Histomorphological Teratogenic Effects of In-Utero Exposure to Carbamazepine on the Cerebral Cortices of Albino Rats (*Rattus Norvegicus*)

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Abstract: The *in-utero*teratogenic effects of carbamazepine on the histomorphogenesis, cellular distribution and the histological thickness of different cerebral cortical layers of fetal brain when exposed at varied doses and on different gestational periods has not been well elucidated. This study set out to evaluate the teratogenic effects of in-utero exposure to varied doses of carbamazepine on the thickness and cellular organization of the cerebral cortices at different gestation periods. In carrying out the study, a static group experimental study design was adopted. A Sample size of 30 albino rat dams (*Rattus norvegicus)* weighing between 200-250grams were used in the study where they were divided into two broad study groups of 3 rats control and 27 rats experimental. These 27 animals in the experimental group were further subdivided into three study groups of 9 rats as follows; (i) Low carbamazepine group [LCG-20.7mg/kg/bw] (ii)Medium carbamazepine [MCG-72.3mg/kg/bw], (iii) High carbamazepine group [HCG-124mg/kg/bw]. the 9 rats in each of the three dose categories were further sub-divided into three groups of 3 rats according to trimesters as follows; (i) Trimester I-(3rats); (ii) trimester II-(3rats) and (iii) trimester III-(3rats) respectively. Fetal brains were harvested and processed for routine microscopy and examined at different magnifications of X10, X40, and X100. The findings of the study showed remarkable disaggregation, sparse distribution of the various cellular components and remarkable reduction in cortical thickness of all the six cerebral cortical layers of the fetal brain across all the treatment groups compared with the controls. The critical period of carbamazepine teratogenicity was established to be 1st and 2nd trimesters with a critical dose of 124mgs/Kg/bw showing highest teratogenicity effects.

Keywords: Carbamazepine, Anticonvulsant, Teratogenic, Histomorphology, Histostreology

1.1 Introduction

Carbamazepine, a commonly used an anticonvulsant medicine has been shown to negatively influence the normal morphogenesis of the fetal nervous system when exposed *in-utero.* However, its influence on the cortical thicknesses and distribution of cells in the six cerebral cortical layers of the fetal brain is not will elucidated. In-addition, whether or not the teratogenic effects of carbamazepine on the differentiation of the fetal cerebral cortical layers is time and dose dependent is yet to be established. This is despite Previous studies showing that *In-utero* exposure to carbamazepine is associated with some of adult neurological conditions like seizures, bipolar disorders among others during pregnancy, (Matlow & Koren, 2012); (Wlodarczyk *et al.*, 2012). It is therefore important to evaluate how carbamazepine influences the cytodifferention, cellular distribution as well as the cerebral cortical thickness as the cerebral cortices as the cerebral cortices are concerned with receiving all somato- sensory and visceral information that is coupled with programing, coordination and execution of all motor functions for both voluntary and involuntary functions of the body. Previous studies on carbamazepine have shown that the mechanism of carbamazepine teratogenicity is due to its active metabolite (*carbamazepine 10 epoxide*) accumulation in maternal blood plasma (Etemad *et al.,* 2012) that readily crosses the blood maternal placental barrier accumulating in the fetal tissues hence interfering with various stages of neurilation, cell differentiation and maturation of the fetal nervous system. Coupled with the carbamazepine low molecular weight of 236.27g/mol, it enhances it to readily cross the maternal blood placenta barrier, accumulates in fetal brain tissue, interfering with the neuro-developmental events including; neurogenesis, synaptogenesis, cell proliferation, migration, synaptogenesis, axonal sprouting, gliogenesis, myelination among others that leads to physiological apoptotic cell death of the fetal brain tissue and oxidative stress (Ikonomidou *et al.*, 2010). While a previous study by Hill *et al.*, (2011) linked the in-utero teratogenic

disturbances of carbamazepine to the fetal organs including the brain with fetal genetic factors, another study by Fujimura *et al.*, (2017) liked genetic factors of the mother as well as environmental factors to the argument. Data from this study on effects of carbamazepine on perturbations of normal histomorphology of fetal brain structurers like the cerebral and cerebellar cortical thickness as well as the cellular distributions is of paramount importance as it can help in explaining the cause of some of the structural, behavioral and functional mental disorders observed both in childhood and in adulthood whose cause is yet to be established (Nie *et al.*, 2016). The present study therefore aims at evaluating the cerebral cortical histomorphological thickness as well as the cellular distributions effects of in-utero exposure to carbamazepine and whether these effects depend on the time of exposure and the dose administered.

1.2 Study Objectives

1.To establish the pregnancy outcomes following *in-utero* exposure to varied doses of carbamazepine at different gestation periods.

2.To evaluate the histo-morphological outcomes that occur to the developing fetal brain following *in-utero* exposure to varied doses of carbamazepine at different gestation periods

3.To evaluate the histo-stereological changes that occur to the developing fetal brain following *in-utero* exposure to varied doses of carbamazepine at different gestation periods on development of fetal brain structures

4.To determine whether the teratogenic histo-stereological effects of carbamazepine on the developing fetal brain structures are time and dose dependent.

1.3 Hypothesis (H_0)

Prenatal exposure to carbamazepine is not associated with teratogenic effects to the developing fetal brain in albino rats and the effects are not time and dose dependent.

1.4 The study assumptions

In carrying out this study it was assumed that the albino rat (*Rattus Norvegicus)* model would replicate the actual teratogenic induction scenario that would occur in humans due to the known close association of this kind of rat species with human biological and functional outcomes when exposed.

2.0 Materials and Methods

2.1 Study site/Location

All experiments that included breeding, handling, weighing, carbamazepine administration and measurements of fetal parameters as well as the fetal brain was done at the Small Animal Facility for Research and Innovation (SAFARI) situated in Jomo Kenyatta University of Agriculture and Technology (JKUAT). Histological procedures were carried out in Human Anatomy labs.

2.2 Study Design

A static group laboratory based experimental study design was adopted

2.3 Description of Albino rats used in the study

Female albino dams used in the study were of the 3rd series breed and weighed between 200-250g.They were used because of the following known scientific facts; (i)They have a large litter size,(ii)Low incidence of spontaneously occurring congenital defects, (iii)a relatively short gestational span,(iv)low cost of maintaining the animals and, (v)considerable amount of the reproductive data on the rat is already available (Bailey *et al.,* 2014; Pritchett & Corning, 2016).

2.4. Acquisition and feeding of the dams

The albino rats were purchased from the Small animal facility for research and innovation (SAFARI) animal house, located in Jomo Kenyatta University of Agriculture and Technology (JKUAT) main campus. They were fed on a standard diet as determined by American institute of nutrition (2011) that included rodent pellets from UNGA meals limited (Thika), and water *adlibitum*. They were kept in spacious polycarbonate plastic cages in the animal house as determined by (Allen *et al.,* 2016).

2.5Sample size calculation

In calculation of the sample size, resource equation was applied to get 30 albino ratsas determined by (Arifin *et al*., 2017). The formula states that the measured value 'E' which is the degree of freedom of analysis of variance (ANOVA) based on a decided sample size value ('E') should lie between 10 and 20 animals according to this equation. Therefore, a value less than 10 necessitates adding more animals which increases the chance of getting significant results while a value more than 20 has been shown to increase the cost of the study without increasing the significance of the results.Therefore, total number of groups=10 while the total number of animal sis 30. E=Total number of Animals-Total number of groups. E is therefore is 30-10 which is 20

2.6 Grouping of animals

After confirmation of pregnancy, the rats were assigned into two broad study groupsof 3 rats in control group and 27 rats in experimental group. The 27 rats in the experimental group were further divided into three sub-groups of 3 rats each assigned according to the dose administered as low (LCG), Medium (MCG) and High carbamazepine group (HCG).Each of the subgroups of the LCG, MCG and HCG were further subdivided into smaller sub-groups according to the time of administration as first (TM_1), second(TM_2) and third(TM_3) trimesters comprising of 3 rats each.

2.7 Determination and acquisition of carbamazepine

A simple guide for conversion of human to animal dosages was used as determined by (Nair & Jacob, 2016)formula as follows; The correction factor (Km) is estimated by dividing the average body weight (kg) of species to its body surface area (m2). For example, the average human body weight is 60 kg, and the body surface area is 1.62 m2. Therefore, the Km factor for human is calculated by dividing 60 by 1.62, which is 37. The Km factor values of a rat is used to estimate the HED as: HED mg / kg = Rat dose mg / kg Animal K /Human K Eq. As the Km factor for each species is constant, the Km ratio is used to simplify calculations. Hence, Equation is modified as: HED mg / kg = Animal dose mg / kg K ratio Eq. The Km ratio values are already provided and are obtained by dividing human Km factor by animal Km factor or vice versa.Carbamazepine tablets from Novartis Farma Pharmaceuticals, batch number TL787 were obtained from a local chemist in Thika and were used to make the reconstitutions and administration was done using an oral gavage needle gauge 16.

2.8 Administration of carbamazepine

All rats in first trimester (TM_1) group in Low, Medium and High dose categories received carbamazepine from gestation day GD_1-GD_{20}while the rats in second trimester (TM_2) group in Low, Medium and High dose categories received carbamazepine from gestation day GD_7-GD_{20}. Rats in third trimester (TM_3) group in Low, Medium and High dose categories received carbamazepine from gestation day GD_{14}-GD_{20}

2.9 Determination of fetal growth parameters

Fetal growth parameters that included fetal and organ weights, crow-rump lengths, head circumference, head lengths and bi-parietal diameters were taken on the day of delivery and recorded. This was obtained by use of a digital weighing scale Vernier caliper.

2.10 Procedure for harvesting the fetal brains

After the Fetuses were removed from the maternal uterine horns, they were euthanised by use of concentrated carbon dioxide. Then the following procedure was followed to harvest their brains; (i) Fetuses were mounted onto the dissection board using mounting pins -dorsal sidefacing the board, (ii) using a pair of scissors and forceps lateral bonders along the lower margin of the temporal bone was opened and the skull cap removed, (iii) Using a magnifying glass, the whole fetal brain was identified, (iv) To avoid damaging the fetal brain, the meninges was opened along the superior sagittal sinus retracted up carefully since the brain lies within the meninges, (iv) The entire brain was excised/ scooped at the level of foramen magnum, (v) Each brain was examined for general external features and obvious congenital malformations (vi) Brain weights were taken by use of a digital weighing scale and their weights to body weight ratio were calculated (vii) The brains were immersed in the formaldehyde, to proceed with processing either for light or histostreology for 12 hours

2.11 Tissuepreparation for light microscopy

In preparation of tissues for light microscopy, the following procedure was followed; (i)The brains were fixed in Zenkers' solution for 24 hours, (ii)They were dehydrated in an ascending concentration of alcohol (50%, 60%, 70%, 80%, 90%, 95% and 100% (absolute) each for one hour, (iii) They were cleared by immersion with cedar wood oil for 12 hours, (iv) They were then infiltrated with paraplastwax for 12 hours at 56^0c, (v) The brain tissue was then orientated in the longitudinal axis (frontal to occipital lobe), (vi) They were then embedded in paraffin wax on the wooden blocks, (vii) Excess wax was trimmed-off till the entire length of the brain tissue was exposed, (viii) 5µm thick longitudinal sections were cut from head to tail regions with Leitzsledge rotary microtome, (ix) The cut sections were floated in water at 37^0 to spread the tissue, (x)The sections were stuck onto glass slides using egg albumin, applied as thin film with a micro-dropper, (xi)The slides were then dried in an oven at 37^0 for 24 hours,(xii)Blinding was done by coding all the slides by the research assistant in absence of the researcher(xiii)They were stained with different stains including: -Haematoxylin and Eosin (H&E), based on the cellular structures that needed to be studied.

2.12 Histomomphological analysis

20-25 slides were chosen using systematic uniform random sampling guided by the co-efficient of error calculations. The slides were observed under a BP Olympus microscope at different magnifications and photomicrographs of the precentral gyri of the frontal lobe were taken using a 32megapixel digital camera.They were then exported to the computer screen where Adobe fireworks software was used for labelling, and were analysed through observations.

2.13 Ethical Approval

All procedures for animal handling, feeding, humane sacrificing and harvesting of organs were performed as per laid down protocols, with approval from Animal Ethics Committee Jomo Kenyatta University of Science and Technology as well as the laid down protocols and regulations by International Animal Research Institute (IARI) of USA as outlined by (Gomez *et al.,* 2010).The study went through the regal and administrative requirements as required by JKUAT and the laws of Kenya, (See document attached in the appendices; REF: JKU/2/4/896A**).**

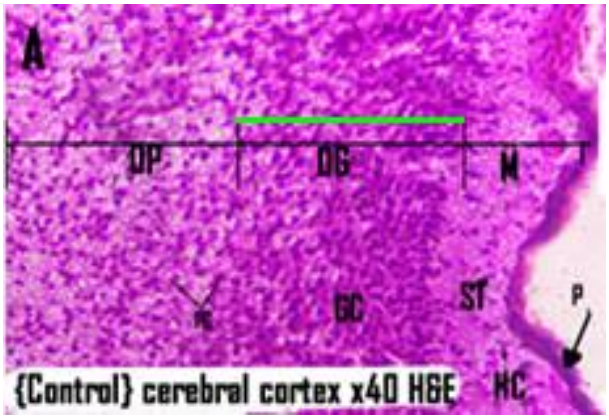
3.0 Results

The histological changes observed in carbamazepine treatment a group includes; reduction of the cerebral cortical thickness as well as cellular disaggregation. These effects were observed to be time and dose dependent as they were more prominent in all dose groups when carbamazepine was administered during the first and second trimesters. Trimester three had no significant outcomes except when administered on high doses. The results were as illustrated below;

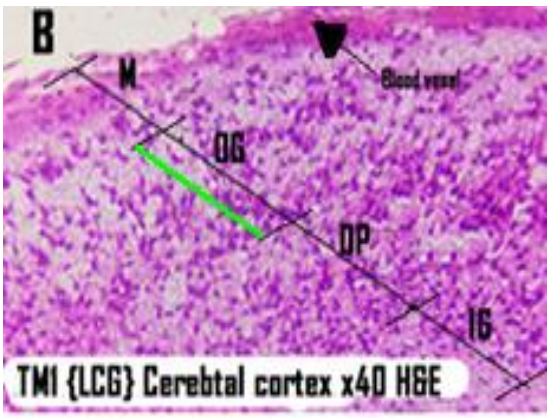
3.1. Shows how carbamazepine influenced the reduction in size of cortical layer and cellular distributions in relation to doses administered at trimester one (TM_1).

It was observed that the cortical thicknesses of the six cerebral cortical layers including (i) Molecular layer (ii) Outer granular layer (iii) Outer pyramidal layer (iv) Inner granular layer (v) Inner pyramidal layer (vi) Multiform layer in that order from outside to the inside were seen to variably differ in their differentiation thickness based on the dose of carbamazepine exposure as wells as with the time of exposure when treatment was done. For instance, when carbamazepine was administered during trimester one (**TM_1**), cortical thickness and the accompanying cell

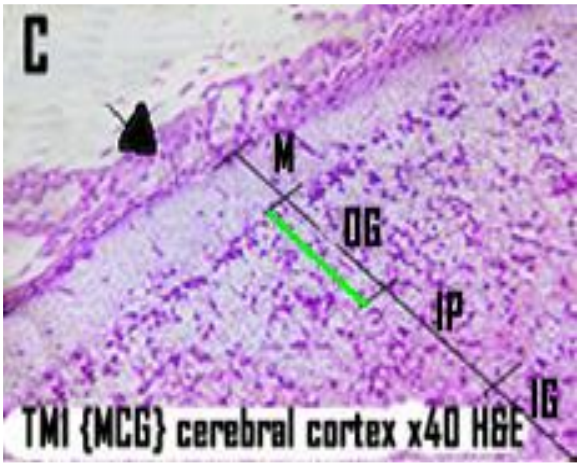
distribution and cellular densities per each of the six cortical layers were seen to reduce appreciably among all the carbamazepine treated groups (LCG,MCG, HCG) figure 3.1.1 to figure 3.1.2 respectively.



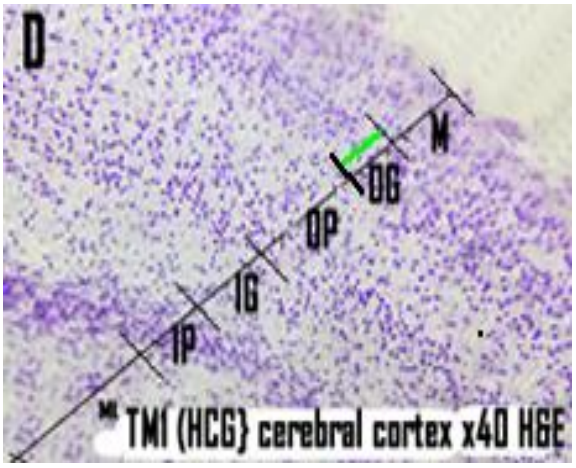
A:Control: Showing the cortical thickness, the cellular densities and cellular distribution in the (a) outer Molecular layer -M, (b) outer granular layer OG and (c) OP-outer pyramidal layers. Note other layers are not visible in this field x40 while the cells are densely packed



B: The LCG: showing the reducing cortical thickness, the cellular densities and cellular distribution in the (a) outer Molecular layer -M, (b) outer granular layer OG and (c) OP-outer pyramidal layers. Note the inner Granular layer (IG) has now become visible in the same field of magnification of x40 and cellular densities are reducing.

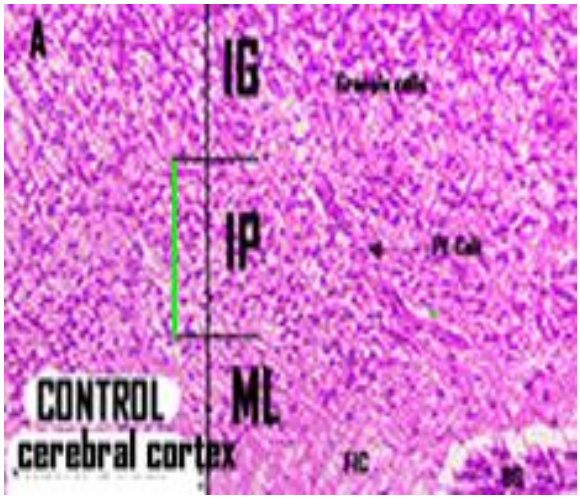


C: MCG: shows further reduction in the various cortical layers thickness of (a) outer Molecular layer -M, (b) outer granular layer OG and (c) OP-outer pyramidal layers. And inner granular layer. Note further reduction in cellular densities in each of the layers.



D: HCG: shows highly reduced cerebral cortical thicknesses in all layers that are now all visible; (a) outer Molecular layer -M, (b) outer granular layer OG and (c) OP-outer pyramidal layers. (d) Inner Granular layer (IG), e) inner pyramidal-IP; and (f) multiform layer. (Magnification of x40).

Figure 3.1.1: The TM_1 comparative thicknesses of the outer cortical layers with their cellular distribution: (a) Control (b) LCG, (c) MCG, and (d) the HC



A: Control: Showing the inner layers cortical thickness, the cellular densities and cellular distribution in the (a) inner granular-IG; (b) inner pyramidal-IP; and Multiform layers ML Note other layers are not visible in this field x40 while the cells are densely packed

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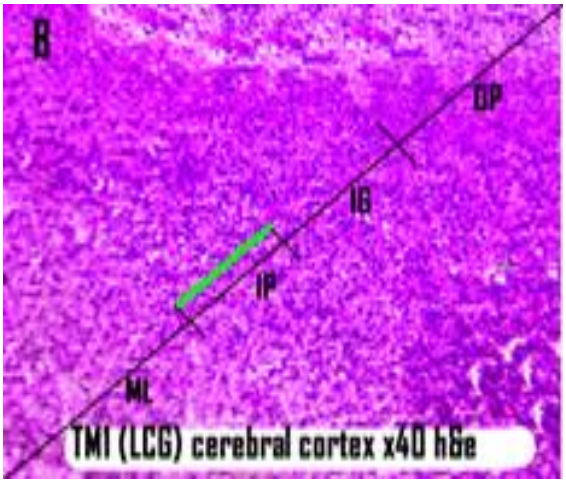
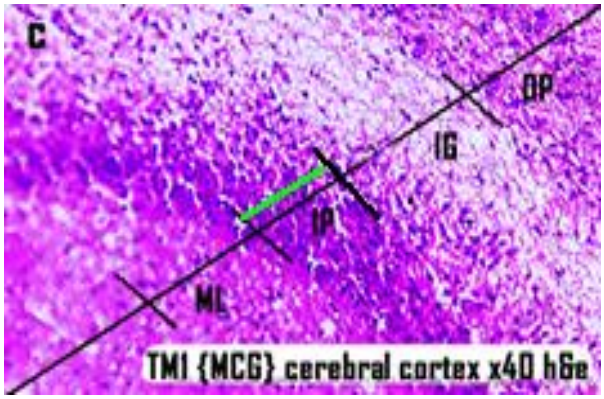
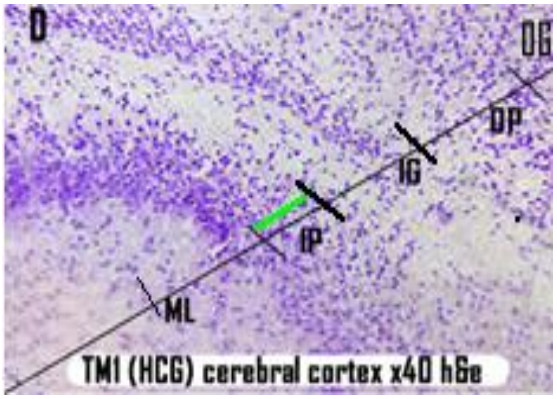


B: LCG: showing the relative reduction of the Inner cortical histological layers with IP layer in the middle (green line). Note some of the other outer layers are visible (mag x40). The outer cortical layer can as well be observed due to the cortical reduction in thickness and cellular densities



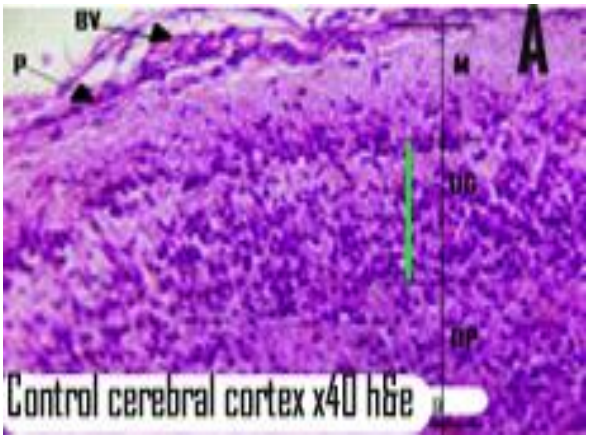
C:MCG: showing further reduction in the inner histological cortical layers and further reduction in cellular densities and sparse distribution of the cell in each inner layers (green line) (mag x40). Four further reduced layers can be observed as well as sparse and disorganized cells



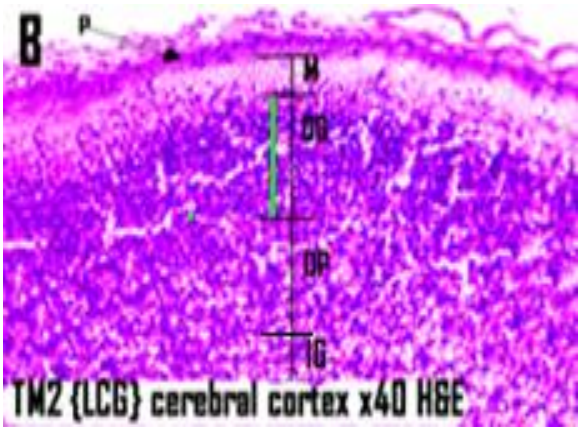
D-HCG: The most reduced inner histological cortical layers at **TM_1, HCG** (mag x40). The IP layer is much more reduced (green line) and two outer cortical layers; OP and OG are visible due to the thinness of layers. The cells are the most disorganized and sparsely distributed

Figure 3.1.2: The TM_1 comparative thicknesses of the inner cortical layers and their cellular densities/distribution:(a) control (b) LCG, (c) MCG, and (d) HCG.Influence of carbamazepine on the morphological thicknesses and cellular distributions of the cerebral cortical layers at TM_2

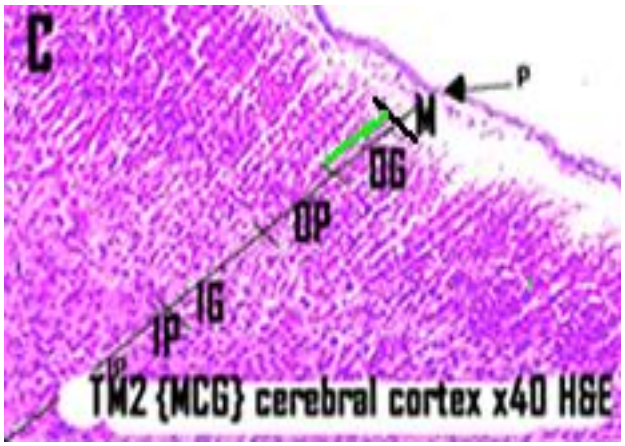
When carbamazepine was administered at **TM_2** where both the outer and the inner cortical layers reduced with the dose of exposure (figure 3.1.3 to figure 3.1.4 respectively).



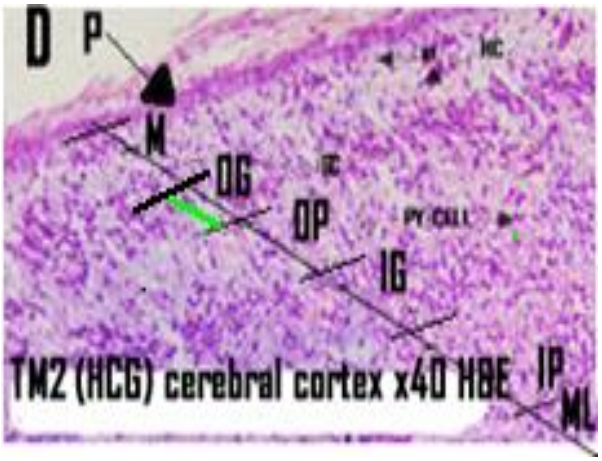
A: Control: A: Showing the cortical thickness, the cellular densities and cellular distribution in the (a) outer Molecular layer -M, (b) outer granular layer OG and (c) OP-outer pyramidal layers. Note other layers are not visible in this field x40 while the cells



B: <u>The LCG</u>: showing the reducing outer cortical thickness, the cellular densities and cellular distribution in the (a) outer Molecular layer -M, (b) outer granular layer OG and (c) OP-outer pyramidal layers. Note the inner Granular layer (IG) has now become visible in the same field of magnification of x40 and cellular densities are reducing

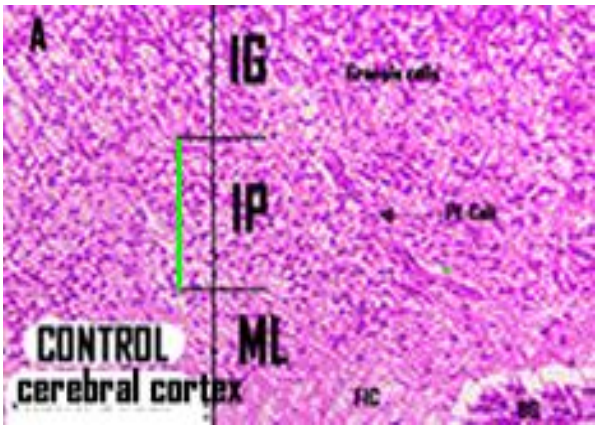


C: <u>MCG</u>: Further reduction in the thickness of outer histological layers at **TM_2, MCG** (magnification x40) depicted by the outer granular layer (green line) with further sparsely distributed cells. IG and IP layers are visible due to reduction of layers

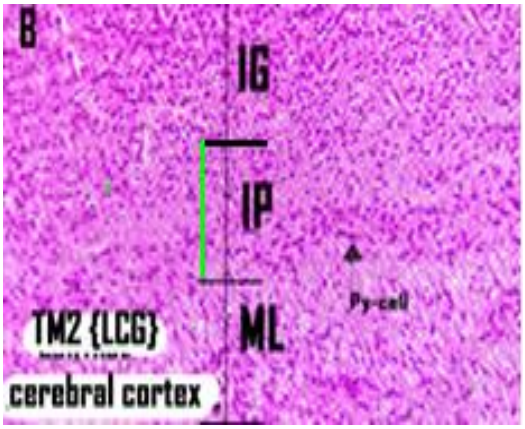


D: <u>HCG</u>: showing the highest reduction in the outer cortical thickness and cellular densities in the outer cortical histological layers at **TM_2, HCG** (mag x40) as depicted by OG layer. In addition, all the 6 cerebral cortical layers are visible and distributed cells.

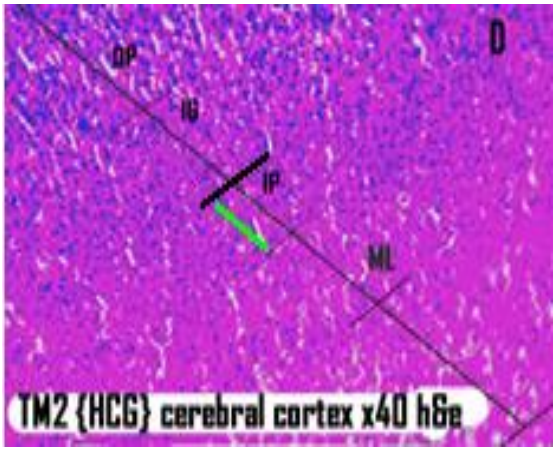
Figure 3.1.3: The TM_2 comparative thickness of the outer cortical layers, plus their cellular densities and distribution: (a) control (b) LCG, (c) MCG, and (d) the HC



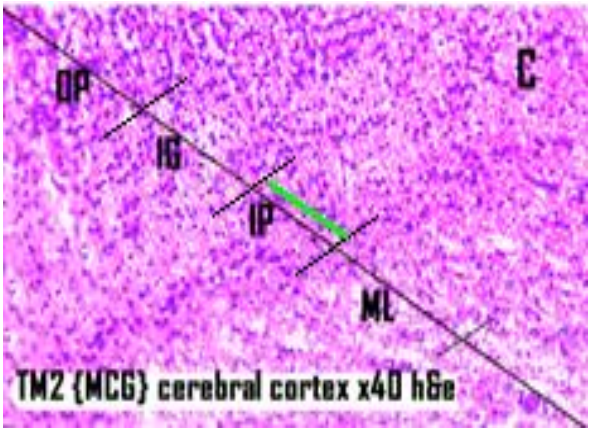
A:Control. Showing the normal inner thickness and cellular distribution in the inner cortical layers of the cerebral cortex included; (1G) Inner granular, (IP) Inner pyramidal and (ML) multiform layer in **Control group** (mag x40). The cells are also densely packed and evenly distributed



B:LCG: showing relatively reduction in thickness and cellular densities in the Inner histological layers of the cortex when at **TM_2, LCG** (mag x40). The cells are also relatively sparse as compared with those of the control



C MCG: showing further reduction and sparse distribution of cells in the inner histological cortical layers at **TM_2**, (mag x40). The cells are becoming fewer and sparsely distributed



D: HCG: Shows the least reduction and sparse distribution of cells in the inner histological layers of the cerebral cortex at **TM_2,** (mag x40) and sparsely distributed cells

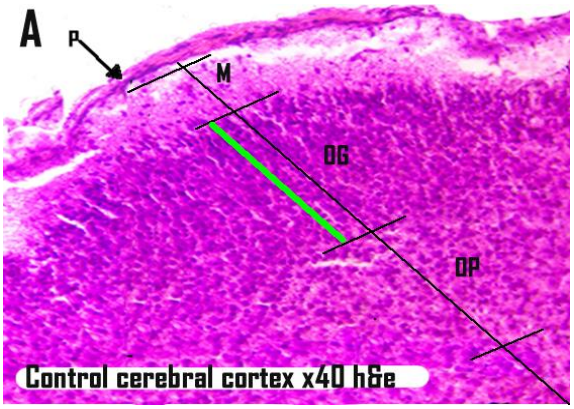
Figure 3.1.4: The TM_2 comparative thicknesses of the inner cerebral cortical layers with their cells distribution: (a) control (b) LCG, (c) MCG, and (d) the HCG.

3.1 Influence of carbamazepine on the morphological thicknesses and cellular distributions of the Cerebral Cortical layers at TM_3

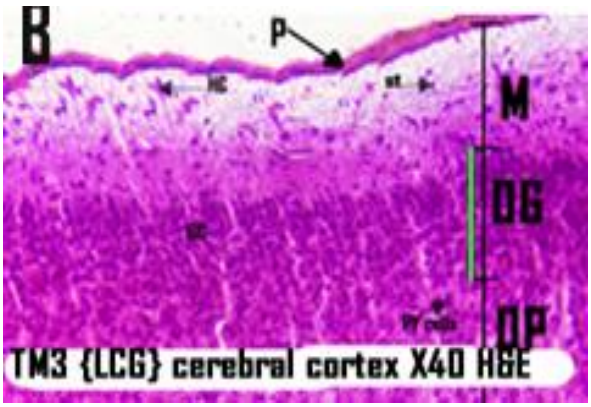
When the treatment was done at **TM_3,** there was no marked significance difference in both the outer and the inner cortical layer

thickness as well as in cellular organization between the carbamazepine treated groups against the control (figure 3.1.5 to

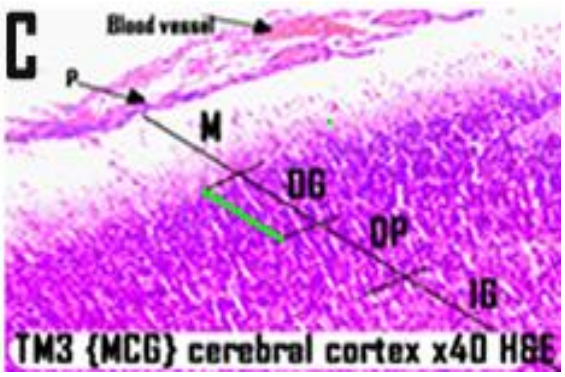
Figure 3.1.6 respectively) except when high dosages were administered



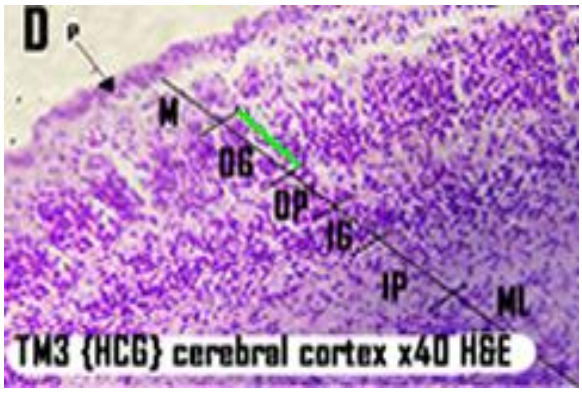
A: Control: Normal thickness of the outer three cerebral cortical layers; M-molecular, OG-outer granular and OP-outer pyramidal layers (mag x40). The densely packed outer cerebral cortical layers for control group.



B: <u>LCG</u>: no much noticeable differences in the outer Inner cortical histological layers as depicted by the IP layer (green line) at **TM_3, LCG** (mag x40).Cells are slightly relatively sparse in the axonal

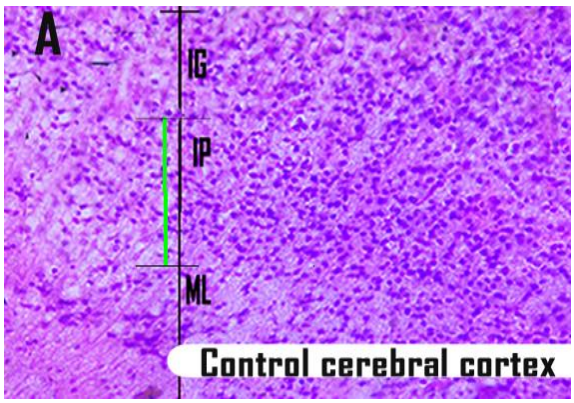


C: <u>MCG</u>: Further slight reduction in the thickness of outer histological layers at **TM_3, in MCG** (magnification x40)

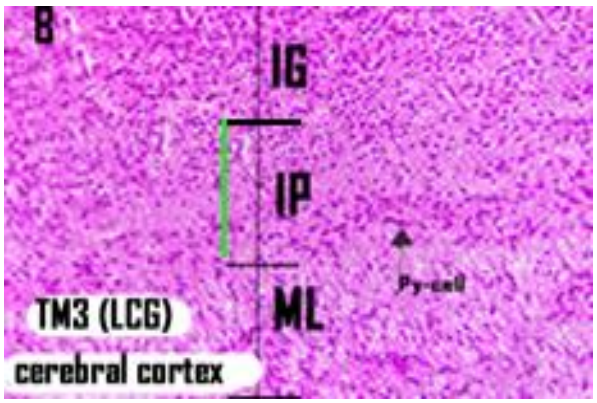


D: <u>HCG</u>: The most reduced outer cortical histological layers at **TM_3, HCG** depicted by OG layer. In addition, all the 6 cerebral cortical layers are

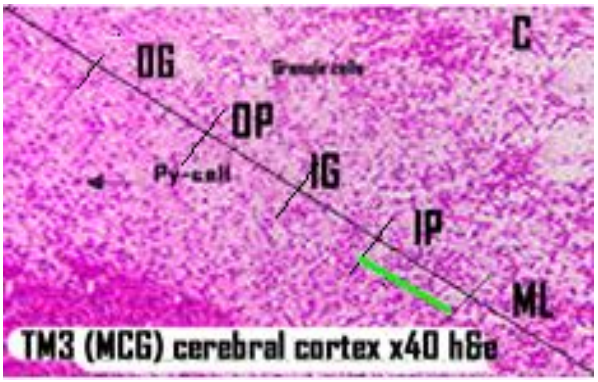
Figure 3.1.5: The TM3 comparative thickness of the outer cerebral cortical layers and their cellular densities/distribution; (a) control (b) LCG, (c) MCG, and (d) the HCG



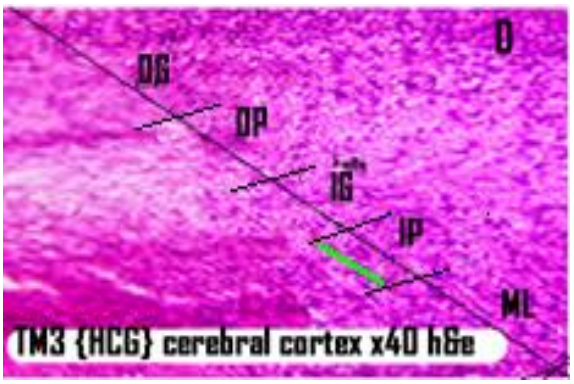
A: Control: showing the normal cortical thickness of inner three histological layers namely; (1G) Inner granular, (IP) Inner pyramidal and (ML) multiform layer (mag x40).



B:LCG: showing no reduction in thickness in the inner cortical histological layers at **TM_3**, (mag x40). The cells are also relatively sparse as compared with those of the control



C: MCG: showing **slight reduction** on the inner histological cortical layers at **TM_2, MCG** as depicted by the IP layer (green line) (mag x40). OG and OP layers can be observed as a result of thinness of layers as well as sparse and disorganized cells



D: HCG: showing **marked reduction** in thickness of the **i**nner histological cortical layers at **TM_3, HCG** (mag x40). The 6 cortical are visible due to the thinness of layers. The cells are the most disorganized and sparsely distributed

Figure 3.1.6: The TM_3 comparative thickness in the inner cerebral cortical layers with their cellular densities in (a) the control (b) LCG, (c) MCG, and (d) HCG.

4.0 Discussion

The current study established that exposure to carbamazepine reduces the histological cerebral cortical thickness of all the six cerebral cortical layers that includes (i) Molecular layer (ii) Outer granular layer (iii) Outer pyramidal layer (iv) Inner granular layer (v) Inner pyramidal layer (vi) Multiform layer in that order from the outer to the inner layer. The findings of this study corroborate with findings of another studies by Agarwal, *et al., (*2010) ;Ahmed, (2017) who observed that cortical layers of the developing brain can be suppressed following the suppressive inhibitory effects of anticonvulsants.The current study also established that the reduction in cortical layers thickness variably

differed based on their differentiation on the dose of carbamazepine exposure as well as with the time of exposure. For instance, the outer cerebral cortical thickness wasobserved to reduce appreciably among all the carbamazepine treated groups (LCG, MCG, HCG) when treatment was done in trimester one (TM_1) and trimester two (TM_2). Similarly, this was also replicated in the inner cortical layers that reduced with increase with the dose of exposure, (Figure 3.1.1 to 3.1.4). The study also established that when the treatment was done at trimester three (TM_3), there was no marked significance difference in both the outer and the inner cortical layer thicknesses between the carbamazepine treated groups against the control (figure 3.1.5 and figure 3.1.6) except when high carbamazepine dosages were administered. These findings are in line with another study by Gedzelman & Meador (2012) who also reported reduction in all cortical layers of the brain cortex upon administration of oxycarbazine, a medicine in the same class with carbamazepine. Similarly, the study findings also demonstrated time dependent relationship in that early exposure to oxycarbazine at TM_1 and TM_2, there was associated altered neuronal proliferation, migration process, synaptogenesis and apoptosis of the fetal brain tissue, processes that are vital for normal neural development that led to cortical dysplasia.

The current study has established that *in-utero* administration of carbamazepine leads to cerebral cortical cellular disorganization as it suppresses the differentiation and development of the neuroblast cells as well as the neuroglial cells. The current study findings corroborate with a previous studyby Ayano *etal.,*(2016)whose findings associated anticonvulsants with perturbations of the developing neuronal structures as they induce apoptosis especially in neural tube cells, with production of the free radicals such as epoxide during metabolism. This hypothesis was also supported by a study by Badaway *et al*(2019), whose findings showed that gabapentin, an anticonvulsant for seizure management was associated with a highly significant decrease in brain weight, alteration of the cerebral cortex and hippocampus cellular layers, vacuolated neuropil and massive cell degeneration with cavity formation in the brain tissue.

5.0. Conclusion

In conclusion of the study has established carbamazepine use during pregnancy is teratogenic to the developing fetal cerebral cortices of the fetal brain particularly when administered during the first and second trimester regardless of the dosage as indicated by the histomorphological feature. When administered in trimester three the effects are not significant except when administered on high doses. The most vulnerable window period for carbamazepine teratogenicity in addition established to be the first trimester while the most critical dose was 124mg/kg/bw.

6.0 Recommendations

The study recommends that;
1.The use carbamazepine during pregnancy should be avoided by all means as it has been shown to beteratogenic to the developing cerebral cortices of the fetal brain particularly in trimester one (TM_1) and trimester two (TM_2) by seeking appropriate alternatives that are safer to the fetus.

2. Should expectant mothers be on chronic use of carbamazepine and the drug cannot be withdrawn because of associated withdrawal side effects to the mother, the doses should be adjusted to the minimal effective dosages that would confer the maximum maternal benefits and reduce the teratogenic risks to the developing cerebral cortices of the fetal brain.

3. Due to time and dose dependent teratogenic effects of carbamazepine, health care workers including clinicians, nurses, midwives and others, need to be educated on how they will need to be educating women of reproductive age and are on chronic usage of carbamazepine of its teratogenicity during pregnancy, on the need for early planning of their pregnancies for effective introduction of alternative medicines, to enable them avoid use of carbamazepine during pregnancy.

4. Further studies be carried out in non-human primates that have close phylogenetic relations to humans, to ascertain its teratogenicity to the cerebral cortices in relation to doses.

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