine were used in the study. This sample size was determined by use of the resource equation for One Way Analysis of Variance method (ANOVA). The 30 albino rats in each of these two study categories of levetiracetam and lamotrigine were first broadly divided into two study groups of 3 rats' control and 27 rats' treatment group. To evaluate whether the teratogenic effects of both medicines are dose dependent, the 27 rats in their treatment groups were further subdivided into three study sub groups of 9 rats as follows; (i) 9 rats for low doses of lamotrigine and levetiracetam group{103mg/kg bw and 3mg/kg respectively}, (ii) 9 rats for medium doses of levetiracetam and lamotrigine group {207mg/kg and 24mg/kg respectively}, (iii) 9 rats for the high levetiracetam and lamotrigine group {310mg/kg and 52mg/kg respectively}. To further evaluate whether the observed teratogenic effects are time dependent, the 9 rats in each of the three dose categories were further sub-divided into three subgroups groups of 3 rats each according to the trimesters of exposure as follows; (i) 3 rats for trimester I (TM_1); (ii) 3rats for trimester II (TM_2) and (iii) 3 rats for trimester III (TM_3). All rats were fed on standard rodent pellets and water *ad-libitum* throughout the gestational period and sacrificed on day 20. The fetal brains were harvested for both histomorphological and stereological analysis. Qualitative histomorphological data was collected using a swift 3.0 microscope digital camera 20mega pixels, then exported to swift 3.0 software for analysis and labelling. Discrete data was analysed using chi-square test for independence. Quantitative data was collected using structured checklists, stored and coded in excel spreadsheets windows 10, version 2019, then was exported for analysis into SPSS programme for windows version 25 for analysis (Chicago Illinois). The findings were expressed as mean$\pm$ SD for all values. Intra and intergroup comparisons were done by one-way ANOVA followed by Tukey's post hoc multiple comparison t- tests, while MANOVA was used as a test of interaction effects, main effects as well as pairwise comparisons. The findings whose $P<.05$ were considered significant. The findings of this study shown that both lamotrigine and levetiracetam are teratogenic to the development of fetal memory circuitry pathways structures including the prefrontal cortex and medial temporal lobe structurers that includes the entorhinal cortex, the subicular complex, the hippocampus, the dentate gyrus and the amygdaloid nucleus. The observed teratogenic effects for both medicines depicted a similar pattern of causing significant reduction($P<.05$) in cellular density, sparse distribution of cells, atrophic changes to all cortical layers and the reduction in volume and volume density in all the cellular components in a dose and time dependent manner particularly at TM_1 and TM_2, with lamotrigine having more deleterious effects than levetiracetam. It is then recommended that high dosages of the two medicines where possible should be avoided at TM_1 and TM_2. Further studies with non-human primates are also recommended to help corroborate these findings to humans.

CHAPTER ONE

INTRODUCTION

1.1 Chapter Introduction

This chapter starts by giving a brief introduction into the organization of the memory circuitry pathways in the brain and how it contributes into the survival of a human being. This is followed by a broad account on disruptive teratogenic mechanisms of anticonvulsant medicines, then highlights the missing teratogenic gaps of the levetiracetum and lamotrigine, followed by the problem statement, justification and significance of the study, research questions and the study objectives, the hypothesis of the study, the aim of the study, the scope of the study, assumptions of the study, the limitation and the delimitations of the study.

1.2 Background Information

The organization of memory circuitry pathway that starts with prefrontal cortex, connecting to medial temporal lobe by entorhinal cortex through the subiculum, then to the hippocampus and dentate gyrus, then lastly to the amygdaloid nuclei, forms an important survival component in human life as they encode, store, consolidate and retrieve information (Barbas *et al.*, 2018; Raslau *et al.*, 2015).These structures play an important role in the cognitive abilities of human beings in learning, performing, and controlling important survival functions (Cowan, 2014; Barrouillet *et al.*, 2011).

With increasing cases of infantile and adult mental disorders that are characterized by typical symptoms of systematic deterioration of cognitive and motor abilities, memory loss, inability to study effectively and increasing cases of suicidal attempts among the youths is a worrying tread globally, (Cowan, 2014; Barrouillet *et al.*, 2011). Since all anticonvulsants are known to be teratogenic, there is need therefore to evaluate the teratogenic disruptive effects that could be emanating from use of commonly used maternal anticonvulsant medicines like lamotrigine and levetiracetam with a view to establishing the association of *in-utero* exposure, to the development of fetal memory circuitry structures. This data will help to build a

scientific data repository that would guide future researchers and clinicians on the rational use of these anticonvulsant medicines during pregnancy. To that end, the comparative teratogenic indexes between levetiracetam and lamotrigine on the histo-stereological components on the fetal memory circuitry structures cannot be overemphasized (Hill *et al*., 2010; Eroğlu *et al.*, 2008; Kaplan, 2004).

The lack of a teratogenic data repository on how the commonly used anticonvulsant medicines impact on the developing fetal viscera will not only pose a challenge to increasing cases of mental disorders being observed in the society, but will continue to pose an intricate challenge to clinicians in making rational choices of the best anticonvulsant medicines to use in the management of maternal convulsive disorders like epileptic seizures, neuralgic disorders, among others during pregnancy (French & Gazzola, 2011). The teratogenic vulnerability of these anticulvulsant medicines on to the developing fetal tissues is further increased by the fact that many of these medicines are known to cross the blood-placental barrier, resulting in their accumulating in the fetal blood and developing fetal tissues with probable disruption of normal fetal organ morphogenesis. The blood-placental closure of these medicines is usually induced by fluctuating levels of the drug-metabolizing enzymes during pregnancy, coupled with their low molecular weights (Bank *et al.*, 2017; Syme *et al.*, 2004; Semczuk-Sikora & Semczuk, 2004).

Currently, lamotrigine and levetiracetam that are second generation anticonvulsant medicines, are the most preferred during pregnancy particularly in the third world countries like Kenya because of the associated efficacy and tolerability (Reimers and Brodtkorb 2012; Talati *et al.*, 2011). However, the American Food and Drug Administration (FDA) categorizes them as class C medicines (Van Norman, 2020; Thind & Kowey, 2020). This means that they need to be applied with care during pregnancy, Dal Pan (2015), necessitating the need to carry out thorough teratogenic studies to establish their safety indices, since not much has been documented on their teratogenic effects to the developing fetal brain and particularly not many studies have been done focussing on the fetal memory circuitry pathway structures (Bansal *et al.*, 2018; Veronika *et al.*, 2017; Yasama *et al.*, 2016).

Furthermore, though, FDA has indicated that all anticonvulsants have some degree of teratogenicity in the developing fetal viscera and the nervous system, there are no specific teratogenic studies that have been done to evaluate their effects to the specific structures of the brain including those concerned with memory (Darrow *et al*., 2020; French & Gazzola, 2011; Prakash *et al.,* 2007).Generally, previous studies have shown that the teratogenic effects of *in-utero* exposure to a part or the whole chemical constituents of any anticonvulsant medicines during pregnancy can result in both short-term and long-term alteration of the fetal memory circuitry pathways, as has been reported in children born from epileptic mothers who after birth manifest with either structural or behavioural abnormalities at childhood or in adulthood (Kamali *et al.,* 2020; Hill *et al.,* 2010; Marchi *et al.,* 2001). As such, there is a paucity of data on the comparative histomorphological and histostereological teratogenic effects of *in-utero* exposure to levetiracetam and lamotrigine, on the fetal memory circuitry structures for both the short and long-term memory.

1.3 Statement of the Problem

Today, the use of lamotrigine and levetiracetum during pregnancy in management of maternal conditions like epileptic seizures, neulargia, bipolar disorders among others has gained popular usage in developing countries (Abou-Khalil, 2022; Hesdorffer & Kanner, 2009). However, their comparative histostereological teratogenic effects on the developing fetal memory circuitry structures, as well as determining whether or not their teratogenic effects on fetal memory circuitory structures are dose or time dependent is yet to be established (Abuga *et al.,* 2021). This is at a time when the cognitive neuropsychiatric disorders are on the increase worldwide affecting about 50 million people, and are estimated to be leading to intellectual disabilities, memory loss and ultimately poor quality of life. The burden is estimated to increase, with numbers rising to 78 million by 2030 and about 139 million in 2050. East and North Africa and middle East are predicted to have the highest numbe (WHO 2019, Maussa *et al.,* 2015; Verrotti *et al.,* 2015).

Lack of comparative histo-stereological teratogenic data on the two anticonvulsants that are currently being commonly applied is not only posing teratogenic risk to the

developing fetuses in the mothers' womb but also continues to be a challenge to the clinicians interms of making rational decisions on which medicine would be safer, the dosages to apply and at what time of exposure during pregnancy. Since all the anticonvulsant medicines are classified under class C by the American food and drug administration (FDA), meaning that they should be given to pregnant women with caution if the benefits to the mother outweighs the risk to the fetus, there is need to carry and in-depth histo-stereological analysis of the two medicines as the comparative neuroteratogenic effects in the development of fetal memory circuitry structures remains unclear (Hesdorffer & Kanner, 2009).

1.4 Study Justification

The current problem of increasing cases of memory loss and cognitive disorders across all age groups in our society as well as the dilemma facing clinicians in making rational decision on the application of either lamotrigeine or levetiracetum in management of maternal neurological conditions like epileptic seizures, bipolar disorders, among others, will continue being a challenge should a scientific data repository on their teratogenicity is not established. Further-more, there will be continued increase in these lifelong disabilities on young people, hindering them to engage in important life functions like learning, memory and execution of various survival functions (Ijff & Aldenkamp, 2013; Eddy *et al.,* 2011). As such, the establishment of a data repository on the comparative neuroteratogenic histomorphological and histostereological effects on the perinatal exposure to varied doses of lamotrigine and levetiracetam on the fetal memory circuitry structures is key in determining the safety indices of these two medicines during pregnancy.

In addition, lack of histostereological comparative teratogenic data that clearly shows the most vulnerable teratogenic periods as well as the most critical doses of lamotrigine and levetiracetam teratogenicity will also keep on causing confusion to clinicians on which among two medicines is more beneficial in management of some maternal medical conditions like mania, bipolar disorders among others.

1.5 Study Significance

The findings of this study will serve as baseline histostereological teratogenic data on the specific memory components including prefrontal cortex, entorhinal cortex, subiculum, presubiculum and parasubiculum connecting to medial temporal lobe, then hippocampus, dentate gyrus and lastly the amygdaloid nuclei that stores the encoded long-term memory. It will elucidate which structures in the memory circuitry pathway is highly affected, by which medicine, and at which dosage. The data will also serve as a clear pointer on comparative time vulnerability on the two medicines. The data will as well serve as a platform for future teratogenic studies in non-human primates that have closer relations to humans, with a view to carrying more advanced teratogenic studies at that level, in-order to guide the clinicians in making informed choices on the safest types of anticonvulsive medicines to use, the appropriate doses and which times is safe to use them or when they need to be avoided during pregnancy.

Further, data obtained from this study will also serve as primordial guide to clinicians when making choices of the first line anticonvulsant medicine to use during pregnancy, that is safe to the mothers and with less effects to the developing fetal memory circuitry structurers. The findings would therefore contribute to a wealth of knowledge on the known teratogenic agents that are currently contributing to postnatal cognitive effects on the brain. This data will in the long run contribute either directly or indirectly in curbing the rising cases of childhood and adult mental health conditions of unknown causes like poor memory retrieval abilities in school, acute mania, suicide ideation among others that are on the increase worldwide.

1.6 Study Objectives

1.6.1 Broad Objective

To comparatively evaluate the histomorphological and histostereological teratogenic effects of prenatal exposure to lamotrigine and levetiracetam on the development of the fetal memory circuitry structures in albino rat (*Rattus norvegicus*)

1.6.2 Research Questions

1. What are the comparative effects of *in-utero* exposure to varied doses of lamotrigine and levetiracetam on the maternal and fetal pregnancy outcomes in albino rats when exposed at different trimesters?
2. What are the comparative histomorphological teratogenic effects of *in-utero* exposure to varied doses of lamotrigine and levetiracetam on the fetal memory circuitry structures in albino rats when exposed at different trimesters?
3. What are the comparative histo-quantitative teratogenic effects of *in-utero* exposure to varied doses of lamotrigine and levetiracetam on the development of fetal memory circuitry structures in albino rats when exposed at different trimesters?
4. Are the histomorphological and histo-stereological teratogenic effects of *in-utero* exposure to lamotrigine and levetiracetam on the fetal memory circuitry structures both time and dose dependent?

1.6.3 Specific Objectives

1. To comparatively evaluate the effects of *in-utero* exposure to varied doses of lamotrigine and levetiracetam on the maternal and fetal pregnancy outcomes when exposed at different trimesters.
2. To comparatively evaluate the histomorphological teratogenic effects of

in-utero exposure to varied doses of lamotrigine and levetiracetam on the fetal memory circuitry structures in albino rats when exposed at different trimesters.

3. To comparatively evaluate the histostereological teratogenic effects of

in-utero exposure to varied doses of lamotrigine and levetiracetam on the fetal memory circuitry structures in albino rats when exposed at different trimesters.

4. To comparatively determine whether the observed histomorphological and

histo-stereological teratogenic effects of *in-utero* exposure to varied doses of lamotrigine and levetiracetam on the fetal memory circuitry structures are both time and dose dependent

1.7 Study Hypothesis

Both null and alternative hypothesis were adopted as follows: -

1.7.1 The Null Hypothesis (Ho)

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

1.7.2 Alternative Hypothesis (H1)

There are significant comparative differences in the histomorphological and the histo-stereological teratogenic effects of lamotrigine and levetiracetam on the development of the fetal memory circuitry structures when exposed in varied doses and at different gestation periods in albino rats (*Rattus norvegicus*).

1.8 Aim of the Study

To comparatively determine whether there are significant teratogenic differences in the histomorphological and the histo-stereological teratogenic effects in the fetal memory circuitry structures when exposed prenatally to varied doses of lamotrigine and levetiracetam at different trimesters in albino rats (*Rattus norvegicus*).

1.9 Assumptions Study

It is assumed that the albino rat (*Rattus Norvegicus)* model memory structurers resemble those of human being because rat species are close to human being. Teratogenic effects of lamotrigine and levetiracetam in rats therefore, depict a similar scenario as compared to humans.

1.10 The Study Model Assumptions

In carrying out this study, it was assumed that this breed of the albino rats (*Rattus Norvegicus)* model used in the animal experimentation would mirror-image a similar histomorphological and histostereological teratogenic effects in the development of the fetal memory circuitry structures, to what would occur to humans due to the known scientific close relationship of this rat species to the human biological and functional teratogenic outcomes when exposed *in utero*.

1.11 Study Limitations

Some of the anticipated study limitations included; failure of some rat does to become pregnant at the same time with the rest, following the introduction of the males in the cages and death of the animals along the experimental process following mishaps in drug administration into the lungs instead of the stomach while administering levetiracetam and lamotrigine using the gastric gavage needle.

1.12 Study Delimitations

To overcome these challenges, the following delimitation measures were applied:

(i) The rats (dams) that did not become pregnant the first day of the experiment were separated from those that got pregnant the first day, put in separate cage then a male rat reintroduced overnight to give chance for conception to take place. If prove of pregnancy was established, their treatment was done separately as they had different gestational days with the ones that got pregnant the first day.

(ii) For the rats that became sick or died in the course of the experimentation, their study groups were noted as per the drug, the dosage and the time of exposure. Post-mortems were conducted to establish the cause of death then repeat experiments on those rats that died or became sick were done after the main experiment was completed.

(iii) A pilot study was done to test the study protocol and to minimize chances of operational and process errors as much as possible

CHAPTER TWO

LITERATURE REVIEW

2.1 Chapter Introduction

This chapter starts by giving a brief introduction on the two medicines in terms of their classes, chemical formula, solubility, mode of action and excretion. It then gives their mode of their teratogenic mechanisms on the fetal nervous system structures. This is followed by the comparative description of the organization of the fetal memory circuitry system between rats and humans, then the histo-morphological organization of fetal memory circuitry system, the memory flow from the prefrontal cortex to the amygdaloidal nucleus, and the comparative morphogenesis of the fetal prefrontal cortex in humans and rats. This is followed by the comparative neurogenesis of the medial temporal lobe between rats and humans, the comparative organization of the prefrontal cortex and medial temporal lobe between rats and humans, and lastly the dose and time effects on the teratogenic outcomes of known anticonvulsant medicines.

2.2 Brief Description of the Class, Structure, Mode of Action and Excretion of both Lamotrigine and Levetiracetam.

Lamotrigine and levetiracetam are both classified under the second generation of anticonvulsants medicines, categorized under the class C as per the US FDA classification of medicines, that should be used with caution during pregnancy (Abou-Khalil, 2022; Abou-Khalil, 2019). Lamotrigine is commonly sold under trade name of *lamictal* and is structurally made up of organic compound that are under the phenyl-triazine class. It has a molecular formula of $C_9H_7C_{l2}N_5$ and molecular weight of 263.09 g/mol, (Goa *et al.,* 1993). It is slightly different from other antiepileptic drugs in the same class (AEDs), as it has a solubility of 0.17 and 4.1 mg/ml at 25°C in water and 0.1 in methyl cyclohexyl isocyanate (MHCl) respectively. 94% of total drug is excreted in urine and 2% in faeces, with an excretion half-life of 25 hours (Fitton & Goa, 1995). Lamotrigine was first marketed in 1994 (Marchi *et al.,* 2001).

Levetiracetam on the other hand, is sold under the brand name *keppra, elepsia, spritam* among others, it is a racetam anticonvulsant with a chemical formula of $C_8H_{14}N_2O_2$ and a molecular weight of 170.21 g/mol (Abou-Khalil, 2008). It is directly soluble in water (104.0 g/100 mL) and freely soluble in chloroform (65.3 g/100 mL), in methanol (53.6 g/100 mL), in ethanol (16.5 g/100 mL) and sparingly soluble in acetonitrile (5.7 g/100 mL). Levetiracetam is excreted in urine with elimination half-life of 6-8hrs (Crepeau & Treiman, 2010). Levetiracetam was approved for medical use in 1999 (Deshpande & Delorenzo, 2014). The mode of action of lamotrigine is that it acts by inhibiting sodium currents flow on the cell membrane by selective binding to the inactive sodium channel, suppressing the release of the excitatory amino acid- glutamate (Yasam *et al.*, 2016). On the other hand, the novel mechanism of action of levetiracetam is modulation of synaptic neurotransmitter release through binding to the synaptic vesicle protein (SV2A) in the brain, (Kumar *et al.*,2022; Abou-Khalil, 2008).

2.3 The Comparative Teratogenic Mechanism of Lamotrigine and Levetiracetam on the Brain Structures of the Fetal Nervous System

Previous studies have shown that the teratogenic mechanisms of both lamotrigine and levetiracetam are similar to other anticonvulsant medicines in the same class, where their neuro-teratogenicity is induced by the concentration of their metabolites namely LAMTG 2-N-glucuronide conjugate and letiracetam carboxylic acid (UCB L057), (Hernández-Díaz and Levin, 2014). These metablolites usually accumulate in the developing fetal tissues after they close the maternal placental blood barrier from the maternal blood plasma, Luciano & Shorvon, (2007; Tomson *et al.*, (2007), coupled with their low molecular weights of 256.091g/mol and 170.209 g/mol respectively (Betchel *et al.*, 2022; Carreno, 2007; Prakash *et al.*, 2007). These two aspects enhance the two medicines to cross the maternal blood placenta barrier easily and rapidly get into the developing fetal tissues early enough during organogenesis (Wlodarczyk *et al.*, 2012; De Santis *et al.*, 2011).

Further, based on the chemical nature of lamotrigine, it inhibits dihydrofolate reductase, a critical enzyme in folate metabolism that catalyzes the reduction of dihydrofolate to tetrahydrofolate, critical cofactors for single-carbon metabolism in biological processes including DNA synthesis, regulation of gene expression, and synthesis of amino acids, neurotransmitters, and myelin (Sajjad *et al,* 2019; Cecilie *al.,* 2018). On the other hand, levetiracetam free radicals interferes with the endogenous bioelectric mechanisms and voltage gradients that function as instructive cues guiding cell division, programmed cell death, cell positioning and orientation in the developing fetal brain (Hernández-Díaz & Levin, 2014). Through the described inhibition or interference of the above-mentioned cellular processes, these two medicines hence are able to cause observable phenotypic and functional teratogenicity to the developing fetal nervous system that includes the fetal memory circuitry system alongside other fetal viscera.

2.4 The Comparative Organization of the Fetal Memory Circuitry System between Rats and Humans

Previous studies have shown that there is a close comparative organization of memory circuitry structures in both the fetal and the adults between rats and humans (Semple *et al.,* 2013; Petrides *et al.,* 2012). It has been established that in both rats and humans, the prefrontal cortex is the first part of the memory circuitry structures of the brain that processes recent events as well as in the executive functioning and control of higher cognitive processes before they are stored in the medial temporal lobe, (Kolb *et al.,* 2012). It is also the last part of the fetal memory circuitry structures to develop in both the rat and human (Donahue *et al.,* 2018; Yeterian *et al.,* 2012).

Functionally and structurally, it is demarcated into different regions including the dorsolateral, dorsomedial, ventrolateral, ventromedial, and orbitofrontal regions in humans (Bergmann *et al.,* 2016). Further, this dorsolateral prefrontal cortex in humans is located in the middle portion of the frontal lobe while in the rats they only have a medial prefrontal cortex that is subdivided into four regions: the anterior cingulate, medial precentral, infralimbic and prelimbic cortices usually considered to

be homologous to the dorsolateral prefrontal cortex (Gao *et al.,* 2013). Both dorsolateral prefrontal cortex in humans and medial prefrontal cortex in rats are comprised of spatial selective neurons with neural circuitry, that encompasses the entire range of sub-functions necessary to carry out an integrated response (executive functions/cognitive processes) including control of emotional, working memory, planning and attention, with connections to other brain regions (Funahashi , 2017).

The second structures of the fetal and the adult memory circuitry system is the entorhinal cortex (EC), also known as cortex entorhinalis (Garcia & Buffalo, 2020; Coutureau *et al.,* 2009). This is the area of the brain's allocortex that is located in the medial temporal lobe in both the rats and in the humans (Takehara-Nishiuchi, 2014; Schultz *et al.,* 2014). The entorhinal cortex (EC) is the main interface between the hippocampus and neocortex whose functions includes memory formation, memory consolidation, and memory optimization in sleep (Simic *et al.,* 2022). It is the one that receive inputs from the prefrontal cortex including other cortical areas, especially the associational, perirhinal and parahippocampal cortices (Staresina *et al.,* 2011). In humans, the entorhinal cortex it is located at the rostral end of the temporal lobe and stretches distolaterally in the temporal lobe while in rats, the EC is located at the caudal end of the temporal lobe, (Piguet *et al.*, 2018).

The entorhinal cortex and the perirhinal cortex that has a major role in recognition and in storing information (memories) about objects, has direct and indirect connections to different regions (Rolls *et al*., 2006). It attaches inferiorly to the hippocampus as well as being the major connection to other memory circuitry structurers in the temporal lobe (Navarro *et al.,* 2015). Inferiorly and caudally, it is bordered by the postrhinal cortex or the parahippocampal cortex (the homologous regions in rats and humans, respectively) and ventrally and medially by the entorhinal cortex (Ku *et al.,* 2021)

The hippocampus is the third structure in the memory circuitry pathway in both the rats and in humans (Opitz, 2014). In both the rats and the humans, the hippocampus has a basal position in the telencephalon and similarly regarding its histological structure and cellular arrangement, they are very much alike in both humans and the

rats, (Eichenbaum, 2017). Hippocampus is the part of the memory circuitry system that is functionally important in processing long-term memory that starts in the entorhinal cortex via the subiculum, Cornu Ammonis, dentate gyrus and back and to the entorhinal cortex forming what is commonly known as the classical trisynaptic pathway (Lisman *et al.,* 2017; Wible, 2013). The rat's hippocampus is a continuous structure that changes its cranial dorsal position to a lateroventral location in the more caudal parts where it eventually reaches the ventral surface of the brain (Schröder *et al.,* 2020).

2.5 The Histomorphological Organization of Fetal Memory Circuitry Structurers

The histomorphological organization of the fetal memory circuitory structures starts with the prefrontal cortex (PFC) that is constituted of six histological layers namely (I) the molecular or the plexiform layer (II) external granular layer (III) external pyramidal layer (IV) internal granular layer (V) internal pyramidal layer and (VI) multiform (fusiform) layer, that can clearly be distinguished from each other in a routine histological staining with haematoxylin and Eosin (H&E) staining technique (Song & Moyer, 2018, Teffer & Semendeferi, 2012). The molecular layer (ML) is further organized to have the upper portion (layer I a) that contains large neurons called Cajal-Retzius cells; and the lower portion (layer I b) that is constituted of horizontally oriented nerve fibers. The external granular layer contains many, tightly packed granule cells and Golgi type II cells that are round to ovoid in shape representing the extensions of what is commonly reffered to as the mossy fibres (Silbereis *et al.,* 2016).

The external pyramidal layer contains predominantly small and medium-size pyramidal neurons as well as non-pyramidal neurons with vertically oriented intra-cortical axons, while granule cells predominate the internal granular layer that receives the afferent connections from the thalamus and from other cortical regions and sends connections to the other layers above it. On the other hand, the internal pyramidal layer consists predominantly of the medium-sized and large pyramidal cells whose axons leave the cortex and connect with subcortical structures including

the basal ganglia, while multiform (fusiform) layer contains mostly fusiform cells with less dominant pyramidal cells and interneurons (Wang *et al.,* 2019). All these prefrontal cortical cells act as primary innate cells that are involved in processing and encoding of short term (working) memory from sensory memory, then transmit signals to structurers of medial temporal lobe which they synapse with for storage (Preston & Eichenbaum, 2013).

The structures of the medial temporal lobe constitute the other structure of the memory circuitry system and is a region of multiple structures with intersections of neuronal networks, reflecting the multi-layered nature of memory, (Insausti *et al.,* 2017). Components of medial temporal lobe involved in memory processing, storage and retrieval includes the hippocampus, connected to a set of immediately adjacent structurers including; parahippocampal cortices, entorhinal cortices and perirhinal cortices, subiculum, presubiculum and parasubiculum, dentate gyrus, and amygdaloid nucleus, (Patel *et al.,* 2022; Kiernan, 2012). Histological organization entails an interface between prefrontal cortex to perirhinal and entorhinal cortices with six neuronal layers, parahippocampal gyrus, interphase to hippocampus with subiculum, pre and parasubiculum structurers and memory storage structures like the hippocampus with six layered neuronal laminae (neocortex), dentate and amygdaloid nucleus structurers (Jin *et al.,* 2022; Lech & Suchan, 2013).

2.6 The Memory Flow from the Prefrontal Cortex to the Amygdaloid Nucleus

Memory processing entails acquiring new information, sorts and processes this information in the prefrontal lobe then sends this information for storing, retaining, and later retrieving information in the medial temporal lobe that includes the entorhinal cortex, the para hippocampus, hippocampus, subiculum, pre and parasubiculum, the dentate and amygdaloid nucleus. These strucures are charged with processing of the memories that start with an initial neural representation of the newly encountered experience, then consolidate them into an organized and optimized codded form for future retrieval when cued by a stimulus associated with the initial experience (Zlotnik & Vansintjan, 2019; Camina, & Güell, 2017; Bisaz *et al.,* 2014; Schacter, 2013; Yoon *et al.,* 2008).

The memory network activity associated with organization, encoding, storage and retrieval of memories involves unique anatomical organization and interconnections from the prefrontal cortex that encodes for the task relevant information in working (short-term) memory (Lara & Wallis, 2015). In the dorsolateral side of prefrontal cortex, information about objects and events that one comes across or experiences, and the places where they occur (declarative memory), is processed and then sent through steamed pathways (reciprocal connections) to medial temporal lobe (MTL) involved in event memory storage (Straube, 2012; Van Strien *et al.,* 2009).

In the medial temporal lobe, memory structurers including rhinal cortices (entorhinal and perirhinal), piriform cortices hippocampal and parahippocampal cortices are essential for long-term declarative memory processing of events, facts and relations (recollection) and hence are labelled the medial temporal lobe memory system, with each brain region playing instinct role (Jin & Maren, 2015). Perirhinal cortex and the lateral entorhinal area are engaged by specific object stimuli and signals the familiarity of those items, whereas the parahippocampal cortex and the medial entorhinal area are involved in processing the spatial contexts in which memorable events occur (Nilssen *et al.,* 2019; Coutureau & Di Scala, 2009).

The hippocampus is involved in encoding individual events within the context and locations in which they occurred, ‘what’ and ‘where’ (Eldridge *et al* 2000). It consciously retrieves previously learned information including its temporal and spatial context, with a high degree of certainty (Lech & Suchan, 2013). Outputs of the hippocampus return to the cortical areas from which inputs arose via perirhinal to lateral entorhinal cortex and parahippocampal and finally medial entorhinal cortex (Wiltgen *et al.,* 2010; Buchanan, 2007).

2.7 The Comparative Morphogenesis of the Fetal Prefrontal Cortex in Humans and Rats

During the evolution of the fetal brain, the observed prefrontal cortical advances in both humans and in rats show similar morphogenetic patterns where in the initials stages of its development it starts with marked increase in the surface area and the introduction of new cytoarchitectonic areas among which the prefrontal cortex (PFC)

is considered to be the substrate of highest cognitive functions, (Kolk & Rakic, 2022). The structural development of the various subdomains of the PFC is a meticulous process starting with a massive expansion of the most proximal part of the developing neural tube (Friedman & Robbins, 2022). The first step in the expansion of the cortical surface during development starts with an increase in the number of symmetrical divisions of neural stem cells in the ventricular zone (VZ) before the onset of neurogenesis and the formation of the subventricular (SVZ), intermediate (IZ) and subplate (SPZ) zones and cortical plate (CP) below the marginal zone (MZ) (Jiang & Nardelli, 2016).

Although neurons of the PFC are generated before birth, the differentiation of its neurons and development of synaptic connections in humans extend to the 3rd decade of life, (Stiles & Jerniga, 2010). During this period, synapses as well as neurotransmitter systems including their receptors and transporters, are initially overproduced followed by selective elimination (Tau & Peterson, 2010). Recent advanced methods applied to human and animal models, have enabled investigation of the cellular mechanisms and role of specific genes, non-coding regulatory elements and signalling molecules in control of prefrontal neuronal production and phenotypic fate, as well as neuronal migration to establish layering of the PFC (Molnár *et al.,* 2019).

Likewise, various genetic approaches in combination with functional assays and immunohistochemical and imaging methods reveal roles of neurotransmitter systems during maturation of the PFC (Molnár *et al.,* 2019). Disruption, or even a slight slowing of the rate of neuronal production, migration and synaptogenesis by genetic or environmental factors like prenatal exposure to lamotrigine and levetiracetam, can induce gross as well as subtle changes that eventually can lead to cognitive impairment. An understanding of the neuroteratogenic effects of prenatal exposure to lamotrigine and levetiracetam on the development and evolution of the PFC will provide an insight into the pathogenesis and treatment of congenital neuropsychiatric diseases as well as idiopathic developmental disorders that cause intellectual disabilities (Rustom *et al.,* 2022).

2.8 The Comparative Neurogenesis of the Medial Temporal Lobe between Rats and Humans

Understading the comparative neurogenesis of the medial temporal lobe between rats and humans is of importance as the medial temporal lobe (MTL) structures are key in terms of memory storage and retrieval systems in humans (Ghetti *et al.,* 2010). The developmental processes of the medial temporal lobe structurers that includes neurogenesis, gliogenesis, oligodendrocyte maturation and synaptogenesis in both human and rats depicts similar key sequential events, although the time scale of their occurrence is not the same (Semple *et al.,* 2013). In both human and rats, magnetic resonance imaging (MRI) images have demonstrated that white matter increases linearly as age advances beginning towards end of second trimester and continues up to the third decade of life, while grey matter follows a linear development up to age 16-17 and begins to decline thereafter, explaining the dementia associated with aging (Giorgio *et al.,* 2010).

The process of cell proliferation in human and rat's medial temporal lobe structures that includes hippocampus, dentate gyrus, amygdaloid nucleus among others is also parallel with different time scales. In humans, it begins during intrauterine development with a subplate zone that contains glutamatergic and Gamma-Aminobutyric neurons that becomes a source of new dispersed neurons, up to the age of two and a half (2.5) years postnatally. On the other hand, rodents have a single compact layer of cells that develops at gestation date of 9.5 and peaks at gestation date 14-17 (Bordiuk *et al.,* 2014).

In both human and rats, neurons in medial temporal structurers begin to arborize (form synapses) and have synaptic response prenatally with their density increasing drastically in the early months after delivery, that as well coincides with astrogenesis (Zeiss, 2021). In humans, it begins at approximately 20^{th} gestational week and is 50% higher by 2 years of age, while in rats, it also peaks at the 10^{th} day postnatally. These synapses however decrease with increase in age (Pressler & Auvin, 2013).

Formation of myelin sheath in both human and rats is of paramount importance since it determines the speed of neurotransmission and increases the white matter volume. In the medial temporal lobe, the preoligodendrocytes (oligodendrocyte percussors) that does the myelination role occurs 18-28 weeks postnatally in humans while in rats, at postnatal day 1-3 and peaks at postnatal day 10, (Banko *et al.,* 2011; Südhof, 2018).

2.9 The Comparative Organization of the Prefrontal Cortex and Medial Temporal Lobe between Rats and Humans

Both the gross and the histological organization of the prefrontal cortex in both the rats and humans shows that the prefrontal cortex (PFC) is the part of the frontal lobe that is the largest of the cortical regions of the brain constituting 29% of the whole cerebral cortex, (Le Merre *et al.,* 2021; Petrides *et al.,* 2012). It is histologically comprised of six layers that can clearly be distinguished from each other by their features (Teffer & Semendeferi, 2012). From inside to outside, the laminae/layers are as follows; (i) Lamina zonalis-This zone contains few horizontal cells of cajal with axons of Martinotti cells being located at deep layers. The last branches of the afferent nerve fibers extend to this lamina. (ii) Lamina granularis externa-this zone contains small pyramidal cells and granular cells. (iii) Lamina pyramidalis externa-this layer contains loosely arranged pyramidal cells that increase in size from outside to inside.

The axons of these cells traverse the white matter and reach other cortical regions and make up the ipsilateral and contralateral cortico-cortical connection, (iv) Lamina granularis interna- this is the layer with the highest number of cells and contains stellate pyramidal cells and granular cells. (v) Lamina pyramidalis interna-this zone contains a smaller number of cells in comparison to the other laminae. It harbours well-developed Martinotti cells and pyramidal cells. Axons of the pyramidal cells located in this layer send projection fibers to the basal ganglia. (Vi) Lamina multiformis-this zone harbours Martinotti cells, fusiform cells and pyramidal cells (Petanjek *et al*., 2008).

The medial temporal lobe on the other hand is a region of multiple structures with intersections of neuronal networks, reflecting the multilayed nature of memory (Insausti *et al.,* 2017). Components of medial temporal lobe includes the hippocampus, connected to a set of immediately adjacent structurers including, perirhinal, entorhinal parahippocampal cortices among others, (Kiernan, 2012). Histological organization entails an interface between structurers like the hippocampus with parahippocampal gyrus, three layered neuronal laminae (orchidocortex), perirhinal and entorhinal cortices composed of the six neuronal layers' structurers (Lech & Suchan, 2013). The volumetric analysis of the total brain (TBV) and the intracranial volume (ICV) and volume density of both prefrontal cortex and medial temporal lobe depict a linear relationship, (Kijonka *et al.,* 2020).

2.10 The Histo-Quantitative Teratogenic Effects of Anticonvulsants on Developing Fetal Brain Structurers in Albino Rats

Previous studies done on the histo-quantitative injurious effects to fetal brain structures upon administration of second-generation anticonvulsants in the same class with lamotrigine and levetiracetam have shown that they have effects on neuro-development where neurons showed pyknotic and chromatolytic nuclei while the cytoplasm had rarefied with swollen organelles (Badawy *et al.,* 2019). In another study done and aimed to clarify the histopathologic effects of prenatal topiramate exposure, a second-generation anticonvulsant on the cerebral cortex and the hippocampus of new-born rats during pregnancy reported that the granules and pyramidal cells in the cerebral cortex and hippocampus were disorganized with signs of degeneration in both the cerebral cortex and hippocampus (Hagar, 2014).

Similary, in-utero exposure to pregabalin showed potential teratogenic effects on the vertebral column even in lower doses, though it had less intensity than other anticonvulsants (Etemad *et al.,* 2013). A study on effects of oxycarbazine on the on the cerebral cortex showed neuro-degenerative changes, that were marked with neuronal cell degeneration, disorganization of the brain tissue, numerous pyknotised cells and vacuolization of the neuropil (Hamdi *et al.,* 2017).

2.11 The Dose and Time Effects on the Teratogenic Outcomes of Known Anticonvulsant Medicines

Previous studies done to establish the effects of doses and the time of exposure to some known first and second-generation anticonvulsants like the carbamazepine, phenobarbital, phenytoin, pregabalin and others that are more or less have the same mechanisms of action with lamotrigine and levetiracetam demonstrated that the observed fetal brain teratogenic effects upon *in-utero* exposure affected the fetal nervous system development throughout the gestation period (Elshama *et al.,* 2015). The most deleterious effects were subsequently observed on higher dosages as compared to lower dosages in all the anticonvulsant medicines studied (Etemad *et al.,* 2013). Other previous study results by Hill *et al.,* (2010) showed that the patterns of exposure in causing brain anomalies varies, with topiramate, a second-generation anticonvulsant causing major structural malformations.

Other previous studies by Holmes et al., (2011) and Kuluga et al., (2011) on comparison between results on monotherapy versus polytherapy anticonvulsants administered to expectant mothers showed that administering one anticonvulsant doubles the risk of malformations while many anticonvulsants triple the effects. Further, a previous study aimed at comparing which among first generation and second-generation anticonvulsants are associated with high teratogenicity risk went further and concluded that older medicines such as phenobarbital and valproate, first generation anticonvulsants are associated with a range of teratogenicity as compared with second generation anticonvulsants (Tomson et al., 2019; Güveli et al., 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Chapter Introduction

This chapter outlines the entire methodological procedures used in carrying out the study. It begins by describing the study setting, followed by study design, the study subjects, the sample size determination, the grouping of the animals, inclusion and exclusion criteria, the feeding of albino rats, breeding and confirmation of pregnancy, determination, calculation and administration of levetiracetum and lamotrigine, prenatal duration of levetiracetum and lamotrigine dose exposures, the humane sacrificing of pregnant albino rats, harvesting of fetuses, harvesting of the fetal brains, histomorphological and stereological procedures, data analysis, ethical considerations and approvals.

3.2 Study Location/ Setting

All animal experimental procedures that included breeding, mating, daily weighing, administration of both lamotrigine and levetiracetam, general observations of the rats, humane sacrificing of the rats, measurement of fetal growth and developmental parameters including crown rump length (CRL), bi-parietal diameter (BD) and fetal body weights, were all carried out in the School of Biomedical Science, situated in the University of Nairobi (UON), Chiromo campus. Processing for light microscopy and stereology was carried out in the department of Human Anatomy based in Jomo Kenyatta University of Agriculture and Technology (JKUAT), Juja main campus.

3.3 The Study Design

This study adopted a post-test-only control experimental study design. This study design was considered suitable for the study because it aimed at establishing the teratogenic effects of the fetal memory circuitry pathways structurers after prenatal exposure of the female albino rats to both lamotrigine and levetiracetam.

3.4 Study Subject

A total of 30 nulliparous albino rat dams of the species *Rattus Norvegicus* from a pure colony of the 3rd series breed weighing between 210 and 240 grams were used as the animal experimental model. These rats were sourced from lower Kabete research institute in Nairobi County. The use of these albino rat dams was guided by the following known scientific facts; (a) they have a large litter size of between 3-16 fetuses, (b) they have low incidence of spontaneously occurring congenital defects, (c) they have a relatively short gestational span, making it easier to get study subjects or a pure bleed colony (d) the low cost of maintaining the animals , (e) they are plentiful, (f) considerable amount of the reproductive data on the rat is already available, (g) they are relatively small and easy to care for and handle during an experiment (h) they are relatively resilient in terms of withstanding a wide range of study medicines (Bailey *et al.*, 2014).

Albino rats were the first mammalian species domesticated for scientific research (Sengupta, 2013). By appearance, both the male and female albino rats are red eyed and have white fur resembling the '*Japanese hooded rats'*, hence essentially genetically identical from a common ancestor, (Pritchett & Corning, 2016). They live about 2-3.5 years (average 3 years). In adulthood, every day of the albino rat is approximately equivalent to 34.8 human days (i.e., one rat month is comparable to three human years), (Andreollo *et al.*, 2012). Albino rats develop rapidly during infancy and become sexually mature at about 4-5 weeks in females and at around postnatal dates 45-48 in males. This is defined by vaginal opening (females) or balanopreputial separation (males) (Quinn, 2005).

Reproductive senescence in female rats occurs between 15 and 20 months of age (Sengupta, 2013). Their gestation period is roughly estimated at from 21 to 23 days during which the fetuses are viable. Gestation period has 3 trimesters, with trimester one being the first 7 days after conception, second trimester from day 7-14 and third trimester from day 14 to day 21. Pregnancy is detectable at about 2 weeks by feeling the abdomen, noticing weight gain or mammary (breast) development and pregnant

females making a nest. Tissue paper provides excellent material for nesting (Windsor & Bate, 2019).

The usual litter size is 3 to 16 pups (Parra-Vargas *et al.*, 2023). When baby rats are born, they are deaf and blind. Weaning occurs about 21 days after birth. Adult female and male rats typically weigh 12 to 16 ounces (350 to 450 grams) and 16 to 23 ounces (450 to 650 grams), with male rats being larger than females and are about 9 to 11 inches long. Male albino rats from a pure colony were used for mating purposes, (Frohlich, 2020).

3.5 Sample Size Determination

A sample size of the 30 albino rat dams was used in the study, determined by use of resource equation method for One-way Analysis of Variance (ANOVA) (Arifin & Zahiruddin, 2017; Charan & Biswas, 2013; Charan & Biswas, 2013). This was guided by the fact that it was not possible to assume the standard deviation and the effect size. It was therefore determined as follows;

- ✓ The acceptable range of degrees of freedom (DF for the error term in the analysis of variance (ANOVA) is usually between 10 to 20, where 20 is considered as being sufficient, since 10 cannot give significant results.
- ✓ DF=Total number of rats-total number of groups=20
- ✓ Formula **(n = DF/k + 1),** where

- ❖ k = number of groups=10
- ❖ n = number of rats per group

- ➢ n=20/10+1 =3.
- ➢ Each group therefore was allocated 3 rat dams
- ➢ Since the total number of groups were 10 and each group was allocated 3rats, therefore, (10 groups x 3rats) =30 rat dams.

Since each rat dam normally gives birth to 3 to16 litter size (Pritchett-Corning *et al.,* 2009), the fetuses from each rat were ordered according to their body weight from the lowest to the highest. By use of systematic uniform random sampling method, 3 fetuses were chosen from each of the 30 rats to make a total of 90 fetuses.

3.6 Breeding, Confirmation of Mating and Confirmation of Pregnancy

3.6.1 Breeding

For breeding purpose, one sexually mature male albino rats from the 3rd series breed of a pure colony were introduced into a translucent polycarbonate cage, containing two female albino rats. They were allowed to mate for 1200-hours light and 1200-hours dark cycle with onset at 0700 hours and offset at 0700hours the following day (Pritchett-Corning, 2009). The males were removed and returned to their separate cages except for the rats that had not conceived after pregnancy confirmation, that were allowed for one extra attempt.

3.6.2 Confirmation of Mating

Mating was confirmed by taking swabs from the females' vaginal canal, smeared on glass slides and observed under the light microscope. Presence of spermatozoa confirmed that coitus had taken place, (Kohn & Clifford, 2002).

3.6.3 Confirmation of Pregnancy

(a) Materials Used in Confirmation of Pregnancy

- ✓ 0.85%phosphate buffered saline
- ✓ Microscope slides
- ✓ Ethanol (95%)
- ✓ Absolute alcohol
- ✓ 10mls blunt tipped disposable pipettes
- ✓ Giemsa stain

(b) Procedure Used in Confirmation of Pregnancy

- With a gauze holder against the body, rats were restrained
- Using a blunt tipped disposable pipette, 1ml of saline was introduced into the vaginal cavity
- Phosphate buffered saline was gently inserted into the vaginal cavity by use of a cotton tipped moist swab
- Gently, the swab was rolled in the vaginal canal before withdrawing
- The moist swab was then rolled onto a clean glass slide
- 95% ethanol was sprayed to fix the specimen
- The slides were subsequently dipped in 100% alcohol to air dry
- Giemsa stain was used for staining
- The stained slides were observed under a microscope

(c) Observation Made;

Fertilization was denoted by presence polyhedral scattered epithelial cells with many neutrophils on the smear. At least 99% of the rats tested positive and this was counted as the first day of their gestation period. 1% of the rats that never conceived the first attempt were given only one additional attempt with males to mate after which those who never tested positive were excluded in the study and replaced.

3.7 Selection Criteria

3.7.1 Inclusion Criteria

- ✓ Rats that conceived after mating
- ✓ Healthy rats with no signs of sickness
- ✓ Live fetuses at the time of sacrificing

3.7.2 Exclusion Criteria

- ❖ Rats with a negative pregnancy test after one exxtra exposure to the males for mating
- ❖ All fetuses whose mother had an underlying disease state during pregnancy.

3.8 Grouping of Female Rats (Dams)

The female rat dams were assigned into either control group of 3 rats or 27 experimental group. In order to determine whether the teratogenic effects of lamotrigine and levetiracetam are dose dependent, the experimental category of 27 rats were further sub-divided into three sub-groups of 9 rats for low lamotrigine/ levetiracetam group; medium lamotrigine/ levetiracetam group and high lamotrigine/ levetiracetam group.

Similarly, to determine whether the effects of lamotrigine/ levetiracetam are time dependent, the 3 study categories were further subdivided into three subgroups of 3 rats for 1^{st} trimester, 3 rats for 2^{nd} trimester and 3 rats for 3^{rd} trimester (Figure 3.1).

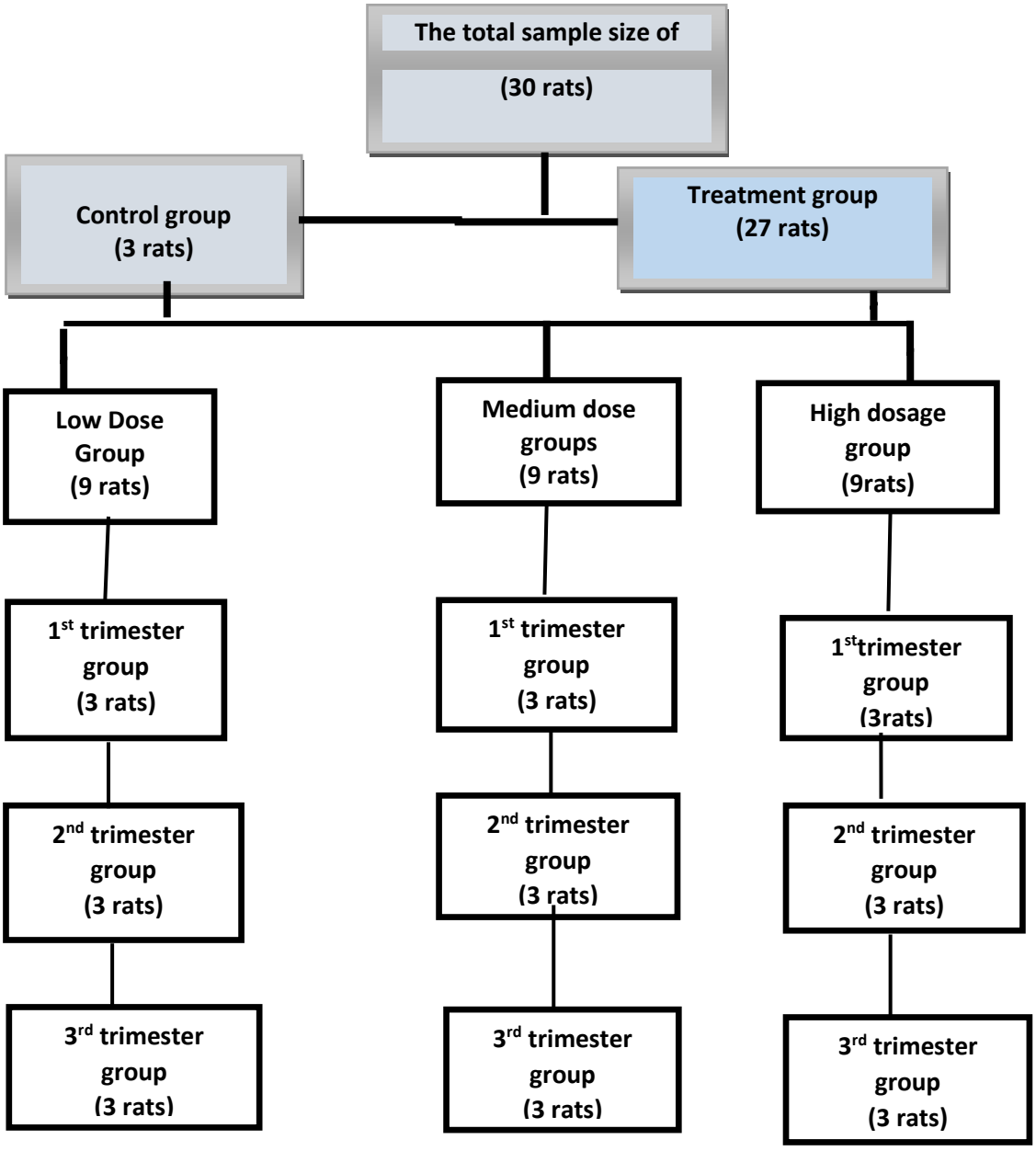

Figure 3.1 : Illustration on How the Grouping of the 30 Albino Rat Dams in Each of the Study Categories of Levetiracetum and Lamotrigine was done

3.9 Feeding and Handling of Albino Rats

Any procedure carried out was according to the laid down guidelines for care of laboratory animals, (Ahmadi-Noorbakhsh *et al.*, 2021; Couto & Cates, 2019; Jones-Bolin, 2012). All rats were fed on rodent pellets obtained from Nairobi Unga Limited and water *ad-libitum* as described by Willems, (2009), as well as folate supplementation *throughout* the gestation period. Weighing of the pregnant rats was

done on daily bases at 0930 hours using Scout Pro model SPU4001 S/N B519923500 digital weighing scale from Uhaus Corporation, USA (Figure 3.2)

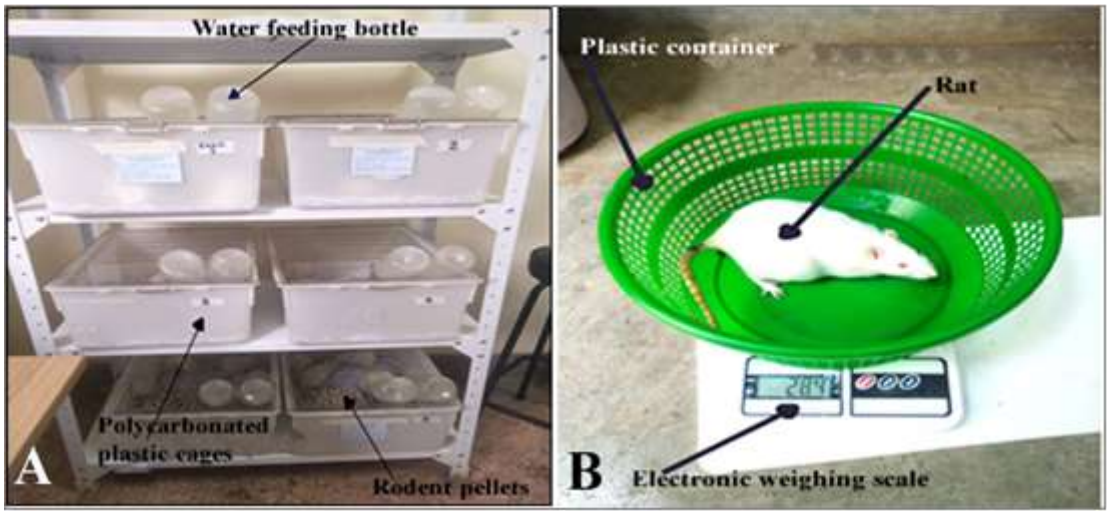

Figure 3.2: An Illustration of how feeding and weighing of the rats was done

Key
A: Polycarbonated plastic cages with rodent pellets and water
B: Illustration of weighing of the rat using an electronic weighing scale

3.10 Determination, Calculation and Administration of Lamotrigine and Levetiracetam

The adult lamotrigine dosages in human ranges between 25mg-500mg per day while levetiracetam ranges between 1000-3000mg in divided dosages (Abou-Khalil, 2008; Warshavsky *et al.,* 2016). Both medicines were obtained from a government chemist in Nairobi, taking into consideration their batch number and both were reconstituted using distilled water.

3.10.1 Determination Lamotrigine and Levetiracetam Doses in Rats

Lamotrigine and levetiracetam rat dosages were determined by a conversion formula from human dosages to animal dosages (Nair & Jacob, 2016). According to the

formula, the Km factor (constant value based on surface area to volume ratio) for each species is constant, and is used to simplify calculations.

- ✓ Km is estimated by dividing the average body weight (kg) of species to their body surface area (m2).
- ✓ **The Km ratio values** for the rats are already provided and are obtained by dividing human Km factor by animal Km factor, which is 6.2.

The formular is as follows;

- ❖ Animal equivalent dose AED (mg / kg) = Human dose (mg / kg) × K_m ratio
- ❖ The Km factor for rats is already provided as 6.2, then we multiply human equivalent dose in mg/kg by a constant ratio of 6.2
  - o For example, if the maximum dose of a particular drug in human is 10 mg/kg, the AED is calculated by multiplying the HED by 6.2
  - o AED is therefore 62 mg/kg (Reagan-Shaw *et al.*, 2008)

3.10.2 Calculation of Lamotrigine and Levetiracetam Doses for the Rats

- ✓ The maximum lamotrigine dose in humans is 25mg, medium dose is 235.7 mg and high dose is 500mg.
- ✓ The maximum levetiracetam dose in humans is 3,000mg, medium dose is 2000 mg and high dose is 1000mg.
- ✓ The average weight of an adult human is approximately 60kg.

i) Calculation of lamotrigine dosages

a) Low dose lamotrigine group

Humans have an average weight of 60kg

The low dose lamotrigine is-25mg

25mg = 60kg

X=1kg

X=1x25/60 =0.417mg/kg

AED = HED X Km factor

Therefore, 0.417mg/kg x 6.2 =**<u>3mg/kg bw</u>**

b) Medium dose lamotrigine group

The medium dose of lamotrigine-235.7mg

235.7mg = 60kg

X=1kg

X=1x235.7/60 =325.7mg/kg
AED = HED X Km factor
Therefore, 3.92mg/kg x 6.2 =**24mg/kg bw**

c) High dose lamotrigine group
The high dose of lamotrigine-500mg
500m= 60kg
X=1kg
X=1x500/60 =20mg/kg
AED = HED X Km factor
Therefore, 8.3mg/kg x 6.2 =**52mg/kg bw**

ii) Levetiracetam dosages

a) Low dose levetiracetam group
The low dose of levetiracetam is-1000mg
1000mg= 60kg
X=1kg
X=1x1000/60 =16.667mg/kg
AED = HED X Km factor
Therefore, 16.667mg/kg x 6.2 =**103mg/kg bw**

b) Medium dose levetiracetam group
The low medium levetiracetam dose-2000mg
2000mg = 60kg
X=1kg
X=1x2000/60 =33.333mg/kg
AED = HED X Km factor
Therefore, 33.333mg/kg x 6.2 =**207mg/kg bw**

c) High dose levetiracetam group
The high dose levetiracetam-3000mg
3000mg = 60kg
X=1kg
X=1x3000/60 =50mg/kg
AED = HED X Km factor
Therefore, 50mg/kg x 6.2 =**310mg/kg bw**

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

iii) Calculation of specific rat dosages
If for example the weight of the rat is **200g** and low lamotrigine dose -**52mg/kg,** then calculation is done as follows;
(52mg/kg/1000) =**0.052mg/g**
0.052mg/g x200g=**10.4mg**
If lamotrigine tablet is **100mg,** and reconstitution is done in **10ml** of distilled water, **then** 100mg=10ml

10.4mg=
10.4mgx10ml=**1.04ml**
100mg

3.10.3 Administration of Lamotrigine and Levetiracetam

Both lamotrigine and levetiracetam were administered by the researcher on daily basis at 0900hrs using the gavage needle gauge 16.

(i) Materials used in administration of lamotrigine and levetiracetam

- Pregnant dams (30)
- Lamotrigine tablets
- levetiracetam tablets
- Gavage needle gauge 16
- 20 ml beaker for dilution
- Syringes-2ml and 5m
- Distilled water
- A table cloth

(ii) Procedure for administering lamotrigine and levetiracetam

- Using the left hand, rats was held at the neck region
- To avoid the rats from soiling the investigators clothing's during the procedure, they were wrapped with a piece of cloth
- With the rats' mouth facing the investigator, the tail was rested against the body
- A gavage needle gauge 16 was gently inserted into the mouth of the rat, turning it gentry to pass the oesophageal constrictions and the cardiac sphincter
- The treatment bolus was put in the stomach of the animal
- The gavage needle was gentry be removed

3.11 Duration of Lamotrigine Levetiracetam and Administration

The duration of rats' pregnancy is 21 days and is divided into three trimesters, with each trimester having seven days. Trimester one (TM_1) rats' category received lamotrigine and levetiracetam (low, medium and high) dosages from the first day of gestation ($GD_{1)}$ to the last day of medication (GD_{20}). Trimester two (TM_2) rats' category received lamotrigine and levetiracetam (low, medium and high) dosages from the seventh day of gestation ($GD_{7)\ to}$ the last day of medication (GD_{20}), while trimester three (TM_3) rat category received lamotrigine and levetiracetam of low, medium and high dosages from the fourteenth day of gestation ($GD_{14)}$ to the last day of medication (GD_{20}).

3.12 Humane Sacrificing of the Pregnant Albino Rats

All rats were humanely sacrificed on the 20^{th} day of gestation period, just one day before delivery, by use of concentrated carbon dioxide in lid-fitting bell-jar.

(i) Materials used for the humane sacrificing of rats

- The pregnant rat dam of gestation date 20
- Concentrated carbon dioxide (CO2)
- Cotton wool
- Bell jar
- Physiological saline 0.85% concentration
- Mounting board
- Mounting pins
- A pair of scissors
- A pair of forceps (toothed)
- Scalpel blade
- Scalpel blade handle
- Fixative- 10% formaldehyde
- 2 drip sets
- Normal saline
- Hypodermic needle gauge 20
- Clean gloves

- ❖ Electronic weighing scale
- ❖ Specimen collection bottles

(ii) Procedure of humane sacrificing of the pregnant albino rat dams

- ✓ Concentrated carbon dioxide was introduced into a bell jar
- ✓ The pregnant rats were put into the bell jar (Figure 3.3)
- ✓ The bell-jar was covered by a tight-fitting lid
- ✓ The rat was waited for 10-15 minutes to be anaesthetized
- ✓ The rat was removed from the bell jar and mounted onto the board using mounting pins with dorsal side on the board (Figure 3.3)
- ✓ Using a pair of scissors and forceps the rat was given an incision in the ventral medial side along the linear alba (Figure 3.3)
- ✓ The perfusion needle was inserted to the left ventricle of the heart while connected to the perfusion set containing 400mls of normal saline
- ✓ The blood was cleared from the rat with physiological saline (200mls of 0.85mol/litre) through the left ventricle of the heart (saline flew by force of gravity from the drip-set)
- ✓ After sufficiently clearing, the saline drip was removed (the needle was left in position of the heart and the 10% formaldehyde fixative was introduced.
- ✓ The firmness of the tail was checked as a sign of effective fixation of tissues
- ✓ The drip was disconnected and the perfusion needle removed from the heart

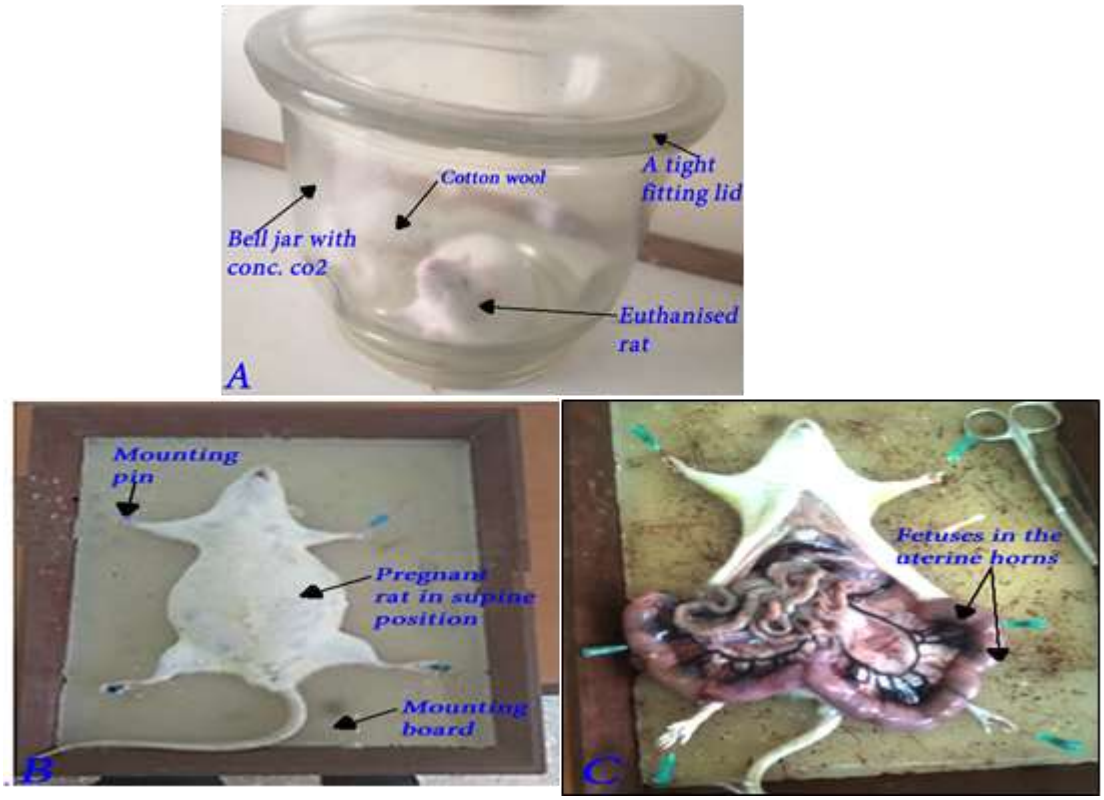

Figure 3.3: An Illustration on How Humanne Sacrificing of the Albino Rats Was Done

Key

A*- pregnant dam at 20th gestation date inside a tight-fitting lid containing concentrated carbon dioxide (co2),* ***B;*** *pregnant rat mounted on a board,* ***C;*** *Sacrificed rat portraying fetuses in the uterine horns.*

3.13 Harvesting of the Fetuses

- ✓ The anterior abdominal wall of the anaesthetized rats was incised in the ventral medial side along the linear alba from the symphysis pubis to xiphisternal joint
- ✓ Fetal positions were observed within the uterine horns
- ✓ The number of live and dead fetuses was determined by use of a gentle probe.
- ✓ Where fetal movements were observed, they were counted as live litter size
- ✓ Where fetal movements were not observed, they were counted as dead fetuses

- ✓ The number of devoured endometrial glands and resorbed fetus were counted and recorded (sample of resorbed endometrial gland and resorbed gland (figure 3.4).
- ✓ To expose the fetuses, 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.
- ✓ The general fetal morphology, and abnormalities of the fetus was examined.
- ✓ Placenta weight were taken and recorded
- ✓ Fetal weight measurements were taken by use of electronic weighing scale, crown-lump length measurements were taken using a calibrated ruler beginning from the tip of the nose (snout) to the root of tail (anus) (Figure 3.5).
- ✓ Head length taken from the external occipital protuberance of the occipital bone to the extremity of the nose, while bi-parietal diameters taken from the right to left mastoid processes of the temporal bone (using a digital Vernier calliper from Hercules from sealing Product-Japan model 1.13.2017) (Figure 3.5).
- ✓ Head circumference measurements were taken using a piece of thread from above the glabella, though the temporal bone (mastoid process to the external occipital protuberance) and were measured against a calibrated ruler (Figure 3.6)
- ✓ All fetuses were inserted in 10% formaldehyde to continue with fixation.

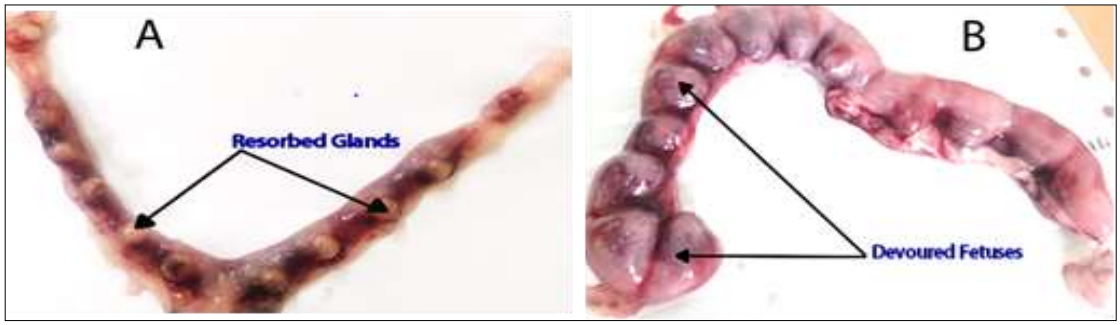

Figure 3.4: An Illustration of Samples of Resorbed Glands and Devoured Fetuses

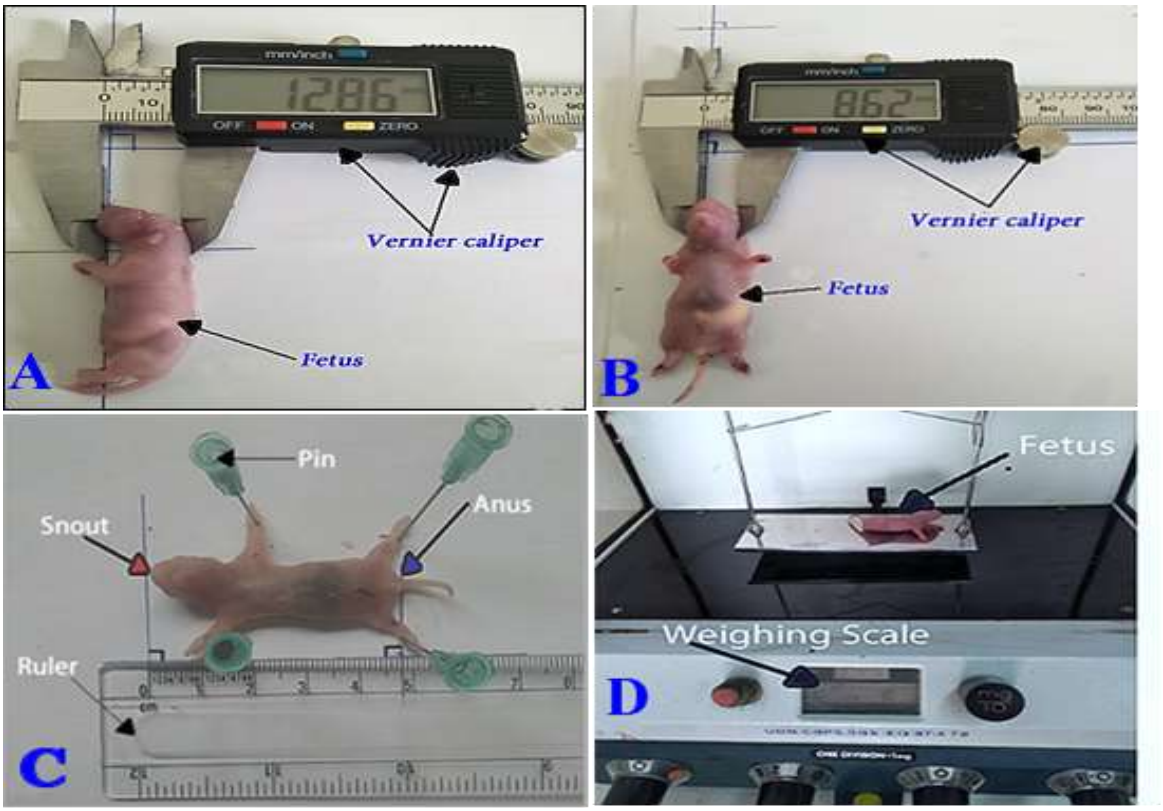

Figure 3.5: An illustration on how the measurements of fetal weight, crown-rump length, head length and crown-rump length was done

Key: ***A****-how crown-rump (CRL) measurements were taken*
B*-how bi-parietal diameter (BD) measurements were taken*
C*-how head length (HL) measurements were taken*
D*-fetuses were weighed*

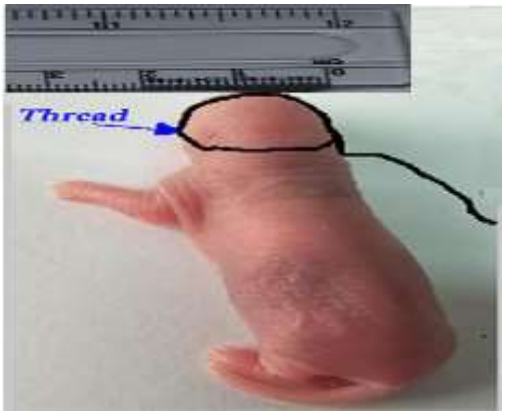

Figure 3.6: An Illustration on Measurements of the Fetal Head Circumference

3.14 Harvesting the Fetal Brains

From the sample of the tree fetuses selected, their brains were harvested for both histomorphological and histostereological analysis

a) Procedure for harvesting fetal brains

- With the ventral side facing the board, all fetuses were mounted on dissection board
- The lower margin of the temporal bone was opened using a pair of scissors and forceps and the skull cap was removed
- The entire fetal brain was identified by use of a magnifying glass.
- The meninges were opened along the superior sagittal sinus and retracted carefully
- The brain was scooped at the level of foramen magnum
- The external features congenital malformations were examined
- Brain parameters that include weight were taken using an electronic weighing scale, N B519923500 from Uhaus Corporation, USA (scout pro model SPU4001 S/, while brain length and width were taken using and a calibrated ruler (Figure 3.7)
- Fixation was done by immersing the brains in 10% formaldehyde for 24 hours

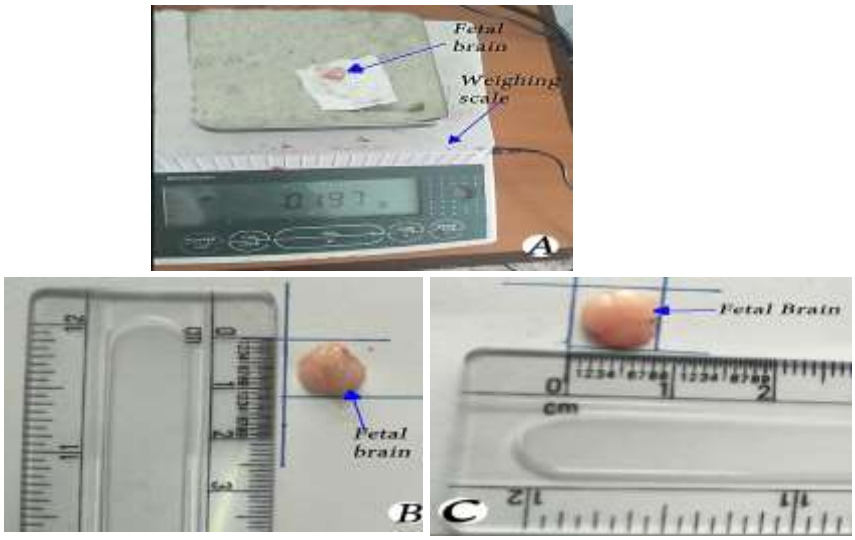

Figure 3.7: An Illustration on how the Messurements of Various Parameters of the Fetal Brain Was Done.

Key: -

A*-Measurements of the brain weight*
B*-Measurements of the brain length*
C*-Measurements of the brain width*

b) Processing fetal brain for light microscopy and stereology

- ✓ Fetal brains were fixed in Zenkers' solution for 24 hours
- ✓ Dehydration was done in ascending grades of alcohol (50%, 60%,70%, 80%, 90%, 95% and 100% each for one hour.
- ✓ They were immersed in cedar wood oil for 12 hours.
- ✓ Infiltration was done with paraffin wax for 12 hours at 56^0c
- ✓ The brain was oriented in longitudinal axis
- ✓ Embedding was done using paraffin wax on the wooden blocs
- ✓ Edges were trimmed-off the excess wax to expose the entire length of the fetal brain tissue
- ✓ Leitz sledge rotary microtome was used to cut 5µm thick longitudinal sections
- ✓ To spread the tissue, they were floated in water at 37^0
- ✓ The stuck slides were dried in an oven at 37^0 for 24 hours
- ✓ In absence of the researcher, blinding was done by a research assistant by coding all the slides

- ✓ Haematoxylin and Eosin were used for staining.

3.15 Qualitative Analysis

Qualitative analysis entailed taking photographs at magnification of x400, by a 20-megapixel digital microscope camera and qualitative analysis by use of Swift 3.0 software

i) Materials and procedure of taking photographs

- ❖ A 20-megapixel swift digital microscope camera
- ❖ A light microscope
- ❖ A Swift 3.0 software
- ❖ Glass slide

ii) Procedure of taking and labelling of photomicrographs using a 20 megapixel digital camera and qualitative analysis by use of Swift 3.0 software

- ➢ A digital camera 20 megapixel was inserted on the eyepiece using an over-eyepiece mount adapter.
- ➢ The adapter had an in-built magnifying lens
- ➢ The microscope USB plug was connected to the computer
- ➢ The slides with brain tissue were mounted in the microscope
- ➢ Images were automatically reflected on the computer in the swift 3.0 software
- ➢ Since calibration had been done on the computer, for any magnification, the output (thickness) measured was automatically labelled in the image (Figure 3.8)

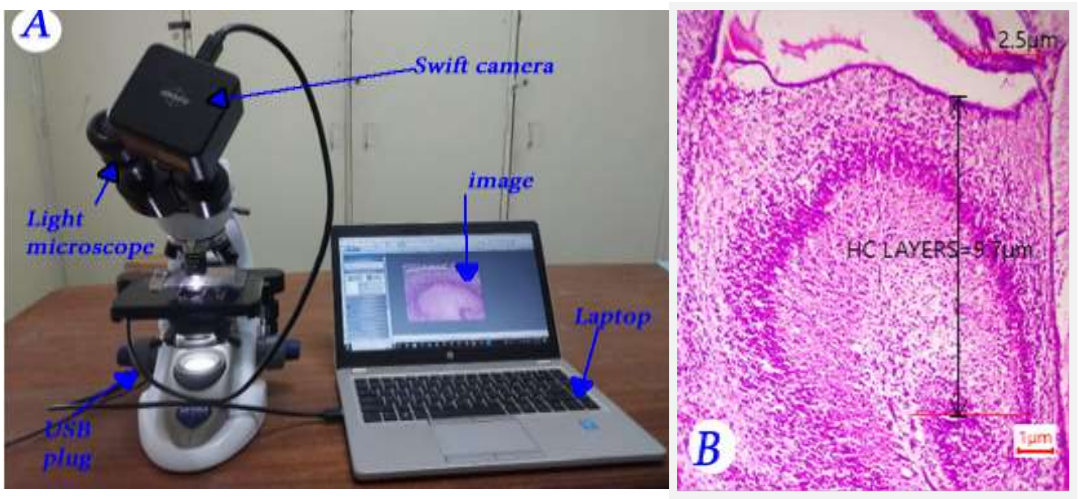

Figure 3.8: An Illustration on How the Calibration of Images Using a 20-Megapixel Swift 3.0 Camera Fixed on a Light Microscope Was Done

Key

A: *The 20-megapixel swift 3.0 camera fixed on a light microscope*
B: *Calibrated image*

3.16 Quantitative Stereological Analysis

The quantitative stereological analysis included; (i) the means of fetal brain weight, length and widths of the fetal brains as shown in figures 3.16 A to C; then the the initial total brain volumes using the Archimedes' displacement methods by use of a digital plethysmometer as shown in figure 3.16A. This was then followed by calculation of the total fetal brain volumes before they were immersed in the fixative, then followed by calculation of the actual terminal brain volume by use of Cavalieri point counting method.

The mean volume difference was established between the initial and the terminal volume (shrinkage) to determine the effects of fixatives; lastly the volume density of fetal memory circuitry structurers was also determined by use of Cavalieri point counting method applying the same steps and procedures like was the same case for the total brain volume with point counting method.

3.16.1 Determination of Total Brain Volume Using Archimedes Principle by Displacement Method Using a Digital Plethysmometer

The initial fetal brain volumes were determined by immersing the fetal brains in plethysmometer containing normal saline and that applies the Archimedes' principle. After immersion of the brain, the recordings on the amount of normal saline displaced digitally appeared automatically to represent the initial brain volume (Figure 3.9)

Figure 3.9: An illustration of how the calculation of total brain volume was done using the Archimedes law of displacement.

Key
A*- the digital plethysmometer with initial readings*
B*- The digital plethysmometer after putting in the fetal*

3.16.2 Determination of Total Brain Volume by Use of Cavalieri Point Counting Method

The following steps was followed in calculation of total brain volume using Cavalieri point counting method

- Brain sections of (5µm) thick sections were prepared
- Spacing for the point probe was selected
- In each section, a point probe was tossed randomly

- All points that hit the region of interest were counted keeping a tally of counts per section
- Cavalieri formula was used to calculate the volume.

Systematic uniform random sampling with a simple random start was used to select twenty sections of 5µm thickness from each longitudinal section of a brain (Zhang *et al.*, 2008). The entire brain slice was viewed at magnification of X100, using the microscope's stage Vernier. Digital images were captured and uploaded in the computer screen and superimposed in a STEPanizer tool for point counting.

A guard area was set to be consistent throughout the entire experiment. All the fields of the prefrontal and medial temporal lobe memory circuitry structurers were selected and images projected on a computer screen. 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, (Altunkaynak *et al.*, 2009) (Figure 3.10)

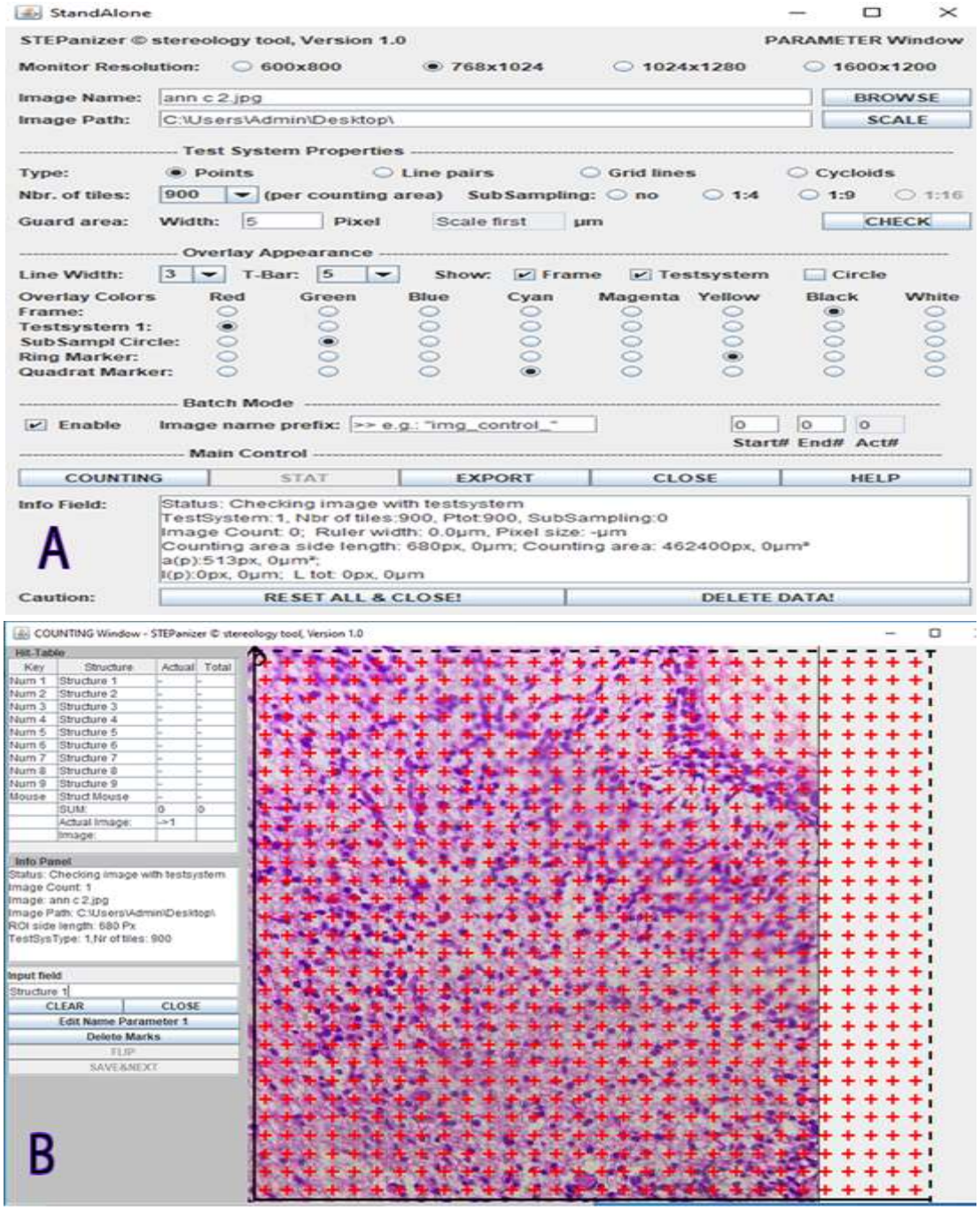

Figure 3.10: An Illustration of a Histological Section on how the Stepanizer Stereologytool Was Used in the Quantification of Fetal Brain Circuitry Structures with an Equidistant Point Grid

Key

A*-stand-alone window,*

B*- brain slice imageX40 superimposed in the counting frame*

The Cavalieri formula applied to calculate the total brain volume was as follows (figure 3.11)

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

Where;

- $\hat{V}$ = Is the Cavarieli volume
- **A_P:** is the area of a point
- **m':** is the section evaluation interval
- **t bar:** Is the thickness of the cut section
- ∑-Means summation
- **p_i:** Are the points counted on the grid from the first (i) to the last (n) (Golub *et al.*, 2015).

Figure 3.11: Illustration of the Formula Used in Cavarieli Point Counting Method

3.16.3 Correction for Brain Tissue Shrinkage

To calculate the percentage of brain tissue shrinkage as a result of histological procedures, fresh brain volume was obtained by use of Archimedes principal method of displacement. Cavalieri method of tissue processing was used to obtain brain volume after sectioning, and shrinkage calculated as per the following formula;

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

Where;

Volume before: Is the Archimedes volume

Volume after-Is the Cavalieri volume (Chung *et al.*, 2018).

3.16.4 Determination of Volume Density of Memory Circuitry Structurers Using Cavalieri Method of Point Counting

In determining the volume density of the prefrontal cortex and medial temporal lobe memory structurers, Cavalieri method of point counting using the STEPanizer tool was used. The number of points falling on the area of interest were counted

and compared with the points falling on the entire brain, and the following formula was finally applied;

$$\textbf{Est Vv} = \frac{\textbf{P (Part)}}{\textbf{P (Ref)}},$$

Where;

Est Vv -Estimated volume density

P (part)- All points that fell in the area of interest (Prefrontal lobe and medial temporal lobe)

P (Ref)-All points that fell on the entire brain (Zhang *et al.*, 2008).

3.17 Data Collection and Analysis

The qualitative histomorphological data was collected by taking histophotomicrographs by use of a digital Swift 3.0 camera under various magnifications, uploaded in a swift 3.0 software where measurements and labelling. Quatitative data on the other hand that entailed data on the maternal and fetal in-utero outcomes and histostereological outcomes was collected using structured checklists and stereological data sheets respectively, stored and coded in excel spreadsheets windows 10, version 2019, then was exported for analysis into SPSS programme for windows version 25 for analysis (Chicago Illinois).
Continuous data was computed by use of one-way analysis of variances (ANOVA) followed by Tukey's post hoc multiple comparison t-tests. Multiple Analysis of Variance (MANOVA) was done to analyse the interaction effects as well as to obtain the mean difference results between lamotrigine and levetiracetam. The findings were expressed as mean$\pm$ standard deviation (SD) for all values, and thoses whose $P<.05$ were considered to be statistically significant. Parametric data was presented in form of tables. Discrete data was analysed by Fishers exact test statistic of independence. Data was presented in form of histophotomicrographs, graphs and tables.

3.18 Study Ethical Approval

All animals used in the study as well as all procedures carried out in handling the animals were done in accordance with the guidelines of the National Institutes of Health Animal Care and the animal research and approval was sought and approved by the Animal Care and Use Committee based in the University of Nairobi (UON), Faculty of Veterinary medicine, Department of veterinary Anatomy and Physiology, before initiation of the study (REF: FVM BAUEC/2021/321 appendix 2).

CHAPTER FOUR

RESULTS

4.1 Chapter Introduction

This chapter outlines the findings of the study and are presented in line with the study objectives, however, the findings of the 4th objective that was meant to evaluate whether or not the observed histomorphological and histostereological teratogenic effects on the fetal memory circuitry structures were dose and time dependent are integrated in the findings of the first three obejcetives. [***NB> Some tables are big and extends beyond the margins and as well from one page to the next].***

4.2 The Maternal and Fetal Pregnancy Outcomes

Objective 1: The findings on how the two medicines comparatively influenced the maternal and fetal pregnancy outcomes following the *in-utero* exposure of varied doses of lamotrigine and levetiracetam at different gestational periods.

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

Level I: The comparative effects on how the two medicines influenced the maternal weight gain treads during pregnancy, and;

Level II: The comparative effects on how the two medicines influeneced the fetal pregnancy outcomes as follows: -

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

The comparative maternal pregnancy outcomes include: (i) the comparative maternal weight gain trends and (ii) the maternal terminal weight, weight gain and placenta weight

4.2.1.1 The Comparative Daily Maternal Weight Gains Trends for both the Lamotrigine and Levetiracetam against the Control

Upon monitoring the daily maternal weight gain trends, it was observed that in all the the treatment groups of both the lamotrigine and the levetiracetam, the daily maternal weight gain treads were remakarbly lower as compared with the controls across trimesters one, two and three (TM_1, TM_2 & TM_3). On further juxtaposistion as to how the trends differed between the lamotrigen and the levetiracetum treated groups, it was notable that the rats in the lamotrigen treated groups had relatively lower mean daily maternal weight gain trends as compared with those rats in the letiracetum treated groups across all the trimesters (Figure 4.1.1 to 4.1.3).

In terms how the dosages influenced the maternal weight gain trends, it was notable that the rats that received the low, medium and high doses in all the treatment groups at TM_1 TM_2, and TM_3, they all first depicted a sudden weight drop immediately after the intiation of the treatments [*probably as a cope-up mechanism with the medicine*] then followed by steady daily weight gains untill the end of the gestational period day 20 (GD_{20}).

With regards to the total terminal weights, it was notable that for the rats that received their treaments in TM_1 and TM_2, they had a significantly lower daily maternal weight trends than those that received their treatment at TM_3, a phenomenon that could be attributed to the the longer periods of nutritional disturbances or a probable prolonged irritation to the GIT occasioned either of the two medicines ver-Oall, it was clear that lamotrigine has a more inimical influence on the daily maternal weight gain treNds as compared to levetiracetum across all the trimesters of exposure (TM_1, TM_2 &TM_3 (Figure 4.1-4.3).

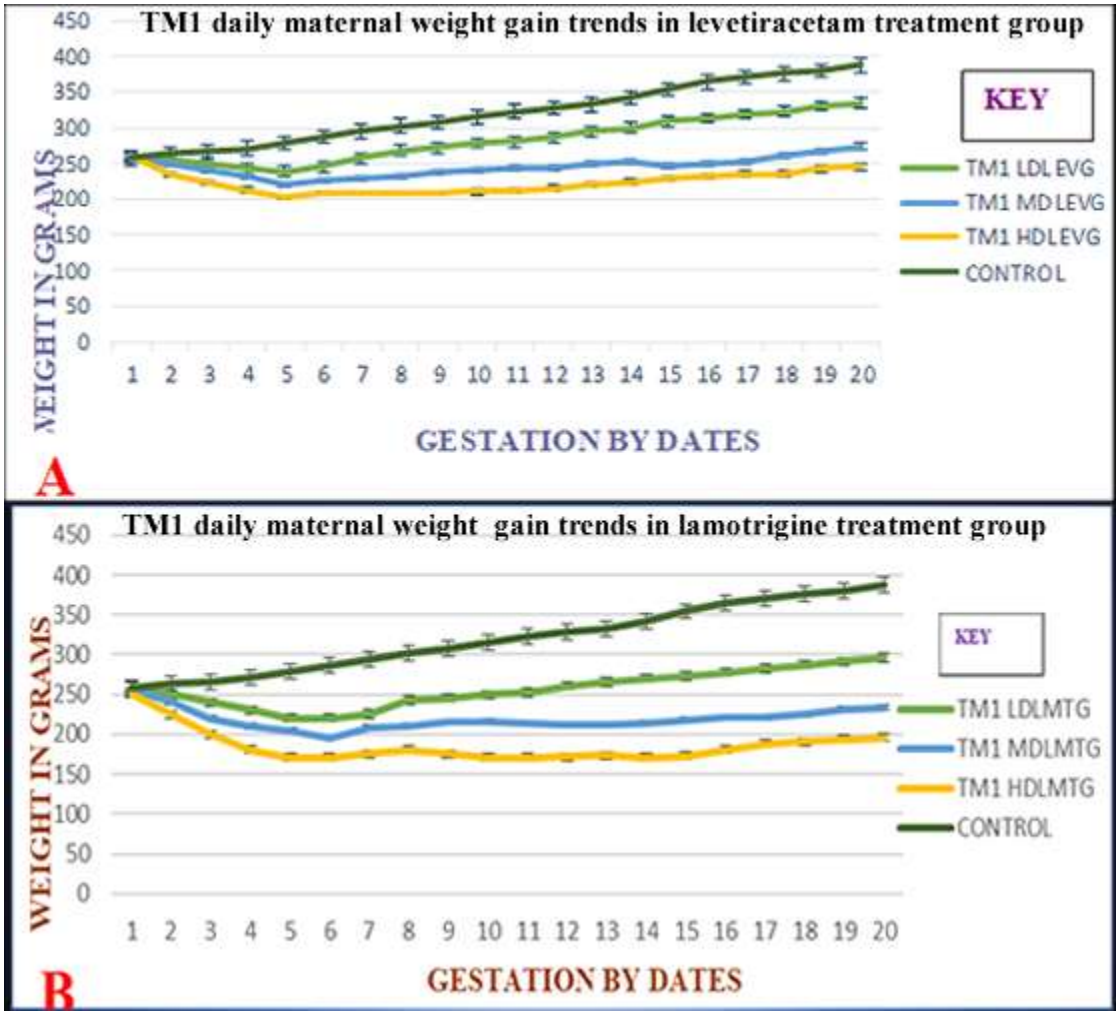

Figure 4.1: The TM_1 Comparative Maternal Weight Gain Trends between Lamotrigine and Levetiracetum Treated Groups against the Control.

KEY

(A) The levetiracetum treated groups

- ✓ *TM_1LDLEVG-Trimester 1, Low-dose levetiracetum treated group*
- ✓ *TM_1MDLEVG-Trimester 1, Medium-dose levetiracetum treated group*
- ✓ *TM_1HDLEVG-Trimester 1, High-dose levetiracetum treated group*

(B) The Lamotrigine Treated groups

- ✓ *TM_1LDLEVG-Trimester 1, Low-dose lamotrigine treated group*
- ✓ *TM_1MDLEVG-Trimester 1, Medium-dose lamotrigine treated group*
- ✓ *TM_1HDLEVG-Trimester 1, High-dose lamotrigine treated group*

Figure 4.2: The TM_2 Comparative Maternal Weight Gain Trends between Lamotrigine and Levetiracetum Treated Groups against the Control.

KEY

(C) The levetiracetum treated groups

- ✓ *$TM_2LDLEVG$-Trimester 2, Low-dose levetiracetum treated group*
- ✓ *$TM_2MDLEVG$-Trimester 2, Medium-dose levetiracetum treated group*
- ✓ *$TM_2HDLEVG$-Trimester 2, High-dose levetiracetum treated group*

(D) The Lamotrigine Treated groups

- ✓ *$TM_2LDLEVG$-Trimester 2, Low-dose lamotrigine treated group*
- ✓ *$TM_2MDLEVG$-Trimester 2, Medium-dose lamotrigine treated group*
- ✓ *$TM_2HDLEVG$-Trimester 2, High-dose lamotrigine treated group*

Figure 4.3: The TM$_3$ Comparative Maternal Weight Gain Trends between Lamotrigine and Levetiracetum Treated Groups against the Control.

KEY: (E) The levetiracetum treated groups

- ✓ *TM$_3$LDLEVG-Trimester 3, Low-dose levetiracetum treated group*
- ✓ *TM$_3$MDLEVG-Trimester 3, Medium-dose levetiracetum treated group*
- ✓ *TM$_3$HDLEVG-Trimester 3, High-dose levetiracetum treated group*

(F) The Lamotrigine Treated groups

- ✓ *TM$_3$LDLEVG-Trimester 3, Low-dose lamotrigine treated group*
- ✓ *TM$_3$MDLEVG-Trimester 3, Medium-dose lamotrigine treated group*
- ✓ *TM$_3$HDLEVG-Trimester 3, High-dose lamotrigine treated group*

4.1.1.2 The Comparative Findings on How Each Individual Drug Influenced the Maternal Pregnancy Outcome Parameters across Their Own Dose Categories at TM1, TM2 &TM3 Using ANOVA.

Upon carrying out a one way analysis of variances(ANOVA) to statistically determine how the three maternal pregnancy outcome paramenters were influenced by the doses and the time of exposure within their own dose categories of low medium and high of both lamotrigine and levetiracetam, it was observed that all the three dose groups had a statisticaly significant reduction(P<.05) in all the means of the three maternal pregnancy outcome parameters when compared with the control (Table 4.1) as follows; (**a**) **mean terminal weight (**F, (18,38) = 292.324, P=.001) (**b**) the **means of the maternal weight gain** values of (F,(18,38) = 281.553, P=.021) while; (c) the **mean placenta weight** (F (18,38) =18.434, P=.018).

On further differential analysis on how the trimesters of exposure influenced the three maternal pregnancy outcomes, it was notable that the three maternal pregnancy outcomes parameters were greatly affected when the treatments were instituted at TM_1 and TM_2 in both the lamotrigine and levetiracetum treated groups. On the dosages it was further noted that the worst deleterious outcomes were associated with both the medium and high treatment doses administred at TM_1. However, overall, lamotrigine has more deleterious effects than levetiracetum (Table 4.1)

Table 4.1: The comparative ANOVA table on how each individual medicine influenced the maternal pregnancy outcome parameters

The study groups	Study groups and dosage levels.	The time of exposure to treatment	The comparative mean terminal weight, weight gain and placenta weight for various study groups		
			Mean terminal weight (g) ± SD)	Mean weight gain (g) ± SD)	Mean placenta weight (g) ± SD)
Control.	Control (C) (**no treatment)**	None.	388.33±2.08	131.00±5.57	5.61±0.03
Levetiracetam treatment groups	Low dosage group **(103mg/kg/bw)**	TM1	334.33±6.03*	71.00±4.36*	4.95±0.39*
		TM2	351.67±1.53	111.00±2.65*	5.28±0.02
		TM3	371.33±1.53	119.67±1.53	5.39±0.04
	Medium dosage group **(207mg/kg/bw)**	TM1	274.33±2.31*	20.00±4.36	4.66±0.06*
		TM2	310.67±2.08*	67.00±2.65*	5.10±.007*
		TM3	350.33±4.51	99.00±6.00	5.37±0.02
	High dosage group **(310 mg/kg/bw)**	TM1	245.33±3.79*	-15.00±3.00*	4.27±0.03*
		TM2	256.67±2.89*	2.00±1.55*	4.73±.003*
		TM3	275.67±2.52*	32.00±2.65*	5.24±0.03*
Lamotrigine treatment groups	Low dosage group **(3mg/kg/bw)**	TM1	296.33±1.15*	36.00±4.58*	3.54±.003*
		TM2	333.33±1.15*	73.00±11.00*	4.12±.001*
		TM3	325.33±0.88*	75.00±1.53*	4.45±0.01*
	Medium dosage group **(24mg/kg/bw)**	TM1	233.33±2.08*	-21.67±4.16*	3.48±0.05*
		TM2	266.67±2.52*	56.00±3.48*	4.03±0.01*
		TM3	311.67±3.08*	74.00±1.15*	4.23±0.16*
	High dosage group **(52mg/kg/bw)**	TM1	195.67±1.53*	-55.00±1.73*	3.23±0.02*
		TM2	233.67±1.15*	-25.00±2.89*	3.65±0.01*
		TM3	235.67±2.47*	-24.00±18.36*	3.93±0.06*
Overall comparison between lamotrigen and levetiracetum by ANOVA [F, P values]			**F (18,38) =292.324 P=0.001**	**F (18,38) =281.553 P=0.021**	**F (18,38) =18.434 P=0.018**

Key: *All values that bear (*) indicates that they depict a statistical significance difference] (p<.05) when compared with the control using three- way ANOVA with Tukey post-hoc multiple comparison t-tests*

futher comparative multivariate analysis using MANOVA to evaluate how the two medicines influenced the three maternal pregnancy outcome parameters, the findings are presented at three levels as follows: -

(i) The level I findings are the global **results of jointed** independent variables of the drugs, dose and time acting together against an amalgamated effect on the three maternal dependent variables of preganacy outcomes with a view to establishing the global picture on whether or not the observed effects were due to treaments or due to chance.

(ii) The level II findings are the main plus the interaction effects of the three independent variables [i.e the drug, dose and time] against each of the three maternal dependent variables acting individually, or when they were combined with each another, or when all were combined together. This was with a view to establishing the contributory effects of each them either individually, when combined with each other or when all the three were combined.

(iii) The level III findings are the pair-wise comparison results between lamorigen and levetiracetum at the same dosage levels against the three maternal pregnancy outcomes variables with a view to establishing which among the two medicines has more deleterious negative teratogenic influence on maternal and fetal developmental structures.

The level I findings: The global comparative results on how the drug, dose and time of exposure influenced the three maternal pregnancy outcome parameters using MANOVA.

Upon carrying out the MANOVA level one analysis to establish how the drugs, dosages and time of exposure globally influenced the three maternal pregnancy outcomes, it was observed that all the the three independent variables had a remarkable contributory role in the reduction of all the means of the three maternal pregnancy otcomes parameters as shown by the the *P* values in the 2nd right column (**bolded**) (Table 4.2).

This clearly shows that the observed mean reduction in the three maternal pregnancy outcome parameters were not due to chance but due to either the main effects treatments/drugs, dosages, time of exposure/trimesters plus their interactions (Table 4.2).

Table 4.2: The Level 1 Manova Table on how Globally the Two Medicines, Dosages and Time of Exposure Plus Their Interactions Influenced the Three Meternal Outcome Parameters.

		The multivariate statistical tests parameters applied					
The comparative global effects assessed	**The parameters used**	**MANOVA test statistics (Wilks' Lambda)**	**Statistics (F)**	**Hypothesis degree of freedom**	**Error degree of freedom**	**P-Sig.<.05**	**Proportion of variance (Partial Eta Squared)**
Assessment of whether or not the observed overall effects were due to drugs (either lamotrigine or levetiracetam)	**Drugs**	.005	2376.119[b]	3.000	36.000	**<.001**	.995
Assessment of whether or not the observed overall effects were due to varied doses of lamotrigine and levetiracetam	**Dosages**	.007	128.438[b]	6.000	72.000	**.003**	.915
Assessment of whether or not the observed overall effects were due to differing time of exposure (TM_1, TM_2, &TM_3)	**Trimesters**	.005	166.245[b]	6.000	72.000	**.001**	.933
Assessment of whether or not the observed overall effects were due to interaction between varied doses and the drugs	**Drugs * Dosages**	.131	29.858[b]	6.000	72.000	**.003**	.672
Assessment of whether or not the observed overall effects were due to interaction between drugs and differing trimesters.	**Drugs * Trimesters**	.125	21.914[b]	6.000	72.000	**<.001**	.646
Assessment of whether or not the observed overall effects were due to interaction between dosages with differing trimesters.	**Dosages *Trimesters**	.077	13.030[b]	12.000	95.539	**.001**	.605
Assessment of whether or not the observed overall effects were due to the two drugs and the dosages as well as the trimesters	**Drugs * Dosages * Trimesters**	.062	14.866[b]	12.000	95.539	**<.001**	.673

***Key:** (*) indicates interaction effects, while ([b])indicates exact statistics using MANOVA*

Upon evaluating how the drugs, dosages and time of exposure globally influenced each of the specific maternal pregnancy outcomes parameters either at individual level or when combined with each other or or when they were all combined, it was observed that:

(i) At individual level each of the three independent variables of drug, dose and time of exposure had a significant contributory role ($P<.05$) in the observed reduction in maternal pregnancy outcomes variables as indicated by Partial Eta squared ($\eta^2 \geq 94\%$, (Table 4.3.)

(ii) It was further established that at duo or tripple combination levels, either as; (a) dosages*trimesters; (b)drugs*trimesters,(c) drugs*dosages & (d) drugs*dosages*trimesters, it was observed that their contribution to mean reduction of the three maternal parameters was not as significant like when it was at indidual level (Partial Eta squared (η^2) .408 to .777), the interaction effects were not as much unlike when the dosages were increased alone, or when exposed at early gestation and when it came to the type of medicine acting alone. As such, it was clear that the combinations had a lesser contributory effect on the three maternal dependent variables than when independent variables were combined (Table 4.3).

Table 4.3: The Level 2 MANOVA Table on How the Drugs, Doses and Time of Exposure Plus their Interations Influenced each of the Three Maternal Pregnancy Outcome Parameters

The groups being tested	The three dependent variables.	Measurements of the variability in the depended variables (Type III Sum of square)	Degree of freedom	The ratio Type III Sum of square to its corresponding degree of freedom. (Mean Square)	(F Statistics)	Sig. (<.05)	Proportion of variance (Partial Eta Squared)
Drugs	Terminal Weight	19078.241	1	19078.241	646.912	**<.001**	.945
	Weight gain	23814.000	1	23814.000	680.741	**<.001**	.947
	Placenta Weight	17.771	1	17.771	6813.857	**<.001**	.994
Dosages	Terminal Weight	81261.148	2	40630.574	1377.717	**<.001**	.986
	Weight gain	82188.926	2	41094.463	1174.716	**<.001**	.984
	Placenta Weight	1.861	2	.931	356.814	**<.001**	.949
Trimesters	Terminal Weight	17929.926	2	8964.963	303.987	**<.001**	.941
	Weight gain	23564.593	2	11782.296	336.806	**<.001**	.947
	Placenta Weight	5.083	2	2.541	974.429	**<.001**	.981
Drugs* dosages	Terminal Weight	110.259	2	55.130	1.869	**.003**	.590
	Weight gain	107.444	2	53.722	1.536	**.012**	.750
	Placenta Weight	111.004	2	42.002	1.714	**.006**	.536
Drugs* trimesters	Terminal Weight	2176.593	2	1088.296	36.902	**<.001**	.660
	Weight gain	3201.333	2	1600.667	45.756	**<.001**	.707
	Placenta Weight	.027	2	.013	15.133	**.003**	.513
Dosages* trimesters	Terminal Weight	2693.741	4	673.435	22.835	**<.001**	.706
	Weight gain	2356.407	4	589.102	16.840	**<.001**	.639
	Placenta Weight	.068	4	.017	6.551	**<.001**	.408
Drugs* dosages* trimesters	Terminal Weight	3895.074	4	973.769	33.019	**.002**	.777
	Weight gain	1623.222	4	405.806	11.600	**<.001**	.550
	Placenta Weight	.216	4	.054	20.735	**.001**	.686

Key: *(*) indicates interaction effects*

Upon carrying out a pair-wise comparative analysis on how the three maternal pregnancy outcome parameters of the mean terminal weights, total maternal weight gain and placental weight at the same dosage levels at TM1, TM2 and TM3, it was

observed that the effects on the the three maternal pregnancy outcome parameters following the exposures to all the dose levels of low, medium and high lamotrigen groups, they were significantly different from those of the levetiracetum treatment groups as indicated by the significance column (Sig (P<.05) plus the lower bound and upper values in table bound columns (Table 4.4).

As such, all the means of the maternal pregnancy outcome parameters were significantly lower (P<.05) for the lamotrigen than for levetiracetam treated groups indicating that lamotrigine has more inhibitory effects in maternal pregancy parameters than for the levetiracetam treated groups (Table 4.4).

Table 4.4: The Level 3 MANOVA Pairwise Comparison Table on How the Two Medicines Influenced the Four Maternal Pregnancy Outcomes When Exposed When Exposed Within the Same Dosage Levels

Multiple/Pairwise Comparisons

Dependent Variable	Dosages Mg/kg	Trimesters	Levetiracetam (LEV)	Lamotrigine (LAM)	Mean Difference (LEV-LAM)	Std. Error	Sig[d] (<.05)	95% Confidence Interval for Difference[d] Lower Bound	Upper Bound
Terminal Weight	LD	TM1	LEV	LAM	38.000*	4.434	**0.001**	29.024	46.976
		TM2	LEV	LAM	38.333*	4.434	**0.011**	29.357	47.310
		TM3	LEV	LAM	26.000*	4.434	**0.003**	17.024	34.976
	MD	TM1	LEV	LAM	40.667*	4.434	**0.001**	31.690	49.643
		TM2	LEV	LAM	18.976*	4.434	**0.023**	19.976	17.976
		TM3	LEV	LAM	83.667*	4.434	**0.001**	74.690	92.643
	HD	TM1	LEV	LAM	49.667*	4.434	**<0.001**	40.690	58.643
		TM2	LEV	LAM	23.000*	4.434	**<0.001**	14.024	31.976
		TM3	LEV	LAM	40.000*	4.434	**<0.001**	31.024	48.976
Weight Gain	LD	TM1	LEV	LAM	35.000*	4.829	**<0.001**	25.244	44.776
		TM2	LEV	LAM	36.000*	4.829	**0.003**	26.224	45.776
		TM3	LEV	LAM	46.000*	4.829	**<0.001**	36.224	55.776
	MD	TM1	LEV	LAM	41.667*	4.829	**0.001**	31.890	51.443
		TM2	LEV	LAM	11.000*	4.829	**0.028**	1.224	20.776
		TM3	LEV	LAM	84.667*	4.829	**<0.001**	74.890	94.443
	HD	TM1	LEV	LAM	40.000*	4.829	**<0.001**	30.224	49.776
		TM2	LEV	LAM	27.667*	4.829	**<0.001**	17.890	37.443
		TM3	LEV	LAM	56.000*	4.829	**0.002**	46.224	65.776
Placenta Weight	HD	TM1	LEV	LAM	1.413*	0.42	**<0.001**	1.329	1.498
		TM2	LEV	LAM	1.161*	0.42	**0.001**	1.077	1.246
		TM3	LEV	LAM	.935*	0.42	**<0.001**	.851	1.020
	MD	TM1	LEV	LAM	1.180*	0.42	**<0.001**	1.095	1.264
		TM2	LEV	LAM	1.071*	0.42	**0.003**	.986	1.155
		TM3	LEV	LAM	1.141*	0.42	**<0.001**	1.056	225
	HD	TM1	LEV	LAM	1.034*	0.42	**<0.001**	.950	1.119
		TM2	LEV	LAM	1.085*	0.42	**0.002**	1.001	1.170
		TM3	LEV	LAM	1.305*	0.42	**<0.001**	1.221	1.390

Key *-(*) Means that mean difference is statistically significance at P<.05*

4.2.2 The Comparative Effects on How the Two Medicines Influenced the Fetal Pregnancy Outcomes

The fetal pregnancy outcomes were assessed at two levels;

level 1: The fetal preganacy outcome before the fetuses were harvested/removed from the uterine horns: - [i.e *the liter zises, embryolithlites/ the numbers of dead fetuses, resorbed endomentrail*; and the *devoured fetuses]*

level 2: **The gross features of each an individual fetus after they were removed/harvested them from the uterine horms as follows**: - [*fetal body weight (BW), crown rump length (CRL), head circumference (HC), bi- parietal diameter (BD) and (v) the head length (HL*)]

4.2.2.1 Level 1: The Comparative Intra-Uterine Fetal Outcomes for both the Levetiracetam and Lamotrigine Treated Groups against the Control.

The parametes evaluated included; the litter sizes, embryolethality, resorbed endomentrial glands/devoured fetuses. On the **litter zises**, it was notable that the rats in the control groups had the highest total litter sizes of between 12-16 fetuses per rat with a total of 40 in the control group, while in the treatment groups the number of the litter sizes ranged between 2-9 across the three dose groups of low, medium and high lamotrigine groups and a total of 29. In the levetiracetum treated group however, the number of fetuses ranged between 3-11 fetuses per rat and a total of 31 [Figure 4.4 (A)]

On **resorbed endometrial glands** and **the devoured foetuses,** the numbers were noted to range between 1-17 in levetiracetum treated groups and 1-25in the lamotrigine treated groups across all the dose groups. The control recorded no

resorptions. These numbers were also noted to have a direct dose and time-response-relationship in that when high and medium dosage were done at trimester one (TM_1) and trimester two (TM_2), the number was higher as compared to the [Figure 4.4 (B)].

Concerning the **total numbers of the dead fetuses or the intrauterine- embryo-lethalities, it was** observed that the treatment groups in both lamotrigine and levetiracetam had remarkably higher numbers of dead fetuses when compared with the controls. With regards as to how the two medicines compared in relation to the doses applied, both the two drugs indicated a similar direct-dose response relationship in that they both reconded similar numbers of dead fetuses/embryolitalites with increasing dosages with the high dosages recording the highest numbers of dead fetuses, followed by the medium and lastly the low dose groups, [Figure 4.4 (C)]

On the trimesters of exposure, the two medicines were observed to depicted an inverse-time-relationship on thenumber of dead fetuses in that, the earlier was the time of exposure the higher were the deleterious outcomes, particulary when exposed at TM_1, followed by those exposed at TM_2 and lastly the TM_3 treatment groups (Figure 4.4).

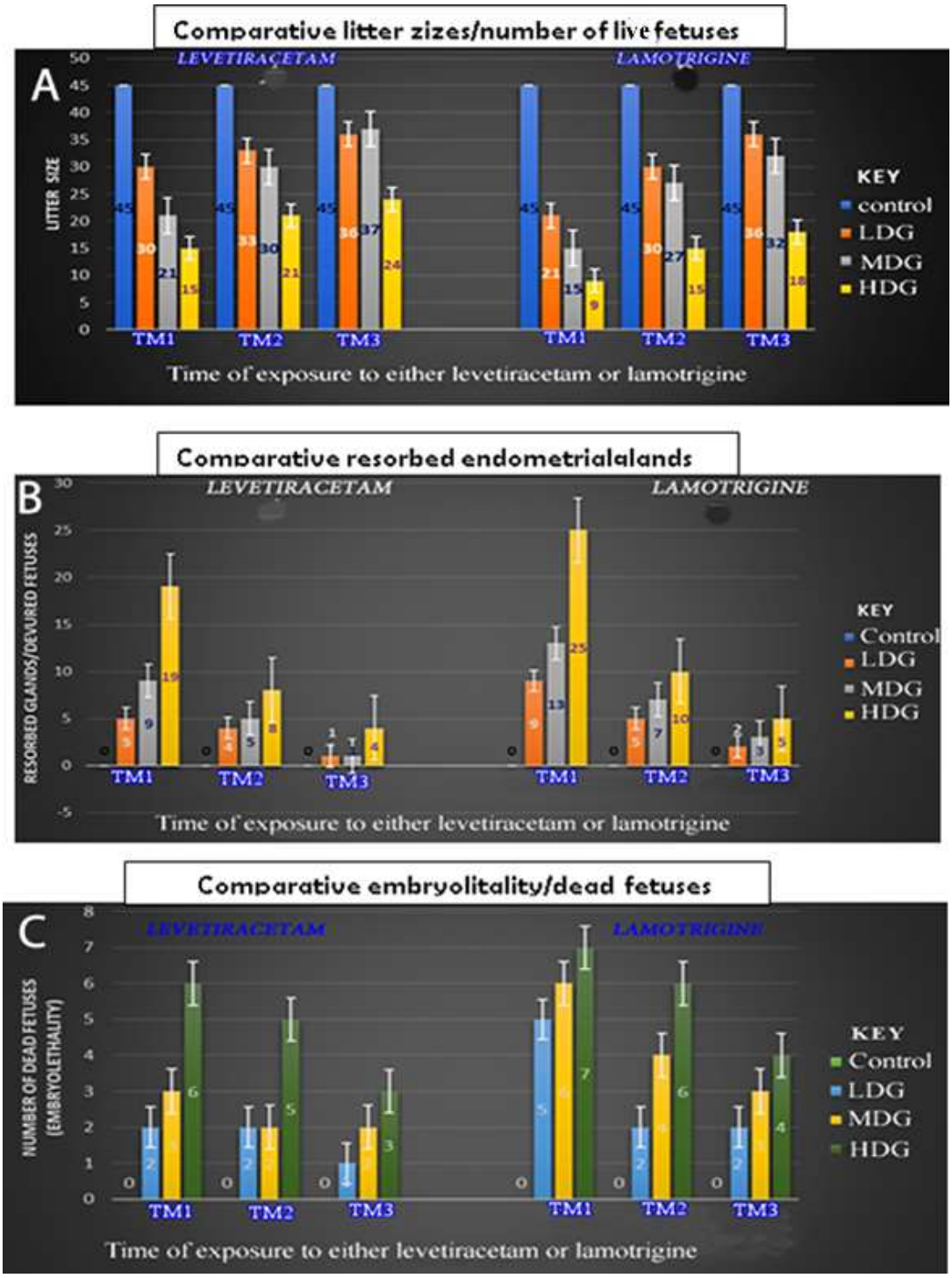

Figure 4.4: The Comparative Intrauterine Fetal Pregnancy Outcomes

KEY

A- *the comparive litter sizes between levetiracetum and the lamotrigine treated groups*

B- *the comparative resorbed endomentrial glands/ devored fetuses*

C- *the comparative embryolithalities/dead fetuses*

4.2.2.2 Level 2: The Comparative Fetal Growth Outcomes and Development In-Utero.

In assessing the fetal growth and development in-utero, the following parameters were evalutaed; (i) fetal body weight (BW), (ii) crown rump length (CRL), (iii) head circumference (HC), (iv) bi-parietal diameter (BPD), and (v) the head length (HL).When the global effects of individual medicines were evaluated, it was observed that they both depicted deleterious effects in all the fetal growth and development parameters with lamotrigine having more detrimental effects than levetiracetam in causing inhibitory outcome to the fetal growth and development parameters in-utero as follows, (a)fetal body weight ($F(18,38) = 221.774$, $P = .031$) and (b) crown -lump length) ($F(18,38) = 765.698$, $P = .011$), head circumference ($F(18,38) = 229.774$, $P = .001$), bi-parietal diameter ($F(18,38) = 441.779$, $P = .047$) and head length ($F(18,38) = 682.764$, $P = .039$) (table 4.5)

With regards as to how the time of exposure influeneced the fetal growth and development, it was observed that all the four fetal growth and development parameters were greatly affected when the treatments were instituted at TM1 and TM2 in both the lamotrigine and levetiracetum treated groups. On the dosages administered, it was noted that the worst deleterious outcomes were associated with both the medium and high treatment doses (Table 4.5).

Table 4.5: The Comparative ANOVA Table on how the Two Medicine Influenced the Fetal Growth and Development Parameters In-Utero.

The study groups	Study groups and dosage levels.	The time of exposure to treatment	The comparative means fetal weight, crown rump length, head circumference, bi-parietal diameter and head length for various study groups				
			Mean fetal weight (g) ± SD)	**Mean crown-rump length (cm) ± SD)**	**Mean head circumference (mm) ± SD)**	**Mean bi-parietal diameter (mm) ± SD)**	**Mean head length (g) ± SD)**
Control.	Control (C) **(no treatment)**	None.	7.75±0.46	7.98±0.02	4.20±0.05	3.30±0.06	1.54±0.01
Levetiracetam treatment groups	LDG **(103mg/kg/bw)**	(TM1)	7.01±0.05*	7.32±0.30*	3.69±0.09*	2.74±0.02*	1.46±0.01*
		(TM2)	7.47±0.07	7.45±0.02*	3.83±0.01	2.89±0.06*	1.50±0.01
		(TM3	7.64±0.01	7.75±0.02	4.04±0.01	3.07±0.08	1.52±0.04
	MDG **(207mg/kg/bw)**	TM1	6.43±0.01*	6.88±0.07*	3.47±0.05*	2.46±0.07*	1.32±0.05*
		TM2	6.65±0.01	7.13±0.01*	3.71±0.04*	2.41±0.07*	1.34±0.02*
		TM3	6.84±0.01	7.50±0.08	3.84±0.03	2.56±0.06	1.36±0.01
	High dosage group **(310 mg/kg/bw)**	TM1	5.57±0.05*	5.45±0.08*	3.01±0.07*	2.30±0.06*	1.27±0.01*
		TM2	6.11±0.06*	6.06±0.01*	3.61±0.07*	2.33±0.01*	1.30±0.03*
		TM3	6.33±0.01*	6.44±0.05*	3.54±0.02*	2.43±0.01*	1.32±0.02*
	Low dosage group **3mg/kg/bw)**	TM1	6.44±0.01*	4.13±0.02*	3.26±0.029*	2.52±0.10*	1.27±0.02*
		TM2	6.59±0.01*	4.45±0.01*	3.50±0.029*	2.76±0.04*	1.31±0.01*
		TM3	6.68±0.24*	4.55±0.05*	3.61±0.038	2.90±0.01	1.32±0.01
Lamotrigine treatment groups	Medium dosage group **(24mg/kg/bw)**	TM1	6.34±0.08*	3.88±0.07*	3.03±0.06*	2.44±0.06*	1.28±0.03*
		TM2	6.44±0.02*	4.16±0.01*	3.25±0.03*	2.39±0.04*	1.31±0.03*
		TM3	6.56±0.02*	4.44±0.05*	3.55±0.03*	2.47±0.02*	1.33±0.01*
	High dosage group **(52mg/kg/bw)**	TM1	5.44±0.03*	3.38±0.04*	2.40±0.02*	2.20±0.04*	1.23±0.01*
		TM2	5.95±0.01*	4.05±0.01*	3.07±0.04*	2.27±0.03*	1.27±0.01*
		TM3	6.24±0.02*	4.40±0.02*	3.45±0.04*	2.36±0.03*	1.30±0.02*
Overall comparison by ANOVA [F, P values]			**F (18,38) =221.774 P=0.031**	**F (18,38) =765.698 P=0.011**	**F (18,38) =229.774 P=0.001**	**F (18,38) =441.779 P=0.047**	**F (18,38) =682.764 P=0.039**

***Key**: All values that bear (*) indicates that they depict a statistical significance difference (p<.05), when compared with the control, using one-way ANOVA with Tukey post-hoc multiple comparison t-test*

On futher analysis using multiple analysis of variances (MANOVA) to evaluate how the two medicines influenced the four fetal growth and development parameters in utero, the findings are presented at three levels as follows: -

Level 1: The MANOVA analysis on how the two medines plus their inetractions globally influenced the four fetal growth and developmental parameters.

Level 2: The MANOVA analysis on how the individual drug, dose and time of exposure plus their interations influenced each of the for fetal growth and development parameters *in-utero*

Level 3: The MANOVA pairwise comparison results on how the two medicines

Influenced the four fetal outcomes when exposed at the same and in the same trimesters.

Level 1: The MANOVA analysis on how glolly the two medicines, dosages and trimesters plus their interactions influenced the four fetal growth parameters *in-utero.*

Upon carrying out a multivariate analysis of variences (MANOVA) to evalute how the two medicines globally influeneced the four fetal growth and development parameters *in-utero,* via checking the overall individual main effects and their interaction effects (*) of the independent variables (drugs, dosages & trimesters), it was observed that these three independent variable depicted statistical significant effects, meaning that they contributed to the total mean reduction of the four fetal pregnancy outcome parameters (i.e the dependent variables) in a varied proportions (Partial Eta squared (η^2) as follows;

(i) At the individual level there was statistical significant oveall main effects of; (a) dugs (*F* (3, 36) = 3127.134, *P*<.001); Wilkis' lambda (Λ) =.001; Partial Eta squared (η^2 =.996), (b) dosages (*F* (6, 72) =383.296, *P*<.001); Wilkis' lambda (Λ) =.003; Partial Eta squared (η^2 =.970), and (c) trimesters (*F* (6,72) = 112.256, *P*=<.001); Wilkis' lambda (Λ) =.008; Partial Eta squared (η^2 =.911), (Table 4.6).

(ii) At the two way combinations there was statistical significant interaction effects of (a) drugs & dosages: (F (6,72) = 111.696, p<.001); Wilkis 'lambda (Λ) =.009; Partial Eta squared (η2 =.90) (b) drugs & trimesters (F (6, 72) = 19.983, *P*=.001); Wilkis'Λ =.141; Partial Eta squared (η^2 =.63), and (c) dosages & trimesters (F (12, 95.539) = *P*<.001); Wilkis'Λ =.049; Partial Eta squared (η^2 =.63), (Table 4.6).

(iii)There was statistically significant three-way combination i.e when all were combined i.e three-way interactions among, drugs*dosages*trimesters, (*F* (12,95.539) = 13.046, *P*=.002); Wilkis' lambda (Λ) =.077; Partial Eta squared (η^2 =.58) (Table 4.6).

Table 4.6: The Level 1 MANOVA Table on How Globally the Two Medicines, Dosages and Trimesters Plus their Interactions Globally Influenced the Four Fetal Growth and Developmental Parameters In-Utero.

		The multivariate statistical tests parameters applied					
The comparative global effects assessed	**The parameters used**	**MANOVA test statistics (Wilks' Lambda)**	**Statistics (F)**	**Hypothesis degree of freedom**	**Error degree of freedom**	**Sig.<.05**	**Proportion of variance (Partial Eta Squared)**
Assessment of whether or not the observed overall effects were due to drugs (either lamotrigine or levetiracetam)	**Drugs**	.003	7302.517 [b]	2.000	37.000	**.<001**	.997
Assessment of whether or not the observed overall effects were due to varied doses of lamotrigine and levetiracetam	**Dosages**	.003	327.560 [b]	4.000	74.000	**<.001**	.947
Assessment of whether or not the observed overall effects were due to differing trimesters (TM_1, TM_2, &TM_3)	**Trimesters**	.012	148.030 [b]	4.000	74.000	**.002**	.889
Assessment of whether or not the observed overall effects were due to interaction between varied doses and the drugs	**Drugs * dosages**	.005	256.380 [b]	4.000	74.000	**.011**	.933
Assessment of whether or not the observed overall effects were due to interaction between drugs and differing trimesters.	**Drugs * trimesters**	.038	137.086 [b]	4.000	74.000	**.002**	.681
Assessment of whether or not the observed overall effects were due to interaction between dosages with differing trimesters.	**Dosages *trimesters**	.044	124.636 [b]	8.000	74.000	**<.001**	.789
Assessment of whether or not the observed overall effects were due to the two drugs and the dosages as well as the trimesters	**Drugs * dosages * trimesters**	.063	138.326 [b]	8.000	74.00	**<.001**	.774

***Key:** (*) indicates interaction effects, while([b])indicates exact statistics using MANOVA*

Level 2: The MANOVA results on how globally the drugs, doses and time of exposure plus their interations influenced each of the four (4) fetal growth and development parameters *in-utero*

Upon carrying out the MANOVA analysis to evaluate globally how the individual drug, dose and time of exposure plus their interations influenced each of the four fetal growth and development parameters *in-utero*, it was established that;

(i) The individual levels contribution of the drug, dose and time of exposure to each of the four independent fetal growth and developmental variables of (i) fetal body weight (BW), (ii) crown rump length (CRL), (iii) head circumference (HC), (iv) bi-parietal diameter (BPD), and (v) the head length (HL), were at varied proportionate (Partial Eta squared (η^2) (Table 4.7).

(ii) The two-way interaction effects of the drug, dose and time of exposure to each of the four fetal growth parameters when combined as follows; (a) drug*dosages, (b)drugs*trimesters &; (c) dosages*trimesters, were found to have statistically significant interaction effects to each of the four fetal parameters with the comination of drug and dose having the highest contribution, then argumented by the time of exposure at varying proportions (Partial Eta squared (η^2), **(**Table 4.7).

(iii) When the three independent variables were combined against each of the four fetal growth and development parameters, it was evedent that though statistically significant, the observed effects on the fetal growth and development were not more due to their combinations, but due to the types of medicine, the dosage applied, and the time of exposure as enumerated; (a) fetal weight, (F (4,38) = .116, P<.001); Partial Eta squared (η^2 =.63), (b) crown-rump length, (F (4,38) = .149, P=.004); Partial Eta squared (η^2 =.63), (c) bi-parietal diameter; (F (4,38) = .008, P=.001; Partial Eta squared (η^2 =.72, & (c) head length (F (4,38) = .004, P<.001); Eta squared (η^2 =.84) (Table 4.7).

Table 4.7: The Level 2 MANOVA Table on How Globally, the Drugs, Dosageand Time of Exposure Plus their Interations Influenced Each of the Four (4) Fetal Growth and Development Parameters In-Utero

Tests of Between-Subjects Effects

The groups being tested	The three dependent variables.	Measurements of the variability in the depended variables (Type III Sum of square)	Degree of freedom	The ratio Type III Sum of square to its corresponding degree of freedom. (Mean Square)	The ration of the mean square for the independent variable to the mean square for error (F Statistics)	Sig. (<.05)	Proportion of variance (Partial Eta Squared)
Drugs	Fetal Weight	1.880	1	1.880	1368.128	**<.001**	.973
	Crown -rump length	100.467	1	100.467	14991.733	**<.000**	.997
	Head circumference	2.166	1	2.166	942.434	**<.001**	.961
	Bi-parietal diameter	.095	1	.095	22.378	**.002**	.371
	Head length	.142	1	.142	7899.034	**<.001**	.995
Dosages	Fetal Weight	9.675	2	4.837	3520.048	**<.001**	.995
	Crown -rump length	9.158	2	4.579	683.275	**<.001**	.973
	Head circumference	2.090	2	1.045	454.573	**<.001**	.960
	Bi-parietal diameter	2.314	2	1.157	271.232	**.001**	.935
	Head length	.154	2	.077	4277.641	**<.001**	.996
Trimesters	Fetal Weight	2.211	2	1.105	804.352	**<.001**	.977
	Crown -rump length	4.115	2	2.057	307.019	**<.001**	.942
	Head circumference	2.624	2	1.312	570.887	**.003**	.968
	Bi-parietal diameter	.383	2	.191	44.862	**<.001**	.702
	Head length	.030	2	.015	833.946	**<.001**	.978
Drugs * dosages	Fetal Weight	1.235	2	.617	449.182	**<.001**	.959
	Crown -rump length	3.235	2	1.618	241.388	**<.001**	.927
	Bi-parietal diameter	<.001	2	<.001	.088	**.016**	.505
	Head length	.072	2	.036	8.397	**.001**	.306
	Fetal Weight	.059	2	.029	1636.053	**<.001**	.989
Drugs * trimesters	Fetal Weight	.108	2	.054	39.278	**<.001**	.674
	Crown -rump length	.029	2	.014	2.160	**.012**	.523
	Bi-parietal diameter	.116	2	.058	25.129	**.002**	.569
	Head length	.004	2	.002	.501	**.010**	.526
	Fetal Weight	.002	2	.001	53.277	**.001**	.737
Dosages * trimesters	Fetal Weight	.483	4	.121	87.839	**<.001**	.902
	Crown -rump length	.609	4	.152	22.733	**<.001**	.705
	Head circumference	.471	4	.118	51.195	**.011**	.843
	Bi-parietal diameter	.142	4	.036	8.336	**<.001**	.467

	Head length	.001	4	.000	11.690	**<.001**	.552
Drugs * dosages * trimesters	Fetal Weight	.116	4	.029	21.119	**<.001**	.690
	Crown -rump length	.087	4	.024	17.636	**.004**	.630
	Head circumference	.149	4	.037	16.223	**<.001**	.631
	Bi-parietal diameter	.008	4	.003	34.193	**.001**	.720
	Head length	.004	4	.001	50.376	**<.001**	.841

Key: *(*) indicates interaction effect*

level 3: The MANOVA pairwise comparison results on how the two medicines influeneced the four fetal growth and development parameters when exposed within the same dosages and the same trimesters.

Upon carrying out the pairwise MANOVA comparative analysis between lamotrigen and levetiracetum in the same dose groups and the same trimesters of exposure to establish how the two medicins influeneced the four fetal growth and developmental parameters, it was notable that, there was a remarkable statistical significance difference ($P<.05$) in how the same dose levels of lamotrigine *visavis* similar dose levels of levetiracetum influenced the four growth and developmental parameters.

It was clear that in all dose levels of low, medium and high lamotrigine against the same dose levels of lamotrigine, the effects were more pronounced in the lamotrigine treated groups as compared with the levetiracetum treated groups across all the trimesters (Table 4.8).

Table 4.8: The Level 3 MANOVA Pairwise Comparison Table on how the Two Medicines Influenced the Four Fetal Growth and Development Parameters when Exposed Within the Same Dosages and the Same Trimesters

Multiple/Pairwise Comparisons									
								95% Confidence Interval for Difference[d]	
Dependent Variable	**Dosages (MG/KG BW)**	**Trimesters**	**(LEV)**	**(LAM)**	**Mean Difference (LEV-LAM)**	**Std. Error**	**Sig[d] (<.05)**	**Lower Bound**	**Upper Bound**
Fetal weight (g)	LD	TM1	LEV	LAM	.567*	.030	**.002**	.506	.629
		TM2	LEV	LAM	.876*	.030	**<.001**	.815	.938
		TM3	LEV	LAM	.954*	.030	**<.001**	.892	1.015
	MD	TM1	LEV	LAM	.085*	.030	**.008**	.024	.147
		TM2	LEV	LAM	.399*	.030	**.001**	.338	.460
		TM3	LEV	LAM	.095*	.030	**.003**	.034	.156
	HD	TM1	LEV	LAM	.127*	.030	**<.001**	.066	.189
		TM2	LEV	LAM	.161*	.030	**<.001**	.100	.222
		TM3	LEV	LAM	.093*	.030	**.004**	.032	.155
Crown-lump length (mm)	LD	TM1	LEV	LAM	3.185*	.067	**.001**	3.049	3.320
		TM2	LEV	LAM	3.004*	.067	**.002**	2.869	3.139
		TM3	LEV	LAM	3.205*	.067	**<.001**	3.069	3.340
	MD	TM1	LEV	LAM	2.999*	.067	**.001**	2.864	3.134
		TM2	LEV	LAM	2.976*	.067	**<.001**	2.841	3.111
		TM3	LEV	LAM	3.067*	.067	**.002**	2.932	3.202
	HD	TM1	LEV	LAM	2.069*	.067	**<.001**	1.934	2.205
		TM2	LEV	LAM	2.010*	.067	**<.001**	1.875	2.146
		TM3	LEV	LAM	2.037*	.067	**<.001**	1.902	2.173
Head circumference	LD	TM1	LEV	LAM	.423*	.039	**<.000**	.344	.502
		TM2	LEV	LAM	.337*	.039	**<.001**	.258	.416
		TM3	LEV	LAM	.431*	.039	**<.001**	.352	.510
	MD	TM1	LEV	LAM	.431*	.039	**<.001**	.352	.510
		TM2	LEV	LAM	.462*	.039	**<.001**	.382	.541
		TM3	LEV	LAM	.296*	.039	**<.001**	.217	.376
	HD	TM1	LEV	LAM	.606*	.039	**<.001**	.527	.685
		TM2	LEV	LAM	.529*	.039	**<.001**	.450	.609
		TM3	LEV	LAM	.089*	.039	**.028**	.010	.169
Bi-Parietal diameter	LD	TM1	LEV	LAM	.225*	.053	**<.001**	.117	.333
		TM2	LEV	LAM	.133*	.053	**.017**	.025	.241
		TM3	LEV	LAM	.165*	.053	**.004**	.057	.273
	MD	TM1	LEV	LAM	.000*	.053	**.005**	.108	.108
		TM2	LEV	LAM	3.469*	.053	**.001**	.108	.108
		TM3	LEV	LAM	.012*	.053	**.019**	.120	.096
	HD	TM1	LEV	LAM	.100*	.053	**.049**	.008	.208

	HD	TM2	LEV	LAM	.067*	.053	**.009**	.041	.175
		TM3	LEV	LAM	.079*	.053	**.047**	.029	.187
		TM1	LEV	LAM	.187*	.003	**<.001**	.180	.194
Head length	LD	TM2	LEV	LAM	.193*	.003	**<.001**	.186	.200
		TM3	LEV	LAM	.207*	.003	**<.001**	.200	.214
		TM1	LEV	LAM	.003*	.000	**.070**	.084	.003
	MD	TM2	LEV	LAM	.063*	.003	**<.001**	.056	.070
		TM3	LEV	LAM	.050*	.003	**<.001**	.043	.057
		TM1	LEV	LAM	.093*	.003	**<.001**	.086	.100
	HD	TM2	LEV	LAM	.037*	.003	**<.001**	.030	.044
		TM3	LEV	LAM	.017*	.003	**<.001**	.010	.024

Key*-(*) indicates that the mean difference is significant at .05 level*

4.3 The Histomorphological Findings

4.3.1 Objective 2: The Comparative Histomorphological Findings on How the Prenatal Exposure to Varied Doses of Lamotrigine and Levetiracetam Influenced the Development of the Fetal Memory Circuitry Pathway-Structures.

The histomorphological results on the fetal memory circuitry pathways are presented in a step wise manner in line with the way the structures of the fetal memory circuitory pathway are organized starting with;

The prefontal cortex: where the sensory memory inputs are perceived and programmed into either short term or the long-term memory, then followed by other memory processing structures including;

- The entorrhinal cortex
- The subiculum,
- The hippocampus,
- The dentate gyrus, and lastly,
- The amygdaloid nucleus.

4.3.1.1 The Histomophological Effects on Pre-Frontal Cortex.

The histomorphological findings on the prefrontal cortex are presented at two levels including: -

A:-The global comparative histo-architecture of the prefrontal cortical layers at low, medium and high dosage level

B: -The comparative thickness of the prefrontal cortical layers at TM1, TM2 and TM3

A: -The global comparative histo-architecture findings of the prefrontal cortical layers at low, medium and high dosage level

The comparative histo-architecture findings of the prefrontal cortical layers are presented in two namely as follows;

Level 1: - The supragranular layer; that constitutes the upper three layers of prefrontal cortex including (i) the plexiform molecular/layer (ML), (ii) outer granular (OG) and, (iii) the outer pyramidal (OP) layers. The supra granular layer is responsible for perceptions, awareness, planning, thought processing, language, consciousness, and conding of all sensory information into short- and long-term memory.

Level 2: - The infragranular layer: that constitutes of (i) the inner granular layer (IG), (ii) the inner pyramidal (IP), and (iii) the multifom layer (ML). The principal role infra-granular cortex in memory ciruitory pathway is to serve as the inner processor and the connector of sensory output pathways to the entorrhinal cortices and the hippocampus. It is hence formed of the cellular components and the nerve axonal output fibre bundles.

Level- 1: -The global comparative histo-cyto-architecture of the three supragranular histological layers in the prefrontal cortex.

In assessing ***the molecular layer at a global scale*** without considering specific drugs and dosages, it was overally observed that the horizontal cells of Cajal and Retzius that are primary involved in the programming and the lamination of incoming sensory information in this layer reduced remarkably in their density. Their morphological shapes and sizes were disrupted as well as the cells becoming sparsely distributed in all the dose groups except for the low dose levetiracetum treated groups at TM_3 **[*Figure 4.5-the cells that are marked as C-R in the four photomicrographs*]**.

In ***the outer granular layer at a global scale,*** it was further observed that the granule/stellate cells that are the key memory cells whose primary role is the spatial sensory memory processing in the graular layer were similary seen to remarkably reduce in their density, their histomorphological shapes and sizes, and they became sparsely distributed with increasing doses of the two medicines. This was particulary when exposed to lamotrigen medication at (TM_1) and (TM_2) **[*Figure 4.6- the cells that are marked as the CG in the four photomicrograph*s]**

In *t**he outer pyramidal layer,*** the small pyramidal cells that are key memory cells in this layer and whose role in memory is to provide the major output loops to the entorhinal cortex and the hippocampus, were also seen to appreciably reduce in their density and also became sparsely distributed with increasing doses and when exposed early in (TM_1) and (TM_2). However, the effects were more in the lamotrigine treated groups as compared to both the levetiracetum treated groups and the control. [*Figure 4.7 -the cells marked as the PC in the four photomicrographs*]

The molecular/plexiform layer

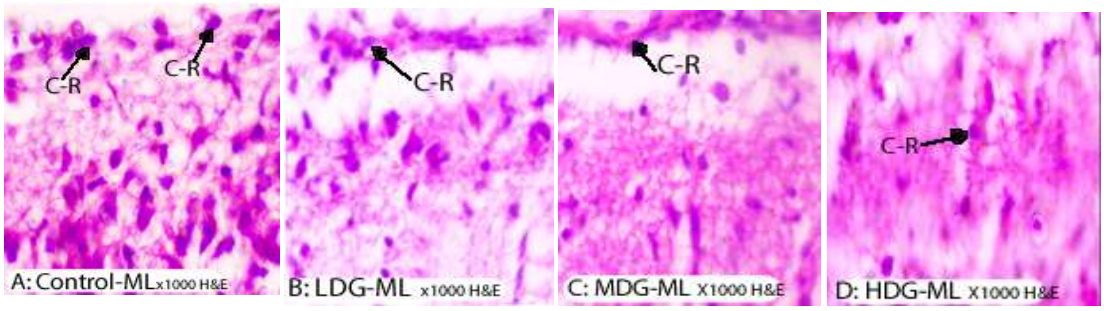

Figure 4.5: The Global Comparative Histo-Cyto-Architecture of the Molecular/Plexiform Prefrontal Cortical Layer in Low, Medium and High Dosage Groups Against Control

Key

*A-Control –Molecular layer **(ML)**, **B**-Low dose group-molecular layer **(LDG-ML)**, **C-**Medium dose group-molecular layer **(MDG-ML)**, **D-** High dose group-molecular layer **(HDG-ML)**, **C-R**-Cajal- Retzius cell*

The outer granular layer

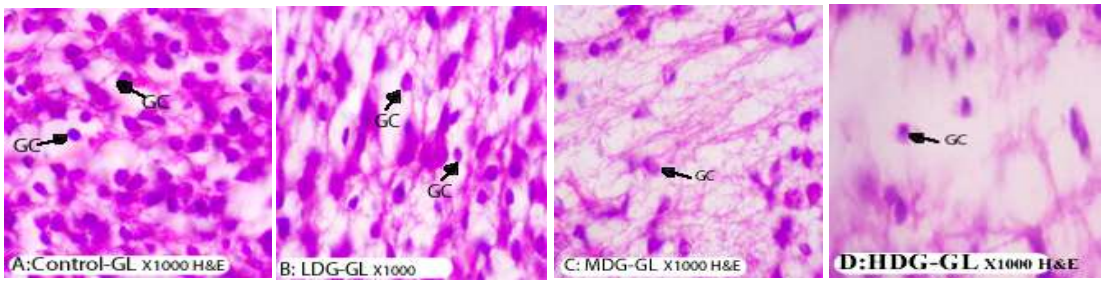

Figure 4.6: The Global Comparative Histo-Cyto-Architecture of the Outer Granular Prefrontal Cortical Layer in Low, Medium and High Dosage Groups Against Control

Key

*A-Control –Outer granular layer **(OGL)**, **B**-Low dose group-outer granular layer **(LDG-OGL)**, **C-**Medium Dose group-granular layer **(MDG-GL)**, **D-** High dose group-outer granular layer **(HDG-OGL)**, **GC**-Granule cell*

The outer pyramidal layer

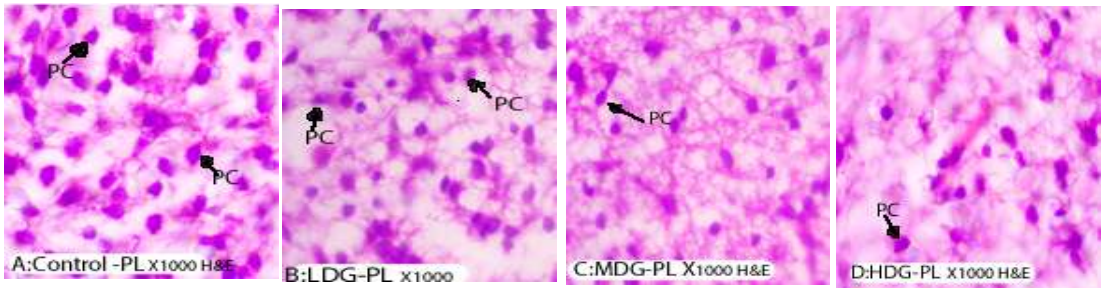

Figure 4.7: The Global Comparative Histo-Cyto-Architecture of the Outer Pyramidal Pref Rontal Cortical Layers in Low, Medium and High Dosage Groups Against the Control

Key

*A-Control –Molecular layer **(ML)**, **B**-Low dose group-molecular layer **(LDG-ML)**, **C-**Medium dose group-molecular layer **(MDG-ML)**, **D-** High dose group-molecular layer **(HDG-ML)**, **PC**-Pyramidal cell*

Level 2:-The global comparative histo-cyto-architecture of the three infra-granular histological layers of prefrontal cortex.

In assessing ***the inner granular layer at a global scale*** without considering specific drugs and dosages it was overally observed that, the stellate cells (STC) and the small pyramidal cells (SPC) that form the key memory processing cells in this layer were also seen to remarkably reduce in their density and shapes. The output fibre bundles (*Ofb*) were also seen to be thinner in sizes and disaggregated *[Figure 4.8-the cells marked as STC and SPC and the output fibre bundles marked (OfP) in the four photomicrographs]***.**

In the inner pyramidal layer, large sized pyramidal cells (Betz-cells) *(LSPC),* **t**he medium-sized (*MSPC),* plus the corticofugal fibre budles *(CffB)* were similary seen to bear significant teratogenic reduction in all the dose groups of both lamotrigen and levetiracetum treated groups interms of their histomorphological shapes and sizes, the cellular density as well as their reduced dispersion. All these componets were seen to be highly affected in the high doses of both the levetiracetum and lamotrigine treated groups as compared with the control. *[Figure 4.9 - the cells marked as MSPC and LSPC and the corticofugal fibre bundles marked as (CffB) in the four photomicrographs]*

In the multiform layer, the fusiform cells (*FC*) that were seen as the predominat cells, followed by the less dominant pyramidal cells (*PC*) plus the few seen interneurons (*IN*) were also noted to reduce in their sizes, shapes plus their density in relation to their distribution, with increasing dose levels of exposures in both the two medicines. Similary the axonal bundles of cotical-fugal fibres *(CffB)* that were seen traversing this layer from the supragranular layers above formed the connecting commissural and the projection fibers were to seen to be thinner and disaggregated in the high dose groups of both the lamotrigine and levetiracetum treated groups, [*Figure 4.10 the cells marked as FC and PC and the corticofugal fibre bundlesmarkedas (CffB) in the four-photomicrograph photo micrograph figure]*.

The inner granular layer

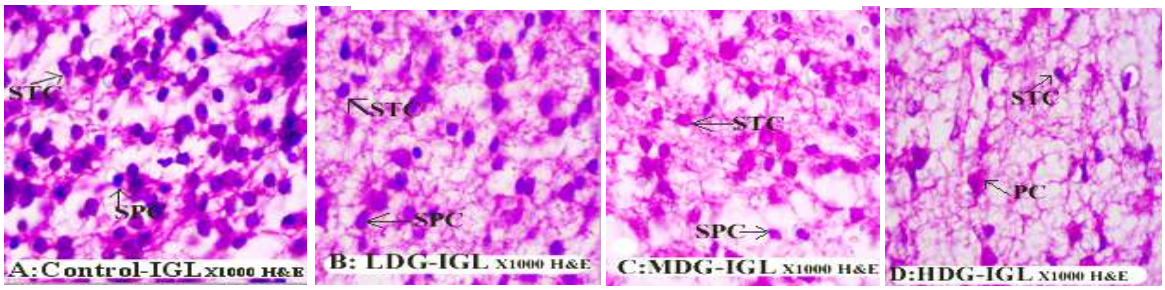

Figure 4.8: The Global Comparative Histo-Cyto-Architecture of the Inner Granular Prefrontal Cortical Layer in Low, Medium and High Dosage Groups Against Control

Key

*A-Control –inner layer (**IGL**), **B**-Low dose group-inner granular layer (**LDG-IGL**), **C-** Medium dose group-inner granular layer (**MDG-IGL**), **D**- High dose group-inner granular layer (**HDG-IGL**), **SPC**-Small pyramidal cell, **STC**-Stellate cell*

The inner pyramidal layer

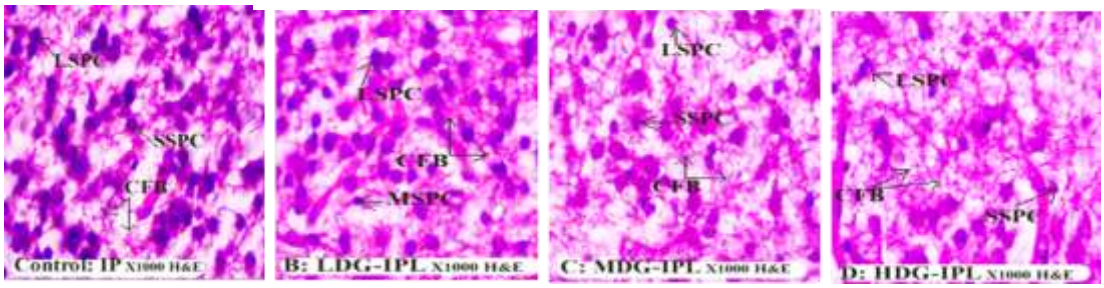

Figure 4.9: The Global Comparative Histo-Cyto-Architecture of the Inner Pyramidal Prefrontal Cortical Layer in Low, Medium and High Dosage Groups against Gainst Control

Key

*A-Control –Inner pyramidal layer (**IPL**), **B**-Low dose group-inner pyramidal (**LDG-IPL**), **C-** Medium dose group-inner pyramidal layer (**MDG-IPL**), **D**- High dose group-inner pyramidal layer (**HDG-IPL**), **SSPC**-Small size pyramidal, cell, **LSPC**-Large size pyramidal cell, **CFB**-Corticofugal bundles*

The multiform layer

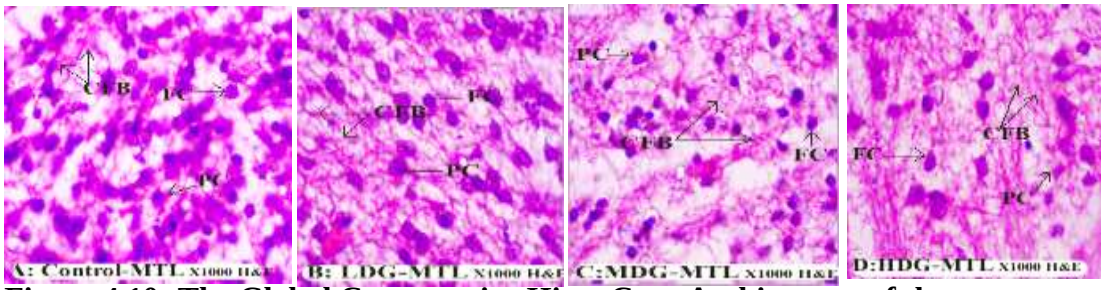

Figure 4.10: The Global Comparative Histo-Cyto-Architecture of the Multiform Layer of the Prefrontal Cortical Layer in Low, Medium and High Dosage Groups Against Control

Key

*A-Control –Multiform layer (**MTL**), **B**-Low dose multiform layer (**LDG-MTL**), **C-** Medium dose group-multoiform layer (**MDG-MTL**), **D**- High dose group-multiform layer (**HDG-MTL**), **FC**-Fusiform cell, **PC**- pyramidal cell, **CFB**-Corticofugal bundles*

B: The comparative cortical thicknesses of prefrontal cortical layers at TM_1, TM_2 and TM_3

The comparative cortical thicknesses of pre-frontal cortex are presented as per the time of exposure as follows: -

At trimester one (TM_1) it was observed that, the cortical thicknesses of the six histological layers in both the supragranular and infragranular layers of the prefrontal cortex were influenced in an inverse-dose-response relationship in that; when the dosages increased, all the histological prefrontal cortical layers plus the cellular histo-cyto-architectural compositions in terms of; their sizes, their numbers and dispersion per layer reduced with increasing dose levels across the three dose levels of low, medium and high in both the levetiracetum and the lamotrigine treated groups. It was however notable that the lamotrigen treated groups across all its dosage levels had more deleterious effects than those of the lamotrigen in the same dosage levels [Figure 4.11].

At trimester two (TM_2) the cortical thicknesss of the histological layers of the prefrontal cotex also depicted the same inverse- dose response relationship like what was seen in trimester (TM_1) in all the three dose groups of low, medium and high in both the levetiracetum and the lamotrigine treated groups. However, at TM2, the medium and high doses of both the two medicines were seen to to affect more the supragranular layers than the infragranular layers that were also marked with high reduction of the cellular density, the cell sizes and the cellular distributions of the key cells in each of the supra granular layer. The lamotrigen treated groups were however seen to have more deleterious effects than for the levetiracetum treated groups (Figure 4.12).

At trimester three (TM_3), All the prefronal cortical thickness were not affected in the low dose groups of the two medicines as well as the medium dose group of the levetiracetum but shown remarkable reduction in all the cortical layers for the medium and high dose groups of the lamtrigen treated category (Figure 4.13).

The comparative prefrontal cortical thickness at TM1

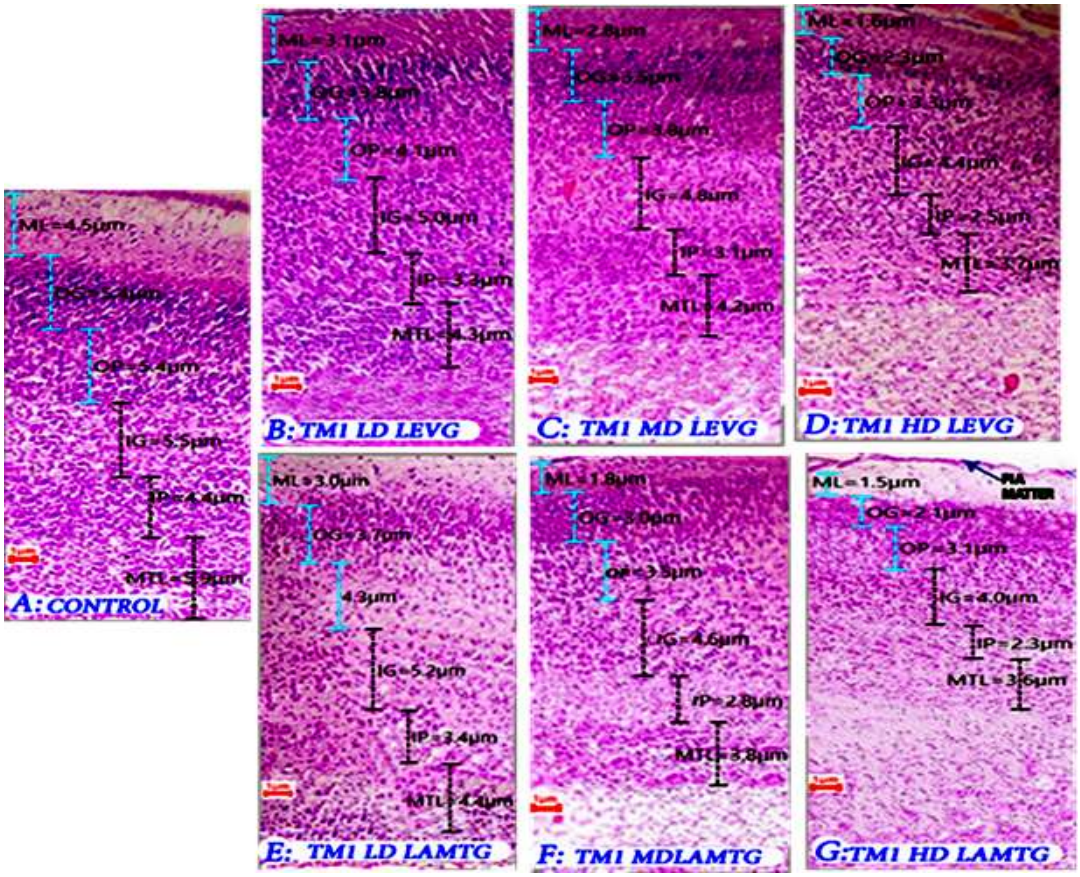

Figure 4.11: The TM1 Comparative Prefrontal Cortical Thicknesses in the Low, Medium, and High Dose Groups of both the Lamotrigine *and* Levetiracetam Treated Groups

Key

A*-control,* ***B-TMI LD LEVG;*** *trimester one low dose levetiracetum treated group,* ***C-TMI MD LEVG;*** *trimester one medium dose levetiracetum treated group,* ***D-TMI HD LEVG;*** *trimester one high dose levetiracetum treated group,* ***E-TMI LD LEVG;*** *trimester one low dose lamotrigine treated group,* ***F-TMI MD LEVG;*** *trimester one medium dose lamotrigine treated group,* ***G-TMI HD LEVG;*** *trimester one high dose lamotrigine treated group,* ***ML****-molecular layer,* ***OGL****-outer granular layer,* ***OPL****-outer pyramidal layer,* ***IPL****-inner pyramidal layer,* ***MTL****-multiform layer*

The comparative prefrontal cortical thickness at TM2

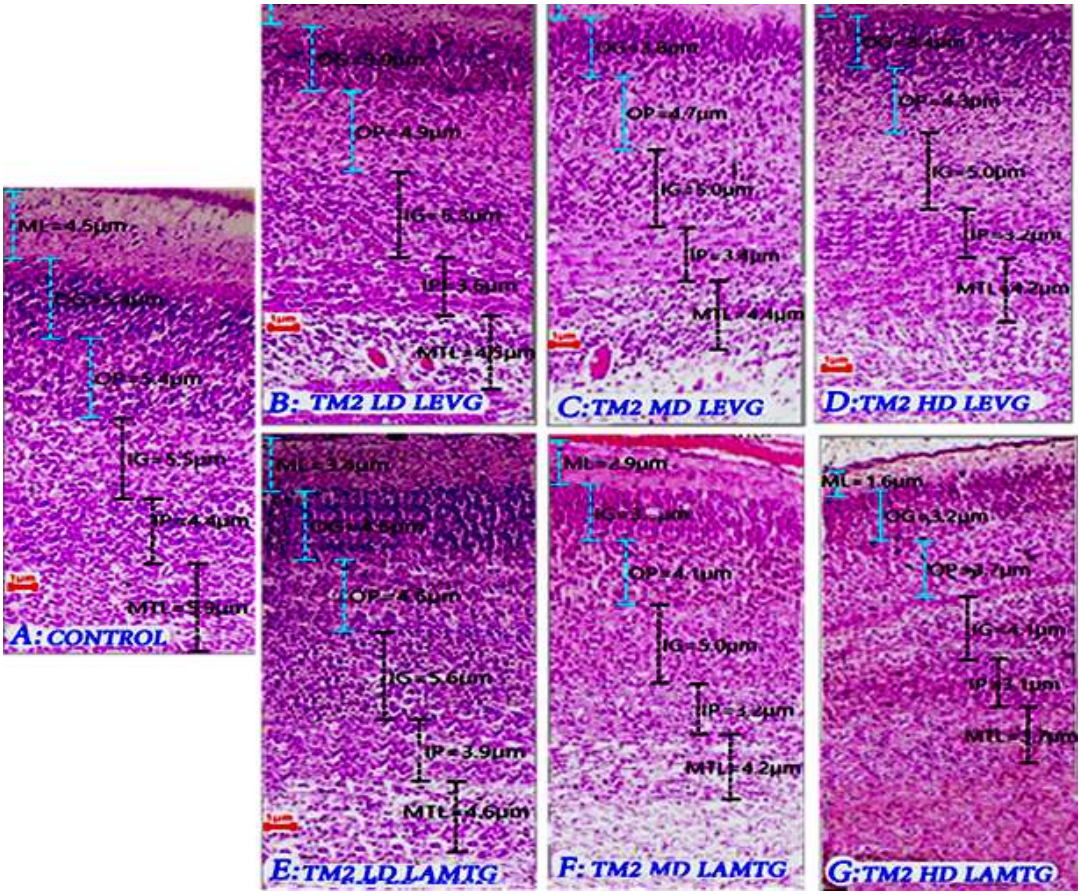

Figure 4.12: The TM2 Comparative Prefrontal Cortical Thicknesses in the Low, Medium, and High Dose Groups of both the Lamotrigine and Levetiracetam Treated Groups

Key

A*-control,* ***B-TMI LD LEVG;*** *trimester one low dose levetiracetum treated group,* ***C-TMI MD LEVG;*** *trimester one medium dose levetiracetum treated group,* ***D-TMI HD LEVG;*** *trimester one high dose levetiracetum treated group,* ***E-TMI LD LEVG;*** *trimester one low dose lamotrigine treated group,* ***F-TMI MD LEVG;*** *trimester one medium dose lamotrigine treated group,* ***G-TMI HD LEVG;*** *trimester one high dose lamotrigine treated group,* ***ML****-molecular layer,* ***OGL****-outer granular layer,* ***OPL****-outer pyramidal layer,* ***IPL****-inner pyramidal layer,* ***MTL****-multiform layer*

The comparative prefrontal cortical thickness at TM3

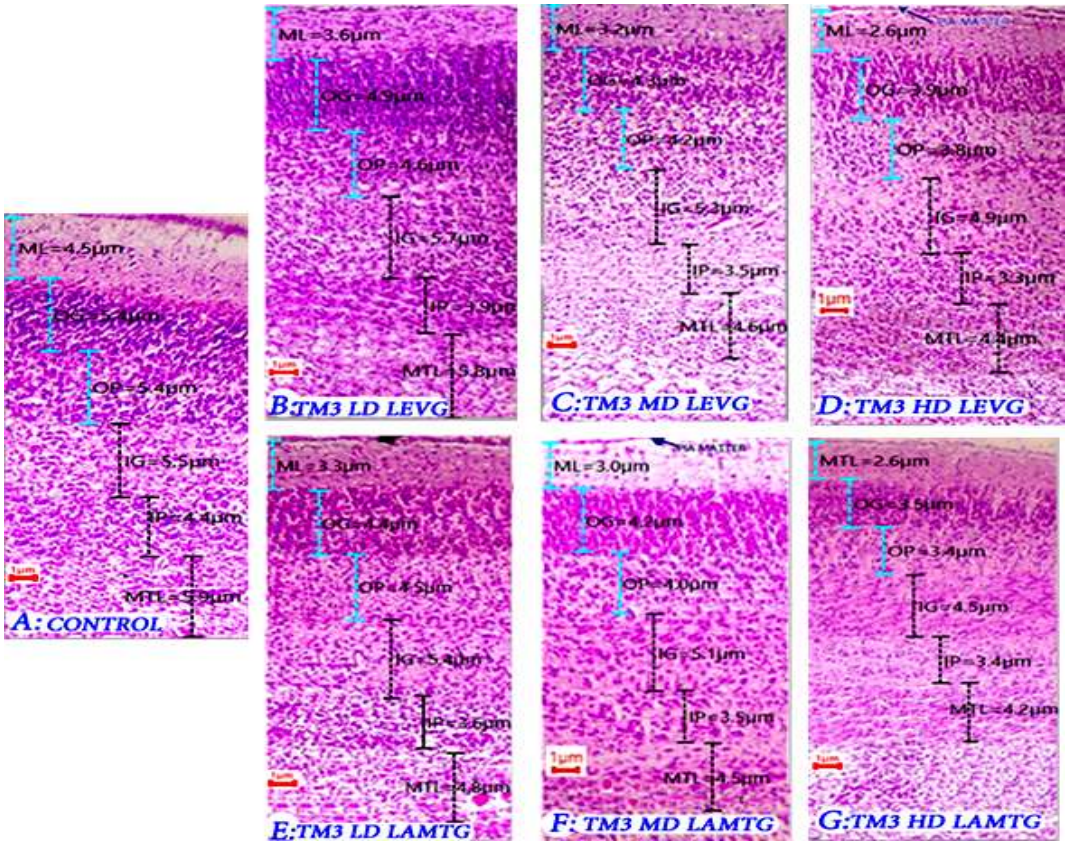

Figure 4.13: The Tm3 Comparative Prefrontal Cortical Thicknesses in the Low, Medium, and High Dose Groups of both the Lamotrigine and Levetiracetam Treated Groups

Key

*A-control, **B-TMI LD LEVG;** trimester one low dose levetiracetum treated group, **C-TMI MD LEVG;** trimester one medium dose levetiracetum treated group, **D-TMI HD LEVG;** trimester one high dose levetiracetum treated group, **E-TMI LD LEVG;** trimester one low dose lamotrigine treated group, **F-TMI MD LEVG;** trimester one medium dose lamotrigine treated group, **G-TMI HD LEVG;** trimester one high dose lamotrigine treated group, **ML**-molecular layer, **OGL**-outer granular layer, **OPL**-outer pyramidal layer, **IPL**-inner pyramidal layer, **MTL**-multiform layer*

The Histomophological Results of the Entorhinal- Cortex.

The entorhinal cortex is the second level structure in the memory circuitry pathway that lies between the prefrontal cortex and the hippocampus, hence forms the interface between the prefrontal cortex and hippocampus. The fetal entorhinal cortex was observed to have two hislogically distinct zones namely;

- **The supra-desiccal cortical zone** that constituties the outer three histological layers namely; (i) the molecular/plexiform layer (ML), (ii) the stratum sterale layer (SS), and the (iii) external principal striatum layer (EPS).
- The **deep infra-desiccal zone of entorhinal cortex** on the other hand were composed of the three deep layers namely; (i) the lamina desiccants layer (LD), (ii) the internal principal striatum layer (IPS), and (iii) multiform layer (MTL). The histomorphological findings are hence presented in line with these two distict entorhinal zones and at two levels as follows: -

Level 1: The histological cyto-archietcture of the the supra-desiccal and the infra-cortical zones entailing the cellular density, the cell distributions, the cell sizes as well as the axonal fibre bundles.

Level 2. The entorhinal cortical thickeness of the six histological layers that constitutes both the supradesiccal and the infradesiccal cortical layers.

Level 1: The histological cyto-archietcture of the the supra-desiccal and the infra- cortical zones

(a) The comparative histo-cyto-arhitecture of the supra-desiccal layers of entorhinal cortex.

In the **supra-deccical cortical layers** of the entorhinal cortex that constitutes the (i) the molecular/plexiform layer (ML), (ii) the stratum sterale layer (SS), and the (iii) external principal striatum layers (EPS), it was observed that these layers were the

most affected following the in-utero exposure to the two medicines but variably in terms of the cell types found in each layer. However, the key memory circuitory cells found in the three supra-desiccal layers included; (i) free neurons with transversely oriented fibres, (ii) the large stellate and modified pyramidal cells, (iii) the loosely arranged medium and large sized pyramidal cells (iv) the head direction cells. The types of cells seen to be disrupted per layer were observed as follows:

(i). In the molecular or the plexiform layer (ML): The granular and the pyramidal cells that are the key cells involved with memory processing in this layer were seen to reduce with increasing dosages of both the two medicines as shown in the photomicrograph ***-Figure 4.14)*** [the cells ***marked as GC and PC in the three photomicrographs of the plexiform layer]*** below,

(ii). In the stratum sterale layer (SS*):* the granular and the pyramidal cells in this layer were simimilary seen to morphologically reduce in their shapes, thier sizes, their numbers as well as in their density with increasing dosages of the two medicines. it was however notable that the medium and the high doses lamortigen were seen to have more deleterious effects specifically to the pyramidal cells than to the glanular cells in this layer. it was further noted that the overall effects of lamotrigen on the cellular components as well as the nerve fiber bundles in its all-dose groups were more deleterious as compared with levetiracetum across the same dose groups. ***-Figure 4.15*** [the cells ***marked as GC and PC in the three photomicrographs***], This was unlike what was observed in the molecular layer where the effects in the cellular componets and the nerve fiber bundles were more or les the same in this layer.

(iii). The external principal striatum layer (EPS), the cells that included the various types of pyramidal cells namely the small, medium size and the large size pyramidal cells were seen to be the ones that were highly affected in their histocyto-architecural arrangement and in their density. the pyramidal cells seemed to be the key target of the lamotrigine teratogenic effects as they are the ones that were also affected more in the lamotrigen treated groups as comared with the levetiracetum treated groups. how ever in both lamotrigen and levetiracetum all the cells in this

layer plus the nerve fibre budles were affected particulary when the treatments were done at trimester one and two. in overall, all cells were observed to reduce in their sizes and in their morphological shapes with increasing dosages of the two medicines as shown in the photomicrograph ***-Figure 4.16*** [***marked as MSPC and LSPC cells]*** *below.*

The molecular layer of entorhinal cortex

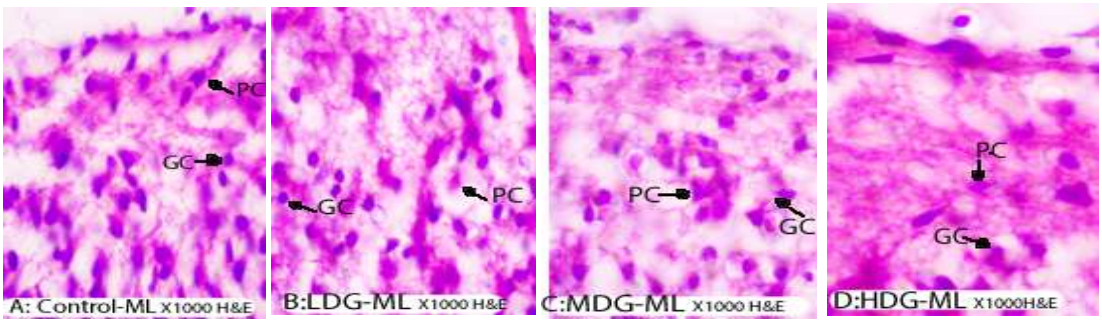

Figure 4.1: The Global Comparative Histo-Cyto-Architecture of the Molecular Layer of the Entorhinal Cortex in Low, Medium and High Dosage Groups Againstcontrol

Key

*A-Control –molecular layer (**ML**), **B**-Low dose group molecular layer (**LDG-ML**), **C-** Medium dose group-molecular layer (**MDG-ML**), **D**- High dose group-molecular layer (**HDG-ML**), **PC**- pyramidal cell, **GC**- granule cell*

The stratum sterale layer of entorhinal cortex

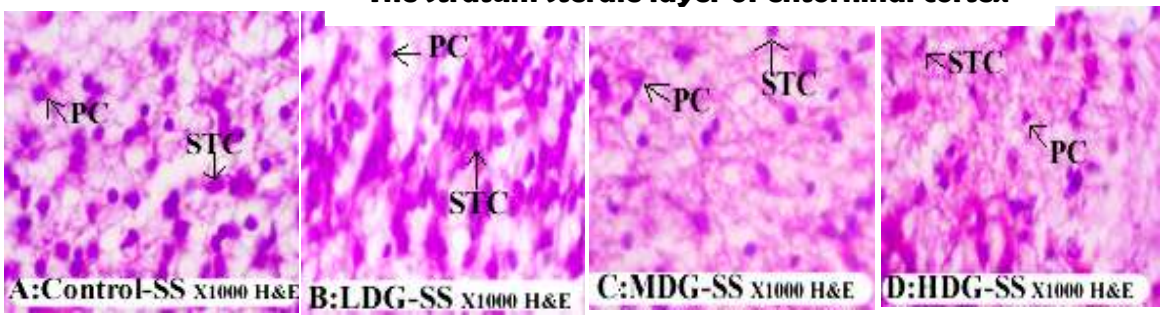

Figure 4.2: The Global Comparative Histo-Cyto-Architecture Of The Stratum Sterale Layer Of The Entorhinal Cortex In Low, Medium And High Dosage Groups Against Control.

Key:

*A-Control –stratum sterale layer (**SS**), **B**-Low dose stratum sterale layer (**LDG-SS**), **C-** Medium dose group- stratum sterale layer, (**MDG-SS**), **D**- High dose group-stratum sterale layer (**HDG-SS**), **PC**- pyramidal cell, **STC**-stellate cell*

The external principal striatum layer of entorhinal cortex

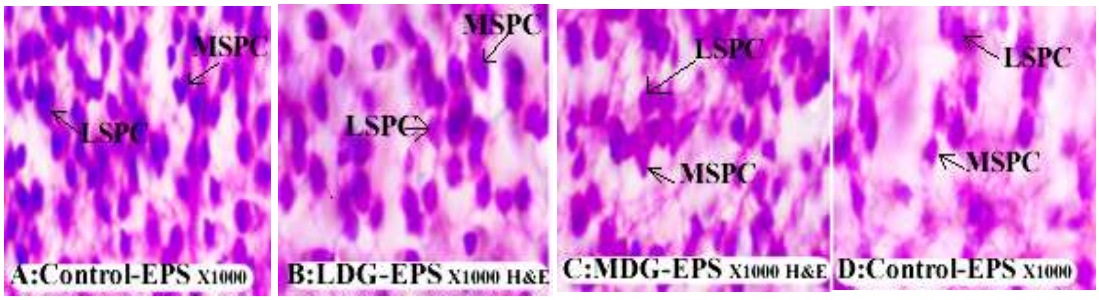

Figure 4.3: The Global Comparative Histo-Cyto-Architecture of the External Principal Striatum Layer of the Entorhinal Cortex in Low, Medium and High Dosage Groups Against Control

Key: A

*Control –external principal striatum layer (**EPS**), **B**-Low dose group external principal striatum layer (**LDG-EPS**), **C-** Medium dose group-external principal striatum layer (**MDG-EPS**), **D**- High dose group-external principal striatum layer (**HDG-EPS**), **MSPC**- medium size pyramidal cell, **LSPC**- large size pyramidal cel*

(b). The histomorphology of the infra-desiccal layers of entorhinal cortex.

In the infra-deccical cortical layers that included the (i) the lamina descicant layer **(LDL),** (ii)the internal principal striatum layer **(IPSL)** and (iii) the multiform layer **(MTL),** it was observed that the histo-cyto-architecutal disruptions of the the cells in terms of the cells shapes, cellular density and the dispersion was not as conspicuous as what was observed in the supra deccical layers in both the treatment groups of lamotrigine and levetiracetam across all the dose groups. The key memory circuitory cells that were noted to be affectd in this zone were the small, medium or large sized pyramidal and stellate cells per layer as follows;

(i) **The lamina descicants layer (LDL): -** the pyramidal cells were observed to reduce in their numbers as well as their morphological shapes with increasing dosages of the two medicines as shown in the photomicrographs in ***-Figure 4.17*** [***marked PC cells]*** *below*.

(ii) **The internal principal striatum layer (IPSL): -** in this layer, the pyramidal cells involved in memory circuit were similarly observed seen to reduce with increasing dosages of the two medicines as shown in the photomicrograph ***-Figure 4.18*** [***marked PC cells]*** *below.*

(iii)the multiform layer (MTL): - in this layer, the pyramidal cells were similarly observed to reduce in their desities. numbers and their morkological shapes and sizes with increasing dosages of the two medicines as shown in the photomicrograph ***-Figure 4.19*** [***marked PC cells]*** *below.*

The lamina descicant layer of entorhinal cortex

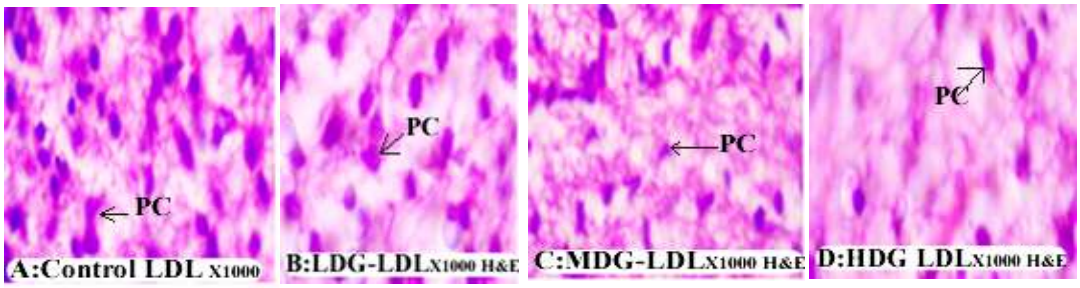

Figure 4.4: The Global Comparative Histo-Cyto-Architecture of the Lamina Descicant Layer of the Entorhinal Cortex in Low, Medium Andhigh Dosage Groups Against Control

Key

*A-Control –stratum lamina descicant **(LDL)**, **B**-Low dose group lamina descicant layer (**LDG-LDL), C-** Medium dose group-lamina descicant layer, **(MDG-LDL), D**- High dose group-lamina descicant layer (**HDG-LDL**), **PC**- pyramidal cell*

The internal principal striatum layer of entorhinal cortex

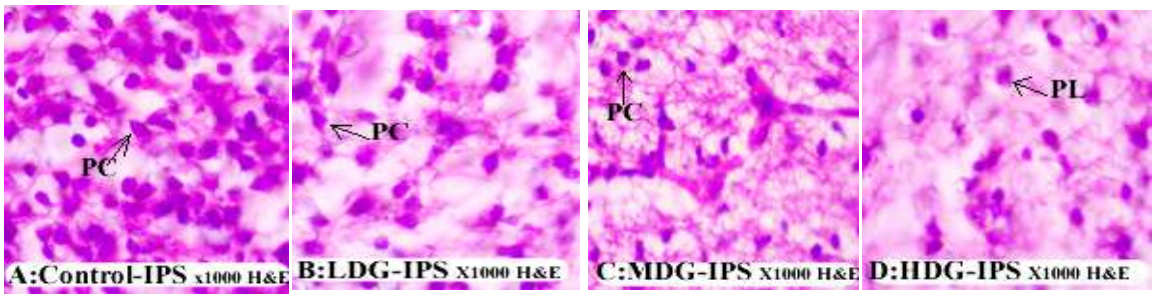

Figure 4.18: The Global Comparative Histo-Cyto-Architecture of the Internal Principal Striatum Layer of the Entorhinal Cortex in Low, Medium and High Dosage Groups Against Control

Key

*A-Control –stratum lamina descicant **(IPSL)**, **B**-Low dose group internal principal striatum layer (**LDG-IPSL), C-** Medium dose group-internal principal striatum layer, **(MDG-IPSL), D**- High dose group-internal principal striatum layer (**HDG-IPSL**), **PC**- pyramidal cell*

The multiform layer

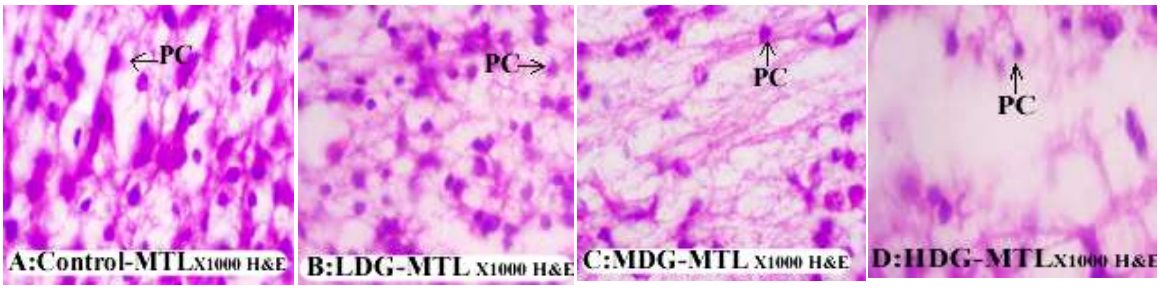

Figure 4.59: The Global Comparative Histo-Cyto-Architecture of the Multiform Layer of Theentorhinal Cortex in Low, Medium and High Dosage Groups Against Control

Key

*A-Control –stratum lamina descicant **(MTL)**, **B**-Low dose group multiform layer (**LDG-MTL), C-** Medium dose group-multiform layer, **(MDG-MTL), D**- High dose group-multiform layer (**HDG-MTL**), **PC**- pyramidal cell*

Level 2: The cortical thicknesses of entorhinal cortical layers at TM_1, TM_2 and TM_3

The histomorphological findings on the cortical thicknesses of entorhinal layers are presented along the trimesters (time) of exposure to the two medicines as follows: -

At trimester one (TM_1) it was observed that, the entorhinal cortical thicknesses of all its six histological layers in both the supradescical and infradescical zonesof entorhinal cortex depicted an inverse dose response relationship in that, as the doses of the two medicines increased it resulted in proportionate reduction in the cortical thicknesses of the histological zones of entorhinal cortex. it was remarkable that at high dosage levels, the thickness of all the entorhinal cortical histological layers were much reduced than in low and medium dosage groups, in both the levetiracetum and the lamotrigine treated groups. It was futher observed that lamotrigen treated groups had more detrimental effects than those of the lamotrigen in the same dosage levels [Figure 4.20].

At trimester two (TM_2) the entorhinal cortical thicknesss of the histological layers similarly were observed to be dose dependant. The high and medium dosage groups were however observed to have the most reduced entorhinal cortical thickness of the histological layers moreso of the supradescical layers as compared to low dosage groups in both lamotrigine and levetiracetam medications. (Figure 4.21).

At trimester three (TM_3), It was observed that the thickness of the entorhinal cortical layers was not affected in the low and medium dose groups in the two medication of lamotrigine and levetiracetam. It was however noted that in the high dosage groups of the two medications, the entorhinal cortical histological layers were remarkably reduced. Across all dosage groups, lamotrigen was onbserved to be associated with more deleterious effects than levetiracetum (Figure 4.22).

The TM1 entorhinal cortical thicknesess in low, medium and high dose

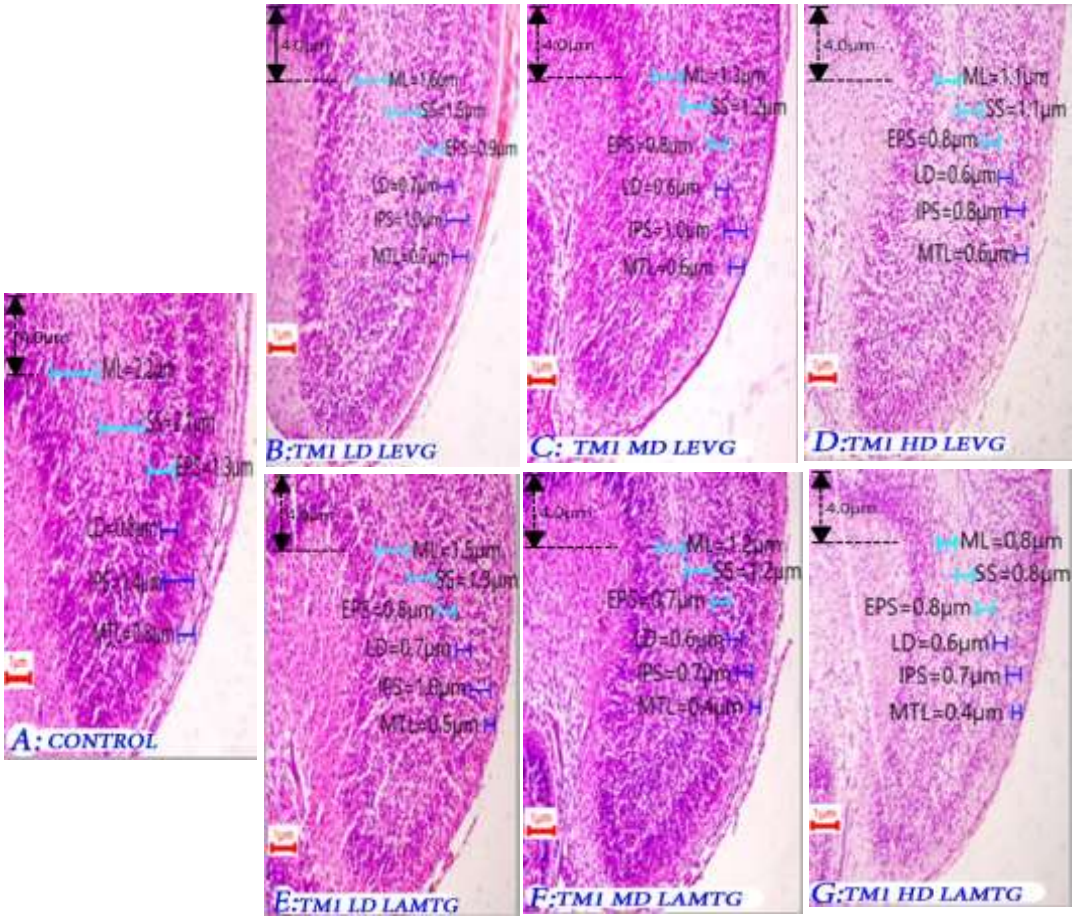

Figure 4.6: The TM1 Comparative Entorhinal Cortical Thicknesses in the Low, Medium, and High Dose Groups of Both the Lamotrigine and Levetiracetam Treated Groups Against the Control.

Key

***A**-control, **B-TMI LD LEVG;** trimester one low dose levetiracetum treated group **C-TMI MD LEVG;** trimester one medium dose levetiracetum treated group, **D-TMI HD LEVG;** trimester one high dose levetiracetum treated group, **E-TMI LD LEVG;** trimester one low dose lamotrigine treated group, **F-TMI MD LEVG;** trimester one medium dose lamotrigine treated group, **G-TMI HD LEVG;** trimester one high dose lamotrigine treated group, **ML**-molecular layer, **SS**-stratum sterale layer, **EPSL**- external principal striatum layer, **LDL**-lamina descicants layer, **MTL**- multiform layer*

The TM2 entorhinal cortical thicknesss in low, medium and high dose

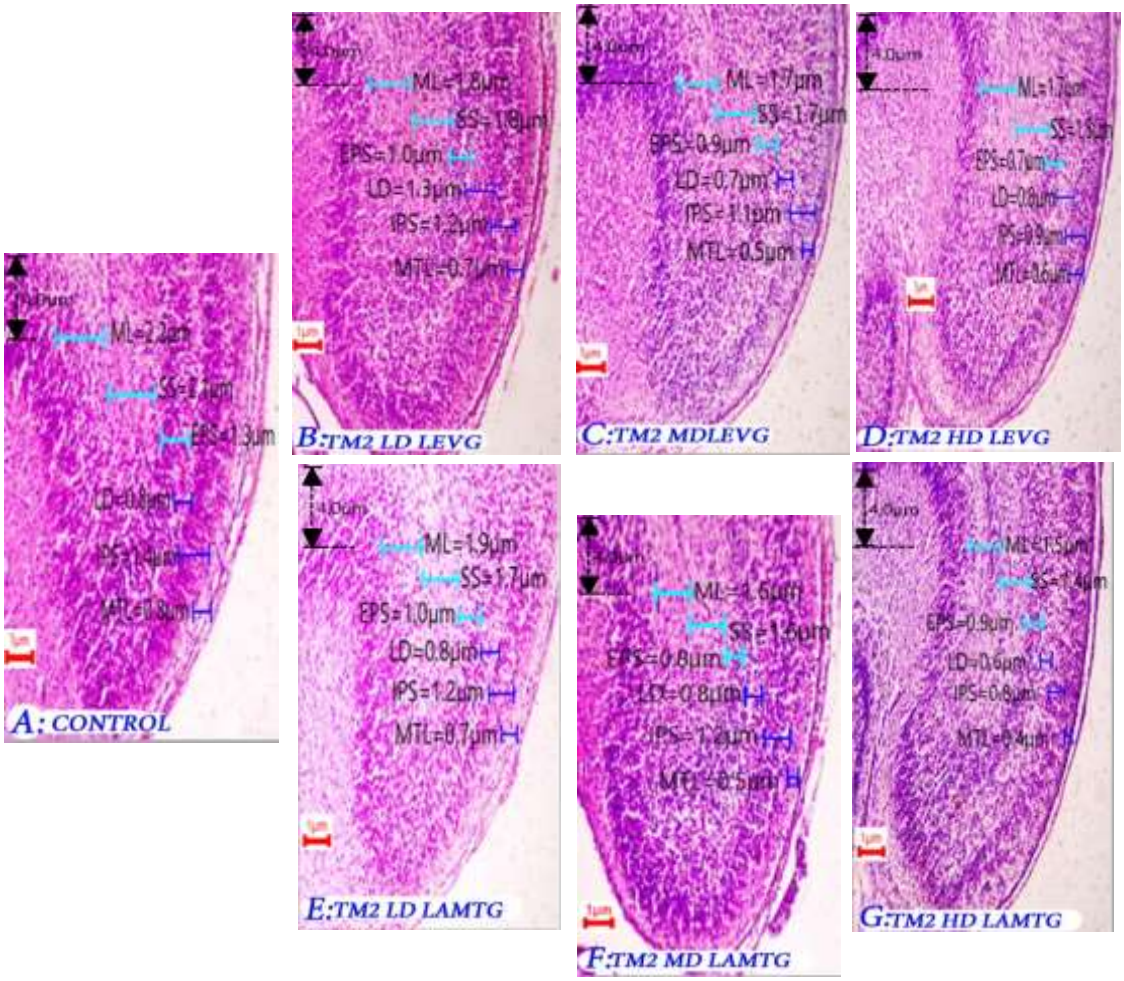

Figure 4.21: The TM2 Comparative Entorhinal Cortical Thicknesses in the Low, Medium, and High Dose Groups of Both the Lamotrigine and Levetiracetam Treated Groups

Key

A*-control,* ***B-TMI LD LEVG;*** *trimester one low dose levetiracetum treated group* ***C-TMI MD LEVG;*** *trimester one medium dose levetiracetum treated group,* ***D-TMI HD LEVG;*** *trimester one high dose levetiracetum treated group,* ***E-TMI LD LEVG;*** *trimester one low dose lamotrigine treated group,* ***F-TMI MD LEVG;*** *trimester one medium dose lamotrigine treated group,* ***G-TMI HD LEVG;*** *trimester one high dose lamotrigine treated group,* ***ML****-molecular layer,* ***SS****-stratum sterale layer,* ***EPSL****- external principal striatum layer,* ***LDL****-lamina descicants layer,* ***MTL****- multiform layer*

The TM3 entorhinal cortical thicknesess in low, medium and high dose

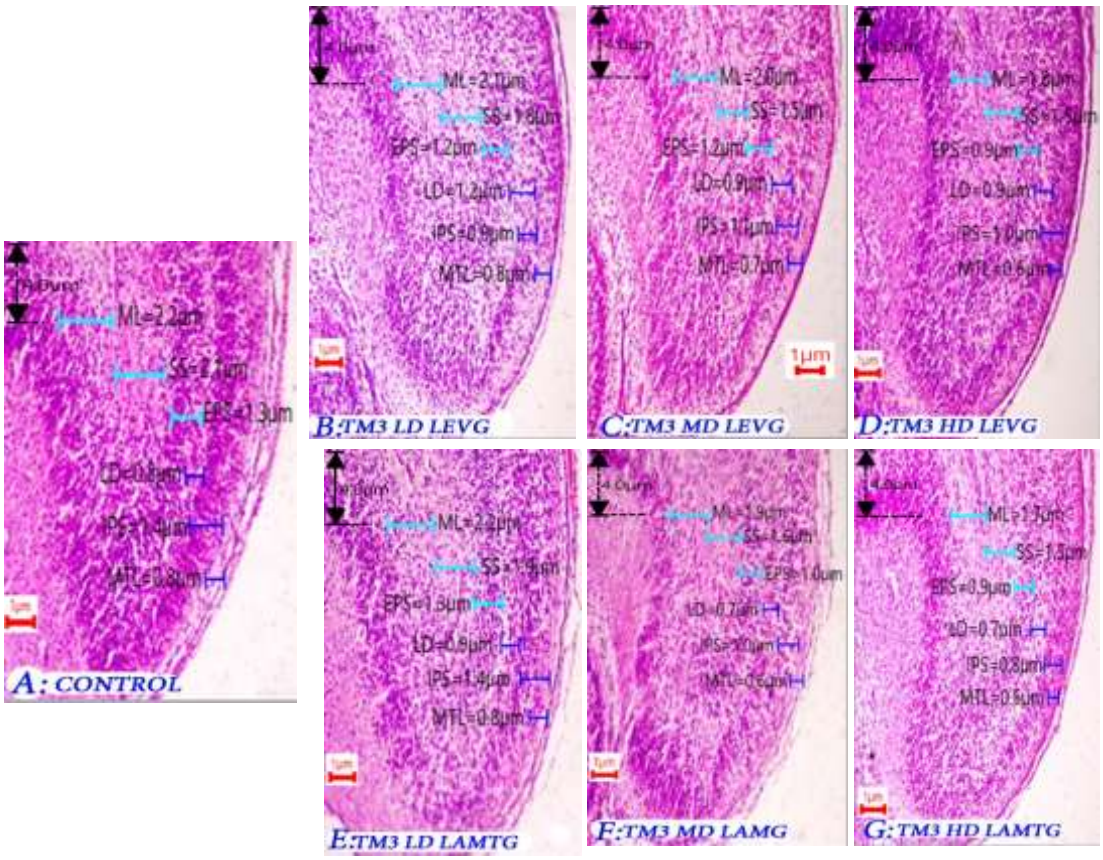

Figure 4.22: The TM3 Comparative Entorhinal Cortical Thicknesses in the Low, Medium, and High Dose Groups of both the Lamotrigine and Levetiracetam Treated Groups

Key

__A__-control, __B-TMI LD LEVG;__ trimester one low dose levetiracetum treated group __C-TMI MD LEVG;__ trimester one medium dose levetiracetum treated group, __D-TMI HD LEVG;__ trimester one high dose levetiracetum treated group, __E-TMI LD LEVG;__ trimester one low dose lamotrigine treated group, __F-TMI MD LEVG;__ trimester one medium dose lamotrigine treated group, __G-TMI HD LEVG;__ trimester one high dose lamotrigine treated group, __ML__-molecular layer, __SS__-stratum sterale layer, __EPSL__- external principal striatum layer, __LDL__-lamina descicants layer, __MTL__- multiform layer

4.3.1.3 The Histomophological Findings on How the Two Medicines Influenced the Histological Cellular Organization of the Subiculum

The subiculum which forms the third group of structurers in the memory circuitry pathway acts to connect the entorhinal cortex with the hippocampus for hippocampal-cortical interactions. The histomorphological findings are presented in two levels as follows;

Level 1 The histological cyto-archietcture of the subiculum

The histo-architecture of the three histological layers; two superficial layers namely; (i) the molecular layer (ML), (ii) the pyramidal cell layer (PCL), and one deep layer namely; (iii) plexiform layer (PFL) as follows: -

(i) In the molecular layer (ML): The sterate and the pyramidal cells that are the key cells involved with memory processing in this layer. They were observed to reduce with increasing dosages of both the two medicines as shown in the photomicrograph *[Figure 4.23 the cells marked as STC and PC in the four photomicrographs of the molecular/plexiform layer]*

(ii)In the pyramidal cell layer (PCL*):* the pyramidal cells in this layer were simimilary seen to reduce with increasing dosages of the two medicines as shown in *-Figure 4.24 [the cells marked as STC and PC in the four photomicrographs of the pyramidal layer]*.

(iii)The plexiform layer (PFL): the pyramidal and the stellate cells in this layer were simimilary seen to reduce with increasing dosages of the two medicines [*Figure 4.25 -the cells marked as STC and PC in the four photomicrographs of the plexiform layer]*

The molecular layer of the subiculum

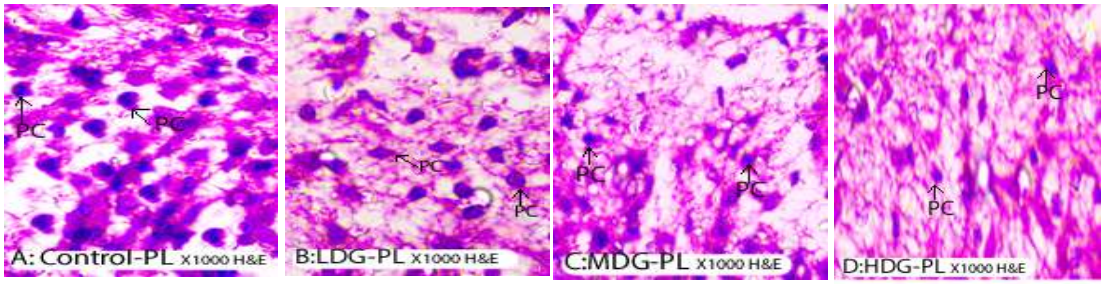

Figure 4.23: The Global Comparative Histo-Cyto-Architecture of the Molecular Layer of the Subiculum in Low, Medium and High Dosage Groups Against Control

Key

*A-Control –molecular layer **(ML)**, **B**-Low dose group molecular layer **(LDG-ML), C-** Medium dose group-molecular laver. **(MDG-ML). D**- High dose aroup-mmolecular laver **(HDG-ML)**. **PC**- ovramidal cell.*

The pyramidal layer of the subiculum

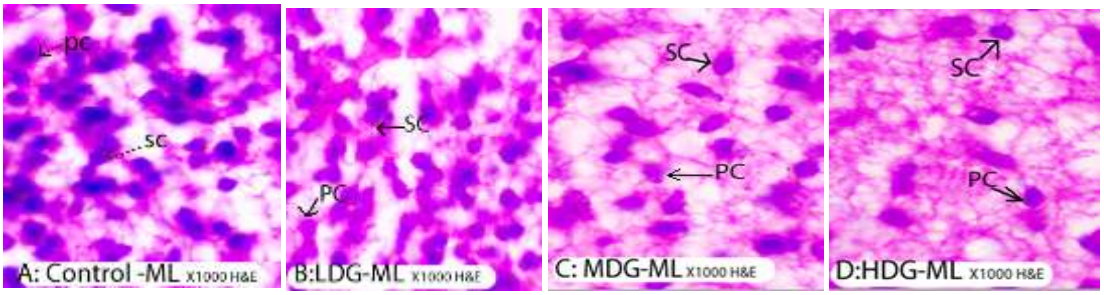

Figure 4.14: The Global Comparative Histo-Cyto-Architecture of the Pyramidal Layer of the Subiculum in Low, Medium and High Dosage Groups Against Control

Key

*A-Control –pyramidal layer **(PL)**, **B**-Low dose group pyramidal layer **(LDG-PL), C-** Medium dose group-pyramidal layer, **(MDG-PL), D**- High dose group-pyramidal layer **(HDG-PL)**, **PC**- pyramidal cell, **SC**-stella*

The plexiform layer of the subiculum

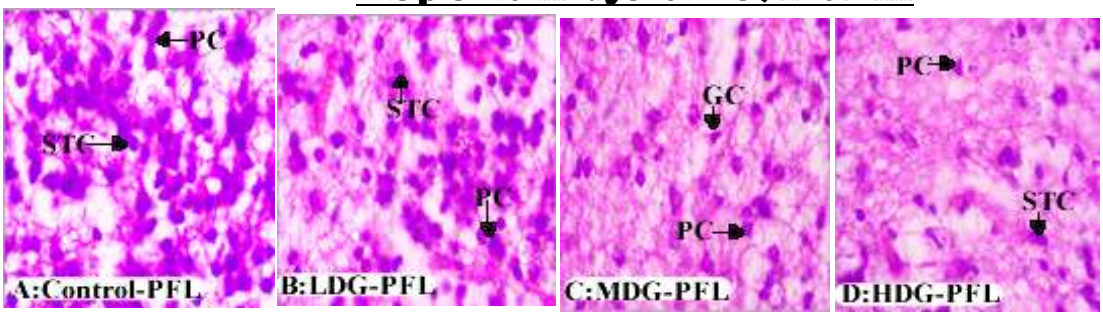

Figure 4.25: The Global Comparative Histo-Cyto-Architecture of the Plexiform Layer of the Subiculum in Low, Medium and High Dosage Groups Against Control

Key

*A-Control –plexiform layer **(PLF)**, **B**-Low dose group plexiform layer **(LDG-ML), C-** Medium dose group-plexiform layer, **(MDG-PFL), D**- High dose group-plexiform layer **(HDG-ML)**, **PC**- pyramidal cell, **GC**-granule cell*

Level 2: The comparative subicular thicknesses of subiculum at TM1, TM2 and TM3

The comparative subicular thicknesses of its histological layers are presented as per the trimester (time) of exposure as follows: -

At trimester one (TM_1) the histomoprhological thicknesses of the three histological layers of subiculum namely; (i) the molecular layer, (ii) the pyramidal cell layer and (iii) the polymorphic/fiber layer were observed to decrease remarkably in a dose a dependant manner. in particular, at medium and high dose groups in both the lamotrigine and levetiracetam treated groups, the three subiculum layers were seen to be the ones highly reduced, then was the case in the low dosage groups. In addition, it was noted that lamotrigen treated groups across all its dosage levels had more detrimental effects than those of the levetiracetam in the histological organization of the three subiculum layers [Figure 4.26].

At trimester two (TM_2) the histological thickness of the three layers of the subiculum combined, they were similarly observed to depict the same reduction in thickneses as was observed in trimester one (TM1) in dose dependent manner. the high and medium dosage groups were noted as well to have the most detrimental effects in effectuating reduction in the thickness of the subiculur histological layers. on further observations, it was notable that, the low dose groups of levetiracetum as well as the medium dose group wthen the treatments were done at TM3 did not have remarable significant difference with those of the control. in overall it was conclusive that lamortigen had more detrimental effects in subicular layers than levetiracetum treated groups across all dose groups with the effects bearing a similar resembalce in the histomicrographs (Figure 4.27).

At trimester three (TM_3), the histological thickness of the three combined layers of subiculum was observed to be affected only by high and medium dosages of levetiracetam as well those of lamotrigine treated groups. The Low dosage groups in the two medication of lamotrigine and levetiracetam did not show any significance reduction in thicknesses (Figure 4.28).

The TM1 comparataive histological thicknesses of the subicular

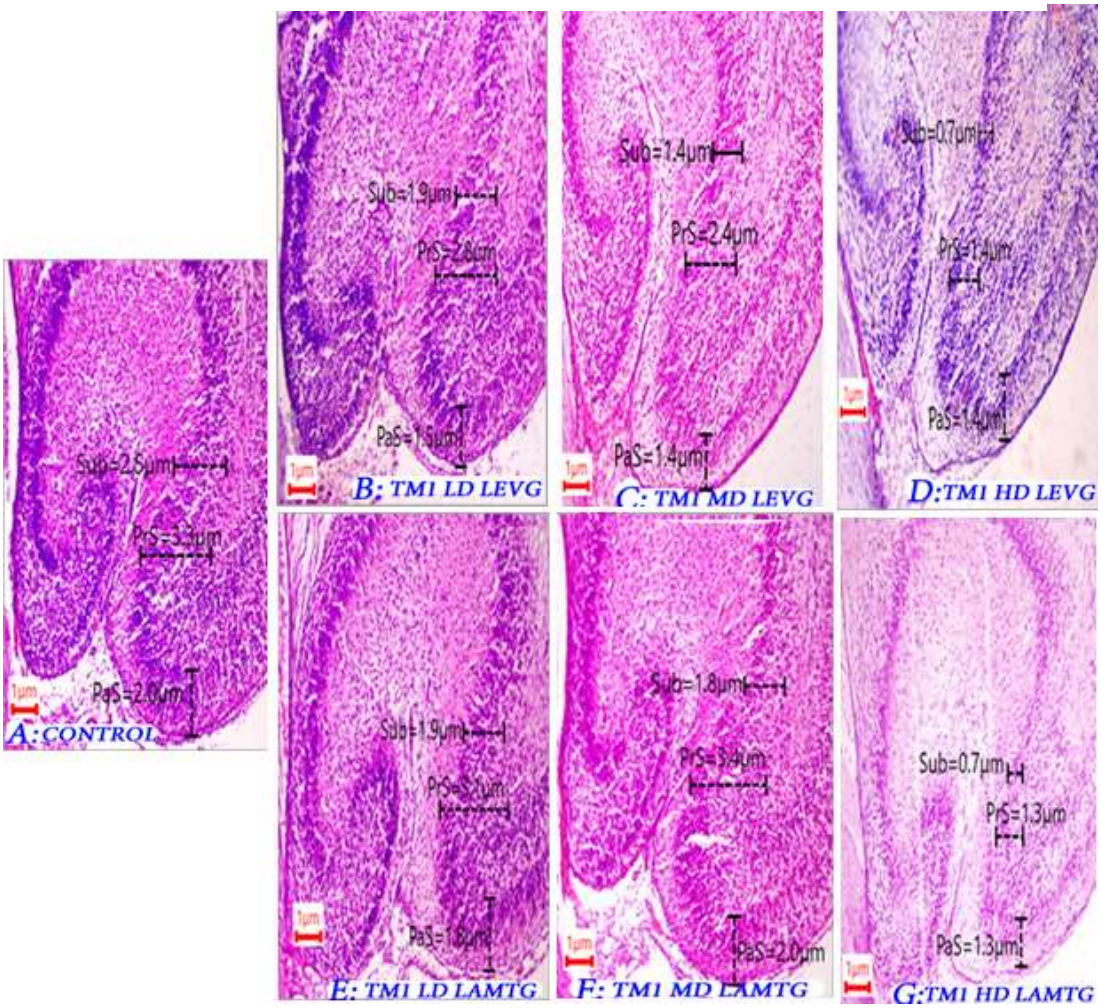

Figure 4.26: The TM1 Comparative Histological Thicknesses of Subiculum, Presubiculum and Parasubiculum in the Low, Medium, and High Dose Groups of Both the Lamotrigine and Levetiracetam Treated Groups

Key

A*-control,* ***B-TMI LD LEVG;*** *trimester one low dose levetiracetum treated group,* ***C-TMI MD LEVG;*** *trimester one medium dose levetiracetum treated group,* ***D-TMI HD LEVG;*** *trimester one high dose levetiracetum treated group,* ***E-TMI LD LEVG;*** *trimester one low dose lamotrigine treated group,* ***F-TMI MD LEVG;*** *trimester one medium dose lamotrigine treated group,* ***G-TMI HD LEVG;*** *trimester one high Dose lamotrigine treated group,* ***Sub****-subiculum,* ***PrS****-presubiculum,* ***PaS****-parasubiculum*

The TM2 comparataive histological thicknesses of the subicular

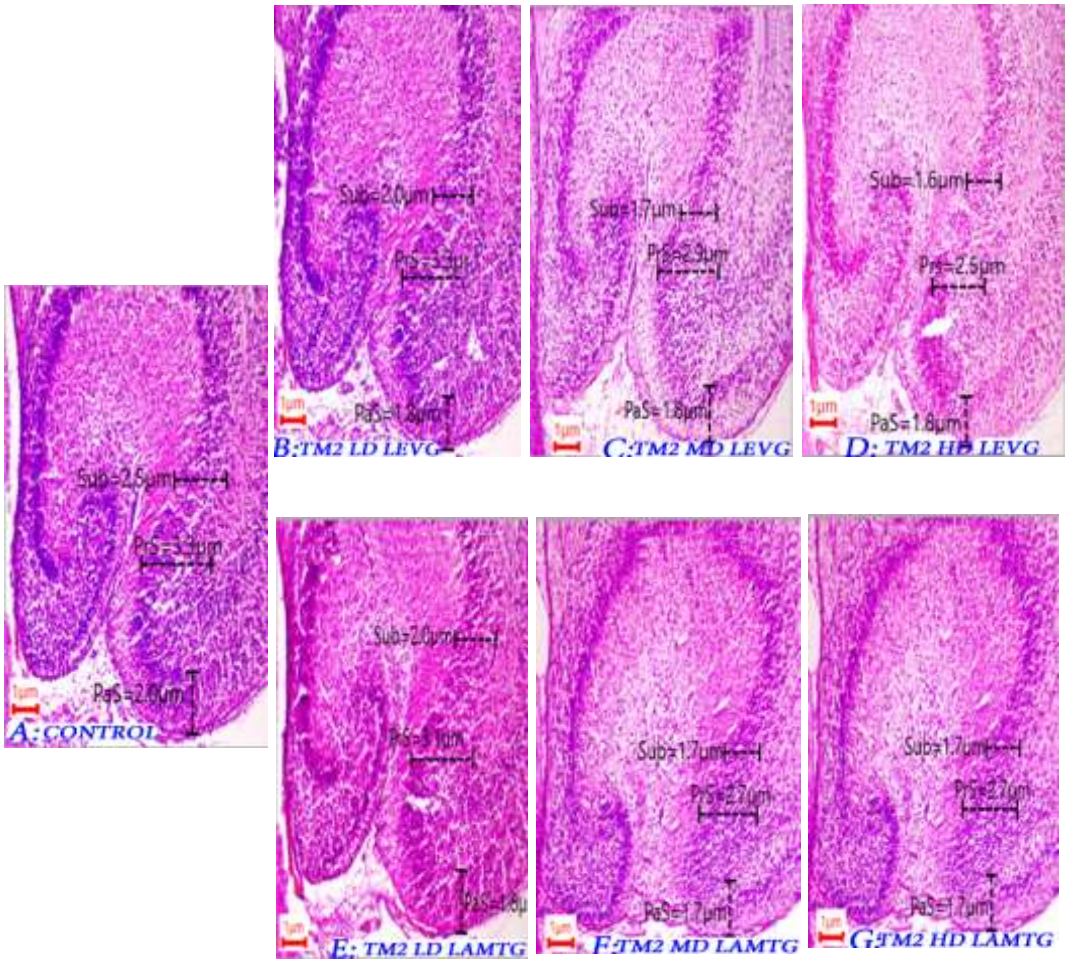

Figure 4.27: The TM2 comparative histological thicknesses of subiculum, presubiculum and parasubiculum in the low, medium, and high dose groups of both the lamotrigine and levetiracetam treated groups

Key

A*-control,* ***B-TMI LD LEVG;*** *trimester one low dose levetiracetum treated group,* ***C-TMI MD LEVG;*** *trimester one medium dose levetiracetum treated group,* ***D-TMI HD LEVG;*** *trimester one high dose levetiracetum treated group,* ***E-TMI LD LEVG;*** *trimester one low dose lamotrigine treated group,* ***F-TMI MD LEVG;*** *trimester one medium dose lamotrigine treated group,* ***G-TMI HD LEVG;*** *trimester one high dose lamotrigine treated group,* ***Sub****-subiculum,* ***PrS****-presubiculum,* ***PaS****-parasubiculum*

The TM3 comparataive histological thicknesses of the subicular

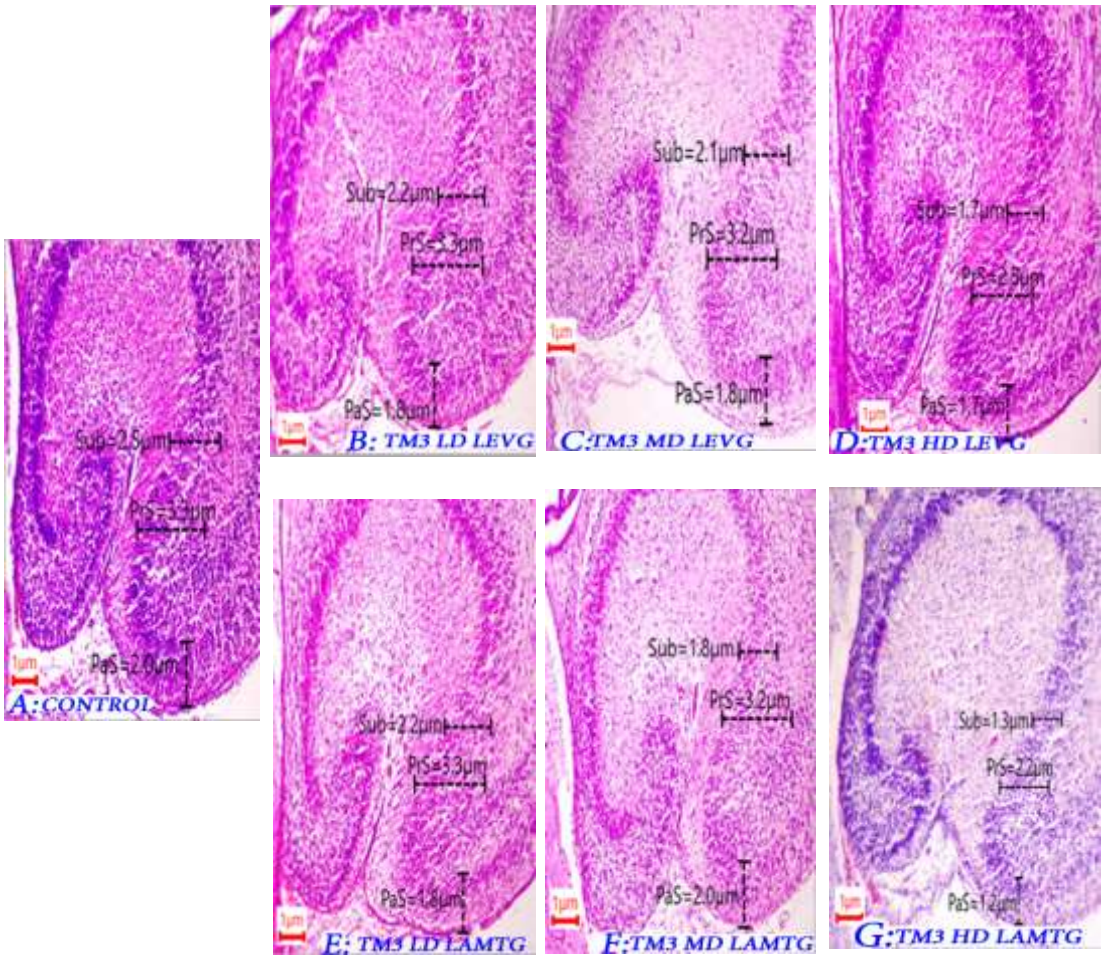

Figure 4.28: The TM3 Comparative Histological Thicknesses of Subiculum, Presubiculum and Parasubiculum in the Low, Medium, and High Dose Groups of Both the Lamotrigine and Levetiracetam Treated Groups against the Control Group

Key

*A-control, **B-TMI LD LEVG;** trimester one low dose levetiracetum treated group, **C-TMI MD LEVG;** trimester one medium dose levetiracetum treated group, **D-TMI HD LEVG;** trimester one high dose levetiracetum treated group, **E-TMI LD LEVG;** trimester one low dose lamotrigine treated group, **F-TMI MD LEVG;** trimester one medium dose lamotrigine treated group, **G-TMI HD LEVG;** trimester one high Dose lamotrigine treated group, **Sub**-subiculum, **PrS**-presubiculum, **PaS**-parasubiculum*

4.3.1.4 The Histomophological Results of the Hippocampus.

The hippocampus is the fouth level structure in the memory circuitry pathway that function to encode and consolidate memory and connects to the dentate gyrus and the amygdaloid nucleus, the momory storage stucturers. The fetal hippocampal histological layers were observed to have two hislogically distinct zones namely **the outer hippocampal zone and the inner hippocampal zone.** The outer hippocampal zone constituties the outer three histological layers namely; (i) the stratum alveus layer (SAL), (ii) the stratum oriens layer (SOL), and the (iii) striatum pyramidale layer (SPL).

The **inner hippocampal zone of** on the other hand is comprised of the two deep layers namely; (i) the stratum radiatum layer SRL), (ii) a combination of stratum lacunosum and stratum moraculare hippocampal layers (SLL/SML). The histomorphological findings are therefore presented in line with these two distict hippocampal zones and at two levels as follows: -

Level 1: The histological cyto-archietcture of the outer and inner layers of the hippocampal gyrus entailing the cellular density, the cell distributions, the cell sizes as well as the axonal fibre bundles.

Level 2. The cortical thickeness of the outer and the inner hippocampal gyrus.

Leve1: The histo-cyto-arhitecture of the hippocampal gyrus

a) The histo-cyto-arhitecture of the inner layers of hippocampal gyrus

In the **outer hippocampal layers** that constitutes the (i) the stratum alveus layer (SAL), (ii) the stratum oriens layer (SOL), and the (iii) striatum pyramidale layer (SPL) were observed to have varying effects following the in-utero administration of either lamotrigine or levetiracetam as follows:

(i) In the stratum alvius layer (SAL): the pyramidal cells involved in memory processing in this layer did not show much effects upon administration of both medications as shown in the photomicrographs (*Figure 4.29*-the cells *marked as PC in the four photomicrographs of the stratum alvius layer]*

(ii)In the stratum oriens layer (SOL): the pyramidal cells in this layer were similary seen to reduce with increasing dosages of the two medicines like was the case in the molecular layer as shown in, *[Figure 4.30-the cells marked PC in the three photomicrographs]*.

(iii)The stratum pyramidale layer (SPL): the pyramidal cells in this layer were similary seen to reduce with increasing dosages of the two medicines just like in the stratum oriens layer, (*Figure 4.31*-the cells *marked as PC in the four photomicrographs*].

The stratum alvius layer

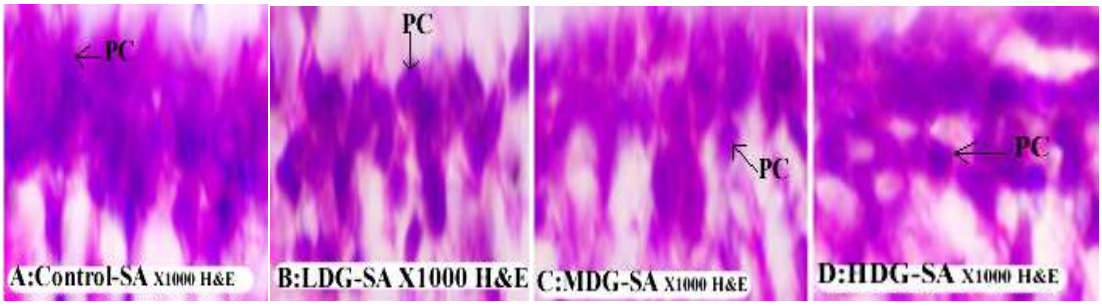

Figure 4.29: The Global Comparative Histo-Cyto-Architecture of the Stratum Alvius Layer of Hippocampus in Low, Medium and High Dosage Groups Against Control

Key

*A-Control –straum alveus layer **(SAL)**, **B**-Low dose group stratum alvius layer **(LDG-SAL)**, **C-** Medium dose group-stratum alvius layer, **(MDG-SAL)**, **D**- High dose stratum alvius layer **(HDG-SAL)**, **PC**-pyramidal cell*

The stratum oriens layer

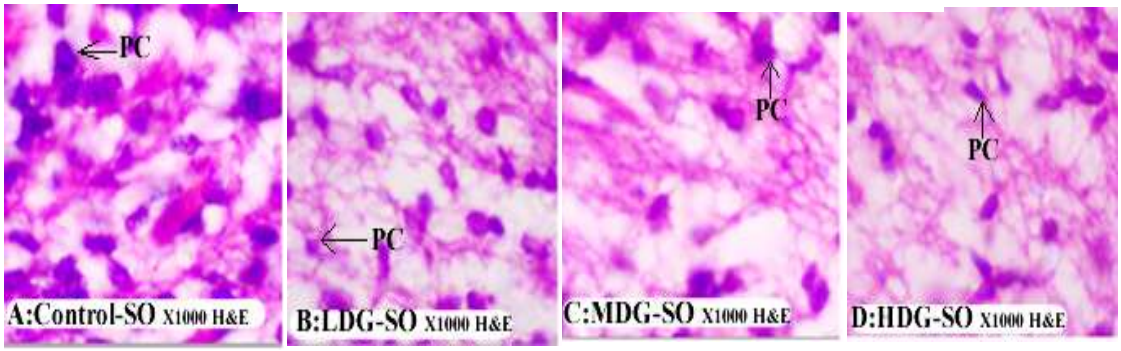

Figure 4.30: The Global Comparative Histo-Cyto-Architecture of the Stratum Alvius Layer of Hippocampus in Low, Medium and High Dosage Groups Against Control

Key

*A-Control –straum oriens layer **(SOL)**, **B**-Low dose group stratum oriens layer **(LDG-SOL)**, **C-** Medium dose group-stratum oriens layer, **(MDG-SOL)**, **D**- High dose stratum oriens layer **(HDG-SOL)**, **PC**-pyramidal cell.*

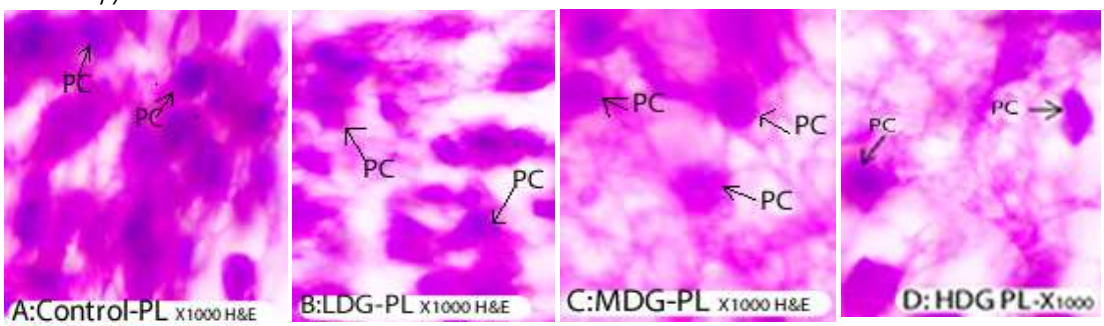

Figure 4.31: The Global Comparative Histo-Cyto-Architecture of the Stratum Pyramidale Layer of Hippocampus in Low, Medium and High Dosage Groups Against Control

Key

*A-Control –straum pyramidale layer **(SPL)**, **B**-Low dose group stratum pyramidale layer **(LDG-SPL)**, **C-***

*Medium dose group-stratum pyramidale layer, (**MDG-SPL), D**- High dose stratum pyramidale layer (**HDG-SPL), PC**- pyramidal cell.*

b) The histo-cyto-arhitecture of the inner layers of hippocampal gyrus

In the inner cortical layers that constitutes the (i) the stratum radiatum layer **(SRL),** and (ii) the stratum lacunosum/moraculare layer **(SLL),** the key memory circuitory cells disrupted were pyramidal, stellate cells and fusiform cells as follows;

(i) **The stratum radiatum layer (SRL): -** in this layer, the pyramidal and the stellate cells were observed to reduce with increasing dosages of the two medicines as shown in the photomicrograph ***-Figure 4.32-cells marked PC and SC]***.

The stratum lacunosum/moraculare layer (SLL):- in this layer, the fusiform cells involved in memory circuit were similarly observed seen to reduce with increasing dosages of the two medicines as shown in the photomicrograph, [***Figure 4.33-cells marked FC]***.

The stratum radiatum layer

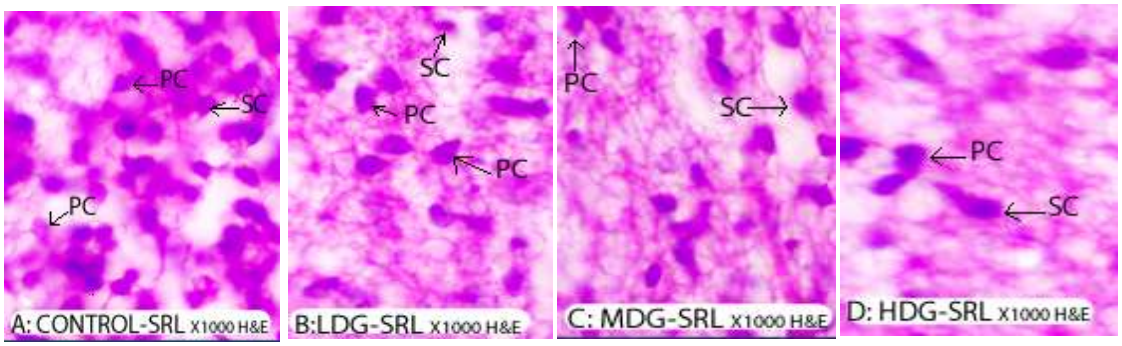

Figure 4.32: The Global Comparative Histo-Cyto-Architecture of the Stratum Radiatum Layer of Hippocampus in Low, Medium and High Dosage Groups Against Control

Key

__A__-Control –straum radiatum layer __(SPL)__, __B__-Low dose group stratum radiatum layer __(LDG-SPL)__, __C__-Medium dose group-stratum radiatum layer, __(MDG-SPL)__, __D__- High dose stratum radiatum layer __(HDG-SPL)__, __PC__- pyramidal cell., __SC__-stellate cell

The stratum lacunosum layer

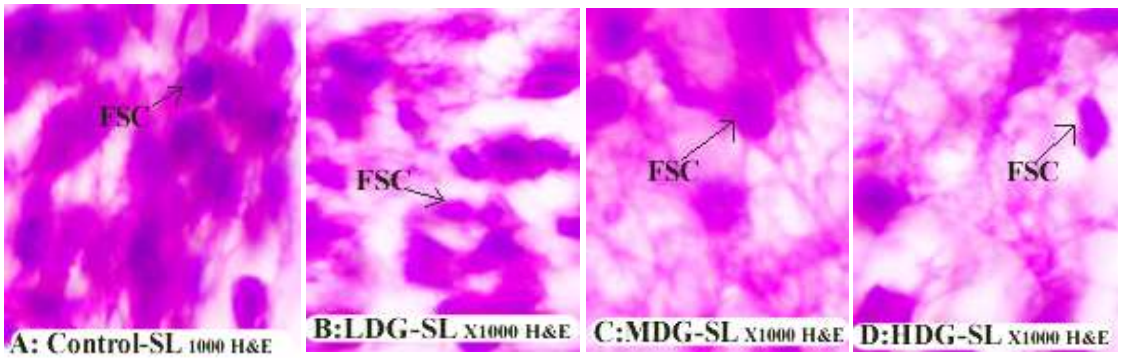

Figure 4.33: The Global Comparative Histo-Cyto-Architecture of the Stratum Lacunosum Layer of Hippocampus in Low, Medium and High Dosage Groups Against Control

Key

__A__-Control –straum lacunosum layer __(SPL)__, __B__-Low dose group stratum lacunosum layer __(LDG-SPL)__, __C__-Medium dose group-stratum lacunosum layer, __(MDG-SPL)__, __D__- High dose stratum lacunosum layer __(HDG-SPL)__, __FSC__- fusiform cell.

Level 2- The comparative cortical thicknesses of hippocampal gyrus at TM_1, TM_2 and TM_3

The comparative thicknesses of the hippocampal histological layers are presented according to the trimester (time) of exposure as follows: -

At trimester one (TM_1) it was observed that, the histological thicknesses of the hippocampal gyrus in both the treatment groups of lamotrigen and levetiracetam depicted an inververse dose response relationship in both its outer and the inner layers, in that, at high dosage levels, theall its histological layers namely (i) the stratum alveus layer (SAL), (ii) the stratum oriens layer (SOL), and the (iii) striatum pyramidale layer (SPL), thickness was observed to be much more reduced than in low and medium dosage groups, in both the levetiracetum and the lamotrigine treated groups. During this trimester, it was futher observed that lamotrigen treated groups had more reduced layers than the levetiracetam group at the same dosage levels, meaning that lamotrigine had more detrimental effects. [Figure 4.34].

At trimester two (TM_2) the hippocampal histological thicknesss of the layers similarly were observed to be dose dependant. High and medium dosage groups were observed to have the most reduced thicknesses of the histological layers than low dosage groups in both lamotrigine and levetiracetam medications. (Figure 4.35).

At trimester three (TM_3), it was observed that the thickness of the hippocampal layers was not affected in the low and medium dose groups in the two medication of lamotrigine and levetiracetam. It was however noted that in the high dosage groups of the two medications, the hippocampal histological layers were remarkably reduced. Across all dosage groups, lamotrigen was onbserved to be associated with more deleterious effects than levetiracetum (Figure 4.36).

The TM1 comparataive histological thicknesses of the hippocampal gyrus

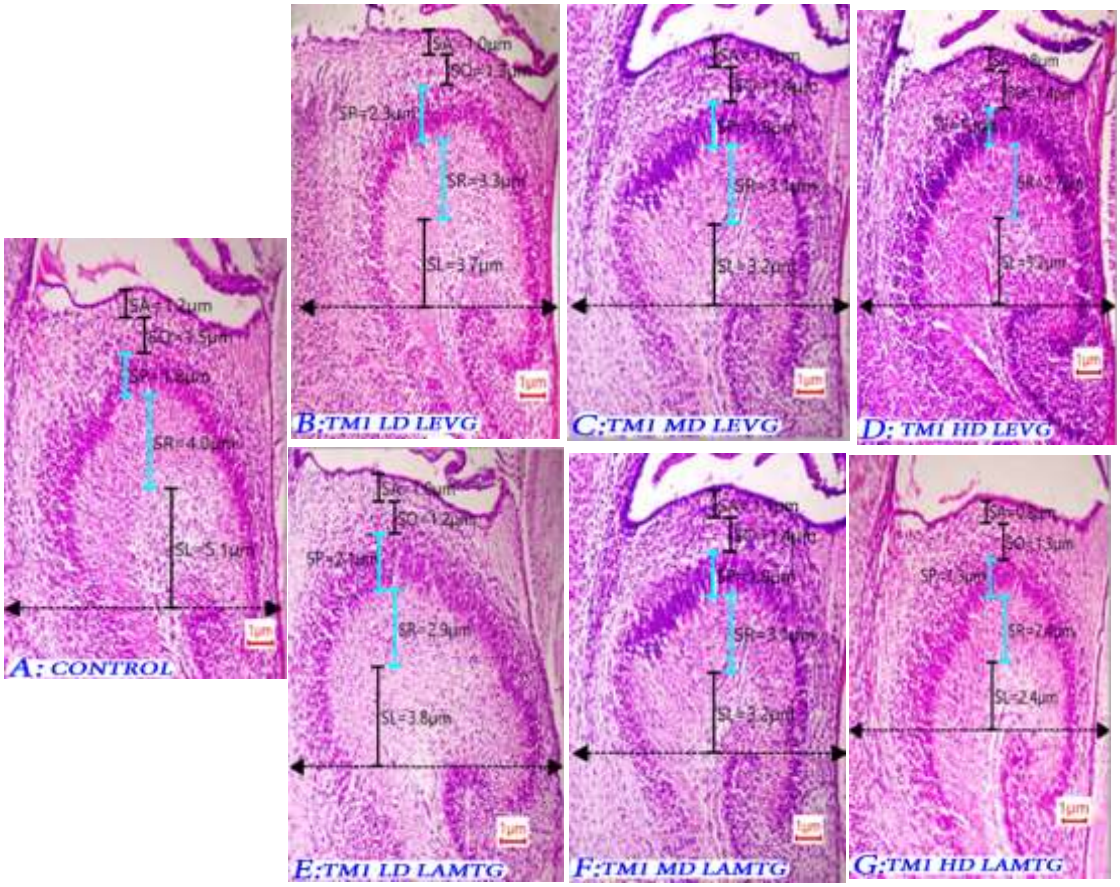

Figure 4.34: The TM1 Comparative Histological Thicknesses of Hippocampal Layers in the Low, Medium, and High Dose Groups of Both the Lamotrigine and Levetiracetam Treated Groups against the Control Group.

Key

*A-control, **B-TMI LD LEVG;** trimester one low dose levetiracetum treated group, **C-TMI MD LEVG;** trimester one medium dose levetiracetum treated group, **D-TMI HD LEVG;** trimester one high dose levetiracetum treated group, **E-TMI LD LEVG;** trimester one low dose lamotrigine treated group, **F-TMI MD LEVG;** trimester one medium dose lamotrigine treated group, **G-TMI HD LEVG;** trimester one high Dose lamotrigine treated group, **SA**-stratum aureus, **SO**-stratum oriens, **SR**-stratum radiatum, **SL**-Stratum lacunosum*

The TM2 comparataive histological thicknesses of the hippocampal gyrus

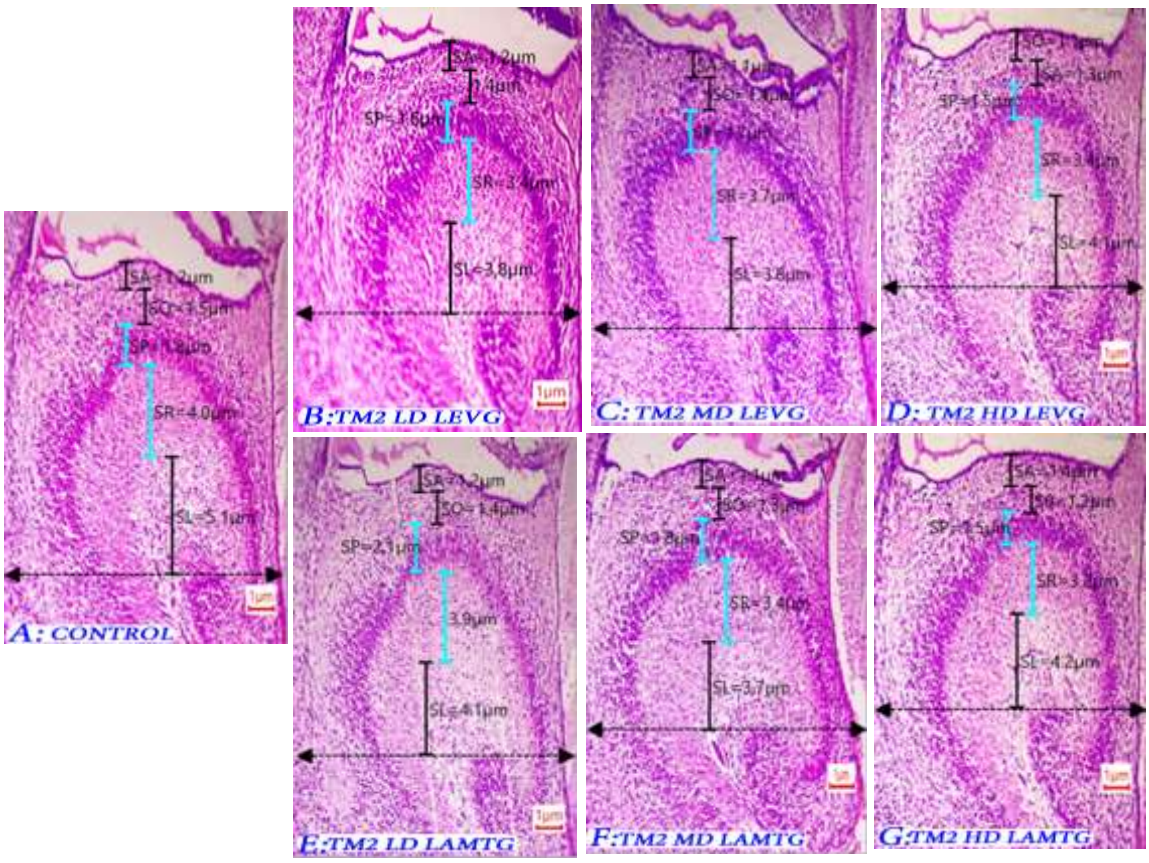

Figure 4.35: The TM2 Comparative Histological Thicknesses of Hippocampal Layers in the Low, Medium, and High Dose Groups of both the Lamotrigine and Levetiracetam Treated Groups against the Control Group.

Key

A*-control,* ***B-TMI LD LEVG;*** *trimester one low dose levetiracetum treated group,* ***C-TMI MD LEVG;*** *trimester one medium dose levetiracetum treated group,* ***D-TMI HD LEVG;*** *trimester one high dose levetiracetum treated group,* ***E-TMI LD LEVG;*** *trimester one low dose lamotrigine treated group,* ***F-TMI MD LEVG;*** *trimester one medium dose lamotrigine treated group,* ***G-TMI HD LEVG;*** *trimester one high Dose lamotrigine treated group,* ***SA****-stratum aureus,* ***SO****-stratum oriens,* ***SR****-stratum radiatum,* ***SL****-Stratum lacunosum*

The TM3 comparataive histological thicknesses of the hippocampal gyrus

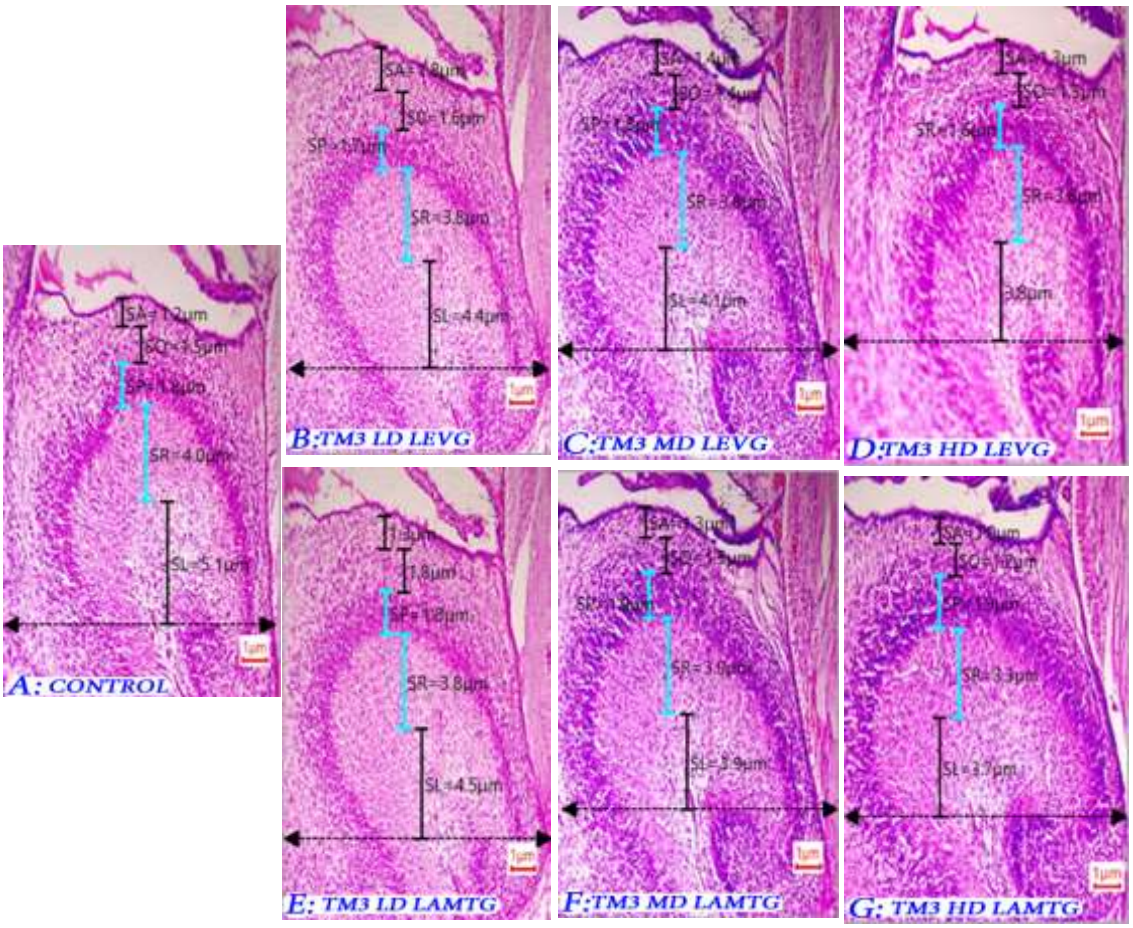

Figure 4.36: The TM3 Comparative Histological Thicknesses of Hippocampal Layers in the Low, Medium, and High Dose Groups of Both the Lamotrigine and Levetiracetam Treated Groups Against the Control Group.

Key

***A**-control, **B-TMI LD LEVG;** trimester one low dose levetiracetum treated group, **C-TMI MD LEVG;** trimester one medium dose levetiracetum treated group, **D-TMI HD LEVG;** trimester one high dose levetiracetum treated group, **E-TMI LD LEVG;** trimester one low dose lamotrigine treated group, **F-TMI MD LEVG;** trimester one medium dose lamotrigine treated group, **G-TMI HD LEVG;** trimester one high Dose lamotrigine treated group, **SA**-stratum aureus, **SO**-stratum oriens, **SR**-stratum radiatum, **SL**-Stratum lacunosum*

4.3.1.5 The Histomophological Results of the Amygdaloid Nucleus and Dentate Gyrus

The amygdaloid nucleus and dentate gyrus forms the fifth group of structurers in the memory circuitry pathway. Dentate gyrus processes the incoming information, and signals hippocampus to encode memory, while amygdaloid nucleus consolidates

longterm-memory related to fear. The histomorphological findings are presented at two levels as follows: -

Level 1: The histological cyto-archietcture of the dentate gyrus and amygdaloid nucleus entailing the cellular density, the cell distributions and the cell sizes.

Level 2. Thickeness of the histological layers of dentate gyrus and amygdaloid nucleus

Leve1: The histo-cyto-arhitecture of the dentate gyrus and amygdaloid nucleus

(i) The molecular layer; the granule cells and the pyramidal cells that are the key cells involved with memory processing in this layer were observed to reduce with increasing dosages of both the two medicines as shown in the photomicrographs, *[Figure 4.37- the cells marked as GC and PC in the three photomicrographs of the molecular/plexiform layer]*

(ii)In the granule layer (GL*):* the granule cells in this layer were simimilary seen to reduce with increasing dosages of the two medicines, (*Figure 4.38-the cells marked as GC in the three photomicrographs of the granular layer]*

(iii)The polymorphic layer (PML), the pyramidal and the stellate cells in this layer were simimilary seen to reduce with increasing dosages of the two medicines, [*Figure 4.39-the cells marked as MC and BC in the three photomicrographs of the polymorphic layer]*

The molecular layer

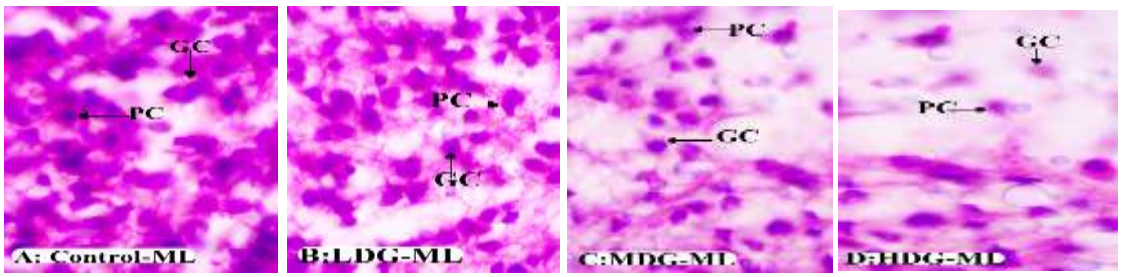

Figure 4.37: The Global Comparative Histo-Cyto-Architecture of the Molecular Layer of Dentate Gyrus and Amygdaloid Nucleus in Low, Medium and High Dosage Groups Against Control

Key

A- ***The granular layer*** *r layer (**LDG-ML**), **C-** Medium dose group-Molecular layer, (**MDG-ML**), **D**- High dose molecular layer (**HDG-ML**), **PC**- pyramidal cell, **GC**-granule cell*

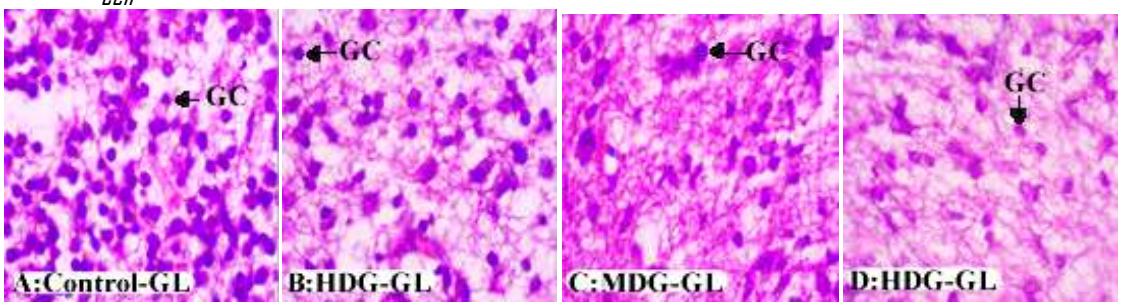

Figure 4.38: The Global Comparative Histo-Cyto-Architecture of the Granularlayer of Dentate Gyrus and Amygdaloid Nucleus in Low, Medium and High Dosage Groups Against Control

Key

***A**-Control –granular layer **(GL)**, **B**-Low dose group granular layer (**LDG-GL**), **C-** Medium dose group-granular layer, (**MDG-GL**), **D**- High dose granular layer (**HDG-GL**), **GC**-granule cell*

The polymorphic layer

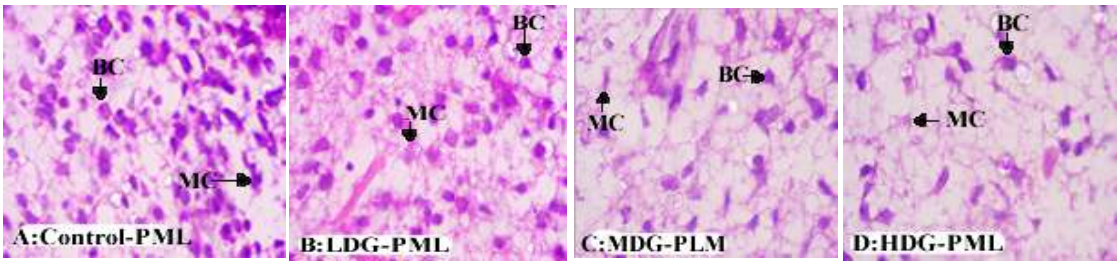

Figure 4.39: The Global Comparative Histo-Cyto-Architecture of the Polymorphiclayer of Dentate Gyrus and Amygdaloid Nucleus in Low, Medium and High Dosage Groups against Control

Key

***A**-Control –granular layer **(GL)**, **B**-Low dose group granular layer (**LDG-GL**), **C-** Medium dose group-granular layer, (**MDG-GL**), **D**- High dose granular layer (**HDG-GL**), **MC**-mossy cell, **BC**-basket cell granular layer, (**MDG-GL**), **D**- High dose granular layer (**HDG-GL**), **GC**-granule cell*

Level 2: the comparative thicknesses of the histological layers of dentate gyrus and amygdaloid nucleus

The comparative thicknesses of histological layers'of dentate gyrus and amygdaloid nucleus are presented as per the trimester (time) of exposure as follows: -

At trimester one (TM_1) it was observed that, the thicknesses of the three histological layers of dentate gyrus and amygdaloid namely; (i) the molecular layer (ML), (ii) the granular layer (GL) and (iii) the polymorphic layer (PML) were all dependant on the dosages exposed. High and medium dosage groups in both lamotrigine and levetiracetam treated groups were associated with the most reduced thickness as compared to low dosage groups. In addition, it was noted that lamotrigen treated groups across all its dosage levels had more deleterious effects than those of the levetiracetam [Figure 4.40].

At trimester two (TM_2) the histological thickness of the three histological layers of dentate gyrus and amygdaloid were observed to similarly portray dose dependency. High and medium dosage groups were observed to have the most reduced thickness of the histological layers than low dosage groups in both lamotrigine and levetiracetam medications. Across all dosage groups, lamotrigen was onbserved to be associated with more deleterious effects in that it was caused more reduction in thicknesses of dentate gyrus and amygdaloid histological layers, than levetiracetum (Figure 4.41).

At trimester three (TM_3), the histological thickness of the three histological layers of dentate gyrus and amygdaloid was observed to be affected only by high dosages of levetiracetam as well as both medium and high dosages of lamotrigine. Low dosage groups in the two medication of lamotrigine and levetiracetam did not show any significance reduction in thicnesses. It was however noted that in the high dosage groups of the two medications, the histological layers were much reduced. Futher, it was observed that lamotrigine treated groups had more reduced thickness than lamotrigine treated groups meaning that lamotrigine had more detrimental effects than levetiracetam (Figure 4.42).

The TM1 comparataive histological thicknesses of dentate gyrus and amygdaloid nucleus

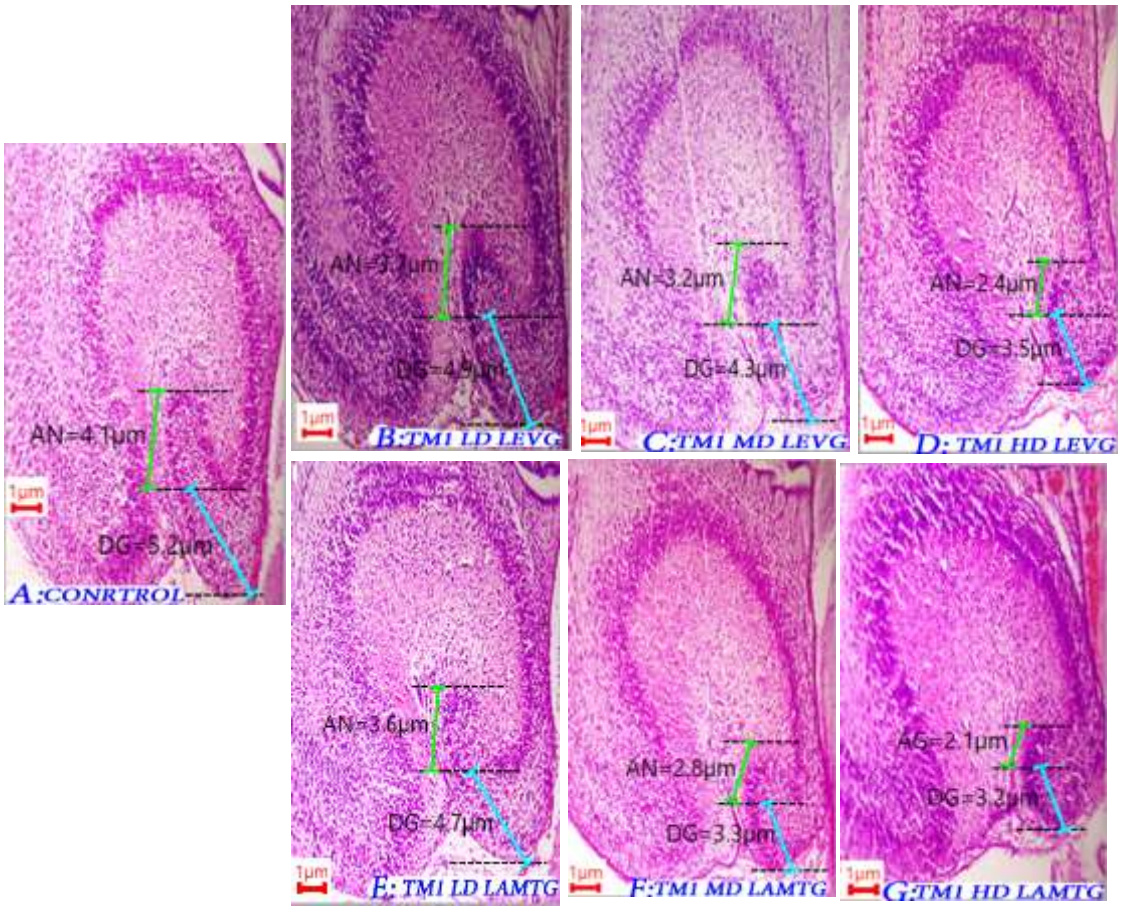

Figure 4.40: The TM1 Comparative Histological Thicknesses of Amygdaloid Nucleus and Dentate Gyrus Histological Layers in the Low, Medium, and High Dose Groups of Both the Lamotrigine and Levetiracetam Treated Groups against the Control Group.

Key

__A__-control, __B-TMI LD LEVG;__ trimester one low dose levetiracetum treated group, __C-TMI MD LEVG;__ trimester one medium dose levetiracetum treated group, __D-TMI HD LEVG;__ trimester one high dose levetiracetum treated group, __E-TMI LD LEVG;__ trimester one low dose lamotrigine treated group, __F-TMI MD LEVG;__ trimester one medium dose lamotrigine treated group, __G-TMI HD LEVG;__ trimester one high Dose amotrigine treated group, __AN__-amygdaloid nucleus, __DG__-dentate gyrus

The TM2 comparataive histological thicknesses of dentate gyrus and amygdaloid nucleus

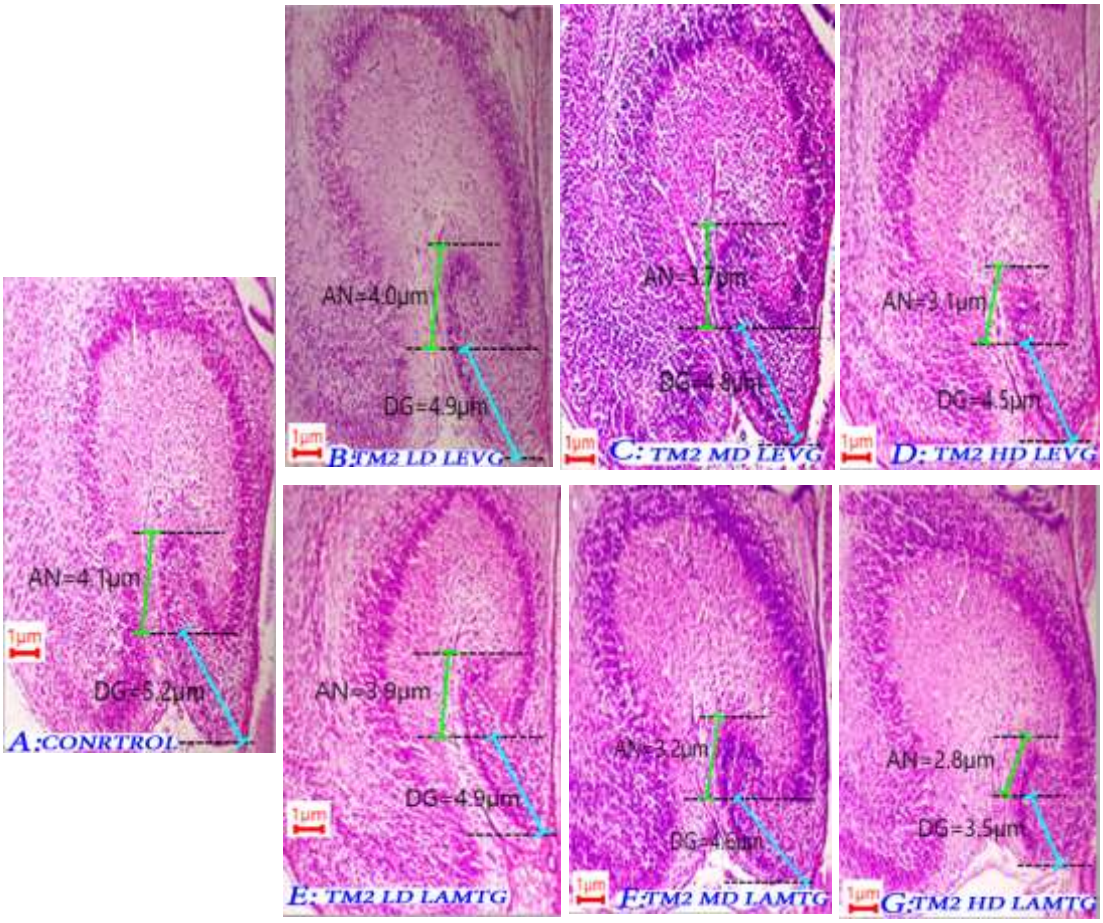

Figure 4.41: The TM2 Comparative Histological Thicknesses of Amygdaloid Nucleus and Dentate Gyrus Histological Layers in the Low, Medium, and High Dose Groups of Both the Lamotrigine and Levetiracetam Treated Groups against the Control Group.

Key

A*-control,* ***B-TMI LD LEVG;*** *trimester one low dose levetiracetum treated group,* ***C-TMI MD LEVG;*** *trimester one medium dose levetiracetum treated group,* ***D-TMI HD LEVG;*** *trimester one high dose levetiracetum treated group,* ***E-TMI LD LEVG;*** *trimester one low dose lamotrigine treated group,* ***F-TMI MD LEVG;*** *trimester one medium dose lamotrigine treated group,* ***G-TMI HD LEVG;*** *trimester one high Dose amotrigine treated group,* ***AN****-amygdaloid nucleus,* ***DG****-dentate gyrus*

The TM3 comparataive histological thicknesses of dentate gyrus and amygdaloid nucleus

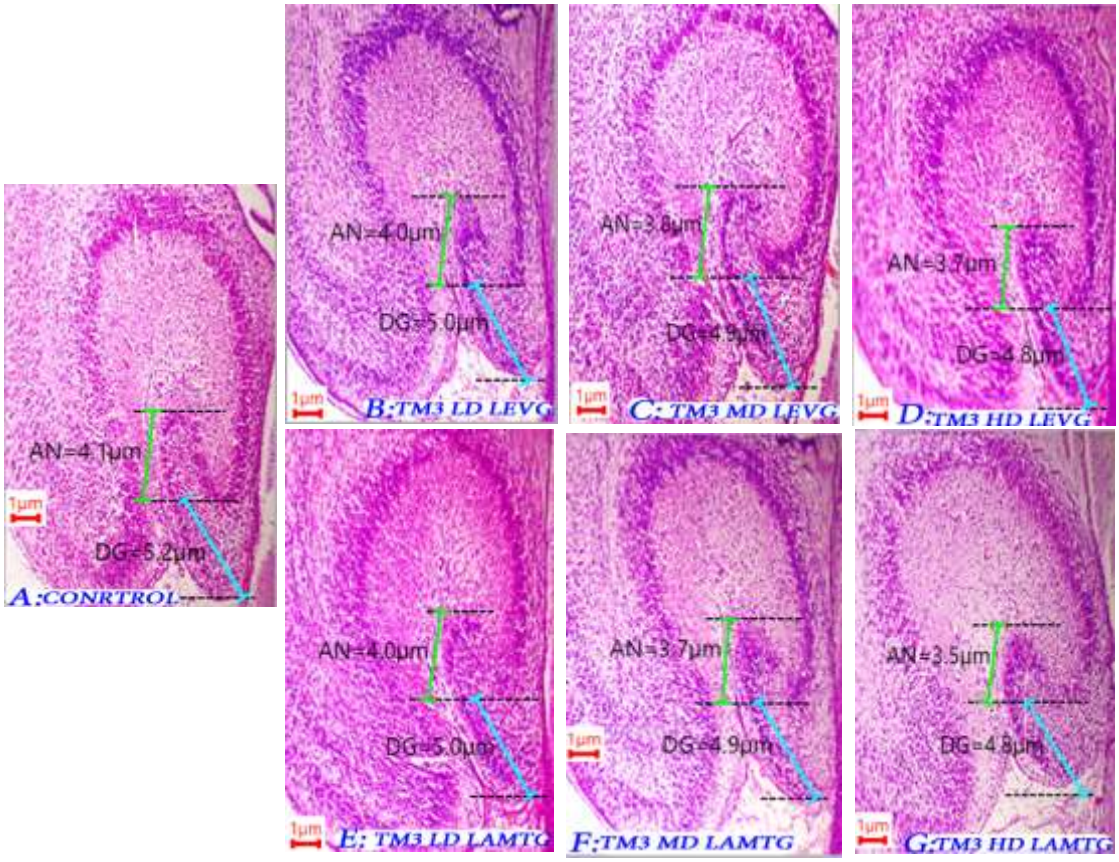

Figure 4.42: The TM3 Comparative Histological Thicknesses of Amygdaloid Nucleus and Dentate Gyrus in the Low, Medium, and High Dose Groups of Both the Lamotrigine and Levetiracetam Treated Groups against the Control Group.

Key

__A__-control, __B-TMI LD LEVG;__ trimester one low dose levetiracetum treated group, __C-TMI MD LEVG;__ trimester one medium dose levetiracetum treated group, __D-TMI HD LEVG;__ trimester one high dose levetiracetum treated group, __E-TMI LD LEVG;__ trimester one low dose lamotrigine treated group, __F-TMI MD LEVG;__ trimester one medium dose lamotrigine treated group, __G-TMI HD LEVG;__ trimester one high Dose amotrigine treated group, __AN__-amygdaloid nucleus, __DG__-dentate gyrus

4.4 The Histostereological Findings

Objective 3: The Comparative Histoquantitative Findings Following Prenatal Exposureto Lamotrigine and Levetiracetam on the Fetal Memory Circuitry Pathways

In follow-up to the principal of teratogenesis that states that "any minor congenital defect observed is usually an indicator of another associated major anomaly" the comparative histostereological findings of the fetal memory circuitry structures was carried at two levels. **The first level** entailed the gross morphometric analaysis of how the two medicines influenced the gross morphometric development of the entire brain (i.e the total brain weights, the brain length and the biparietal brain widths) **while level 2** entailed the histo-stereological assessment of each of the fetal memory circuitory stuctures starting from the prefrontal cortex, the entorhinal cortex, the hippocampus, the subicular complex, the dentate gyrus and the amgdaloid nucleus.

4.4.1 The Comparative Gross Morphometric Findings on how the Two Anticonvulsant Medicines Influenced the Fetal Brain Weight, Length and Widths

To evaluating how the two medicines influenced the gross morphometric development of the fetal brain the following parameters were evaluated, the total brain weights, the occipital-frontalis brain length and the biparietal brain widths and the total brain volumes. As such a univariate, bivariate and multivariate regressional analsyis was carried out by use of ANOVA and MANOVA respectively. This was to determine the deleterious teratogenic contribution of each an individual variable, as well as when they were combined in terms of dosages, drugs and time of exposure (trimesters).

The ANOVA results established that there was a statistically significant reduction in the three morphological brain parameters in both medicines as follows; (i) brain weight (F (18,38) =732.667, P=.001), (ii) brain length, F (18,38) =552.441, *P* =.003) and (iii) brain width, (F (18,38) =332.661, *P* =.001), ***ANOVA (***Table 4.9). The mean reduction was observed to be both dose and time dependent, with the most

deleterious effects being observed at medium and high dosages during TM1 and TM2.

The MANOVA results established that the independent variables that includes; drugs, dosages and trimesters either alone or in combiation of had a contributory role in negatively influencing the observed deleterious effects in causing the reductions in the morphometric sizes and volumes of the fetal brains harvested from the two treatment of groups ascompared with the control (Table 4.9).

On fuher analysis to observe how the two medicines differed globally with each other in influencing the four gross morphometric parameters, it was noted that lmaotrigen in all its three dose levels of low, medium and high casued a more deleterious effects on the total gross morphometric parameters of the fetal brain as compared to the levetiracetum (Table 4.9).

Table 4.9: The Comparative ANOVA Table on How the Two Medicines Influenced the Fetal Brain Gross Morphometric Paremeters of Total Brain Weight, Length, and Width.

The study groups	Study groups and dosage levels.	The time of exposure to treatment	The comparative means of fetal brain weight, length and width for various study groups		
			Mean brain weight (g) ± SD	**Mean brain length (cm) ± SD**	**Mean brain width (cm) ± SD**
Control.	Control (C) (**no treatment)**	None.	1.26±0.04	1.58±0.06	1.32±0.01
Levetiracetam treatment groups	Low dosage group **(103mg/kg/bw)**	Trimester one	1.23±0.04*	1.48±0.06*	1.26±0.06*
		Trimester two	1.24±0.03	1.52±0.03*	1.28±0.02*
		Trimester three	1.25±0.06	1.58±0.01	1.31±0.06
	Medium dosage group **(207mg/kg/bw)**	Trimester one	1.18±0.04*	1.39±0.06*	0.99±0.06*
		Trimester two	1.20±0.07*	1.42±0.03*	1.04±0.07*
		Trimester three	1.21±0.06	1.48±0.02	1.23±0.01*
	L High dosage group **(310 mg/kg/bw)**	Trimester one	1.12±0.03*	1.23±0.01*	1.18±0.05*
		Trimester two	1.13±0.05*	1.30±0.04*	1.15±0.06*
		Trimester three	1.15±0.05*	1.34±0.05*	1.21±0.03*
Lamotrigine treatment groups	Low dosage group **(3mg/kg/bw)**	Trimester two	1.01±0.06*	1.22±0.03*	1.03±0.02*
		Trimester one	1.08±0.03	1.25±0.02*	1.08±0.06*
		Trimester two	1.08±0.01	1.28±0.06	1.09±0.06
	Medium dosage group **(24mg/kg/bw)**	Trimester one	0.94±0.12*	1.13±0.01*	0.94±0.04*
		Trimester two	1.04±0.03*	1.24±0.06*	1.07±0.03*
		Trimester three	1.08±0.06	1.27±0.03	1.08±0.06
	L High dosage group **(52mg/kg/bw)**	Trimester one	0.85±0.02*	1.04±0.06*	0.89±0.03*
		Trimester two	0.95±0.03*	1.15±0.03*	0.96±0.01*
		Trimester three	1.03±0.01*	1.24±0.01*	1.05±0.04*
Overall comparison by ANOVA [F, P values			**F (18,38) =732.667 P=0.001**	**F (18,38) =552.441 P=0.003**	**F (18,38) =332.661 P=0.001**

Key: All values that bear (*) indicates that they depict a statistical significance difference (p<.05), when compared with the control, using one- way ANOVA with Tukey post-hoc multiple comparison t-test

Upon carrying out the fist level of multivariate regression analysis using MANOVA to establish how globally the medicines, drugs and dosages plus thier interaction effects either in two way or in three ways influenced the global mean reduction in the total fetal brain weight, occipital-frontalis length, and the bi-pariatal brain widths, it was notable that the mean reduction in the three fetal brain parameters were contributed at varying proportions (Partial Eta squared (η^2) by the three independent variables as follows;

(i). At the individual levels when each of the individual independent variable of the drug, dose and time] acted alone in influencing the three gross morphometric measurement parameters of gross brain weight, length, width the following were the findings; (a) dugs (F (3, 36) = 1483.511, P<.001); Wilkis' lambda (Λ) =.008; Partial Eta squared (η^2 =.992), (b) dosages (F (6, 72) = 83.840, P<.001); Wilkis' lambda (Λ) =.016; Partial Eta squared (η^2 =.875), and (c) trimesters (F (6,72) = 45.032, P<.001); Wilkis' lambda (Λ) =.044; Partial Eta squared (η^2 =.790), The highest contribution was observed to be from the type of medicine at (99%), then followed by dosages at (88%) and lastly the trimesters effects of exposure at (79%), (Table 4.10).

(ii). At two way combinations i.e the two way intercation effects when each of the two independent variables were combined and their interaction effects evaluated on the global fetal brain gross morphometric measurements, the findings of the two way a combination were as follows i.e (a) drug *dosages, (F (6,72) = 32.061, P<.001); Wilkis 'lambda (Λ) =.074, Eta squared (η^2 =.73), (b) drugs*trimesters, (F (6, 72) =42.834, P=.001); Wilkis'Λ =.043; Partial Eta squared (η^2 =.70) and lastly (c) dosages*trimesters, (F (12,95.539) = 57.053, P=003); Wilkis' lambda (Λ) =.084; Eta squared (η^2 =.83). It was therefore clear that the combinations of the dosages and the trimesters had the highest contribution at 83%, followed by the the combination of the drug and dosages at 73% and finally the drug and trimesters at 70% (Table 4.10).

(ii) In the three-way combinations, i.e when all the three independent variable were all combined together i.e the interaction effects among, [drugs* dosages* trimesters] the findings were as follows, F (12, 95.539) = 24.624, P=.005); Wilkis' lambda (Λ) =.078; Partial Eta squared (η^2 =.63). It was clear that the combinations of the three independent variables had the worst deletious effect when the cominations were done at TM1 and the TM2 (Table 4.10).

Table 4.10: The Level 1 MANOVA Table on How Globally the Two Medicines, Dosages and Trimesters plus Their Interactions Influenced the Three Fetal Fetal BrainMorphological Measurements Parameters

		The multivariate statistical tests parameters applied					
The comparative global effects assessed	**The parameters used**	**MANOVA test statistics (Wilks' Lambda)**	**Statistics (F)**	**Hypothesis degree of freedom**	**Error degree of freedom**	**Sig.<.05**	**Proportion of variance (Partial Eta Squared)**
Assessment of whether or not the observed overall effects were due to drugs (either lamotrigine or levetiracetam)	**Drugs**	.008	1483.511[b]	3.000	36.000	**<.001**	.992
To assess whether or not the observed overall effects were due to varied doses of lamotrigine and levetiracetam	**Dosages**	.016	83.840[b]	6.000	72.000	**<.001**	.875
To assess whether or not the observed overall effects were due to differing trimesters (TM_1, TM_2, &TM_3)	**Trimesters**	.044	45.032[b]	6.000	72.000	**<.001**	.790
To assess whether or not the observed overall effects were due to interaction between varied doses and the drugs	**Drugs * dosages**	.074	32.061[b]	6.000	72.000	**<.001**	.728
To assess whether or not the observed overall effects were due to interaction between drugs and differing trimesters.	**Drugs * trimesters**	.043	42.834[b]	6.000	72.000	**<.001**	.697
To assess whether or not the observed overall effects were due to interaction between dosages with differing trimesters.	**Dosages *trimesters**	.087	57.053[b]	12.000	95.539	**.003**	.828
Whether or not the observed overall effects were due to the two drugs and the dosages as well as the trimesters	**Drugs * dosages * trimesters**	.078	24.624[b]	12.000	95.539	**.005**	.632

Key: (*) indicates interaction effects, while([b])indicates exact statistics using MANOVA

Upon carrying out the second level of MANOVA analysis to establsih how the globally the drugs, dosages and trimesters/time of exposure plus their interations influenced the mean reduction of each of the three fetal brain measurement parameters, it was established that their contributions were as follows;

(i) At one way contributions on how each of the three independent variables of drug, dose and trimesters/time of exposure to the observed fetal brain gross morphometric measurements, the statistical

contributory effects of each an individual independent variable to the three fetal brain gross morphometric parameters collectively at a global level to the three depenedent variables of [(i) fetal brain weight (BW), (ii)brain length (BL) and (iii) brain weight (BW)] was that they each contributed in varied proportions (Partial Eta squared (η^2), with the highest contribution being from the type of drug administered (97%), the dose (94%) and time (89%) (Table 4.11).

(ii) The two-way interaction effects of the drug, dose and time of exposure when combined as follows; (a) drug*dosages, (b)drugs*trimesters &; (c) dosages*trimesters at varied proportionate (Partial Eta squared (η^2), to each of the three fetal brain measurement parameters were found to have statistically significant interaction effects, with the comination of drug and dose having the highest contribution (Table 4.11).

(i) At three way combinations, i.e when the three independent variables of the drugs*dosages*trimesters were acting together, their interaction effects as per the level two MANOVA analaysis was as follows: (a) mean brain weight, (F (4, 38) =13.309, P=0.002; Partial Eta squared (η^2 =.66), (b) mean brain length F (4, 38) =10.265, P<.001; Partial Eta squared (η^2 =.52) and (c) mean brain width (F (4, 38) =.11.641, P=.004; Partial Eta squared (η^2 =.65). It is clear that when the three independent variables were acting together, the worst deleterious effects of the three when acting together was when the time of exposures were at TM1 and the TM2 (Table 4.11).

Table 4.11: The Level 2 MANOVA Table on How Globally, the Drugs, Dosages and Time of Exposure plus Their Interations Influenced Each of the Three (3) Fetal Brain Morphological Measurement Parameters

Tests of Between-Subjects Effects							
Source	**Dependent Variable**	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig[d] (<.05)**	**Partial Eta Squared**
	Brain width	53.500	1	53.500	116619.908	**<.001**	1.000
Drugs	Brain weight	.449	1	.449	1666.566	**<.001**	.978
	Brain length	.572	1	.572	4405.170	**<.001**	.991
	Brain width	.373	1	.373	814.108	**<.001**	.955
Dosages	Brain weight	.110	2	.055	204.939	**<.001**	.915
	Brain length	.254	2	.127	979.881	**<.001**	.981
	Brain width	.261	2	.131	284.863	**<.001**	.937
Trimesters	Brain weight	.060	2	.030	111.413	**<.001**	.854
	Brain length	.099	2	.050	383.044	**<.001**	.953
	Brain width	.107	2	.053	116.199	**<.001**	.859
Drugs * dosages	Brain weight	.001	2	.000	1.105	**.042**	.355
	Brain length	.031	2	.016	121.221	**<.001**	.864
	Brain width	.024	2	.012	26.552	**<.001**	.583
Drugs * trimesters	Brain weight	.025	2	.013	46.802	**<.001**	.711
	Brain length	.007	2	.003	25.851	**<.001**	.576
	Brain width	.008	2	.004	9.171	**.001**	.326
Dosages * trimesters	Brain weight	.006	4	.002	5.895	**.001**	.383
	Brain length	.014	4	.003	26.831	**<.001**	.739
	Brain width	.027	4	.007	14.661	**<.001**	.607
Drugs * dosages * trimesters	Brain weight	.004	4	.001	13.309	**.002**	.658
	Brain length	.005	4	.001	10.265	**<.001**	.519
	Brain width	.013	4	.003	11.641	**.004**	.647

Key: (*) indicates interaction effects

Upon carrying out the level 3 pairwise MANOVA comparative analysis to determine how the two medicines within the same dose groups influenced the three fetal brain morphological measurement parameters, it was notable that, there was a statistical significance difference ($P<.001$) between the same dosage levels of lamotriegen against those of levetiracetum when they were admisntered in the same trimester. in particular, in all dose levels of low, medium and high lamotrigine against the same dose levels of lamotrigine, the effects were more pronounced in the lamotrigine treated groups as compared with the levetiracetum treated groups across the three trimesters (Table 4.12).

Table 4.12: The Level 3 MANOVA Pairwise Comparison Table on How the Two Medicines Influeneced the Three (3) Fetal Brain Morphological Measurement Parameters When Exposed Within the Same Dosages and the Same Trimesters

Multiple/Pairwise Comparisons

Dependent Variable	Dosages (mg/kg bw)	Trimesters	(LEV)	(LAM)	Mean Difference (LEV-LAM)	Std. Error	Sig[d]	95% Confidence Interval for Difference[d] Lower Bound	Upper Bound
Brain Weight (g)	Low	TM_1	LEV	LAM	**.223***	.013	**.000**	.195	.250
		TM_2	LEV	LAM	**.160***	.013	**<.001**	.133	.187
		TM_3	LEV	LAM	**.167***	.013	**<.000**	.140	.194
	Medium	TM_1	LEV	LAM	**.231***	.013	**.011**	.204	.258
		TM_2	LEV	LAM	**.154***	.013	**.003**	.127	.181
		TM_3	LEV	LAM	**.136***	.013	**.001**	.108	.163
	High	TM_1	LEV	LAM	**.272***	.013	**<.001**	.245	.299
		TM_2	LEV	LAM	**.177***	.013	**.001**	.150	.204
		TM_3	LEV	LAM	**.121***	.013	**<.001**	.093	.148
Brain length (cm)	Low	TM_1	LEV	LAM	**.261***	.009	**<.001**	.242	.279
		TM_2	LEV	LAM	**.270***	.009	**<.000**	.251	.289
		TM_3	LEV	LAM	**.270***	.009	**<.001**	.252	.289
	Medium	TM_1	LEV	LAM	**.255***	.009	**.001**	.236	.274
		TM_2	LEV	LAM	**.178***	.009	**.001**	.159	.197
		TM_3	LEV	LAM	**.172***	.009	**.001**	.154	.191
	High	TM_1	LEV	LAM	**.193***	.009	**.003**	.174	.212
		TM_2	LEV	LAM	**.146***	.009	**.002**	.127	.165
		TM_3	LEV	LAM	**.107***	.009	**.001**	.088	.126
Brain width (cm)	Low	TM_1	LEV	LAM	**.226***	.017	**<.001**	.191	.262
		TM_2	LEV	LAM	**.197***	.017	**.003**	.162	.233
		TM_3	LEV	LAM	**.213***	.017	**<.001**	.177	.248
	Medium	TM_1	LEV	LAM	**.240***	.017	**<001**	.205	.276
		TM_2	LEV	LAM	**.144***	.017	**<.001**	.109	.180
		TM_3	LEV	LAM	**.146***	.017	**<.001**	.111	.181
	High	TM_1	LEV	LAM	**.017***	.000	**.002**	.173	.017
		TM_2	LEV	LAM	**.094***	.017	**<.001**	.058	.129
		TM_3	LEV	LAM	**.099***	.017	**<.001**	.063	.134

Key-(*) indicates that the mean difference is significant at .05 level

4.4.2 The Comparative Gross Mophometric Measurement Outcomes of the Fetal Total Brain Volume

In evaluating the teratogenic influences on the total brain voumes, two methods were used; (a) the initial volumes using Archimedes displacement method, and, (b) the terminal total brain volume using Cavalieri point counting method after fixation and taking care of the total mean shrinkage following use of formaldehyde fixatives. This study established in both the Archmedes and the Cavarieli point counting total brain volumes in both the treatment groups of lamotrigine and levetiracetam had stastically

significant lower total brain volumes ($P<.05$) as compared with those of the controls as follows; {(F (18,38) =423.412, $P=.003$) and (F (18,38) =324.653, $P=.001$)} respectively). On assessing the effects of shrinkage on the total brain volumes, it was notable that there was no statistical significance difference, ($P>.063$) in the mean total brain volumes using the two method.

In comparing how the different dosages plus their time of exposure differed between the two medicines, it was noted that the effects of the two medicines in causing reduction of the mean total brain volumes was both dose and time dependent in that; when the doses of the two medicines were increased, they caused subsequent reductions in the mean total brain volumes. On the other hand, with regards to the time of exposure it was noted that the total brain volumes were inversely influenced by the time of exposure in that when treatments were instituted early at trimester one (TM1) and and two (TM2) the total brain volumes reduced appreciably unlike when the treatments were done at trimesr three TM3 (Table 4.13)

Table 4.13: The Comparative ANOVA Table on How the Two Medicines Influenced the Total Fetal Brain Volume

The study groups	Study groups and dosage levels.	The time of exposure to treatment	The comparative means of initial (Archimedes volume, terminal Cavalieri volume and shrinkage for various study groups Mean initial Archimedes brain volume (mm^3) ± SD)	Mean terminal Cavalieri brain volume (mm^3) ± SD)	Mean shrinkage (mm^3) ± SD)
Control.	Control (C) **no treatment**	None.	0.31±0.03	0.314±0.01	0.004±0.03
Levetiracetam treatment groups	Low dosage group **(103mg/kg/bw)**	Trimester one	0.281±0.06*	0.274±0.07*	0.005±0.03
		Trimester two	0.289±0.07	0.288±0.07	0.006±0.01
		Trimester three	0.301±0.01	0.297±0.06	0.008±0.04
	Medium dosage group **(207mg/kg/bw)**	Trimester one	0.258±0.04*	0.256±0.01*	0.006±0.02
		Trimester two	0.271±0.07*	0.264±0.03*	0.007±0.07
		Trimester three	0.281±0.03	0.290±0.06*	0.009±0.05
	High dosage group **(310 mg/kg/bw)**	Trimester one	0.246±0.07*	0.238±0.03*	0.008±0.01
		Trimester two	0.248±0.03*	0.241±0.02*	0.007±0.04
		Trimester three	0.261±0.04*	0.256±0.03*	0.005±0.03
Lamotrigine treatment groups	Low dosage group **(3mg/kg/bw)**	Trimester two	0.269±0.04*	0.264±0.07*	0.004±0.04
		Trimester one	0.278±0.06	0.278±0.05	0.007±0.01
		Trimester two	0.294±0.07	0.290±0.06	0.005±0.07
	Medium dosage group **(24mg/kg/bw)**	Trimester one	0.239±0.04*	0.251±0.02*	0.006±0.06
		Trimester two	0.261±0.07*	0.245±0.07*	0.005±0.07
		Trimester three	0.274±0.03	0.280±0.03*	0.003±0.06
	High dosage group **(52mg/kg/bw)**	Trimester one	0.239±0.04*	0.229±0.07*	0.002±0.03
		Trimester two	0.237±0.06*	0.239±0.05*	0.003±0.07
		Trimester three	0.249±0.02*	0.233±0.04*	0.004±0.04
Overall comparison by ANOVA [F, P values]			**F (18,38) =423.412 P=0.003**	**F (18,38) =324.653 P=0.001**	**F (18,38) =112.543 P=0.073**

Key: *All values that bear (*) indicates that they depict a statistical significance difference (p<.05), when compared with the control, using one-way ANOVA with Tukey post-hoc comparison t-test*

Upon carraying out **the level 1 multivariate analysis using MANOVA** to establish how globally the individual main effects and the interaction effects of drugs, dosages and trimesters influenced the global mean reduction of the total fetal brain volumes, it was noted that the individual main effects of each of the three independent variables of drug, dose and time of exposure, as well as when they were combined in two-ways or three-way interaction effects (*)were statistically significant (P<.05), meaning that they all had a contributory role in causing reduction in the total fetal brain volumes but in varying propotionate manner (Partial Eta squared, η^2), ***MANOVA level*** *1* (table 4.14) as follows;

(i) At individual level, the main contributory effects were as follows; (a) dugs (F (3,36) = 28.634, P<.001); Wilkis' lambda (Λ) =.295; Partial Eta squared (η=.705), (b) dosages (F (6, 72) = 43.948, P<.001); Wilkis' lambda (Λ) =.046; Partial Eta squared (η^2 =.786), and (c) trimesters (F (6,72) = 15.155, P<.001); Wilkis' lambda (Λ) =.046; Partial Eta squared (η^2 =.804), that had the highest contribution (Table 4.14).

(ii) At a two-way combination; the contributory interaction effects were (a) drugs*dosages (F (6,72) = 14. 328, P=.020); Wilkis 'lambda (Λ) =.048; Partial Eta squared (η^2 =.727), (b) drugs*trimesters, (F (6, 72) =12.660 P=0.43); Wilkis'Λ =.072; Partial Eta squared (η^2 =.622) & (c) dosages*trimesters, (F (12,95.539) = 11.195, P=.043); Wilkis' lambda (Λ) =.071; Partial Eta squared (η^2 =.64). The highest contribution was the combination of drugs and dosages (73%, (Table 4.14).

(iii) At three-way combination; the interaction contributory effects among

the three independent variables>drugs*dosages*trimesters (F (12,95.539) = 32.537, P=.008); Wilkis' lambda (Λ) =.041; Partial Eta squared (η^2 =.56), (Table 4.14)

Table 4.14: The Level 1 MANOVA Table on How Globally the Two Medicines, Drugs and Trimesters plus Their Interactions Influenced the Total Fetal Brain Volume

		The multivariate statistical tests parameters applied					
The comparative global effects assessed	**The parameters used**	**MANOVA test statistics (Wilks' Lambda)**	**Statistics (F)**	**Hypothesis degree of freedom**	**Error degree of freedom**	**Sig.<.05**	**Proportion of variance (Partial Eta Squared)**
Assessment of whether or not the observed overall effects were due to drugs (either lamotrigine or levetiracetam)	**Drugs**	.295	28.634[b]	3.000	36.000	**<.001**	.705
To assess whether or not the observed overall effects were due to varied doses of lamotrigine and levetiracetam	**Dosages**	.046	43.948[b]	6.000	72.000	**<.001**	.786
To assess whether or not the observed overall effects were due to differing trimesters (TM_1, TM_2, &TM_3)	**Trimesters**	.195	15.155[b]	6.000	72.000	**<.001**	.804
To assess whether or not the observed overall effects were due to interaction between varied doses and the drugs	**Drugs * dosages**	.048	14.328[b]	6.000	72.000	**.020**	.727
To assess whether or not the observed overall effects were due to interaction between drugs and differing trimesters.	**Drugs * trimesters**	.072	12.660[b]	6.000	72.000	**.043**	.622
To assess whether or not the observed overall effects were due to interaction between dosages with differing trimesters.	**Dosages *trimesters**	.071	11.195[b]	12.000	95.539	**.047**	.638
Whether or not the observed overall effects were due to the two drugs and the dosages as well as the trimesters	**Drugs * dosages * trimesters**	.041	32.537[b]	12.000	95.539	**.008**	.556

Key: (*) indicates interaction effects, while([b])indicates exact statistics using MANOVA

Upon carrying out **the level 2 multivariate analysis** using MANOVA to determine how globally the drugs, doses and trimesters/time of exposure plus their interations (*) influenced the mean reduction in total foetal brain volume by use of either Archimedes' point counting method or terminal Cavalieri point counting method, it was established that their contributions were as follows;

(i) At individual level the contribution effects of the drug, dose and trimesters/time, there was statistically significant contribution ($P<.05$) to total fetal brain volume by use of either Archimedes' point counting method or terminal Cavalieri point counting method at varied proportions (Partial Eta squared, η^2). The highest contribution was from the dosages administered (Table 4.15).

(ii) At two-way interaction effects there was statistically significant contribution as follows; (a) drug*dosages, (b) drugs*trimesters &; (c) dosages*trimesters at varied proportionate (Partial Eta squared (η^2), to the total brain volume, with the comination of drugs and doses having the highest contribution (Table 4.15).

(iii) Statistically significant three-way interaction effects (drugs*dosages*trimesters) as listed (a)initial Archimedes' volume (F (4, 38) =209.353, P=.040; Partial Eta squared (η^2 =.54); (b)terminal Cavalieri volume, (F (4, 38) =12.296, P=.008); Partial Eta squared (η^2 =.63), (c) a non-significance effect on mean shrinkage, (F (4,38) =143.458, P=.163; Partial Eta squared (η^2 =.087), (Table 4.15).

Table 4.15: The Level 2 MANOVA on How the Globally the Drugs, Dosages and the Time of Exposure Plus their Interations Influenced the Total Brain Volume Either By Use of Archimedes Principal or Cavarieli Point Counting Method

Tests of Between-Subjects Effects

Indepenndent Variables	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig[d] (<.05)	Partial Eta Squared
Drugs	Archimedes' volume	.002	1	.002	58.009	<.001	.604
	Cavalieri volume	.002	1	.002	43.261	<.001	.532
	Shrinkage	**1.707E-6**	**1**	**1.707E-6**	**13.308**	**.061**	**.259**
Dosages	Archimedes' volume	.017	2	.008	281.852	<.001	.937
	Cavalieri volume	.018	2	.009	239.658	<.001	.927
	Shrinkage	**1.893E-7**	**2**	**9.463E-8**	**.738**	**.485**	**.037**
Trimesters	Archimedes' volume	.003	2	.002	54.117	<.001	.740
	Cavalieri volume	.004	2	.002	53.564	<.001	.738
	Shrinkage	**1.593E-8**	**2**	**7.963E-9**	**.062**	**.940**	**.003**
Drugs * Dosages	Archimedes' volume	3.060E-5	2	1.530E-5	42.517	.001	.526
	Cavalieri volume	3.863E-5	2	1.931E-5	38.510	.004	.626
	Shrinkage	1.444E-8	**2**	**7.222E-9**	**.056**	**.945**	**.003**
Drugs * Trimesters	Archimedes' volume	.000	2	.000	3.793	.031	.166
	Cavalieri volume	2.336E-5	2	1.168E-5	46.309	.036	.516
	Shrinkage	**1.900E-7**	**2**	**9.500E-8**	**.741**	**.484**	**.038**
Dosages * Trimesters	Archimedes' volume	6.833E-5	4	1.708E-5	44.577	.001	.557
	Cavalieri volume	.000	4	3.253E-5	39.859	.007	.483
	Shrinkage	**1.007E-6**	**4**	**2.519E-7**	**1.964**	**.110**	**.171**
Drugs * Dosages * Trimesters	Archimedes' volume	4.181E-5	4	1.045E-5	209.353	.040	.536
	Cavalieri volume	4.488E-5	4	1.122E-5	12.296	.008	.630
	Shrinkage	**4.622E-7**	**4**	**1.156E-7**	**.901**	**.163**	**.087**

Key: (*) indicates interaction effects

Upon doing the pairwise comparisons to determine how the two medicines influenced the total fetal brain volumes in the same dosage levels using MANOVA, it was notable that, the lamotrigen treated groups across all its dosage levels, the total fetal brain volumes were statistically significant lower (P<.05) as compared with those of the levetiracetum treated groups (Table 4.16)

Table 4.16: The Level 3 MANOVA Pairwise Comparison Table on How the Two Medicines Influenced the Total Fetal Brain Volume Parameters When Exposed Within the Same Dosages and the Same Trimesters

Multiple/Pairwise Comparisons

Dependent Variables	Dosages	Trimesters	LEV	LAM	Mean Difference (LEV-LAM)	Std. Error	Sig[d] <.05	95% Confidence Interval for Difference[d] Lower Bound	Upper Bound
		TM1	LEV	LAM	**.016***	.004	**.001**	.007	.025
	Low	TM2	LEV	LAM	**.008***	.004	**.042**	.001	.017
		TM3	LEV	LAM	**.007***	.004	**.027**	.002	.016
		TM1	LEV	LAM	**.015***	.004	**.002**	.006	.024
Initial brain volume	Medium	TM2	LEV	LAM	**.014***	.004	**.004**	.005	.023
		TM3	LEV	LAM	**.002***	.004	**.009**	.007	.011
		TM1	LEV	LAM	**.017***	.004	**<.001**	.008	.026
	High	TM2	LEV	LAM	**.014***	.004	**.003**	.005	.023
		TM3	LEV	LAM	**.009***	.004	**.043**	<.001	.018
		TM1	LEV	LAM	**.010***	.005	**.036**	<.001	.020
	Low	TM2	LEV	LAM	**.010***	.005	**.006**	.001	.020
		TM3	LEV	LAM	**.006***	.005	**.005**	.004	.017
		TM1	LEV	LAM	**.014***	.005	**.010**	.004	.024
Terminal brain volume	Medium	TM2	LEV	LAM	**.012***	.005	**.021**	.002	.022
		TM3	LEV	LAM	**.011***	.005	**.030**	.001	.021
		TM1	LEV	LAM	**.015***	.005	**.006**	.005	.025
	High	TM2	LEV	LAM	**.007***	.005	**.006**	.003	.017
		TM3	LEV	LAM	**.014***	.005	**.007**	.004	.024
		TM1	LEV	LAM	**.000***	<.001	**.008**	<.001	.001
	Low	TM2	LEV	LAM	**.000***	<.001	**.008**	<.001	.001
		TM3	LEV	LAM	**.001***	<.001	**.022**	<.001	.001
		TM1	LEV	LAM	**.001***	<.001	**<.001**	.001	.001
Shrinkage	Medium	TM2	LEV	LAM	**.000***	<.001	**.001**	.001	.001
		TM3	LEV	LAM	**.000***	<.001	**.019**	<.001	.001
		TM1	LEV	LAM	**.001***	<.001	**.047**	<.001	.001
	High	TM2	LEV	LAM	**.000***	<.001	**.021**	<.001	.001
		TM3	LEV	LAM	**.000***	<.001	**.002**	<.001	.001

Key-(*) indicates that the mean difference is significant at .05 level

4.4.3 The Comparative Histostereiological Findings on How the Two-Anticonvulsant Medicines Influenced each of the Fetal Memory Circuitry Structures.

The histostereological findings are presented along the way the fetal memory circuitry structures are organized starting with the prefrontal cortex, then the

entorhinal cortex, the hippocampus, the sabiculum, the dentate gyrus and the amygdaloid nucleus as follows: -

4.4.3.1 The Comparative Histostereiological Effects on the the Pre-Frontal Cortex:

In assessing the histostereological effects on how the two anticonvulsant medicines influenced the histology of the pre-frontal cortical layers, the volume densities of the key memory cells plus the corresponding histological thicknesses of each of the six histological layers of prefrental cortex were calaculated together using the cavalieri point counting method. The six layers of the prefrontal cortex included; (I) the plexiform molecular/layer (ML), (II) outer granular (OG) and, (III) the outer pyramidal (OP) layers, (IV) the inner granular layer (IG), (V) the inner pyramidal (IP), and (VI) the multifom layer (ML) layer. The univerate, bivariate and multivariate regression analysis was done by use of ANOVA and MANOVA followed by Turkey post-hoc multiple comparative t-tests, to establish how the reductions in cellular numbers, plus the cell volume desnities subsequently influenced the overll all volume densities per each of the prefrontal histological layer.

It was observd that the reduction in the volume density of the key meory cells including the pyramidal, stellate and the granules cells had a direct proportionate reduction in the volume densites of the corresponding histological layers of the prefrontal cortex. This reduction in voulume densites were also noted to cut-across all the dose levels for both medicines and particulary more pronounced with the lamotrigine treated groups at TM_1 and TM_2 as follows (I) **(ML**) (18,38) =322.463,***P*=0.011**) (II)outer granular layer **(OGL**) (F(18,38)=365.635,***P*=.001),** (III) outer pyramidal layer **(OPL)** (F(18,38)=251.009,***P*=.001**), (IV)inner granular layer **(IGL)** (F(18,38)=317.717,***P*=.011)** (V)inner pyramidal layer **(IPL)** (F(18,38)=125.321,***P*=.013),** and (VI) multiform layer **(MTL)** (F(18,38) =252.212,***P*=.001).**

In comparing how the two medicines differed from each other, it was observed that in the lamotrigine treated groups, the mean volume densities of the prefrontal cortical layers were observed to be significaly lower or lather they were affected more than

those of the levetiracetum treated groups particulary when the treatments were done at TM_1 and TM_2. At TM3 there was no marked statistical significance difference ($P<.05$) betweeen the efefcst seen between the lamotrigen and the levetiracetum treated groups (Table 4.17).

Table 4.17: The Comparative ANOVA Table on How the Two Medicines Influenced the Volume Density of the Prefrontal Cortex

The study group	Study groups and dosage levels.	The time of exposure to treatment	The comparative mean volume density of molecular layer, striatum sterale, external principal striatum, lamina desiccant, internal principal striatum and multiform layer for various study groups					
			Mean molecular layer (mm^3) ± SD	Mean striatum sterale (mm^3)± SD)	Mean external principal striatum (mm^3)± SD)	Mean lamina desiccant (mm^3)± SD)	Mean internal principal striatum (mm^3) ± SD)	Mean multiform layer (mm^3) ± SD)
C	Control (C) (**no treatment)**	None.	0.016±0.03	0.011±0.13	0.008±0.07	0.009±0.01	0.008±0.03	0.007±0.01
LEV	Low Dosage group **(103mg/kg/bw)**	TM1	0.010±0.07*	0.007±0.03*	0.006±0.03*	0.007±0.05*	0.004±0.03*	0.004±0.07*
		TM2	0.011±0.06	0.009±0.07	0.007±0.07	0.009±0.06*	0.006±0.03	0.005±0.03*
		TM3	0.015±0.03	0.010±0.06	0.008±0.04	0.009±0.03	0.006±0.06	0.005±0.06
	Medium dosage group **(207mg/kg/bw)**	TM1	0.009±0.02*	0.007±0.01*	0.005±0.01*	0.006±0.02*	0.003±0.01*	0.003±0.03*
		TM2	0.010±0.03*	0.008±0.07*	0.006±0.07*	0.008±0.07*	0.004±0.06*	0.003±0.02*
		TM3	0.012±0.06	0.009±0.03	0.007±0.02*	0.008±0.03*	0.004±0.07	0.003±0.04*
	High dosage group **(310 mg/kg/bw)**	TM1	0.008±0.04*	0.006±0.01*	0.005±0.02*	0.005±0.06*	0.002±0.01*	0.001±0.07*
		TM2	0.009±0.07*	0.007±0.03*	0.006±0.07*	0.007±0.02*	0.003±0.03*	0.003±0.01*
		TM3	0.010±0.03*	0.007±0.06*	0.006±0.05*	0.006±0.03*	0.004±0.07*	0.003±0.04*
LLAM	Low dosage group **(3mg/kg/bw)**	TM1	0.009±0.07*	0.007±0.03*	0.005±0.01*	0.006±0.02*	0.003±0.04*	0.004±0.07*
		TM2	0.010±0.03	0.008±0.02	0.006±0.04*	0.008±0.06*	0.005±0.07*	0.004±0.03
		TM3	0.014±0.04	0.009±0.03	0.007±0.03	0.008±0.04	0.005±0.03	0.004±0.04
	Medium dosage group **(24mg/kg/bw)**	TM1	0.009±0.04*	0.006±0.03*	0.004±0.04*	0.006±0.04*	0.003±0.03*	0.002±0.04*
		TM2	0.010±0.03*	0.007±0.05*	0.005±0.03*	0.007±0.03*	0.004±0.05*	0.003±0.03*
		TM3	0.011±0.07	0.008±0.03	0.006±0.01*	0.006±0.07*	0.004±0.03*	0.003±0.01*
	High dosage group **(52mg/kg/bw)**	TM1	0.007±0.04*	0.005±0.02*	0.004±0.06*	0.004±0.04*	0.001±0.02*	0.001±0.01*
		TM2	0.008±0.07*	0.006±0.07*	0.005±0.07*	0.006±0.03*	0.002±0.07*	0.002±0.03*
		TM3	0.009±0.03*	0.006±0.03*	0.005±0.06*	0.006±0.07*	0.003±0.04*	0.002±0.06*
Overall comparison by ANOVA [F,P values]			**F (18,38) =269.322 P=0.001**	**F (18,38) =311.328 P=0.012**	**F (18,38) =532.603 P=0.001**	**F (18,38) =381.262 P=0.011**	**F (18,38) =562.342 P=0.003**	**F (18,38) =558.332 P=0.001**

Key: All values that bear (*) indicates that they depict a statistical significance difference (p<.05), when compared with the control, using one- way ANOVA with Tukey post-hoc multiple comparison t-test

Upon carrying out the **level 1 MANOVA** alaysis to find out on how globally, the two medicines plus their interactions globally influenced the volume density of the prefrontal cortex, there was an observed statistically significant diffrences on the

individual main effects, two-way and three-way interaction effects (*) (Partial Eta squared (η^2) as follows;

(i) At individual level levels the observed individual main effects of; (a) dugs (F (6, 33) = 18.361, P<.001); Wilkis' lambda (Λ) =.231; Partial Eta squared (η^2 =.769), (b) dosages (F (12, 66) = 27.354, P<.001); Wilkis' lambda (Λ) =.028; Partial Eta squared (η^2 =.833), and (c) trimesters (F (12,66) = 9.759, P<.001); Wilkis' lambda (Λ) =.130; Partial Eta squared (η^2 =.640), with the dosages having the highest contribution (83%), (Table 4.18).

(ii) At two-way statistically the observed interaction effects between; (a) drugs*dosages, (F (12,66) =5.764, P<.001); Wilkis 'lambda (Λ) =.238; Eta squared (η^2 =.542), (b) drugs*trimesters, (F (12,66) = 1.067, P<.001); Wilkis' lambda (Λ) =.702; Partial Eta squared (η^2 =.162), (c) dosages*trimesters, (F (24,166.333) =4.4835, P=0.001); Wilkis'Λ =.102; Partial Eta squared (η^2 =.435) with the highest contributin being combination of drugs and dosages (54%), (Table 4.18).

At three-way interaction when all three idependent varaibles were combined the observed effects of drugs*dosages*trimesters, (F (24,116.333) = 2.899, P<.001); Wilkis' lambda (Λ) =.195; Partial Eta squared (η^2 =.336) (Table 4.18).

Table 4.18: The Level 1 MANOVA Table on How Globally the Two Medicines, Dosages and Trimesters plus Their Interactions Influenced the Volume Density of the Prefrontal Cortex

		The multivariate statistical tests parameters applied					
The comparative global effects assessed	**The parameters used**	**MANOVA test statistics (Wilks' Lambda)**	**Statistics (F)**	**Hypothes is degree of freedom**	**Error degree of freedom**	**Sig.<. 05**	**Proportion of variance (Partial Eta Squared)**
Assessment of whether or not the observed overall effects were due to drugs (either lamotrigine or levetiracetam)	**Drugs**	.231	18.361[b]	6.000	33.000	**<.001**	.769
To assess whether or not the observed overall effects were due to varied doses of lamotrigine and levetiracetam	**Dosages**	.028	27.354[b]	12.000	66.000	**<.001**	.833
To assess whether or not the observed overall effects were due to differing trimesters (TM_1, TM_2, &TM_3)	**Trimesters**	.130	9.759[b]	12.000	66.000	**<.001**	.640
To assess whether or not the observed overall effects were due to interaction between varied doses and the drugs	**Drugs * dosages**	.238	5.764[b]	12.000	66.000	**<.001**	.542
To assess whether or not the observed overall effects were due to interaction between drugs and differing trimesters.	**Drugs * trimesters**	.702	1.067[b]	12.000	66.000	**<.001**	.162
To assess whether or not the observed overall effects were due to interaction between dosages with differing trimesters.	**Dosages *trimesters**	.102	4.4835[b]	24.000	166.333	**<.001**	.435
Whether or not the observed overall effects were due to the two drugs and the dosages as well as the trimesters	**Drugs * dosages * trimesters**	.195	2.899[b]	24.000	116.333	**<.001**	.336

Key: (*) indicates interaction effects, while([b])indicates exact statistics using MANOVA

Upon carrying out the **level II MANOVA** alaysis to find out how globally, the independent variable of the drug, dose and time of exposure plus their interations influenced the volume density of each of the histological layers of the prefrontal cortex, it was established that their contributions at individual levels, or when they were combined at two ways or three ways were as follows;

(i) The statistically significant contribution of the individual independent variable of drug, dose and trimesters/time of exposure (P<.05) to the volume density of the prefrontal cortical layers at varied proportions

(Partial Eta squared, η^2). The highest contribution was observed to be from the dosages administered (Table 4.19).

(iv) The two-way interaction effects of the drug, dose and time of exposure when combined as follows; (a) drug*dosages, (b)drugs*trimesters at varied proportionate (Partial Eta squared (η^2), for the outer five prefrontal cortical layers (layers I, II, III, IV & V) (P<.001), and a non-significant two-way interaction effects between dosages*trimesters for the last layer (layer VI; multiform layer) (F (4, 38) =.908, P<.469; Partial Eta squared (η^2 =.087) (Table 4.19).

(v) Statistically significant three-way interaction effects among drugs*dosages*trimesters for layers I, II, III, IV&V; (I) molecular layer (ML), (F (4, 38) =2.656, P=.047; Partial Eta squared (η^2 =.519); (II) outer-granular layer (OGL) (F (4, 38) =1.827, P=.014; Partial Eta squared (η^2 =.161). (II) outer pyramidal layer (OPL) (F (4, 38) =.1.220, P=.008; Partial Eta squared (η^2 =.544); (IV) inner granular layer (IGL), (F (4, 38) =1.444, P=.038; Partial Eta squared (η^2 =.43); (V) inner pyramidal layer (F (4, 38) =1.217, P=.020; Partial Eta squared (η^2 =.414); and a non-significant effects layer VI (multiform layer) (MTL), (F (4, 38) =.1.101, P=0.370; Partial Eta squared (η^2 =.104) (Table 4.19)

Table 4.19: The Level 2 MANOVA on How Fglobally, the Drugs, Dosages and Time of Exposure Plus their Interations Influenced the Volume Density of Each of the Prefrontal Cortical Layers

Tests of Between-Subjects Effects

Independent Variables	Prefrontal Cortical layers	Type III Sum of Squares	df	Mean Square	F	Sig[d] (<.05)	Partial Eta Squared
	Molecular	1.788E-6	1	1.788E-6	76.130	**.020**	.667
	0uter granular	9.927E-6	1	9.927E-6	76.813	**.046**	.369
Drugs	Outer pyramidal	5.629E-5	1	5.629E-5	2.989	**.002**	.730
	Inner granular	9.744E-6	1	9.744E-6	91.601	**<.001**	.707
	Inner pyramidal	4.980E-6	1	4.980E-6	76.279	**<.001**	.667
	Multiform layer	1.696E-5	1	1.696E-5	48.892	**<.001**	.563
	Molecular	1.333E-5	2	6.664E-6	283.699	**<.001**	.937
	0uter granular	7.262E-5	2	3.631E-5	280.937	**<.001**	.937
Dosages	Outer pyramidal	.000	2	8.716E-5	4.629	**.016**	.196
	Inner granular	9.575E-5	2	4.788E-5	450.064	**<.001**	.959
	Inner pyramidal	3.185E-5	2	1.592E-5	243.880	**<.001**	.928
	Multiform layer	.000	2	.000	340.891	**<.001**	.947
	Molecular	1.961E-6	2	9.807E-7	41.746	**.047**	.207
	0uter granular	1.091E-5	2	5.455E-6	42.206	**<.001**	.690
Trimesters	Outer pyramidal	1.484E-5	2	7.422E-6	.394	**.007**	.620
	Inner granular	1.242E-5	2	6.209E-6	58.369	**<.001**	.754
	Inner pyramidal	6.009E-6	2	3.004E-6	46.014	**<.001**	.708
	Multiform layer	2.920E-5	2	1.460E-5	42.094	**<.001**	.689
Drugs *	Molecular	1.679E-7	2	8.395E-8	3.574	**.048**	.158
Dosages	0uter granular	9.745E-7	2	4.872E-7	3.770	**.032**	.166
	Outer pyramidal	3.079E-5	2	1.539E-5	.818	**.009**	.410
	Inner granular	5.062E-7	2	2.531E-7	2.379	**.006**	.311
	Inner pyramidal	1.790E-6	2	8.952E-7	13.711	**<.001**	.419
	Multiform layer	2.116E-6	2	1.058E-6	3.050	**.059**	.138
	Molecular	7.978E-8	2	3.989E-8	1.698	**.017**	.082
Drugs *	0uter granular	3.886E-7	2	1.943E-7	1.503	**.035**	.073
Trimesters	Outer pyramidal	3.597E-5	2	1.799E-5	.955	**.004**	.480
	Inner granular	1.789E-7	2	8.944E-8	.041	**.039**	.420
	Inner pyramidal	4.560E-7	2	2.280E-7	3.492	**.041**	.155
	Multiform layer	1.365E-7	2	6.825E-8	.197	**.022**	.500
	Molecular	2.520E-7	4	6.301E-8	2.682	**.046**	.220
	0uter granular	1.275E-6	4	3.186E-7	2.465	**.011**	.206
Dosages *	Outer pyramidal	7.509E-5	4	1.877E-5	.997	**.021**	.095
Trimesters	Inner granular	1.932E-6	4	4.829E-7	4.539	**.004**	.323
	Inner pyramidal	3.391E-6	4	8.477E-7	12.983	**<.001**	.577
	Multiform layer	1.788E-7	4	4.470E-8	.129	**.001**	.713
	Molecular	6.943E-7	4	1.736E-7	2.658	**.047**	.519
Drugs *	0uter granular	2.535E-6	4	6.338E-7	1.827	**.014**	.161
Dosages *	Outer pyramidal	9.191E-5	4	2.298E-5	1.220	**.008**	.544
Trimesters	Inner granular	6.144E-7	4	1.536E-7	1.444	**.038**	.432
	Inner pyramidal	1.144E-7	4	2.859E-8	1.217	**.020**	.414
	Multiform layer	**5.692E-7**	**4**	**1.423E-7**	**1.101**	**.370**	**.104**

Key: (*) indicates interaction effects

Upon carrying **out level III MANOVA analysis** on **the pairwise comparisons** to find out how the two medicines influenced the volume density of the prefrontal cortex within the same dosage levels, it was notable that, there was a statistical

significance difference (P<.05) between lamotrigen and the levetiracetam treated groups.

In comparing all the dose levels of low, medium and high between the two medicines, the lamotrigine had more significant deleterious effects as shown by the mean differences of (LEV-LAM) (Table 4.20).

Table 4.20: The Level 3 MANOVA Pairwise Comparison Table on How the Two Medicines Influenced the Volume Density of the Prefrontal Cortex When exposed within the Same Dosages and the Same Trimesters

Multiple/Pairwise Comparisons									
								95% Confidence Interval for Difference[d]	
Dependent Variable (prefrontal cortical layers)	**Dosages (mg/kg bw)**	**Trimesters**	**Levetiracetam (LEV)**	**Lamotrigine (LAM)**	**Mean Difference (LEV-LAM)**	**Std. Error**	**Sig[d] (<.05)**	**Lower Bound**	**Upper Bound**
Molecular layer	Low	TM1	LEV	LAM	**.000***	<.001	**.033**	8.37E-5	.000
		TM2	LEV	LAM	**<.001***	<.001	**.043**	3.84E-6	.001
		TM3	LEV	LAM	**.000***	<.001	**.042**	3.71E-5	.000
	Medium	TM1	LEV	LAM	**.001***	<.001	**<.001**	<.001	.001
		TM2	LEV	LAM	**.001***	<.001	**<.001**	<.0001	.001
		TM3	LEV	LAM	**<.001***	<.001	**.034**	2.207E-5	.001
	High	TM1	LEV	LAM	**.001***	<.001	**<.001**	1<.000	.001
		TM2	LEV	LAM	**<.001***	<.001	**.033**	2.298E-5	.001
Outer granular layer		TM3	LEV	LAM	**<.001***	<.001	**.013**	7.308E-5	.001
	Low	TM1	LEV	LAM	**<.001***	<.001	**.015**	<.001	.001
		TM2	LEV	LAM	**.001***	<.001	**.005**	1.27E-5	.001
		TM3	LEV	LAM	**.001***	<.001	**.004**	8.99E-5	.001
	Medium	TM1	LEV	LAM	**.001***	<.001	**<.001**	.001	.002
		TM2	LEV	LAM	**.001***	<.001	**.000**	.001	.002
		TM3	LEV	LAM	**.001***	<.001	**.019**	<.001	.001
	High	TM1	LEV	LAM	**.001***	<.001	**<.001**	.001	.002
		TM2	LEV	LAM	**.001***	<.001	**.034**	5.052E-5	.001
		TM3	LEV	LAM	**.001***	<.001	**.013**	<.001	.001
Outer pyramidal layer	Low	TM1	LEV	LAM	**<.001***	.004	**.024**	.007	.008
		TM2	LEV	LAM	**<.001***	.004	**.009**	.007	.008
		TM3	LEV	LAM	**.012***	.004	**.002**	.004	.019
	Medium	TM1	LEV	LAM	**.002***	.004	**.017**	.005	.009
		TM2	LEV	LAM	**.001***	.004	**.005**	.006	.008
		TM3	LEV	LAM	**.001***	.004	**.007**	.006	.008
	High	TM1	LEV	LAM	**.001***	.004	**.003**	.006	.008
		TM2	LEV	LAM	**.001***	.004	**.007**	.007	.008
		TM3	LEV	LAM	**.001***	.004	**.005**	.007	.008
Inner granular	Low	TM1	LEV	LAM	**<.001***	<.001	**.047**	-8.61E-	.001

layer								5	
		TM2	LEV	LAM	**.001***	<.001	**.017**	<.001	.001
		TM3	LEV	LAM	**.001***	<.001	**.013**	<.001	.001
	Medium	TM1	LEV	LAM	**.001***	<.001	**.001**	<.001	.001
		TM2	LEV	LAM	**.001***	<.001	**.001**	<.001	.001
		TM3	LEV	LAM	**.001***	<.001	**.011**	<.001	.001
	High	TM1	LEV	LAM	**.002***	<.001	**<.001**	<.001	.002
		TM2	LEV	LAM	**.001***	<.001	**.009**	<.001	.001
		TM3	LEV	LAM	**.001***	<.001	**.002**	<.001	.001
Inner pyramidal	Low	TM1	LEV	LAM	**<.001***	<.001	**.003**	<.001	.001
		TM2	LEV	LAM	**<.001***	<.001	**.053**	6.53E-6	.001
		TM3	LEV	LAM	**1.765E-7***	<.001	**.009**	<.001	.000
	Medium	TM1	LEV	LAM	**.002***	<.001	**<.001**	.001	.002
		TM2	LEV	LAM	**.001***	<.001	**<.001**	.001	.001
		TM3	LEV	LAM	**.001***	<.001	**.009**	<.001	.001
	High	TM1	LEV	LAM	**.000***	<.001	**.034**	3.777E-5	.001
		TM2	LEV	LAM	**.000***	<.001	**.033**	3.818E-5	.001
		TM3	LEV	LAM	**.001***	<.001	**.013**	<.001	.001
Multiform Layer	Low	TM1	LEV	LAM	**.001***	<.001	**.016**	.000	.002
		TM2	LEV	LAM	**.001***	<.001	**.045**	2.445E-5	.002
		TM3	LEV	LAM	**.000***	<.001	**.006**	.001	.001
	Medium	TM1	LEV	LAM	**.001***	<.001	**.006**	<.001	.002
		TM2	LEV	LAM	**.001***	<.001	**.007**	<.001	.002
		TM3	LEV	LAM	**.001***	<.001	**.007**	<.001	.002
	Medium	TM1	LEV	LAM	**.002***	<.001	**<.001**	.001	.003
		TM2	LEV	LAM	**.001***	<.001	**.027**	<.001	.002
		TM3	LEV	LAM	**.001***	<.001	**.010**	<.001	.002

Key-(*) indicates that the mean difference is significant at .05 level

4.4.3.2 The Comparative Histostereiological Effects of the Two Medicines on the the Entorhinal Cortex

In assessing how the two anticonvulsant mendicines i.e lamotrigine or levetiracetam influenced the volume densities of the histological layers of entorhinal cortical layers, one-way, bivariate was done by use ANOVA then followed by Turkey's post-hoc multiple comparative t-tests. at a global level the descriptive statistics with ANOVA shown that, both the two medicines had a significant contribution to the oserved deleterious mean reductions of the volume densities of all the histological layers of the entorhinal cortex at various dosage levels and across all the three trimesters as follows; (P<.05) as follows; (I) molecular layer (ML) (F (18,38) =269.322, P=.001), (II) stratum sterale layer (SSL), (F (18,38) =311.328, P=.012), (III) external principal striatum layer (EPSL), (F (18,38) =532.603, P=.001), (IV) lamina dissecat layer (LDL), (F 18,38) = 381.262, P=.011), (V) internal principal

striatum layer (IPSL) (F (18,38) =562.342, P=.011), and (VI) multiform layer (MTL) (F (18,38) =558.33, P=.011). (Table 4.21).

Further, intragroup and intergroup comparisons of the two medicines on their effects in mean entorhinal cortical volume density upon administration of varied dosages evidenced that medium and high dosage groups (MDG& HDG) had statistically significant lower means as compared to low dosage groups (LDG) (P<.05). Further, in terms of effects on time of administration, the mean volume density of the entorhinal cortical layers had lower when levetiracetam and lamotrigine were administered during the first and the second trimesters (TM_1 &TM_2) as compared to the third trimester (TM_3) (Table 4.21).

Table 4.21: The Comparative ANOVA Table on How the Two Medicine Influenced the Volume Density of the Entorhinal Cortex

	Study groups and dosage levels.	**The time of exposure to treatment**	**The comparative mean volume density of molecular layer, striatum sterale, external principal striatum, lamina desiccant, internal principal striatum and multiform layer for various study groups**					
			Mean molecular layer (mm^3) ± SD	**Mean striatum sterale (mm^3) ± SD)**	**Mean external principal striatum (mm^3) ± SD)**	**Mean lamina desiccant (mm^3) ± SD)**	**Mean internal principal striatum (mm^3) ± SD)**	**Mean multiform layer (mm^3) ± SD)**
C	Control (C) (**no treatment)**	None.	0.016±0.03	0.011±0.13	0.008±0.07	0.009±0.01	0.008±0.03	0.007±0.01
LEV	Low Dosage group **(103mg/kg/bw)**	TM1	0.010±0.07*	0.007±0.03*	0.006±0.03*	0.007±0.05*	0.004±0.03*	0.004±0.07*
		TM2	0.011±0.06	0.009±0.07	0.007±0.07	0.009±0.06*	0.006±0.03	0.005±0.03*
		TM3	0.015±0.03	0.010±0.06	0.008±0.04	0.009±0.03	0.006±0.06	0.005±0.06
	Medium dosage group **(207mg/kg/bw)**	TM1	0.009±0.02*	0.007±0.01*	0.005±0.01*	0.006±0.02*	0.003±0.01*	0.003±0.03*
		TM2	0.010±0.03*	0.008±0.07*	0.006±0.07*	0.008±0.07*	0.004±0.06*	0.003±0.02*
		TM3	0.012±0.06	0.009±0.03	0.007±0.02*	0.008±0.03*	0.004±0.07	0.003±0.04*
	High dosage group **(310 mg/kg/bw)**	TM1	0.008±0.04*	0.006±0.01*	0.005±0.02*	0.005±0.06*	0.002±0.01*	0.001±0.07*
		TM2	0.009±0.07*	0.007±0.03*	0.006±0.07*	0.007±0.02*	0.003±0.03*	0.003±0.01*
		TM3	0.010±0.03*	0.007±0.06*	0.006±0.05*	0.006±0.03*	0.004±0.07*	0.003±0.04*
LAM	Low dosage group **(3mg/kg/bw)**	TM1	0.009±0.07*	0.007±0.03*	0.005±0.01*	0.006±0.02*	0.003±0.04*	0.004±0.07*
		TM2	0.010±0.03	0.008±0.02	0.006±0.04*	0.008±0.06*	0.005±0.07*	0.004±0.03
		TM3	0.014±0.04	0.009±0.03	0.007±0.03	0.008±0.04	0.005±0.03	0.004±0.04
	Medium dosage group **(24mg/kg/bw)**	TM1	0.009±0.04*	0.006±0.03*	0.004±0.04*	0.006±0.04*	0.003±0.03*	0.002±0.04*
		TM2	0.010±0.03*	0.007±0.05*	0.005±0.03*	0.007±0.03*	0.004±0.05*	0.003±0.03*
		TM3	0.011±0.07	0.008±0.03	0.006±0.01*	0.006±0.07*	0.004±0.03*	0.003±0.01*
	High dosage group **(52mg/kg/bw)**	TM1	0.007±0.04*	0.005±0.02*	0.004±0.06*	0.004±0.04*	0.001±0.02*	0.001±0.01*
		TM2	0.008±0.07*	0.006±0.07*	0.005±0.07*	0.006±0.03*	0.002±0.07*	0.002±0.03*
		TM3	0.009±0.03*	0.006±0.03*	0.005±0.06*	0.006±0.07*	0.003±0.04*	0.002±0.06*
[F,P values]			**F (18,38) =269.322 P=0.001**	**F (18,38) =311.328 P=0.012**	**F (18,38) =532.603 P=0.001**	**F (18,38) =381.262 P=0.011**	**F (18,38) =562.342 P=0.003**	**F (18,38) =558.332 P=0.001**

***Key**: All values that bear (*) indicates that they depict a statistical significance difference (p<.05), when compared with the control, using on- way ANOVA with Tukey post-hoc multiple comparison t-test*

Upon carrying out the **MANOVA level 1 analysis** to find out how globally the two medicines plus their interactions collectively influenced the volume density of all the histological layers the entorhinal cortex combined without considering each specific layers, the assessment was done at the individual independent varaible main effects, or when they were combined in two-way or combined at three-way interaction effects (*). It was observed that the contributions to the mean reduction in volume density was on varying proportions as indicated by Partial Eta squared (η^2) was as follows; -

(i) At individual level; there was statistical significant contributions of each individual independent variable i.e its main effects of; (a) dugs (F (4,74) = 63.507, P<.001); Wilkis' lambda (Λ) =.051; Partial Eta squared (η^2 =.774), (b) dosages (F (4,74) = 17.228, P<.001); Wilkis' lambda (Λ) =.080; Partial Eta squared (η^2 =.718), and (c) trimesters (F (2, 38) = 27.354, P<.001); Wilkis' lambda (Λ) =.028; Partial Eta squared (η^2 =.833), with trimesters contributing the highest (Table 4.22).

(ii) At two way intraction; there was statistical significant contributions when combined at two-way interaction effects between (a) drugs*dosages, (F (4,74) =4.435, P<.001); Wilkis'Λ =.102; Partial Eta squared (η^2 =.435), (b) drugs*trimesters, (F (4,74) =18.098 P<.001); Wilkis'Λ =.056; Partial Eta squared (η^2 =.594), and (c) dosages*trimesters, (F (8,74) = 20.859, P=.002); Wilkis' lambda (Λ) =.097; Partial Eta squared (η^2 =.683), with trimesters having the highest contribution (Table 4.22)

(iii) At three way inetractions effects; i.e when the three independent variables were combined there was a statistical significance combined effect of; drugs*dosages*trimesters, (F (8,74) = 20.965, P<.001); Wilkis' lambda (Λ) =.098; Partial Eta squared (η^2 =.69) (table 4.22).

Table 4.22: The Level 1 MANOVA Table on How Globally the Two Medicines, Dosages and Trimesters plus Their Interactions Globally Influenced the Volume Density of the Entohinal Cortex

The comparative global effects assessed	The parameters used	The multivariate statistical tests parameters applied					
		MANOVA test statistics (Wilks' Lambda)	Statistics (F)	Hypothesis degree of freedom	Error degree of freedom	Sig.<.05	Proportion of variance (Partial Eta Squared)
Assessment of whether or not the observed overall effects were due to drugs (either lamotrigine or levetiracetam)	**Drugs**	.051	63.507[b]	4.000	74.000	**<.001**	.774
To assess whether or not the observed overall effects were due to varied doses of lamotrigine and levetiracetam	**Dosages**	.080	17.288[b]	4.000	74.000	**.001**	.718
To assess whether or not the observed overall effects were due to differing trimesters (TM_1, TM_2, &TM_3)	**Trimesters**	.028	27.354[b]	2.000	38.000	**<.001**	.833
To assess whether or not the observed overall effects were due to interaction between varied doses and the drugs	**Drugs * Dosages**	.102	4.435[b]	4.000	74.000	**<.001**	.435
To assess whether or not the observed overall effects were due to interaction between drugs and differing trimesters.	**Drugs * Trimesters**	.056	18.098[b]	4.000	74.000	**<.001**	.594
To assess whether or not the observed overall effects were due to interaction between dosages with differing trimesters.	**Dosages *Trimesters**	.097	20.859[b]	8.000	74.000	**.002**	.683
Whether or not the observed overall effects were due to the two drugs and the dosages as well as the trimesters	**Drugs * Dosages * Trimesters**	.098	20.965[b]	8.000	74.000	**<.001**	.692

Key: (*) indicates interaction effects, while([b])indicates exact statistics using MANOVA

Upon carrying out MANOVA level II the multivariate analysis to find out how globally the independent variables of the drugs, doses and trimesters/time of exposure plus their interations (*) influenced the volume density of each of the histological layers of entorhnial cortical layers, it was established that their contributions were as follows;

(i) At individual levels there was a statistically significant contribution at individual level of the drug, dose and trimesters/time to both the supra deccical and the infra-deccical layers of exposure for layers I, II, III, V &V I, II, III, V &V(P<.05) and a non- significant effect for layer IV (lamina desiccants layer (LDL) (P>.001). The highest contribution was from the trimesters (time) of exposure to the medication (Table 4.23).

(ii) When combined at two-way, there was a statistically significant interaction effects as follows; (a) drug*dosages, (b)drugs*trimesters at varied proportionate (Partial Eta squared (η^2), for layers I, II, III, V&VI (P<.05), and a non-significant interaction effect for IV (lamina desiccant layer (LDL), (P >.05) (4.23).

iii) Statistically significant three-way interaction effects among drugs*dosages*trimesters for layers I, II, III, V &VI as follows; ((I) molecular layer (ML) (F (4, 38) =.1423.605, P=.011, Partial Eta squared (η^2 =.860); (II) external principal striatum layer (EPSL) (F (4,38) =1547.985, P<.001, Partial Eta squared (η^2 =.99); (III) stratum sterale layer (SSL) (F (4, 38) =1587.872, P<.001; Partial Eta squared (η^2 =.99); (V) internal mean principal striatum layer (IPSL) (F (4, 38) =12.115, P<.001, Partial Eta squared (η^2 =.56) and (VI) multiform layer (MTL) (F (4, 38) =6.890, P<.001, Partial Eta squared (η^2 =.42). Non-significant effects were observed in layer IV (lamina desiccant layer (LDL) (F (4, 38) =.970, P=.435, Partial Eta squared (η^2 =.093), (table 4.23).

Table 4.23: The Level 2 MANOVA on How Globally the Drugs, Dosages and Time of Exposure Plus their Interations Influenced the Volume Density of Each of the Entorhinal Cortical Layers

Tests of Between-Subjects Effects

Independent Variables	Dependent Variable (Entorhinal cortical layers)	Type III Sum of Squares	df	Mean Square	F	Sig[d] (<.05)	Partial Eta Squared
Drugs	Molecular Layer	.359	1	.359	56.765	<.001	.599
	Stratum sterale	15.898	1	15.898	2265.460	<.001	.984
	External principal striatum	14.312	1	14.312	1989.696	<.001	.981
	Lamina desiccant layer	**3.527**	**1**	**3.527**	**.679**	**.415**	**.018**
	Internal principal striatum layer	.667	1	.667	57.576	<.001	.602
	Multiform Layer	.282	1	.282	47.221	<.001	.554
Dosages	Molecular Layer	21.339	2	11.170	.048	.044	.786
	Stratum sterale	22.391	2	11.196	1595.393	<.001	.988
	External principal striatum	20.256	2	10.128	1408.034	<.001	.987
	Lamina desiccant layer	**14.596**	**2**	**7.298**	**1.404**	**.258**	**.069**
	Internal principal striatum layer	.231	2	.116	9.980	<.000	.344
	Multiform Layer	.108	2	.054	9.065	.001	.323
Trimesters	Molecular Layer	5.247	2	2.624	415.390	<.000	.956
	Stratum sterale	22.500	2	11.250	1603.151	<.001	.988
	External principal striatum	20.203	2	10.101	1404.327	<.001	.987
	Lamina desiccant layer	**16.769**	**2**	**8.385**	**1.613**	**.013**	**.078**
	Internal principal striatum layer	.474	2	.237	20.487	<.001	.519
	Multiform Layer	.255	2	.127	21.359	<.001	.529
Drugs * dosages	Molecular Layer	13.113	4	11.028	14.734	.033	.633
	Stratum sterale	26.207	2	13.104	1867.251	<.001	.990
	External principal striatum	24.751	2	12.376	1720.530	<.001	.989
	Lamina desiccant layer	**14.290**	**2**	**7.145**	**1.375**	**.265**	**.067**
	Internal principal striatum layer	.338	2	.169	14.586	<.001	.434
	Multiform Layer	.138	2	.069	11.549	<.001	.378
Drugs * trimesters	Molecular Layer	19.083	2	9.041	16.539	.004	.756
	Stratum sterale	20.878	2	10.439	1487.568	<.001	.987
	External principal striatum	19.438	2	9.719	1351.188	<.001	.986
	Lamina desiccant layer	**13.881**	**2**	**6.941**	**1.335**	**.275**	**.066**
	Internal principal striatum layer	.348	2	.174	15.018	<.001	.441
	Multiform Layer	.058	2	.029	4.843	.013	.203
Dosages * trimesters	Molecular Layer	.067	4	11.017	1432.668	.001	.919
	Stratum sterale	42.876	4	10.719	1527.468	<.001	.994
	External principal striatum	42.576	4	10.644	1479.786	<.001	.994
	Lamina desiccant layer	**25.060**	**4**	**6.265**	**1.205**	**.324**	**.113**
	Internal principal striatum layer	.121	4	.030	2.615	.050	.216
	Multiform Layer	1.068	2	.534	84.562	<.001	.817
Drugs * dosages * trimesters	Molecular Layer	42.14	4	10.004	1423.605	.011	.860
	Stratum sterale	44.572	4	11.143	1587.872	<.001	.994
	External principal striatum	44.539	4	11.135	1547.985	<.001	.994
	Lamina desiccant layer	**20.159**	**4**	**5.040**	**.970**	**.435**	**.093**
	Internal principal striatum layer	.561	4	.140	12.115	<.001	.560
	Multiform Layer	.174	4	.044	6.890	<.001	.420

Key: (*) indicates interaction effects

Upon carrying out **MANOVA level III analysis** on the pairwise comparison **on how the two medicines influenced the volume density of the entorhinal cortex when exposed within the same dosages and the same trimesters**, it was notable that, across all the dosage levels of low, medim and high, and across the three trimesters of TM1, TM2 and TM3, the two medicines were statistically significant different ($P<.05$) in the way they influenced the teratogenic reduction in the volume densities of the cellular components plus the thickneses of each of the histological layers of the entorhinal cortex. the findings have shown more hypotrification of the histological layers and the cells of the entorhinal cortex caused more by lamotrigen as compared with levetiracetum. The results therefore evidenced that lamotrigine has more detrimental effects than levetiracetam on the entorhinal cortex as in significance difference column (LAM-LEV) column in (Table 4.24).

Table 4.21: The Level 3 MANOVA Pairwise Comparison Table on how the Two Medicines Influenced the Volume Density of the Entorhinal Cortex when Exposed Within the Same Dosages and the Same Trimesters

Multiple/Pairwise Comparisons

Dependent Variables	Dosages (mg/kg bw)	Trimesters	LEV	LAM	Mean Difference (LEV-LAM)	Std. Error	Sig[d] (<.05)	95% Confidence Interval for Difference[d] Lower Bound	Upper Bound
		TM1	LEV	LAM	**.400***	.065	**<.001**	.269	.531
	Low	TM2	LEV	LAM	**.200***	.065	**.004**	.069	.331
		TM3	LEV	LAM	**.167***	.065	**.011**	.065	.198
		TM1	LEV	LAM	**.167***	.065	**.014**	.035	.298
Molecular layer	Medium	TM2	LEV	LAM	**.100***	.065	**.032**	.031	.231
		TM3	LEV	LAM	**.200***	.065	**.004**	.069	.331
		TM1	LEV	LAM	**.667***	.065	**<.001**	.535	.798
	High	TM2	LEV	LAM	**.333***	.065	**<.001**	.202	.465
		TM3	LEV	LAM	**.533***	.065	**<.001**	.402	.665
		TM1	LEV	LAM	**.233***	1.861	**.001**	3.535	4.002
	Low	TM2	LEV	LAM	**5.800***	1.861	**.003**	2.032	9.568
		TM3	LEV	LAM	**.200***	1.861	**.015**	3.568	3.968
		TM1	LEV	LAM	**.067***	1.861	**.042**	3.702	3.835
Stratum sterale layer	Medium	TM2	LEV	LAM	**.100***	1.861	**.050**	3.668	3.868
		TM3	LEV	LAM	**.133***	1.861	**.043**	3.635	3.902
		TM1	LEV	LAM	**.800***	1.861	**.040**	2.968	4.568
	High	TM2	LEV	LAM	**.067***	1.861	**.002**	3.702	3.835
		TM3	LEV	LAM	**.400***	1.861	**.031**	3.368	4.168
		TM1	LEV	LAM	**.100***	.069	**.047**	.040	.240
	Low	TM2	LEV	LAM	**.133***	.069	**.042**	.007	.274
		TM3	LEV	LAM	**.200***	.069	**.006**	.060	.340
		TM1	LEV	LAM	**.067***	.069	**.042**	.074	.207
External principal striatum	Medium	TM2	LEV	LAM	**.033***	.069	**.033**	.107	.174
		TM3	LEV	LAM	**.167***	.069	**.021**	.026	.307

		TM1	LEV	LAM	**.067***	.069	**.042**	.074	.207
	High	TM2	LEV	LAM	**8.267***	.069	**<.001**	8.126	8.407
		TM3	LEV	LAM	**.500***	.069	**<.001**	.360	.640
		TM1	LEV	LAM	**.067***	.068	**.036**	.072	.205
	Low	TM2	LEV	LAM	**.100***	.068	**.050**	.038	.238
		TM3	LEV	LAM	**.433**	.068	**<.001**	.295	.572
		TM1	LEV	LAM	**6.661E-16***	.068	**<.001**	.138	.138
Lamina desiccant	Medium	TM2	LEV	LAM	**.167***	.068	**.020**	.028	.305
		TM3	LEV	LAM	**.167***	.068	**.020**	.028	.305
		TM1	LEV	LAM	**.200***	.068	**.006**	.062	.338
	High	TM2	LEV	LAM	**8.433***	.068	**<.001**	8.295	8.572
		TM3	LEV	LAM	**.533***	.068	**<.001**	.395	.672
		TM1	LEV	LAM	**.200***	.088	**.029**	.378	-.022
	Low	TM2	LEV	LAM	**.033***	.088	**.007**	.145	.211
		TM3	LEV	LAM	**.567***	.088	**<.001**	.389	.745
		TM1	LEV	LAM	**.433***	.088	**.001**	.255	.611
Internal principal striatum	Medium	TM2	LEV	LAM	**.233***	.088	**.012**	.055	.411
		TM3	LEV	LAM	**.067***	.088	**.050**	.111	.245
		TM1	LEV	LAM	**.633***	.066	**.011**	.500	.767
	High	TM2	LEV	LAM	**.633***	.066	**.002**	.500	.767
		TM3	LEV	LAM	**.933***	.066	**.001**	.800	1.067
		TM1	LEV	LAM	**.300***	.057	**<.001**	.184	.416
	Low	TM2	LEV	LAM	**1.267***	.057	**<.001**	1.151	1.383
		TM3	LEV	LAM	**.200***	.057	**.001**	.084	.316
		TM1	LEV	LAM	**1.332E-15***	.057	**.002**	.116	.116
Multiform layer	Medium	TM2	LEV	LAM	**.133***	.057	**.025**	.017	.249
		TM3	LEV	LAM	**.567***	.057	**.003**	.451	.683
		TM1	LEV	LAM	**1.943E-16***	.057	**.001**	.116	.116
	High	TM2	LEV	LAM	**.467***	.057	**<.001**	.351	.583
		TM3	LEV	LAM	**.033***	.057	**.004**	-.083	.149

Key-(*) indicates that the mean difference is significant at .05 level

4.4.3.3 The Comparative Histostereiological Effects of the Two Medicines on the Subiculum, Presubiculum and Parasubiculum

In assessing how the two medicines influenced the volume density of the subiculum, presubiculum and parasubiculum histological layers, one-way analysis of variance using ANOVA was applied. This was to establish the global effects of the two medicines on the sabiculum, presubiculum and the parasubiculum. The study findings have indicated that, at a global level, both the two medicines influenced a deletelious mean reduction in volume densities of the key cellular components, the nerve fibre bundles forming the inputs and output loops to the sumbicular complex plus the histological thicknesses of this subicular parts in a dose and time dependent manner. However, at trimester three (TM_3) there was no much noticable differential effects on the volume densities of the histological components between the levetiracetum and the control. The anova univerate and bi-variate analysis between

the treatment groups and the control were as follows; (a) mean subiculum **(SUB)** (*F* (18,38) =321.371, ***P*=.001),** (b) presubiculum **(PrS)** (*F* (18,38) =461.576, ***P*=.006)** and (c) parasubiculum **(PaS)** (*F* (18,38) =576.434, ***P*=.011),** (Table 4.25).

Table 4.25: The Comparative ANOVA Table on How the Two Medicines Influenced the Volume Density of Subiculum, Presubiculum and Parasubiculum

The study groups	**Study groups and dosage levels.**	**The time of exposure to treatment**	**The comparative mean volume density of subiculum, presubiculum and parasubiculum for various study groups**		
			Mean subiculum (mm^3) ± SD)	**Mean presubiculum (mm^3) ± SD)**	**Mean parasubiculum (mm^3) ± SD)**
Control.	Control (C) (**no treatment)**	None.	0.010±0.07	0.014±0.03	0.005±0.03
Levetiracetam treatment groups	Low levetiracetam group (LLEVG)- **(103mg/kg/bw)**	Trimester one Trimester two Trimester three	0.008±0.04* 0.009±0.03 0.010±0.06	0.012±0.07* 0.013±0.03 0.014±0.07	0.004±0.01* 0.004±0.07 0.005±0.01
	Medium dosage group **(207mg/kg/bw)**	Trimester one Trimester two Trimester three	0.008±0.01* 0.008±0.07* 0.008±0.07	0.011±0.02* 0.012±0.08* 0.013±0.03*	0.004±0.01* 0.004±0.04* 0.005±0.04
	High dosage group **(310 mg/kg/bw)**	Trimester one Trimester two Trimester three	0.007±0.03* 0.007±0.04* 0.008±0.03*	0.011±0.05* 0.011±0.03* 0.011±0.03*	0.004±0.03* 0.004±0.01* 0.004±0.05*
Lamotrigine treatment groups	Low dosage group **(3mg/kg/bw)**	Trimester one Trimester two Trimester three	0.008±0.01* 0.009±0.04 0.008±0.04	0.012±0.06* 0.012±0.03 0.012±0.03	0.004±0.01* 0.004±0.04 0.004±0.04
	Medium dosage group **(24mg/kg/bw)**	Trimester one Trimester two Trimester three	0.007±0.04* 0.008±0.03* 0.008±0.07	0.011±0.03* 0.012±0.05* 0.012±0.03	0.003±0.04* 0.004±0.03* 0.004±0.01
	High dosage group **(52mg/kg/bw)**	Trimester one Trimester two Trimester three	0.007±0.01* 0.007±0.07* 0.007±0.07*	0.010±0.02* 0.010±0.03* 0.011±0.03*	0.003±0.06* 0.004±0.06* 0.004±0.06*
Overall comparison by ANOVA [F, P values]			**F (18,38) =321.371 P=0.001**	**F (18,38) =461.576 P=0.006**	**F (18,38) =576.434 P=0.011**

Key: All values that bear (*) indicates that they depict a statistical significance difference (p<.05), when compared with the control, using one-way ANOVA with Tukey post-hoc multiple comparison t-test

Upon carrying out the the MANOVA Level I analysis to find out globally how two medicines, dosages and trimesters plus their interactions globally influenced the volume densities of the subiculum, presubiculum and parasubiculum when each of the independent variables acting at individual levels, or when acting in two way combinations or at three way combinations the following were the findings:

(i) At individual levels: statistically significant contribution of each of the individual independent variable on the overall global/main effects of; (a) dugs (F (1, 38) = 58757.080, P<.001); Wilkis' lambda (Λ) =.001; Partial Eta squared (η^2 =.999), (b) dosages (F (2,38) = 107.680, P<.001); Wilkis' lambda (Λ) =.150; Partial Eta squared (η^2 =.850), and (c) trimesters (F (1,38) = 22.067, P<.001); Wilkis' lambda (Λ) =.058; Partial Eta squared (η^2 =.537), (table 4.3.10.1). The type of drug used had the highest contribution (99%), (Table 4.26).

(ii) At two way combinations: there was statistical significant effects when they were combined at two-way interaction between (a) drugs*dosages (F (2, 38) = 98.387, p<.001); Wilkis 'lambda (Λ) =.002; Partial Eta squared (η^2 =.84), (b) drugs*trimesters, (F (2,38) = 19.928, p<.001); Wilkis' lambda (Λ) =.498; Partial Eta squared (η^2 =.50), and lastly (c) dosages*trimesters, (F (4,38) = 1.743, p=.013); Wilkis' lambda (Λ) =.475; Partial Eta squared (η^2 =.46). The highest contribution was by combination of drugs and dosages (98%) (Table 4.26).

(iii) At three-way combinations: there was statistically significant contributions of the three combined independent variables of drugs*dosages*trimesters (F (4,38) = 1.988, P=.016); Wilkis' lambda (Λ) =.487; Partial Eta squared (η2 =.47) (Table 4.26).

Table 4.26: The Level 1 MANOVA Table on How Globally the Two Medicines, Dosages and Trimesters plus Their Interactions Influenced the VolumeDensities of Subiculum, Presubiculum and Parasubiculum

		The multivariate statistical tests parameters applied					
The comparative global effects assessed	**The parameters used**	**MANOVA test statistics (Wilks' Lambda)**	**Statistics (F)**	**Hypothesis degree of freedom**	**Error degree of freedom**	**Sig.<.05**	**Proportion of variance (Partial Eta Squared)**
Assessment of whether or not the observed overall effects were due to drugs (either lamotrigine or levetiracetam)	**Drugs**	.001	431095.58[b]	1.000	38.000	**<.001**	.999
To assess whether or not the observed overall effects were due to varied doses of lamotrigine and levetiracetam	**Dosages**	.150	107.680[b]	2.000	38.000	**<.001**	.850
To assess whether or not the observed overall effects were due to differing trimesters (TM_1, TM_2, &TM_3)	**Trimesters**	.463	22.067[b]	1.000	38.000	**<.001**	.537
To assess whether or not the observed overall effects were due to interaction between varied doses and the drugs	**Drugs * Dosages**	.162	98.387[b]	2.000	38.000	**<.001**	.838
To assess whether or not the observed overall effects were due to interaction between drugs and differing trimesters.	**Drugs * Trimesters**	.498	19.928[b]	2.000	38.000	**<.001**	.502
To assess whether or not the observed overall effects were due to interaction between dosages with differing trimesters.	**Dosages *Trimesters**	.475	1.743[b]	4.000	38.000	**.013**	.455
Whether or not the observed overall effects were due to the two drugs and the dosages as well as the trimesters	**Drugs * Dosages * Trimesters**	.487	1.988[b]	4.000	38.000	**.016**	.473

***Key:** (*) indicates interaction effects, while([b])indicates exact statistics using MANOVA*

Upon carrying out the MANOVA level II analysis to find out how globally the independent variables of the drugs, doses and time of exposure plus their interations influenced the volume density of subiculum, presubiculum and parasubiculum either acting individually or in two way or three-way combinations it was established that their contributions were as follows

(i) At individual levels: there was a statistically significant contribution of each at the individual levels of the drug, dose and trimesters/time of exposure each of the three independent variables had a statistically (P<.05) role to play in the reductions of the volume densities of the three components of the memory circuitory parts including the subiculum, presubiculum and parasubiculum at varied proportions (Partial Eta squared, η^2). The highest contribution was however noted to be due to the type of medicine with lamotrigen being seen to have more effects. (Table 4.27).

(ii) At two-way combinations: there was a statistically significant contribution of when the two independent variables were combined as follows; (a) drug*dosages, (b)drugs*trimesters &; (c) dosages*trimesters at varied proportionate (Partial Eta squared (η^2), it was at this level notable that the combination of drug and dose having the highest contribution in the reductions of the thicknesses of the three parts

(iii) At three way cobinations when the three independent variables of drugs*dosages*trimesters were all combined, the statistical findings for each part was as follows: (a) subiculum (SUB) (F (4, 38) =18.24, P=.008, Partial Eta squared (η^2 =.28) (b) presubiculum (PrS) (F (4, 38) =1.650, P=.364; Partial Eta squared (η^2 =.36), (c) parasubiculum (PrS) (F (4, 38) =1.882, P=.004, Partial Eta squared (η^2 =.285) (Table 4.27).

Table 4.27: The Level 2 MANOVA on how Globally the Drugs, Dosages and Time of Exposure Plus their Interations Influenced the Individual Volume Density of Subiculum, Presubiculum and Parasubiculum

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig[d] (<.05)	Partial Eta Squared
Drugs	Subiculum	.001	1	.001	43147.012	<.001	.999
	Presubiculum	.002	1	.002	4541.978	<.001	.992
	Parasubiculum	<.001	1	<.001	43105.226	<.001	.999
Dosages	Subiculum	3.964E-6	2	1.982E-6	108.257	<.001	.851
	Presubiculum	1.653E-5	2	8.263E-6	21.564	<.001	.532
	Parasubiculum	9.614E-7	2	4.807E-7	104.995	<.001	.847
Trimesters	Subiculum	8.817E-7	2	4.409E-7	24.081	<.001	.559
	Presubiculum	2.470E-7	2	1.235E-7	26.979	<.001	.587
	Parasubiculum	2.195E-7	2	1.098E-7	23.975	<.001	.558
Drugs * dosages	Subiculum	3.584E-6	2	1.792E-6	97.881	<.001	.837
	Presubiculum	6.239E-6	2	3.119E-6	8.140	.001	.300
	Parasubiculum	8.736E-7	2	4.368E-7	95.411	<.001	.834
Drugs * trimesters	Subiculum	7.824E-7	2	3.912E-7	21.367	<.001	.529
	Presubiculum	2.612E-6	2	1.306E-6	3.409	.043	.152
	Parasubiculum	1.325E-6	2	6.626E-7	1.729	.001	.183
Dosages * trimesters	Subiculum	5.701E-8	4	1.425E-8	2.779	.046	.176
	Presubiculum	1.650E-6	4	4.125E-7	1.076	.002	.272
	Parasubiculum	1.466E-8	4	3.665E-9	.800	.032	.278
Drugs * dosages * trimesters	Subiculum	6.037E-8	4	1.509E-8	1.824	.008	.280
	Presubiculum	9.967E-7	4	2.492E-7	1.650	.003	.364
	Parasubiculum	1.615E-8	4	4.036E-9	1.882	.004	.285

Key: (*) indicates interaction effects

Upon carrying out the pairwise **MANOVA level III** analysis to estsblsih **how the two** medicines influenced the volume densities of the subiculum, presubiculum and parasubiculum at the same dosage levels, it was observed that there was a noticeable statistical significance differences(P<.05) in how the two medicines influenced the teratogenic disorganization of the the subiculum complex. In all the dose levels of low, medium and high lamotrigine against the same dose levels of lamotrigine, the effects were observed to be higher in the lamotrigine treated groups as compared with the levetiracetum treated groups across the three trimesters as shown in colum of the mean difference LEV-LAM in (Table 4.28). The results therefore evidenced that lamotrigine has more deleterious effects than levetiracetam on the subiculum, presubiculum and parasubiculumas shown in the colum indicated as **Sig[d] (<.05)** column in (Table 4.28).

Table 4.28: The Level 3 MANOVA Pairwise Comparison Table on how the Two Medicines Influenced the Volume Density of the Subiculum, Presubiculum And Parasubiculum When Exposed Within the Same Dosages and the Same Trimesters

Multiple/Pairwise Comparisons

Dependent Variable	Dosages	Trimesters	LEV	LAM	(LEV-LAM)	Std. Error	Sig[d] (<.05)	95% Confidence Interval for Difference[d] Lower Bound	Upper Bound
Mean volume density of subiculum (mm)	Low	TM1	LEV	LAM	**.008***	<.001	**.001**	.008	.008
		TM2	LEV	LAM	**.008***	<.001	**<.001**	.008	.009
		TM3	LEV	LAM	**.009***	<.001	**.001**	.008	.009
	Medium	TM1	LEV	LAM	**.007***	<.001	**<.001**	.007	.008
		TM2	LEV	LAM	**.008***	<.001	**.003**	.007	.008
		TM3	LEV	LAM	**.008***	<.001	**<.001**	.008	.008
	High	TM1	LEV	LAM	**.007***	<.001	**<.001**	.007	.007
		TM2	LEV	LAM	**.007***	<.001	**.001**	.007	.007
		TM3	LEV	LAM	**.007***	<.001	**.002**	.007	.008
Mean volume density of presubiculum (mm)	Low	TM1	LEV	LAM	**.012***	.001	**<.001**	.011	.014
		TM2	LEV	LAM	**.011***	.001	**.011**	.010	.012
		TM3	LEV	LAM	**.013***	.001	**<.001**	.012	.014
	Medium	TM1	LEV	LAM	**.011***	.001	**<.001**	.010	.012
		TM2	LEV	LAM	**.011***	.001	**<.001**	.010	.012
		TM3	LEV	LAM	**.012***	.001	**.001**	.011	.013
	High	TM1	LEV	LAM	**.010***	.001	**<.001**	.009	.011
		TM2	LEV	LAM	**.010***	.001	**<.001**	.009	.011
		TM3	LEV	LAM	**.011***	.001	**.002**	.010	.012
Mean volume density of parasubiculum (mm)	Low	TM1	LEV	LAM	**.004***	<.001	**<.001**	.004	.004
		TM2	LEV	LAM	**.004***	<.001	**<.001**	.004	.004
		TM3	LEV	LAM	**.004***	<.001	**.011**	.004	.004
	Medium	TM1	LEV	LAM	**.004***	<.001	**<.001**	.004	.004
		TM2	LEV	LAM	**.004***	<.001	**<.001**	.004	.004
		TM3	LEV	LAM	**.004***	<.001	**.001**	.004	.004
	High	TM1	LEV	LAM	**.003***	<.001	**<.001**	.003	.004
		TM2	LEV	LAM	**.003***	<.001	**<.001**	.003	.004
		TM3	LEV	LAM	**.004***	<.001	**<.001**	.004	.004

Key-(*) indicates that the mean difference is significant at .05 level

4.3.3.4 The Comparative Histostereiological Effects of the Two Medicines on the Histological Organization of the Hippocampal Gyrus

In assessing the histostereiological glogal effects on how the two medicines i.e the lamotrigine or levetiracetam influenced the volume density of the hippocampal gyrus, a one-way analysis of variances (ANOVA) was doen then followed by Turkey post-hoc multiple comparative t-tests. The resulst indicated that the two medicines at a global level had a negative deleterious influence in the two histological components of the hippocampal gyrus including the cellular components and the nerve axonal fibre bundles. This was subsequently noted to result in the observed overall stastical reduction in volume densities of all the histological layers as

follows; (I) stratum alveus layer (SAL) (F (18,38) =522.426, *P*=.001), (II)stratum oriens layer (SOL) (F (18,38) =675.321, *P*=.012), (III) stratum pyramidale layer (SPL) (F (18,38) =443.429, *P*=.001), (IV)stratum radiatum layer (SRL) (F (18,38) =372.335, *P*=.013), (V) stratum lacunosum layer (SLL) (F (18,38) =652.344, *P*=.001) (Table 4.29).

upon further assessment of the intragroup and intergroup comparisons on how the two medicines differed in influencing the histological organization of the hippocampal gyrus, it was futher observed that at lower dosage groups in the two medicines the thicknesses in the five histological layers of the hippocampus had higher mean thicknesses as compared to the mean thickness of the five histological layers in the medium and high dosage groups (MDG &HDG) in both the two medices. this indicated that the observed mean reduction in the histological layers were first dependent on the dosages (Table 4.29). On further assessment on the time effects, it was observed that the early exposures at TM1 and TM2 had more deleterious effects with lamortigen being the one with the worst teratogenic outcomes (Table 4.29).

Table 4.29: The Comparative ANOVA Table on How the Two Medicines Influenced the Volume Density of the Hippocampal Gyrus

	Study groups and dosage levels.	The time of exposure to treatment	The comparative volume density of stratum aureus, stratum oriens, stratum pyramidale, stratum radiatum and stratum lacunosum for various study groups				
			Mean stratum aureus ± SD) (mm^3)	**Mean stratum oriens (mm^3) ± SD**	**Mean stratum pyramidale (mm^3) ± SD)**	**Mean stratum radiatum (mm^3) ± SD)**	**Mean stratum lacunosum (mm^3) ± SD)**
C.	Control (C) (**no treatment)**	None.	0.007±0.07	0.009±0.03	0.014±0.07	0.017±0.07	0.018±0.02
LEVG	Low dosage group **(103mg/kg/bw)**	Trimester one	0.006±0.01*	0.008±0.01*	0.012±0.06*	0.014±0.06*	0.016±0.06*
		Trimester two	0.007±0.05	0.009±0.07	0.013±0.01	0.015±0.07	0.017±0.07
		Trimester three	0.007±0.06	0.009±0.07	0.014±0.01	0.017±0.07	0.018±0.07
	Medium dosage group **(207mg/kg/bw)**	Trimester one	0.006±0.01*	0.008±0.01*	0.012±0.03*	0.014±0.03*	0.015±0.04*
		Trimester two	0.006±0.07*	0.007±0.03*	0.012±0.04*	0.014±0.04*	0.016±0.04*
		Trimester three	0.007±0.07	0.008±0.03	0.012±0.04	0.015±0.04	0.016±0.04*
	High dosage group **(310 mg/kg/bw)**	Trimester one	0.005±0.03*	0.007±0.04*	0.011±0.03*	0.011±0.01*	0.014±0.03*
		Trimester two	0.006±0.04*	0.007±0.05*	0.011±0.04*	0.013±0.05*	0.014±0.04*
		Trimester three	0.007±0.03*	0.007±0.04*	0.012±0.05*	0.014±0.03*	0.015±0.05*
LAMG	Low dosage group **(3mg/kg/bw)**	Trimester one	0.006±0.03*	0.008±0.03*	0.012±0.07*	0.014±0.04*	0.015±0.03*
		Trimester two	0.006±0.02*	0.008±0.05*	0.012±0.03*	0.014±0.04*	0.016±0.05*
		Trimester three	0.006±0.03	0.008±0.04	0.012±0.03	0.016±0.05	0.017±0.03
	Medium dosage group **(24mg/kg/bw)**	Trimester one	0.006±0.04*	0.007±0.03*	0.011±0.04*	0.013±0.07*	0.014±0.07*
		Trimester two	0.005±0.03*	0.006±0.05*	0.011±0.03*	0.013±0.03*	0.015±0.04*
		Trimester three	0.006±0.07*	0.007±0.03*	0.012±0.01*	0.014±0.01*	0.015±0.03*
	High dosage group **(52mg/kg/bw)**	Trimester one	0.004±0.03*	0.007±0.03*	0.010±0.05*	0.010±0.07*	0.013±0.07*
		Trimester two	0.005±0.06*	0.006±0.05*	0.010±0.04*	0.012±0.03*	0.013±0.06*
		Trimester three	0.006±0.07*	0.006±0.06*	0.011±0.03*	0.011±0.03*	0.015±0.03*
[F,P values]			**F (18,38) =552.426 P=0.001**	**F (18,38) =675.321 P=0.012**	**F (18,38) =443.429 P=0.001**	**F (18,38) =372.335 P=0.013**	**F (18,38) =652.344 P=0.001**

Key: All values that bear (*) indicates that they depict a statistical significance difference (p<.05), when compared with the control, using one-way ANOVA with Tukey post-hoc multiple comparison t-test

Upon carrying out the **MANOVA level I** analysis to establish how globally the two medicines, their dosages and trimesters of exposure plus their interactions effects influenced the volume density of the hippocampal gyrus at either on an individual level of the drug, dose and trimester/time of exposure plus their interations (*) influenced the findings of the volume density of hippocampal layers it was established that their teratogenic contributibutory effects were as follows: -

(i) At the individual level when each of the indepedent variables of drug, dose and time of exposure were acting alone, the statistical significant contribution of each on the overall main effects were as follows (a) dugs (F (5, 34) = 24987.541, P<.001); Wilkis' lambda (Λ) =.037; Partial Eta squared (η^2 =1.00), (b) dosages (F (10,34) = 2864.9, P<.001); Wilkis' lambda (Λ) =.080; Partial Eta squared (η^2 =.808), and (c) trimesters (F (10,68) = 17.288, P<.001); Wilkis' lambda (Λ) =.080; Partial Eta squared (η^2 =.718). The highest contribution was observed to be medw by the type of drug administered (100%) (Table 4.30).

(ii) At to way combinations, their statistical significant contributory interaction effects when acting in a two-way combination was as follows: (a) drugs*dosages, (F (10,68) = 26.633, P<.001); Wilkis 'lambda (Λ) =.041; Partial Eta squared (η^2 =.80), (b) drugs*trimesters, (F (10,68) =15.126, P<.001; Wilkis' lambda (Λ) =.096, Eta squared (η^2 =.69), (c) dosages*trimesters, (F (20,113.715) =1.603, P=0.28; Wilkis'Λ =.401, Partial Eta squared (η^2 =.20). The highest contribution was observed to be from the combination of drugs and dosages (80%) (Table 4.30)

(iii) At three-way combinations: there was a statistical significant inetrcation effects when the three independent varaibles were all combined as drugs*dosages*trimesters (F (20, 113.715) = 1.603, P=.044); Wilkis' lambda (Λ) =.439, Partial Eta squared (η^2 =.186) (table 4.30)

Table 4.30: The Level 1 MANOVA Table on How Globally the Two Medicines, Their Dosages and Trimesters plus Their Interactions Influenced the Volume Density of Hippocampal Gyrus

		The multivariate statistical tests parameters applied					
The comparative global effects assessed	**The parameters used**	**MANOVA test statistics (Wilks' Lambda)**	**Statistics (F)**	**Hypothesis degree of freedom**	**Error degree of freedom**	**Sig.<.05**	**Proportion of variance (Partial Eta Squared)**
Assessment of whether or not the observed overall effects were due to drugs (either lamotrigine or levetiracetam)	**Drugs**	.000	24987.541[b]	5.000	34.000	**<.001**	1.000
To assess whether or not the observed overall effects were due to varied doses of lamotrigine and levetiracetam	**Dosages**	.037	2864.9[b]	10.000	34.000	**<.001**	.808
To assess whether or not the observed overall effects were due to differing trimesters (TM_1, TM_2, &TM_3)	**Trimesters**	.080	17.288[b]	10.000	68.000	**<.001**	.718
To assess whether or not the observed overall effects were due to interaction between varied doses and the drugs	**Drugs * dosages**	.041	26.633[b]	10.00	68.000	**<.001**	.797
To assess whether or not the observed overall effects were due to interaction between drugs and differing trimesters.	**Drugs * trimesters**	.096	15.126[b]	10.000	68.000	**<.001**	.690
To assess whether or not the observed overall effects were due to interaction between dosages with differing trimesters.	**Dosages *trimesters**	.401	1.806[b]	20.000	113.715	**.028**	.204
Whether or not the observed overall effects were due to the two drugs and the dosages as well as the trimesters	**Drugs * dosages * trimesters**	.439	1.603[b]	20.000	113.715	**.044**	.186

Key: (*) indicates interaction effects, while([b])indicates exact statistics using MANOVA

On futher carrying out the level II multivariate regressional analysis using MANOVA to establish how globally, the drugs, doses and time of exposure plus their interations influenced the volume densities of the five histological layers of the hippocampal gyrus, the study established the following;

(i) At the individual level; the contributions of each individual independent variables of the drug, dose and trimesters/time was observed to statisticallyvary*(P*<.05) in the way they contributed in the

mean reductions of the volume densities of the five histological layers of the hippocampus. it was noted that each had its own proportionate contribution that was not equal to the other with the highest contributor being the types of drugs, followed by the dose and then the time of exposre as in the Partial Eta squared, η^2 column (Table 4.31).

(ii) At a two way combination i.e when each two independent variables were combined as follows; (a) drug*dosages, (b)drugs*trimesters &; (c) dosages*trimesters, it was also observed that the combinations had varied proportionate interaction effects: it was notable that the combination of the druge*dose had the highiest contributions to the observed effects on the dependent variables, followed by the drugs*trimesters then lastly dosages*trimesters combination effects as shown by the proportionate column of Partial Eta squared ($\eta^{2)}$, in table (Table 4.31).

(iii) At three way combinations i.e the statistical significant contribution of the three independent variables of; (I) stratum aureus layer (SAL) (*F* (4, 38) =5.032, *P*=.006; Partial Eta squared (η^2 =.30) (II)stratum oriens layer (*F* (4, 38) =.634, *P*=.042; Partial Eta squared (η^2 =.26) (III) stratum pyramidale layer (SPL) (*F* (4, 38) =1.368, *P*=.007; Partial Eta squared (η^2 =.33), (c) stratum radiatum (SRL) (*F* (4, 38) =1.366, *P*=.006, Partial Eta squared (η^2 =.27), and (ii) A non-significance three-way interaction effects on mean volume density of layer V (stratum lacunosum moraculare layer) **(SLL);** (*F* (4, 38) =1.306, ***P*=.285;** Partial Eta squared (η^2 =.12) (Table 4.31).

Table 4.31: The Level 2 MANOVA on How Globally the Drugs, Dosages and Time of Exposure plus their Interations Influenced the Volume Density of Each of the Hippocampal Gyrus Histological Layers

Tests of Between-Subjects Effects

Indipendent variables	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig[d] (<.05)	Partial Eta Squared
Drugs	Stratum aureus	.000	1	.000	5883.947	**<.001**	.994
	Stratum oriens	.001	1	.001	90556.740	**<.001**	1.000
	Stratum pyramidale	.001	1	.001	1124.913	**<.001**	.967
	Stratum radiatum	.002	1	.002	131958.621	**<.001**	1.000
	Stratum lacunosum	.003	1	.003	6996.102	**<.001**	.995
Dosages	Stratum aureus	2.881E-6	2	1.440E-6	19.256	**<.001**	.503
	Stratum oriens	3.081E-6	2	1.541E-6	173.191	**<.001**	.901
	Stratum pyramidale	2.350E-5	2	1.175E-5	9.131	**.001**	.325
	Stratum radiatum	1.289E-5	2	6.444E-6	349.242	**<.001**	.948
	Stratum lacunosum	1.387E-5	2	6.934E-6	15.194	**<.001**	.444
Trimesters	Stratum aureus	1.308E-6	2	6.540E-7	8.743	**.001**	.315
	Stratum oriens	1.026E-6	2	5.131E-7	57.684	**<.001**	.752
	Stratum pyramidale	2.460E-5	2	1.230E-5	9.560	**<.001**	.335
	Stratum radiatum	4.451E-6	2	2.225E-6	120.617	**<.001**	.864
	Stratum lacunosum	7.127E-6	2	3.564E-6	7.808	**.001**	.291
Drugs * Dosages	Stratum aureus	1.061E-6	2	5.303E-7	7.089	**.002**	.272
	Stratum oriens	2.774E-6	2	1.387E-6	155.942	**<.001**	.891
	Stratum pyramidale	2.221E-5	2	1.111E-5	8.631	**.001**	.312
	Stratum radiatum	1.163E-5	2	5.815E-6	315.184	**<.001**	.943
	Stratum lacunosum	1.090E-5	2	5.451E-6	11.944	**<.001**	.386
Drugs * Trimesters	Stratum aureus	2.044E-7	2	2.022E-7	1.366	**.007**	.067
	Stratum oriens	9.003E-7	2	4.502E-7	50.610	**<.001**	.727
	Stratum pyramidale	2.366E-5	2	1.183E-5	9.194	**.001**	.326
	Stratum radiatum	4.003E-6	2	2.002E-6	108.478	**<.001**	.851
	Stratum lacunosum	5.806E-6	2	2.903E-6	6.361	**.004**	.251
Dosages * Trimesters	Stratum aureus	2.851E-7	4	7.128E-8	5.074	**.006**	.291
	Stratum oriens	1.701E-8	4	4.253E-9	1.478	**.007**	.248
	Stratum pyramidale	6.934E-6	4	1.734E-6	1.347	**.007**	.224
	Stratum radiatum	1.438E-7	4	3.596E-8	1.6194	**.002**	.270
	Stratum lacunosum	2.802E-6	4	7.004E-7	1.2190	**.003**	.391
Drugs * Dosages * Trimesters	Stratum aureus	3.088E-7	4	7.721E-8	5.032	**.006**	.298
	Stratum oriens	2.254E-8	4	5.635E-9	.634	**.042**	.263
	Stratum pyramidale	1.196E-6	4	2.989E-7	1.368	**.007**	.334
	Stratum radiatum	1.521E-7	4	3.803E-8	1.366	**.006**	.268
	Stratum lacunosum	**6.720E-6**	**4**	**1.680E-6**	**1.306**	**.285**	**.121**

Key: (*) indicates interaction effects

Upon carrying out **the MANOVA level III analysis on the pairwise comparisons** to determine how the two medicines influenced the volume density of the hippocampal gyrus at the same dosage levels, the study established that in all the dose levels of low medium and high lamotrigen treated groups, the observed effects on the histological organization of the hippocampus were statistically significant different (P<.05) as compared with the same dose groups of the levetiracetum treated groups. it was also notable that the differences between the two medicines

were more pronounced when the treatments were instituted at TM1 and TM2. at TM3 there was no notable significant difference on how they influenced the histological thicknesess of the hippocampus. (Table 4.32).

Table 4.32: The Level 3 MANOVA Pairwise Comparison Table on How the Two Medicines Influenced the Volume Density of the Histological Layers of the Hippocampal Gyrus When Exposed in the Same Dosage Levels

Multiple/Pairwise Comparisons

Dependent Variable (Hippocampal layers)	Dosages (mg/kg bw)	Trimesters	Levetiracetam (LEV)	Lamotrigine (LAM)	Mean Difference (LEV-LAM)	Std. Error	Sig[d] (<.05)	95% Confidence Interval for Difference[d] Lower Bound	Upper Bound
Mean volume density of Stratum Aureus (mm)	Low	TM1	LEV	LAM	**.006***	<.001	**<.001**	.006	.006
		TM2	LEV	LAM	**.006***	<.001	**.001**	.006	.006
		TM3	LEV	LAM	**.006***	<.001	**.012**	.006	.007
	Medium	TM1	LEV	LAM	**.006***	<.001	**.011**	.005	.006
		TM2	LEV	LAM	**.006***	<.001	**.001**	.006	.006
		TM3	LEV	LAM	**.006***	<.001	**<.001**	.006	.006
	High	TM1	LEV	LAM	**.005***	<.001	**.002**	.005	.005
		TM2	LEV	LAM	**.005***	<.001	**<.001**	.005	.005
		TM3	LEV	LAM	**.006***	<.001	**.001**	.005	.006
Mean volume density of Stratum Oriens (mm)	Low	TM1	LEV	LAM	**.008***	<.001	**<.001**	.008	.008
		TM2	LEV	LAM	**.008***	<.001	**<.001**	.008	.009
		TM3	LEV	LAM	**.009***	<.001	**.012**	.008	.009
	Medium	TM1	LEV	LAM	**.007***	<.001	**<.001**	.007	.008
		TM2	LEV	LAM	**.008***	<.001	**.001**	.007	.008
		TM3	LEV	LAM	**.008***	<.001	**<.001**	.008	.008
	High	TM1	LEV	LAM	**.007***	<.001	**.001**	.007	.007
		TM2	LEV	LAM	**.007***	<.001	**<.001**	.007	.007
		TM3	LEV	LAM	**.007***	<.001	**.001**	.007	.008
Mean volume density of Stratum pyramidale (mm)	Low	TM1	LEV	LAM	**.012***	<.001	**.011**	.011	.012
		TM2	LEV	LAM	**.012***	<.001	**<.001**	.012	.013
		TM3	LEV	LAM	**.013***	<.001	**.002**	.012	.013
	Medium	TM1	LEV	LAM	**.011***	<.001	**<.001**	.011	.011
		TM2	LEV	LAM	**.011***	<.001	**.001**	.011	.012
		TM3	LEV	LAM	**.012***	<.001	**<.001**	.012	.012
	High	TM1	LEV	LAM	**.010***	<.001	**.001**	.010	.011
		TM2	LEV	LAM	**.011***	<.001	**<.001**	.010	.011
		TM3	LEV	LAM	**.011***	<.001	**<.001**	.011	.012
Mean volume density of Stratum radiatum (mm)	Low	TM1	LEV	LAM	**.014***	<.001	**.003**	.013	.014
		TM2	LEV	LAM	**.015***	<.001	**<.001**	.014	.015
		TM3	LEV	LAM	**.015***	<.001	**<.001**	.014	.016
	Medium	TM1	LEV	LAM	**.013***	<.001	**.001**	.013	.014
		TM2	LEV	LAM	**.013***	<.001	**<.001**	.013	.014
		TM3	LEV	LAM	**.014***	<.001	**<.001**	.013	.014
	High	TM1	LEV	LAM	**.011***	<.001	**.002**	.011	.012
		TM2	LEV	LAM	**.012***	<.001	**.011**	.011	.012
		TM3	LEV	LAM	**.013***	<.001	**.001**	.012	.014
Mean volume density of Stratum lacunosum (mm)	Low	TM1	LEV	LAM	**.016***	<.001	**<.001**	.016	.017
		TM2	LEV	LAM	**.017***	<.001	**<.001**	.016	.017
		TM3	LEV	LAM	**.017***	<.001	**<.001**	.017	.018
	Medium	TM1	LEV	LAM	**.014***	<.001	**.003**	.014	.015
		TM2	LEV	LAM	**.015***	<.001	**.002**	.015	.016
		TM3	LEV	LAM	**.016***	<.001	**<.001**	.015	.016
	High	TM1	LEV	LAM	**.013***	<.001	**.001**	.013	.014
		TM2	LEV	LAM	**.014***	<.001	**<.001**	.013	.014
		TM3	LEV	LAM	**.015***	<.001	**.001**	.014	.015

Key-(*) indicates that the mean difference is significant at .05 level

4.4.3.5 The Comparative Histostereiological Effects of the Two Medicines on the Histological Organization of the Dentate Gyrus and the Amygdaloid Nuclei:

In assessing the histostereiological glogal effects on how the two medicines i.e the lamotrigine or levetiracetam influenced the volume density of the denatate gyurs and the amygdaloid nuclei, a one-way analysis of variances (ANOVA) was doen then followed by Turkey post-hoc multiple comparative t-tests. The resulst indicated that the two medicines at a global level had a negative deleterious influence on both histological components of the denatate gyurs and the amygdaloid nuclei including the cellular components and the nerve axonal fibre bundles. This was subsequently noted to result in the observed overall stastical reduction in volume densities of all theier histological layers as follows; (a)amygdaloid nucleus (AN) (F (18,38) = 962.447, P=.011), (b) dentate gyrus nucleus (DG) (F (18,38) =885.355, P=.013 (Table 4.33).

Upon further assessment of the intragroup and intergroup comparisons on how the two medicines differed in influencing the histological organization of the denatate gyrus and the amgygdaloid nuclei, it was futher observed that at lower dosage groups of both the lamorigen and the levetiracetion recorded the least reductions in the histological thicknesses of of the layers of both the dentate gyrus and the amygdaloid nuclei as compared to the mean thickness of their histological layers plus the cellular densities in the medium and high dosage groups (MDG &HDG) of both the two medices. this indicated that the observed mean reduction in the histological layers were first dependent on the dosages (Table 4.33). On further assessment on the time effects, it was observed that the early exposures at TM1 and TM2 had more deleterious effects with lamortigen being the one with the worst teratogenic outcomes (Table 4.33).

Table 4.33: The Comparative ANOVA Table on How the Two Medicines Influenced the Volume Densities of the Histological Components of the Dentate Gyrus and the Amygdaloid Nucleus

The study groups	Study groups and dosage levels.	The time of exposure to treatment	The comparative mean volume density of dentate gyrus and amygdaloid nucleus for various study groups	
			Mean dentate gyrus (mm^3) ± SD	**Mean amygdaloid nucleus (mm^3) ± SD**
Control	Control (C) **(no treatment)**	None.	0.0024±0.03	0.0083±0.03
Levetiracetam treatment groups	Low dosage group **(103mg/kg/bw)**	Trimester one	0.0021±0.06*	0.0062±0.07*
		Trimester two	0.0023±0.05	0.0065±0.03*
		Trimester three	0.0023±0.06	0.0067±0.07
	Medium dosage group **(207mg/kg/bw)**	Trimester one	0.0019±0.01*	0.0056±0.01*
		Trimester two	0.0020±0.07*	0.0058±0.03*
		Trimester three	0.0021±0.07	0.0059±0.03
	High dosage group **(310 mg/kg/bw)**	Trimester one	0.0018±0.01*	0.0053±0.04*
		Trimester two	0.0018±0.04*	0.0054±0.03*
		Trimester three	0.0020±0.03*	0.0057±0.04*
Lamotrigine treatment groups	Low dosage group **(3mg/kg/bw)**	Trimester one	0.0019±0.01*	0.0060±0.01*
		Trimester two	0.0022±0.04	0.0062±0.03*
		Trimester three	0.0023±0.03	0.0065±0.04
	Medium dosage group **(24mg/kg/bw)**	Trimester one	0.0017±0.06*	0.0054±0.06*
		Trimester two	0.0019±0.03*	0.0055±0.05*
		Trimester three	0.0020±0.07	0.0057±0.03
	High dosage group **(52mg/kg/bw)**	Trimester one	0.0016±0.04*	0.0051±0.04*
		Trimester two	0.0017±0.06*	0.0052±0.03*
		Trimester three	0.0019±0.07*	0.0055±0.06*
Overall comparison by ANOVA [F, P values]			**F (18,38) =885.355 P=0.013**	**F (18,38) =962.447 P=0.001**

***Key**: All values that bear (*) indicates that they depict a statistical significance difference (P<.05), when compared with the control, using one-way ANOVA with Tukey post-hoc multiple comparison t-test*

Upon carrying out the **MANOVA level I analysis to determine how globally** the two medicines plus their interaction effects globally influenced the volume densities of the dentate gyrus and Amygdaloid nucleus on either at an individual level of the drug, dose and trimester/time of exposure plus their interations (*) influenced the findings on the volume densities of the dentate gyrus and the amygdaloid nucleus, it was established that their teratogenic contributibutory effects were as follows: -

(i) At the individual level when each of the indepedent variables of drug, dose and time of exposure were acting alone, the statistical significant contribution of each on the overall main effects were as follows; (a) dugs (F (2,37) = 453727.066, P=<.001); Wilkis' lambda (Λ) =1.000; Partial Eta squared

(η^2 =.1000), (b) dosages (*F* (2,37) = 166.713, *P*<.001); Wilkis' lambda (Λ) =.047; Partial Eta squared (η^2 =.783), and (c) trimesters (*F* (4,74) = 19.200, *P*<.001); Wilkis' lambda (Λ) =.241; Partial Eta squared (η^2 =.509). The highest contribution was from the type of drug that was bing administered (100%) (Table 4.34)

(ii). At a two way combinations when either of the two independent varaibles were combined with each other at two-way interactions, their contributions were as follows: - (a) drugs*dosages, (*F* (4,74) = 63.507, *P*<.001); Wilkis 'lambda (Λ) =.051; Partial Eta squared (η^2 =.77) (b) drugs*trimesters, (*F* (4,74) =18.098, *P*<001); Wilkis' lambda (Λ) =.056; Partial Eta squared (η^2 =.60), (c) dosages*trimesters (*F* (4,74) =20.391, *p*<.001; Wilkis'Λ =.097; Partial Eta squared (η^2 =.69). The highest contributory combinations were noted to be from drugs and dosage (77%), folowed by dose and time of exposure 69%, then dose and and trimester effects (60%) (Table 4.34)

(iii). At three-way when the interaction effects of the comibanation of the three independent variables were evaluated, their statistically significant interaction effects of drugs*dosages*trimesters, (*F* (8,76) = 20.662, *P*<.001); Wilkis' lambda (Λ) =.096; Partial Eta squared (η^2 =.69). It was hence notable that at three-way cominations their worst deleterious effects were when all were acting at TM1 and two as the combination at these times of exposre gave rise to the worst deleterious effects (Table 4.34)

Table 4.34: The Level 1 Manova Table on How Globally the Two Medicines, Dosages and Trimesters Plus their Interactions Influenced the Volume Density of Amygdaloid Nucleus and Dentate Gyrus

		The multivariate statistical tests parameters applied					
The comparative global effects assessed	**The parameters used**	**MANOVA test statistics (Wilks' Lambda)**	**Statistics (F)**	**Hypothesis degree of freedom**	**Error degree of freedom**	**Sig.<.05**	**Proportion of variance (Partial Eta Squared)**
Assessment of whether or not the observed overall effects were due to drugs (either lamotrigine or levetiracetam)	**Drugs**	1.000	45327.066[b]	2.000	37.000	**<.001**	1.000
To assess whether or not the observed overall effects were due to varied doses of lamotrigine and levetiracetam	**Dosages**	.047	66.713[b]	4.000	74.000	**<.001**	.783
To assess whether or not the observed overall effects were due to differing trimesters (TM_1, TM_2, &TM_3)	**Trimesters**	.241	19.200[b]	4.000	74.000	**<.001**	.509
To assess whether or not the observed overall effects were due to interaction between varied doses and the drugs	**Drugs * dosages**	.051	63.507[b]	4.000	74.000	**<.001**	.774
To assess whether or not the observed overall effects were due to interaction between drugs and differing trimesters.	**Drugs * trimesters**	.056	18.098[b]	4.000	74.000	**<.001**	.595
To assess whether or not the observed overall effects were due to interaction between dosages with differing trimesters.	**Dosages *trimesters**	.097	20.391[b]	8.000	74.000	**<.001**	.691
Whether or not the observed overall effects were due to the two drugs and the dosages as well as the trimesters	**Drugs * Dosages * Trimesters**	.096	20.662[b]	8.000	74.000	**<.001**	.691

Key: (*) indicates interaction effects, while([b])indicates exact statistics using MANOVA

Upon carrying out the MANOVA analysis on how globally, the drugs, dosages and trimesters/time of exposure plus their interations (*) influenced the mean reduction

in amygdaloid nucleus and dentate gyrus histological layers, it was established that their contributions were as follows;

(i) At the individual level when each of the indepedent variables of drug, dose and time of exposure (trimesters) were acting alone, the statistical significant contribution of each on the overall main effects statistically significant ($P<.05$) to amygdaloid nucleus and dentate gyrus histological layers at varied proportions (Partial Eta squared, η^2). The highest contribution was from the type of drug administered at 99% followed by dose at 79% and the time of exposure being 46% (Table 4.35).

(ii) In a two-way combinations; there was statistically significant contributions of a two-way combination when each of any two independent variables were combined were as follows; (a) drug*dosages, (b)drugs*trimesters &; (c) dosages*trimesters at varied proportionate (Partial Eta squared (η^2), to the volume density of dentate gyrus and amygdaloid nucleus. The highest contribution was noted to be the combination of drug and dosages at 80%, followed by drug and trimesters of exposure at 71%, then lastly dosage and trimesters at 56% (Table 4.35).

(iii). In the three-way combinations, there was statistical significant contributions when the three independent variables were all combined as follows; [drugs*dosages*trimesters] for; (i) amygdaloid nucleus (AN) (F (4, 38) =65.982, $P<.001$; Partial Eta squared (η^2 =.87), and dentate gyrus (DG) (4, 38) =1.988, $P=.016$; Partial Eta squared (η^2 =.373). In overall for both the dentate guyrus and the amygdaloid nucleus, the effects of the three combined was at TM1 and TM2. (Table 4.35).

Table 4.35: The Level 2 MANOVA on How Globally the Drugs, Dosages and Time of Exposure plus their Interations Influenced the Volume Density of Amygdaloid Nucleus and Dentate Gyrus Histological Layers

Tests of Between-Subjects Effects

The independent variables	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig[d] (<.05)	Partial Eta Squared
Drugs	Amygdaloid nucleus	5.830E-5	1	5.830E-5	10837.597	**<.001**	.997
	Dentate gyrus	.000	1	.000	43109.576	**<.001**	.999
Dosages	Amygdaloid nucleus	5.420E-7	2	2.710E-7	50.375	**<.001**	.726
	Dentate gyrus	2.218E-6	2	1.109E-6	107.680	**<.001**	.850
Trimesters	Amygdaloid nucleus	1.221E-7	2	6.106E-8	11.351	**<.001**	.374
	Dentate gyrus	4.546E-7	2	2.273E-7	22.067	**<.001**	.537
Drugs * dosages	Amygdaloid nucleus	6.441E-7	2	3.221E-7	59.871	**<.001**	.759
	Dentate gyrus	2.027E-6	2	1.013E-6	98.387	**<.001**	.838
Drugs * trimesters	Amygdaloid nucleus	3.258E-7	2	1.629E-7	30.286	**<.001**	.614
	Dentate gyrus	3.941E-7	2	1.970E-7	19.128	**<.001**	.502
Dosages * trimesters	Amygdaloid nucleus	1.199E-6	4	2.998E-7	55.742	**<.001**	.854
	Dentate gyrus	7.181E-8	4	1.795E-8	1.743	**.041**	.555
Drugs * dosages * trimesters	Amygdaloid nucleus	1.420E-6	4	3.549E-7	65.982	**<.001**	.874
	Dentate gyrus	8.191E-8	4	2.048E-8	1.988	**.016**	.373

Key: *(*) indicates interaction effects*

Upon carrying out **the MANOVA level III analysis on the pairwise comparison** on how the two medicines influenced the volume densities of the various histological components of the dentate gyrus and the amygdaloid nucleus when exposed within the same dosages levels, the study established that in all the dose levels of low medium and high lamotrigine treated groups, the observed effects on the histological organization of both the dentate gyrus and the amygdaloid nuclei were seen to be statistically significant different (P<.05) as compared with the same dose groups of the levetiracetum treated groups. it was also notable that the differences between the two medicines were more pronounced when the treatments were instituted at TM1 and TM2. at TM3 there was no notable significant difference on how they influenced the histological thicknesess of the the dentate gyrus and the amygdaloid nucleus (Table 4.36).

Table 4.36: The Level 3 MANOVA Pairwise Comparison Table on How the Two Medicines Influenced the Volume Density of the Amygdaloid Nucleus and Dentate Gyrus When Exposed Within the Same Dosage Levels

Multiple/Pairwise Comparisons									
								95% Confidence Interval for Difference[d]	
Dependent Variable	**Dosages (mg/kg bw)**	**Trimesters**	**Levetiracetam (LEV)**	**Lamotrigine (LAM)**	**Mean Difference (LEV-LAM)**	**Std. Error**	**Sig[d] (<.05)**	**Lower Bound**	**Upper Bound**
	Low	TM_1	LEV	LAM	**.003***	<.001	**.001**	.003	.003
		TM_2	LEV	LAM	**.001***	<.001	**.001**	.001	.002
		TM_3	LEV	LAM	**.001***	<.001	**.012**	.001	.001
Mean amygdaloid nucleus volume density	Medium	TM_1	LEV	LAM	**.002***	<.001	**.001**	.002	.002
		TM_2	LEV	LAM	**.002***	<.001	**.001**	.002	.002
		TM_3	LEV	LAM	**.002***	<.001	**.001**	.002	.003
	High	TM_1	LEV	LAM	**.002***	<.001	**.001**	.002	.002
		TM_2	LEV	LAM	**.002***	<.001	**.001**	.002	.002
		TM_3	LEV	LAM	**.002***	<.001	**.001**	.002	.002
	Low	TM_1	LEV	LAM	**.006***	<.001	**.011**	.006	.006
		TM_2	LEV	LAM	**.006***	<.001	**<.001**	.006	.006
		TM_3	LEV	LAM	**.006***	<.001	**<.001**	.006	.007
Mean dentate gyrus volume density	Medium	TM_1	LEV	LAM	**.006***	<.001	**.002**	.005	.006
		TM_2	LEV	LAM	**.006***	<.001	**<.001**	.006	.006
		TM_3	LEV	LAM	**.006***	<.001	**<.001**	.006	.006
	High	TM_1	LEV	LAM	**.005***	<.001	**.002**	.005	.005
		TM_2	LEV	LAM	**.005***	<.001	**.002**	.005	.005
		TM_3	LEV	LAM	**.006***	<.001	**.002**	.005	.006

***Key-**(*) indicates that the mean difference is significant at .05 level*

CHAPTER FIVE
DISCUSSION, CONCLUSION AND RECOMMEDATIONS

5.1 Objective 1: Comparative Findings on How the Prenatal Exposure to Lamotrigine and Levetiracetam Influenced the Maternal and Fetal Pregnancy Outcomes

The maternal pregnancy outcomes parameters that were the focus of this study included; the daily maternal weight gain treads, the terminal weight, the total terminal weight gain and the placental weights. This study established that, when the two anticonvulsant medicines were exposed prenatally at varied doses and at different gestation periods of TM1, TM2 and TM3, the four maternal pregnancy outcome parameters were all noted to be significantly lower as compared with the control

In particular, **the daily maternal weight gains treads** in both the levetiracetam and the lamotrigen treated groups were observed to be sluggish in the entire gestation period especially in medium and high dosage groups as compared with the control These findings on the sustained linear reduction in the mean daily maternal weight gain treads in the treatment groups as compared with the controls in the entrire gestation period are in tardem with some previous findings by Mwangi *et al* (2019) and Wlodarczyk *et al* (2012) whose study findings showed that upon prenatal exposure to. carbermazepin and phenytoin respectively that has similar mode of action with lamotrigine and levetiracetam, they resulted in sutained maternal nutrition pertabations that subsequently impacted on the fetal growth and development in-utero, due to their effects on the placenta that served as the source of nutrients to the fetus. This ultimately was delineated by low daily maternal weight gain trends in the study groups.

With regards to how the two medicines influenced **the terminal weights and the total weight gains** of the mothers that serves as an indicator on how long the purtabations of the maternal nutritional status were sustained in the entire gestaional period, it was observed that the means were statistically significantly lower ($P<.05$)

in both lamotrigine and levetiracetam treatment groups as compared with the control. It was further notable that the percentage ranges of the total weight gain for the rats in the treatment groups was between 10-38% while for the rats in the control group ranged between 40- 46%. This range in the control group was in line with the findings of a standard normal total weight gain in albino rats that was reported by Paronis *et al.,* (2015) who noted that it is usually approximately 41%, and was low in the treatment groups.

Upon carrying out the multivariate analysis (MANOVA) to compare how the two medicines, their dosages and the time of exposure differed interms of influencing the maternal terminal weight and terminal maternal weight gain. This analysis was done to assess to what extent the maternal nutrition status was perturbed by both the individual and the combinations of these indepent variables. From the findings, it was established that both their main effects plus their interaction effects of drugs, dosages and trimesters plus their combinations of either of the two or combination of the three independent variables were statistically significant *(P*<.05) .This means that they influenced the mean reduction of the maternal terminal weight and terminal maternal weight gain in varied proportions with the most reductions being associated with lamotrigine, meaning that it caused more purtabaions to the maternal nutritional status in the entire gestation period as well as making the fetal growth environment *in-utero* to be toxic and hence the observation made .

It was further noted that, these findings on the maternal nutritional purtabations that were occasioned by prenatal exposure to lamotrigen and levetiracetam are interdem with study findings by (Elshama *et al.,* 2015). In their article, they obsrerved that all anticonvulant medicines could be having similar mode of maternal nutritional disturbances whether in the first, second or third generation. In addition, study findings by Khouri *et al.,* (2005) showed that exposure to 100mg/kg bw of topiramate caused reduction in the number of implantation sites as well as the number of the viable developing foetuses with resultand decrease in maternal weight.

The terminal placental weight is an important paramenter in maternal pregnancy outcomes, as it serves as an indicator on the total size/surface area in the maternal

placental- blood barrier where the nutrinional exchange takes place between the mother and the fetus. In this study, the total terminal weight of the placenta for both the treatment groups and the control were evaluated. The study established that the total terminal weight of the placentas from both the treatment groups of lamotrigen and levetiracetam were statistically significantly small in sizes and lighter in weight ranging between 3.23-5.39 milligrams against the controls that ranged between 5.1-5.61 milligrams.

Upon carrying out the multivariate analysis using MANOVA to campare the main effects of the two medicines plus their interaction effects with dosages and the time of exposure, it was affirmed that the two drugs plus their interaction effects with the dosages and the time of exposure had a role to play in the observed reducations in placental weight in the treatment groups. Futher affirmations were made on the deferentails on how the two medicines differed in their influences to the reductions in the palcental weight, where lamotrigen was observed to have more statistically significant deleterious effects than was the case for the levetiracetam treated group ($P<.05$).

These findings in reduction in the sizes and the terminal placental weights served as poineter to the observed small litter sizes as well as the stunted growths in the individual fetuses harvested from the uterine horns of the mother rats from the treatment groups. The current findings are interdem with the study results of Semczuk-Sikora and Semczuk (2004). Their publication results demonstrated that one of the anticonvulsant medicine valproic acid with similar mode of action with lamotrigine and levetiracetam crosses the maternal placenta barrier and cause adverse effects to the placenta that includes atrophied syncytiotrophoblast as well as degeneration of microvascular cytoplasm leading to atrophy and necrosis.

On the fetal preganacy outcomes; the parameters that were assessed included the litter size/numbers, embryo-lethality, resorbed endometrial glands/devoured fetuses, dead fetuses, the fetal body weight (BW), crown rump length (CRL), head circumference (HC), bi- parietal diameter (BD) and the head length (HL). With regards to the intra-uterine fetal outcomes that included the **litter size/numbers,**

embryo lethality, the number of resorbed endometrial glands/devoured fetuses and the numbers of dead fetuses, the study established that the total numbers of these intrauterine parameters in the treatment groups were significantly lower especialy in medium and high dosage groups at TM_1 &TM_2 as compared with the control group .This reduction in the litter sizes, increased number of the dead fetuses, devoured endomentrial glands and devoured fetuses served as an indicator to the inhibitory caused by the two medicines during the process of implantation, cellular differentiation, and tissue organization during organogenesis. This denoted the levels of toxixcy in the fetal growth environment in-utero that could be attributed to some form of teratogenic purtabations in the process of fetal growth and development perinatally, (Ypsilantis *et al.*, 2009).

Further, previous study findings by Hill *et al.*, (2010) correlated the increased number of resorptions, devoured and dead fetuses observed in the treatment groups with un-intentional occurrences, associated with the teratogenic effects of all anticonvulsant medications whether in first, second or third generation during the process of implantation, organogenesis to maturation of the fetal organ systems. In addition, the current study results are in agreement with those of Cansu *at al.*, (2020) and Etemad *et al.,* (2013), whose findings demonstrated that upon administration of valproic acid and pregabaline medicines respectively, they both were observed to inhibit prenatal embryo implantation in the uterus by inducing death of the stroma and swellings of the mitochondria, with resultant endometrial gland resorptions and embryo-lethality. The current study results however contradict the findings of Morse, (2016). In his findings, he reported that upon prenatal exposure to varying doses of pregabalin, there was no evidence of toxicity, malformations to the implantation sites or embryo-letality. The contradicting results could be attributed to the small sample size used in his study.

Upon carrying out the univeriate, bivariate and multivariate analysis by use of ANOVA and MANOVA to establish how the individual effects of each medicine plus their interactions effects of drug, dose and time of exposure influenced the fetal growth and development parameters that included the means of **fetal body weight, crown-rump length, head circumference, bi-parietal diameter and head length**,

the current study results depicted statistically significant reduction in all these fetal growth and development parameters from both the treatment groups across all the dosages of low, medium and high treatment at TM1, TM2 and TM3 as compared with the control.It was further observed that lamotrigine has more deleterious effects on these fetal growth and development parameters *in-utero* than it is with levetiracetam when administered at the same dosage levels.These current study findings are interdem with those of Bath & Scharfman, (2013) and Prakash *et al., (*2008) that reported deleterious effects on foetal growth and development parameters upon administration of phenytoin, phenobarbitone and valproate, anticonvulsant medicines in the fist and second generation.

Further, results by López-Escobar *et al.,* (2020) are interdem with the current study results since lacosamide anticonvulsant medicine was observed to decrease fetal head circumference, bi-parietal diameter, brain weight and resultant neuro-developmental effects. In the contrary, the findings by Montouris (2005) and Eisenschenk (2006) contradicted the findings of the current study. According to their findings, fetuses born to mothers exposed to oxcarbazepine medicine had no associated effects. These contradictory findings could be attributed to the small sample size used, though the authors recommended for further follow-up studies using a bigger sample size in order to come up with more varied conclusions.

5.2 Objective 2: The Comparative Findings on How the Two Anticonvilsant Medicines Influenced the Cyto-Architecture and the Histomorphological Development of the Fetal Memory Circuitry Structurers

Upon evaluating how the two anticonvulsant medicines (i.e lamotrigen and levetiracetam) influenced the histo-cyto-architectural development of the fetal memory circuitry structures, the study focused on the pre-frontal-cortex, the entorhinal cortex, the hippocampus, the subiculum, the dentate gyrus, and the amygdaloid nucleus. This current study established that when the two medicines were prenatally exposed in varied doses and at different gestaional periods i.e TM1, TM2, and TM3, they depicted variances in the way they influenced the histo-cyto-arhitectural arrangement of the cells in each of the above mentioned fetal memory

circuitry strauctur, as well as how they influenced the histomorphological thickness of the different histological layers per each of the components of the said fetal memory circuitrory structure as follows:-.

On the prefrontal cotex, the findings of this study established that the key cells involved in memory processing that included the granular cells, the pyramidal cells, the horizontal cells of Cajal and Reitzius, the fusiform cells and the stallete cells were remarkably reduced in their densities, the histomorphological sizes and shapes, and they were also noted to be sparsely distributed within their respective histological layers of the prefrontal cortex. In particular, the pryamical cells (the small, and the medium), the granular cells as well as the stellate cells in the fisrt three layers that included; (I) the molecular layer, (II) the outer granular and (III) the outer pyramidal layer were seen to be the ones higly targeted by the deletelious teratogenic effects of the two medicines as they were highly reduced in their numbers and sizes.

In the inner three layers of the prefrontal cortex that included (IV) the inner pyramidal, (V) the inner granular and (VI) multiform layer whose key memory cells observed were the medium and large pyramidal /Betz cells, the granular cells and the fusiform cells, they were noted to be the ones that were largely affected by the *in-utero* exposre to these two anticonvulsant medicines in terms of their distribution, reduction in the cell sizes and numbers plus their general histomorphological apperances .In these inner layers, it was further notable that it had conspicuous interconnecting axonal nerve fibre bundles that interconnected the lower inner structures of the memory circuitory pathway as well the other parts of the brain. These nerve fibre bundles were simillary observed to be reducing in their sizes in both the treaetment groups of lamotrigen and levetiracetam in comparison to those of the control.

It was futher notable the the histomorphological teratogenic effects seen in form of disorganization of the cellular components and in the reduction of the histological layers of prefrontal cortical layers were both dose and time dependent, where the high and the medium doses of the two medicines when exposed at TM1 and TM2 recorded the worst deleterious effects as compared to the low doses whe they were

exposed at TM3.In addition, lamotrigen was seen to have more deleterious teratogenic effects than levetiracetam.

This observed disorganisation of the memory cells and histological layers of the prefrontal cortex in the current study could be attributed probably by the fact that both lamotrigine and levetiracetam have low molecular weight, hence are able to penetrate the maternal placenta barrier and cause effects to the foetal brain structurers that includes the prefrontal cortex. The current study results are in agreement with those of Badawy *et al.*, (2019), whose results showed that upon administration of gabapentin that is a 2nd generation anticonvulsant medicine during organogenesis period, it caused alteration of the cerebral cortical layers of the frontal lobe as well as of the hippocampal gyrus of the medial temporal lobe.

On the entorhinal cortex, this study established that the histomorphological organization of the key memory cells in both the supra-deccical and infra-deccical zones that included; the granular, small and medium sized pyramidal cells, the stellate cells plus the interconnecting nerve fibre bundles were negatively affected by the prenatal exposure to the two anticonvulant medicines .**On the supra-deccical layers** that included the; (I) molecular/plexiform (ML), (II) stratum sterale (SS), and the (III) external principal striatum (EPS), the pyramidal and the granules cells were the ones that were mostly affected.. On the other hand, in the infra-deccical layers that included (IV)lamina desiccants (LD), (V) internal principal striatum (IPS), and (VI) multiform layer (MTL), the key cells seen to be affected more were the the granule and the the stallate cells plus the nerve axonal fiber bundles that were a key component on this inner layers.

In overall, the cellular organization, the cell distributions, the densities of all the key memory cells, the axonal fibre bundles that forms the ineter-connections superioly and inferioly to the hippocampus were all noted to be affected by the prenatal exposure to the two medicines in a time and dose response relationship, where the prenatal exposures in high and medium doses had the worst observed teratogenic effects particulary in early exposures of TM1 and TM2.

Further, the histological thickness of the six layers of the entorhinal corticex were subsequently seen to reduce in size in the treatment groups of both the two medicines in a dose response manner. This reduction in cortical thickneness was hence attributed to the reduction in the cellular numbers and the sizes of the key memory cells per layer, that were also becoming sparsely distributed depending on the dosage and the time of exposures as described above. These effects were observed to be more marked in the lamotrigine treatment groups as opposed to levetiracetam treatment groups. The current study findings are interdem with tose of Badaway *et al.,* (2019), that exhibited disruption and alterations of the cyto-architecture and thickness of the entorhinal and hippocampal layers upon administration of gabapectin, with results in neurodegenerative changes and apoptosis

On the subicular complex that includes; the subiculum, presubiculum and parasubiculum, this study established that the histocyto-architecural organization of the subicular complex was not any different from what was observed in the prefrontal and the entorhinal cortex in that the cellular organization, distributions, densities of its key memory cells that included the pyramidal, stellate and the granular cells equally reduced with the observed reducation in thickenes of its histological layers namely; (I) molecular (ML), (II) pyramidal (PL) and (III) plexiform (PLL) layers .

The current study findings could be attributed to the enhibitory teraotenic effects of the two medicines in the maturation of the subiculum cortical layers, as was reported by Manet *et al.,* (2007). The study established thatwhen pregabalin anticonvulsant medicine is exposed prenatally, it perturbs the morphogenenetic processes of the brain cell maturation in the subicular cortex with subsequent delay in cortical maturation of its histological layers, resulting in disorganization of cellular layers and interfering with neuronal migration and ultimately neuronal death.

On the hippocampus, it was observed that, the cellular compoments that included the pyramidal cells, stellate and the granule cells plus the histological thicknesses of the hippocampal gyrus in both the treatment groups of lamotrigen and levetiracetam

depicted an inververse dose response reduction in both its outer and the inner layers. At medium and the high dosage levels, all the outer histological layers namely; (I) the stratum alveus layer (SAL), (II) the stratum oriens layer (SOL), and the (III) stratum pyramidale layer (SPL), were observed to be much reduced across the three trimesters of TM1, TM2 and TM3 for both the levetiracetum and the lamotrigine treated groups than the inner histological layers; (IV) the stratum radiatum layer (SRL), and the (V) striatum lacunosum/moraculare layer (SLL). However, in the lamotrigen treated groups, all the layers of the hippocampus were more reduced than those of levetiracetam treated groups.

The current study results are in agreement with those of Kaushal *et al.,* (2016) that indicated that upon administration of a wide range of 1st and 2nd generation anticonvulsant medicines *in-utero,* they caused disorganisation of the layers of the hippocampal gyrus and and sparce distribution of cells, with resultant cell apoptosis.

On the dentate and the amygdaloid nucleus: - The comparative thicknesses of histological layers'of the amygdaloid nucleus and the dentate gyrus namely; (I) the molecular layer (ML), (II) the granular layer (GL) and (III) the polymphic layer (PML) and their key memory cells that includes the pryaramidal, stellate and the granular cells were all noted to be reduced in their sizes, number and in their densities in a dose and time dependent manner for both the two medicines .It was further noted that, the high and medium dosage groups in both lamotrigine and levetiracetam treated groups were the ones associated with the most reduction in the cortical thickness of the histological layers of both the dentate gyrus and the amygdaloid nuclei. At trimester one and (TM1 and TM_2), the thickness of the three histological layers were observed to similarly portray remarkable reductions in the thickness of the histological layers.

The results of the current study on the histomorphological organization of the dentate and the amygdaloid nuclei are in line with the findings by Mwangi etal 2019, and González-Maciel *et al.,* (2020) who repoted that upon administration of carbamazepine, there was architectural alteration of the thickness in the hippocampal gyrus as well as thecellular organization of dentate and the amygdaloid nuclei.

5.3 Objective 3: Comparative Histo-Quatitative Findings on Effects of Lamotrigine and Levetiracetam on the Development of Foetal Memory Structurers

The comparative histostereological findings are discussed in two levels as follows;

the gross morphometric effects **on the gross morphometric measurements of the fetal brain** including;(a) the gross brain weight, (b) the occipital-frontalis length and (c) the bi-parietal width; **(ii) the histostereological effects on the histological organization of the fetal memory circuitry pathway structurers** including; (a) the Archimedes and the calculated cavalieri total brain volume, (b) the volume densities of prefrontal cortex, entorhinal cortex, subiculum, hippocampal layers, the dentate gyrus and the amygdaloid nucleus

5.3.1 The Comparative Effects on the Gross Morphometric Measurement of the Fetal Brain (Brain Weight, the Brain Length and the Brain Width).

On evaluating how the two medicines influenced the gross morphology of the entire brain, it was in a view to finding out whether the brain had a translational relationship with the histostereological quantification of the various fetal memory circuitry structures in obeyance to the principle of teratogensis that states that an observed minor defect is a conjent indicator of another major defect. As such, the parameters eveluatued included the total brain weight, the bi-pariental brain width, and the occipital-frontalis length that are of paramount importance since they serve as indicators of brain integrity and rule out neuronal abnormality.

The current study established that the prenatal exposre to the two medicines i.e lamotrigen and levetiracetam had a teratogenic gross morphometric deleterious effect on the three gross mophometric parameters evaluted on the brain as all of them were noted to be statistically significantly low ($P<.05$) as compared with the control. As such, the mean average brain weights, lengths and widths for the treatment groups were ranging as follows; [brain weigts (1.08-1.23g), brain length (1.13-1.48cm), and

brain width (0.99-1.26cm) respectively, while for the control, the range was as follows; brain weight (1.25-1.26g) brain length (1.57-1.58cm) and brain width (1.31-1.32cm). The mean reduction was also noted to depict dose and time relationship in that was lowest when medium and high dosages (MD&HD) of lamotrigine and levetiracetam were administered during the first and the second trimesters (TM_1 &TM_2).

Further, upon carrying out multivariate regressional analysis using MANOVA, it was observed that the drugs, dosages and trimesters of exposure portrayed statististical significant main and interaction effects at two-way and three-way combinations, meaning that they contributed to the mean reduction of the three fetal brain morphological parameters at varying proportions. Pairwise comparisons further depicted that lamotrigine has more deleterious effects than levetiracetam at the same dosage levels.

The current study results concur with previous outcomes by Wairimu *et al.,* (2019) and Elshama *et al.,* (2015), that both reported of reduction in brain weight, length and width, upon administration of carbamazepine, an anticonvulsant medicine with similar mode of action with lamotrigine and levetiracetam due to their effects in cortical and subcortical structurers of the brain. Similarly, study findings by Song *et al.,* (2018) demonstrated decrease in brain weight upon administration of oxycarbazine, a second-generation anticonvulsant medicine like lamotrigine and levetiracetam. In contrary, a neurotoxic study by Erisgin *et al.,* (2019) conveyed that upon administration of second-generation anticonvulsants medicines that included gabapentin and oxycarbazine at aried trimesters, there was no effects observed on means of fetal brain weight, length and width. The study however advocated for further subsequent studies to be carried out, as it had made use of a small sample size.

5.3.2 The Comparative Histostereological Effects of the Two Medicines on the Total Fetal Brain Volume and Volume Densities of the Memory Circuitry Structures

On evaluating how the two medicines influenced the **total fetal brain volume**, it was observed that both lamotrigine and levetiracetam caused reduction of both the initial Archimedes' displacement volume and terminal Cavalieri point counting volume in a dose and time related manner, as compared with the control group. Medium and high dosage groups when medication was administered TM1 and TM2 had statistically significant lower means ($P<.05$), than when high doses were administered at TM3. The MANOVA results depicted that dosages, drugs and trimester contributed to the mean reduction in total brain volume at varying proportions, while pairwise results showed that lamotrigine has more detrimental effects than lamotrigine when administered at the same dosage levels across the trimesters

The current study results agree with those of Bittigau *et al.,* (2003) that evidenced that exposure of drugs with similar mode of action with levetiracetam and lamotrigine including vigabatrin, valproic acid, phenytoin, phenobarbital, diazepam and clonazepam, they resulted in decrease in developing fetal brain mass and volume ascribed by neuronal death in a dose dependent manner. The current study results however contradict those of Glier *et al.,* (2004), whose results indicated that upon dispensation of both varied doses of topiramate that is a second-generation anticonvulsant medicine, there was no reduction in total brain volume as well as volume densities. The study therefore concluded that topiramate has no neurotoxic effects to the developing foetal brain. This could have been attributed by the small sample size used in the study.

Upon carrying out the univariate and bivariate ANOVA as well as multivariate regressional analysis (MANOVA) to assess how the two medicines influenced the histostereological volume densities of the cells and the axonal fibre bundles on the histological layers of **the pre-frontal cortex,** the study established that, the volume densities of the key memory cells including the pyramidal, stellate and the granules cells had a direct proportionate reduction in volume densities of the corresponding

histological thicknesses of each of the six histological layers of prefrental cortex namely (I) the plexiform molecular/layer (ML), (II) outer granular (OG) and, (III) the outer pyramidal (OP) layers, (IV) the inner granular layer (IG), (V) the inner pyramidal (IP), and (VI) the multifom layer (ML) was reduced in treatment groups as compared with the control.

This reduction in voulume densites were also noted to cut-across all the dose levels for both medicines and particulary more pronounced with the lamotrigine treated groups at TM_1 and TM_2**.** It was futher notable that the reducation in volume densities of the six cortical layers affected more the supra granular layers that included (I) the plexiform molecular/layer (ML), (II) outer granular (OG) and (III) outer pyramidal layer (OPL) as compared with the infra granular layers including the inner granular layer (IG), (v) the inner pyramidal (IP), and (vi) the multifom layer (ML) layer.

The MANOVA results depicted that dosages, drugs and trimesters either individually or their interactions when they were combined, contributed to the mean reduction in the volume densities of the prefrontal cortex at varying proportions. Pairwise comparison results showed that lamotrigine has more detrimental effects that lamotrigine when administered at the same dosage levels across the trimesters

The current study results are intendem with those of Magar *et al.,* (2020) that exhibited that upon prenatal exposure to pregabalin, there was reduction in brain volume and volume densities of the cerebral cortex. This is in addition to associated degenerative changes of the axons with depletion of myelin sheath on the developing cerebral cortex of albino rat's offspring.

In carrying out both ANOVA to assess how the two anticonvulsant mendicines influenced the volume densities of various histological layers of the **entorhinal cortex** namely; (I) molecular layer (ML) (II) stratum sterale layer (SSL), (III) external principal striatum layer (EPSL), (IV) lamina dissecat layer (LDL), (V) internal principal striatum layer (IPSL) and (VI) multiform layer (MTL), the current study findings showed they both statistical caused statistical significant reduction of thevolume densities, especially when medium and high medications were administered TM1 and TM2 .

The MANOVA results depicted that dosages, drugs and trimesters either individually as well as their interactions, contributed to the mean reduction of the volume densities of entorhinal cortex at varying proportions. Pairwise comparison results similarly showed that lamotrigine has more baneful effects that lamotrigine when administered at the same dosage levels across the trimesters. The current study results are in agreement with those of Hagar, (2014), that delineated that upon prenatal exposure to topiramate, there was associated cellular disorganisation and reduction cellular numbers observed in both entorhinal cortex and the hippocampal gyrus.

Upon performing the univariate, bivariate and multivariate regressional nalaysis to assess how the two medicines influenced the histosteriological volume densities of the subicular complex involving the **subiculum, presubiculum and parasubiculum** histological layers, the study establsihed that the two medicines caused deletelious mean reduction in volume densities of the key cellular components, the nerve fibre bundles that form the inputs and output loops to the subiculum, presubiculum and parasubiculum with resultant overall reductions in all the histological thicknesses of the histological layers namely; (I) the molecular layer, (II) the pyramidal cell layer and (III) the polymorphic/fiber layer, in a dose and time dependent manner especially when medium and high dosages were administered TM1 and TM2.

Further, the MANOVA results depicted that the independent variables that includes the dosages, drugs and trimesters contributed to the reduction in volume densities of subiculum, presubiculum and parasubiculum at varying proportions. Pairwise comparison results showed that lamotrigine has more baneful effects that lamotrigine when administered at the same dosage levels across the trimesters The current study results are in accordance with findings of Tomson & Perucca (2019), who reported that generally, the first-generation anticonvulsant medicines cause more deleterious effects to the fetal brain structures including cerebral cortex and subcortical structurers than the second-generation anticonvulsant medicines.

In assessing the histostereiological glogal effects on how the two medicines i.e the lamotrigine or levetiracetam influenced the **volume density of the hippocampal gyrus**, a one-way analysis of variances (ANOVA) was done then followed by Turkey post-hoc multiple comparative t-tests. The results indicated that the two medicines at a global level had a negative deleterious influence in the two histological components of the hippocampal gyrus including the cellular components and the nerve axonal fibre bundles of the layers namely; (I) the stratum alveus layer (SAL), (II) the stratum oriens layer (SOL), and the (III) striatum pyramidale layer (SPL), (IV) the stratum radiatum layer SRL), (V) a combination of stratum lacunosum and stratum moraculare hippocampal layers especially when medium and high medications were administered TM1 and TM2

The MANOVA results depicted that the main effects of dosages, drugs and trimesters as well as their interactions when they were combined either at two way or at three ways, they contributed to the mean reduction of volume densities of the layers of the hippocampal gyrus at varying proportions. Pairwise comparison results showed that lamotrigine has more deleterious effects that lamotrigine when administered at the same dosage levels across the trimesters. The current study results are intredem with the findings of (López-Escobar *et al.*, 2020). In their publication, they stated that upon exposure of lacosamide *in-utero* it interfered with cellular organisation of the thickness of the hippocampal layers.

Upon performing ANOVA on the how the two medicines influenced the influenced the histological organization of the volume density of the various histological components of the **denatate gyurs and the amygdaloid nuclei**, the results indicated that the two medicines have a negative deleterious influence on the cellular components the axonal nerve fibres and thicknesses of the histological layers namely; (I) the molecular layer (ML), (II) the granular layer (GL) and (III) the polymorphic layer (PML) especially when medium and high medications were administered TM1 and TM2

The MANOVA results depicted that the three independent variables that includes dosages, drugs and trimesters individually as well as their interactions, contributed to

the mean reduction of the volume densities of denatate gyurs and the amygdaloid nuclei at varying proportions Pairwise comparison results showed that lamotrigine has more detrimental effects than lamotrigine when administered at the same dosage levels across the trimesters The current study results are in agreement with those of Chen *et al.,* 2009 which delineated that upon prenatal exposure to a wide range of anticonvulsant medicines that includes; phenobarbital, clonazepam, carbamazepine, valproate and topiramate for the entire gestation, they resulted in disruptions of cellular distribution pattern and their differentiation toward neuron and glial cells in dentate gyrus, amygdaloid nucleus and hippocampal gyrys. This finally resulted in inhibition of neurogenesis and cell survival.

5.4 Objective 4: Comparative Effects of Lamotrigine and Levetiracetam on the Dose and Time Administration

The current study has established that the comparative teratogenic effects of in-utero exposure to varied doses of both levetiracetam and lamotrigine **are time and dose dependent**. These findings have been affirmed by all the study parameters evaluated including; (i) the maternal and fetal pregnancy outcomes, (ii) the histomorphological findings on the fetal memory circuitry structures; (iii) the univariate, bivariate and the mutivaraite analysis in both the gross morphometric and histostereiological results where all the parameters in all the componets of memory circuitry system were statistaically significant lower in the treatment groups as compared with the controls.

In both treatment groups, the observed deleterious effects on the developing fetal memory circuitry structurers depicted an inverse time response relationship across the three trimesters (TM_1, TM_2 & TM_3) in that when the two medicines were issued during the first and the second trimester (TM_1 & TM_2), they were associated with the most baneful effects, as compared to whn they were issued during the third trimester (TM_3) except at high dosages. The current study findings are in accordance to those of Etemad *et al.,* (2013) that indicated that upon prenatal exposure to pregabalin, the percentage of malformations increased when the medicine was exposed at high dosage during organogenesis. The current study results however contradict those of

Erisgin *et al.,* (2019) that established that upon administration of prenatal gabapentin and oxcarbazepine prenatally, no congenital malformations were observed across the different trimesters.

It is also apparent from the current study results that the studied parameters in both lamotrigine and levetiracetam treated groups depicted a direct dose response relationship across three dosages of low, medium and high. High and medium dosages were observed to have pernicious effects as compared to the low dosage groups in both treatment groups. The current study results coincide with those of Tomson *et al.,* (2019) that exhibited that upon dispensation of anticonvulsant medicines that included valproate, phenobarbital, phenytoin, carbamazepine, and lamotrigine, the associated neurotoxic effects were dose dependant. Past study results by Elshama *et al.,* (2015) similarly delineated that upon administration of carbamazepine, foetal growth parameters as neurodevelopmental were observed in high dosage groups.

5.5 Conclusion

In conclusion, this study has established that;

1. In-utero exposure to lamotrigine and levetiracetam interferes with both the maternal nutritional status as well as the fetal growth and development environment in-utero that in return impacted on the observed deleterious effects on the poor maternal and fetal pregnancy out-comes.
2. In-utero exposure to lamotrigine and levetiracetam leads to the iniminical disorganization of the histological fetal brain components including the key memory cells, their nerve axonal fiber bundles as well as the histological thicknesses of the various layers that constitute the different components of the fetal memory circuitory pathway structures.
3. The prenatal exposure to lamotrigine and levetiracetam leads to the the reduction in both gross morphometric as well as the histostereiological volume densities of the various histological components including the key memory cells, the axonal fiber bundles as well as the histological thicknesses of the various

histological layers that constitute the different componets of the fetal memory circuitory pathway structures.

4. The observed injurious effects of perinatal exposure to lamotrigine and levetiracetam onto the developing fetal memory circuitory structurers were both dose and time dependent with the most toxic teratogenic doses for the two medicines were noted to be medium and high doses of levetiracetam 207/310mg/kg bw and lamotrigine of 24/52mg/kg bw particularly when administered during the first (TM_1) and second trimester (TM_2).
    - Lamotrigine has more teratogenic deleterious effects as compared with levetiracetam regadless of the dosages and the time of exposure.

5.6 Recommendations

The study recommends that;

1. Use of lamotrigine and levetiracetam during pregnancy should be avoided where possible particularly in first and second trimesters by seeking appropriate alternatives that would be safer to the fetus and the nutritional status of the mother.
2. If both the lamotrigine and levetiracetam cannot be avoided and must be be used during pregnancy in management of maternal conditions, the doses should be adjusted to the minimal effective dosages that would confer the maximum maternal benefits and also reduce the teratogenic risks to the developing fetal brain memory circuitory structures.
3. Levetiracetam is safer than lamotrigine at all dosage levels when apllied during pregnancy
4. Further studies should be carried out in non-human primates as they have a close phylogenetic relation to humans, to ascertain teratogenicity of levetiracetam and lamotrigine in relation to the most applicable safe doses during pregnancy.

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APPENDICES

Appendix I: Ethical Approval Form

DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY
P.O. Box 30197,
00100 Nairobi, Tel: 4449004/4442014/ 6
Kenya. Ext. 2300
Direct Line. 4448648

REF: FVM BAUEC/2021/321

Ms. Ann Wairimu Mwangi.
Dept. Human Anatomy,
JKUA & Technology.
10/11/2021

Dear Ann,

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

Comparative histostereological teratogenic effects of in-utero exposure to lamotrigine and levetiracetam on fetal medial temporal lobe and pre frontal cortex in Albino rats.

Ann Wairimu Mwangi HSM401-1220/2020.

We refer to your PhD. proposal submitted to our committee for review and your application letter dated 8th November 2021. We have reviewed your application for ethical clearance for the study. The number of albino rats and protocols used to assess how histomorphological and histostereological Teratogenic effects of in-utero exposure to Lamotrigine and Levetiracetam on fetal medial temporal lobe and pre frontal cortex in Albino rats during first, second and third trimester 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, PhD
Chairperson, Biosafety, Animal Use and Ethics Committee,
Faculty of Veterinary Medicine,
University of Nairobi

Appendix I1: 1st Publication

Journal of Agriculture Science & Technology JAGST 22 (2) 2023, 22-33

Maternal pregnancy outcomes following in-utero exposure to lamotrigine

ORIGINAL RESEARCH ARTICLE

The pregnancy outcomes of female albino rats *(Rattus Norvegicus)* exposed prenatally to varied doses of lamotrigine

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ABSTRACT

The maternal pregnancy outcomes following the *in-utero* exposure to lamotrigine (LAMT), a second-generation anticonvulsant medicine, have not been well elucidated. Lamotrigine is currently being prescribed widely and increasingly as a first-line medicine in the management of maternal conditions such as partial and generalised epileptic seizures, neuromodulators in mood disorders among others. Previous results have not been conclusive on its safety profile when administered to the expectant women, with some study results reporting that it is safe, and others advocating for more research to be carried out since their results are inconclusive. Data on the effects of prenatal exposure to lamotrigine on maternal pregnancy outcomes following prenatal exposure to varying doses of lamotrigine when administered at different trimesters is therefore of key importance, in order to maximise benefits to expectant women while minimising effects on developing

fetuses. A post -test only-control experimental design was adopted using 30 female sexually mature rats weighing 250 ± 30 grammes. These female albino rats were divided into two main groups: three rats in the control group and 27 rats in the experimental group. Excel spreadsheets were used to code the data, which was then analysed in SPSS. The study's findings were presented as mean + standard error of the mean (SEM). $P<0.05$ values were considered statistically significant. Study findings depicted a reduction in daily maternal weight trends, mean maternal weight gain (WG), mean placenta weight (PW), litter size (LS), total number of resorbed glands (RG), and total number of dead fetuses (DF) in a time- and dose-related manner, with the reduction being more pronounced at medium and high lamotrigine dosages, especially when it was administered during the first and the second trimesters. Further studies with higher primates close to humans and clinical trials are recommended to rule out the safety index of lamotrigine during pregnancy.

Keywords: Lamotrigine, gestation period, anticonvulsants, trimester, teratogenic.

Appendix III-2nd Publication

Journal of Agriculture Science & Technology JAGST 22 (3) 2023, 51-63

Quantitative effects of varied doses of lamotrigine on the developing fetal brain

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ORIGINAL RESEARCH ARTICLE

The histostereological teratogenic effects of in-utero exposure to varied doses of lamotrigine on the developing fetal brain in albino rats (*Rattus Norvegicus*)

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ABSTRACT

The histoqualitative teratogenic effects of lamotrigine, a second-line anticonvulsant medicine, on the developing fetal brain structures when exposed *in utero* in a time- and dose-dependent manner remain unclear. On the other hand, lamotrigine is currently being widely prescribed as a first-line medicine in the management of maternal conditions like epileptic seizures and bipolar disorders, among others. The preferential use of lamotrigine is attributed to the considerations of its efficacy, tolerability, and minimal teratogenic effects on fetal organs like the brain, among others, though with insufficient supportive data. The aim of this study was therefore to evaluate the histo-quantitative effects of lamotrigine on the developing fetal brain structures when exposed *in utero* at varying dosages during different trimesters. The study adopted a post-test only experimental study design where a sample size of 30 sexually mature albino rat dams of the species (*Rattus*

norvegicus) weighing between 250 ± 30 grams was used. The rats were divided into two broad groups: 3 control rats and 27 dosage rats. The data collected was coded in Excel spreadsheets and analyzed in SPSS. Results were expressed as the mean ± standard error of the mean (SEM), and values with a $P < 0.05$ were considered to be significant. Study findings depicted a reduction in brain weights, length, width, volumes, and volume densities of cortical and subcortical layers in a dose- and time-dependent manner. High lamotrigine dosages, especially during the first and second trimesters, were observed to be associated with significant mean reductions in the brain weights, length, width, volumes, and volume densities of the developing fetal brain structures. Therefore, further studies with higher primates closer to the human species as well as clinical trials are recommended to rule out the safety index of lamotrigine during pregnancy.

Keywords: Stereology, lamotrigine, anticonvulsants, trimester, teratogenic.

Appendix IV-3rd Publication

Journal of Agriculture Science & Technology JAGST 22 (3) 2023, 136-145

ORIGINAL RESEARCH ARTICLE

The growth and development outcomes of fetuses born of albino rats *(Rattus Norvegicus) prenatally* exposed to varying doses of lamotrigine

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ABSTRACT

The growth and development outcomes of the fetuses born by mothers who prenatally get exposed to lamotrigine (LAMT) have not been well established. Lamotrigine is an anticonvulsant medicine used in the management of acute epileptic seizures, Lennox-Gastaut syndrome, fibromyalgia, schizophrenia, unipolar depression, bipolar I disorder maintenance among others. Though currently lamotrigine is being prescribed as a first line medicine in the management of these maternal conditions, past studies are not conclusive on its teratogenic effects on growth and development of embryos and fetuses upon its in-utero exposure, with some demonstrating no effects, while others recommend further studies. Data on growth and development effects upon administration of lamotrigine at varying dosages at different trimesters will therefore be of help to the expectant mothers who consume lamotrigine, developing embryos and fetuses as well as guide the clinicians on the dosage and when to prescribe lamotrigine. A post-test-only experimental design was

adopted using 30 female sexually mature rats of 250 ± 30grams.These female albino rats were divided into two main groups of 3 rats in the control group and 27 rats in the dosage group. Excel spreadsheets were used to code the data and was analyzed in SPSS. Study findings were expressed as mean ± standard error of the mean (SEM). Values whose $p<.05$ were reported as being statistically significant different. Study findings depicted a reduction in mean fetal weight (FW), mean crown-rump length (CRL), mean bi-parietal diameter (BD), mean head circumference (HC) as well as mean head length (HL) in a time and dose related manner. More reduction in foetal growth and development parameters were observed in high lamotrigine dosages, especially when administrations were done during the first and the second trimesters. Further studies with animals close to human species are recommended to guide on the safety human therapeutic dosages.

Keywords: Lamotrigine, Teratogenic, Anticonvulsants, Gestation period.