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The Histostereological Effects of Varied Doses of Carbamazepine on the Development of Fetal Brain In Albino Rats *(Rattus Norvegicus)*

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Abstract: *The in-utero exposure to carbamazepine, an anticonvulsant medicine has been shown to perturb the normal morphogenesis of the fetal brain when used in management of maternal conditions. However, the anatomical histomomphological teratogenic effects on quantitative histostereological effects when exposed at different gestation periods and at different doses is not well elucidated. The broad objective of this study was therefore to evaluate the histostereological teratogenic effects of exposure to various doses of carbamazepine on the development of fetal brain when exposed at different gestation periods.*

Materials and methods: *In conducting the study, a static group experimental study design was adopted. The animal experimentation was carried out at Small Animal Facility for Research and Innovation (SAFARI) animal house while tissue processing for histology and stereological analysis was done in the department of human anatomy.*

Sample size*: A Sample size of 30 albino rat dams (Rattus norvegicus) weighing between 200-250grams were used in the study as determined by use of the resource equation method. These 30 Albino rats were divided into two broad study groups of 3 rats control and 27 rats experimental. To evaluate the teratogenic histological effects of carbamazepine on differing doses, the 27 rats 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].To further evaluate the teratogenic effects of carbamazepine on differing gestation periods, 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.*

Study findings*: study findings showed that there was dilatation of ventricular system as well as disaggregation of choroid plexus in the lateral ventricle of the fetal brain.*

Conclusion, *carbamazepine is teratogenic to the developing fetal brains and its teratogenicity is time and dose dependent. The study recommends that carbamazepine should not be used during pregnancy and particularly during 1st and 2nd trimesters.*

Keywords: *Carbamazepine, Anticonvulsant, Teratogenic, Histostreology.*

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I. Introduction

In-utero exposure to carbamazepine perturbs the normal morphogenesis and cyto-differention of fetal organs when applied in management of maternal conditions during pregnancy, Matlow, J., & Koren, G. (2012); Wlodarczyk *et al*., (2012), the specific histomomphological perturbation on the ventricular system when various doses of carbamazepine are administered at different gestation periods has not been studied. The morphogenetic perturbations to the developing neuronal tissues in the fetus are believed to be caused by its inhibitory mode of action due to accumulation of its principal metabolite (*carbamazepine 10 epoxide*) in maternal blood plasma, creating a negative osmotic gradient. Coupled with its low molecular weight of 236.27g/mol enhances it to cross the maternal blood placenta barrier, accumulate in fetal brain tissue, interfering with the neuro-developmental events including; cell proliferation and migration, synaptogenesis, axonal sprouting, gliogenesis, neurogenesis, synaptogenesis, myelination among others that leads to physiological apoptotic cell death of the fetal brain tissue and oxidative stress (Ikonomidou *et al*., 2010). As such, these intrauterine disturbances to the developing neuronal tissues in the fetus may cause permanent structural damage to the brain that may manifest in form of some of the behavioural mental conditions seen in adulthood like mild mental retardation, cyclic maniac depressive disorders, suicidal ideation among others, whose causes are yet to be established (Bath & Scharfman,

2013; Fujimura *et al.,* 2017).The present study therefore aim at evaluating histomomphological teratogenic effects of in-utero exposure to various doses of carbamazepine on fibres of corpus callosum when administered at different gestation periods.

II. Materials And Methods

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.

Study Design

A static group laboratory based experimental study design was adopted

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).

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).

Sample size calculation

In calculation of the sample size, resource equation was applied to get 30 albino rats as 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

Grouping of animals

After confirmation of pregnancy, the rats were assigned into two broad study groups of 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.

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.

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}

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.

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 side facing 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

Tissue preparation 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 paraplast wax 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 Leitz sledge 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.

III. Stereological Analysis

Estimation of total brain volume using Archimedes principle

Immediately after the removal of the entire fetal brain from both the control and experimental groups the total brain volumes (Archimedes volume) were determined using the water/fluid displacement methods that employed the Archimedes' principle. Through this method the Archimedes brain volumes were obtained by inserting the whole brain tissue into graduated beakers containing normal saline, and the amount of fluid displacement upward was measured. The normal saline displaced by the brain represented the actual brain volume as described by a study by (Hughes, 2005). The Archimedes volumes were used as the reference volumes when determining the cavalieri stereological densities and volume densities. This method was compared to the cavalieri methods and the mean and standard error of the mean (± SEM) of the measurements were calculated.

Determination of stereological total brain volume and volume densities using cavalieri and point counting methods

The stereological total brain volume and the estimation of the volume densities of both the cortical and subcortical layers of the brain structurers was determined by using a combination of both the cavalieri and point-counting method (Cruz-Orive, 1999). The following steps were followed: i) Preparation of brain cavalieri sections (5µ) thick sections) ii) Selection of the spacing for the point probe iii) The point probe was tossed randomly onto each section iv) The points that hit the region of interest was counted using STEPnizer stereology tool v) All sections were processed keeping a tally of counts per section vi) The volume was then calculated. Twenty sections of 5µm thickness from each longitudinal brain section were selected by systematic uniform random sampling, (Bural *et al.,* 2015). Using the microscope's stage Vernier, images were viewed at

magnification of 10x. The volume was obtained by fully sectioning the brain into a series of cuts which was the product of the sum of the cut areas (starting with the first to the last section). Point counting was done using the STEPnizer software (Bolender & Weibel, 1973). The digital images of the brain tissue were captured using stereological sampling rules with same magnification and saved in the jpeg (joint photograph expert group) file format at adequate resolution. The picture height was ensured that it matched the height of the computer monitor, both defined in pixels. All images captured both for the control and experimental groups were organized appropriately and saved in one folder. A calibrated scale bar was added to one image of a batch to define the real dimensions of the structures under investigation, and placed on left hand side. Where stereological estimation required the use of a guard area it was set and were not be changed in the course of the whole experiment to obtain consistent results.

Point counting using the cavalieri principle was employed to estimate the total fetal brain volume using the formula:

$$_{Est}V = \frac{\sum_{i-1}^{m} P.\ a / p.\ t}{M^2}$$

Where: $_{est}V$= was the estimation of the volume of the brain,
$\sum P$= was the sum of the number of points landing within the various components of the fetal brain profiles,
$^{m}i\text{-}1$=All points from the first to the last
A/p= was the area associated with each point,
t = was the distance between sections and
M= was represent the magnification (Welniak–Kaminska *et al*., 2019)

On each sampled section, five fields were selected in a systematic random manner with the aid of mounted grid scale on the Newscast computer screen projected by the stereology new cast microscope. A transparent test system on the grid was then superimposed on the images projected on the computer screen on the cortical and sub-cortical brain structurers and points hitting these areas counted at a final magnification of x10. Then estimates of their volume density, (Vv) of the in the reference space were obtained using the formula:

EstVv = P (part)/P (ref),

Where **P (part)** and **P (ref)** were the number of test points falling in all structure profiles and in the reference space, respectively (Keller *et al*., 2018)

3.15.3: Stereological correction for brain tissue shrinkage

The following method was applied to quantify the percentage brain tissue shrinkage caused by fixation and histological procedures. The volume of removed fresh brain was calculated by Archimedes displacement method. After tissue processing and exhaustively sectioning, the brain volume was estimated with cavalieri method. The brain volume shrinkage was then calculated as follows (Tran *et al., 2015*);

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

NB/Volume before is Archimedes volume while volume after cavalieri volume.

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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**).**

IV. Results

Influence of carbamazepine on the fetal brain size, weight, length and width

The comparative gross appearance of the fetal brain from the carbamazepine treated groups showed that the gross appearances of fetal brains from the experimental groups looked relatively small in size with poorly defined lobes, sulci and gyri when compared with the control group. Similarly, when the intra and the intergroup fetal brain weights comparisons were done for the experimental groups, there was a marked intra-group and inter-group variances in the total gross weights and brain sizes based on the dose of exposure and the time of exposure. For instance, it was observed that when carbamazepine treatment was done at $\mathbf{TM_1}$, **the mean total** brain weight in (**grams)** was found to be lowest in HCG group at 0.12±0.012 followed by MCG at 0.216±0.008 and LCG at 0.297±0.0033. When carbamazepine was administered in $\mathbf{TM_2}$, the mean totals of the

fetal brain weight in grams was found to be 0.247±0.0071 in the HCG at followed by MCG at 0.300±0.000, then LCG at 0.347±0.0059 as compared with control group (P=0.001). When treatment was done at **TM_3, the mean** brain weight for the HCG group in millimeters was 0.33±0.0028, followed by MCG at 0.370±0.008 and LCG at 0.394±0.0051. The values were found to be statistically significant (p=0.002) when the comparisons were done within and across groups and when compared with the control group **(table 1).**

For the mean brain length and width in (**millimeters),** a similar scenario was observed. Mean brain length at **TM_1** HCG group was at 1.02±0.012 followed by MCG at 1.12±0.008 and LCG at 1.197±0.0033. This was found to be statistically lower as compared with the control group (p=0.001) at 1.295±0.005. At **TM_2**, brain length was found to be lowest in HCG at 1.285±0.1433 followed by MCG at 1.2000±0.000, then LCG at 1.247±0.0033. This was not statistically different as compared with control group at 1.295±0.005 (P=0.783). **At TM_3, the mean** brain length was lowest at HCG group at 1.287±0.0018, followed by MCG at 1.31±0.0024 and LCG at 1.304±0.136. The brain width in millimeters were also seen to follow the same treads (**table 1**).

Table 1: Showing a comparative means fetal brain weight, brain length, and width for LCG, MCG and the HCG treated at TM_1, TM_2 and TM_3 against the control.

Study groups	Period of CBZ treatment	Mean brain weight(g) ± SEM	Mean brain length(mm) ± SEM	Mean brain width(mm) ± SEM
Control group	----------	0.395±0.005	1.295±0.005	1.097±0.003
Low dose carbamazepine group (LCG, 20.7mg/kg)	Trimester one (TM1)	0.297±0.0033 ^{bc*}	1.197±0.0033 ^{b*}	0.997±0.0033 ^{b*}
	Trimester two (TM2)	0.347±0.0059 ^{bc*}	1.247±0.0033 ^{bc*}	1.0711±0.0066 ^{b*}
	Trimester three (TM3)	0.394±0.0051	1.304±0.136	1.09±0.009
Medium dose carbamazepine group (MCG, 72.3mg/kg)	Trimester one (TM1)	0.216±0.008 ^{bc*}	1.12±0.008^{b*}	0.916±0.0079 ^{b*}
	Trimester two (TM2)	0.300±0.000 ^{bc*}	1.2000±0.000^{bc*}	1.023±0.012 ^{b*}
	Trimester three (TM3)	0.370±0.008	1.31±0.0024	1.073±0.009
High dose carbamazepine group (HCG, 124mg/kg)	Trimester one (TM1)	0.12±0.012 ^{b*}	1.02±0.012 ^{bc*}	0.813±0.007 ^{b*}
	Trimester two (TM2)	0.247±0.0071 ^{bc*}	1.285±0.1433	0.948±0.0024 ^{b*}
	Trimester three (TM3)	0.33±0.0028	1.287±0.0018	1.02±0.00696 ^{b*}

Key: All value that bear (*) as a superscript indicates that they depicts a statistical significance differences
(p<0.05) when compared with the control. Values with (^{b}) &$^{(c)}$ superscripts have a statistical significance difference (p<0.05) in the intragroup and intergroup comparisons respectively using one way ANOVA with Turkey post-hoc t-tests

Influence of carbamazepine on the total fetal brain volume and volume densities

The reference and calculated mean total fetal brain volume as determined by use of water displacement method (WIM) and calculated by the cavalieri method was found to depict an inverse dose response relationship in that when the dose of exposure to carbamazepine increased the mean total brain volume had a corresponding decrease in total brain volume and *vice versa,* (**table 2**). On the other hand, when the total brain volume was compared with the time of exposure, it depicted a direct response relationship to the time of exposure in the when carbamazepine treatment was administered at different trimesters (**TM_1, TM_2 TM_3),** the brain volumes decreased directly with the time of exposure. For instance when the carbamazepine treatment was done at **TM_1** the total brain volume **(mm^3**) was lowest in the HCG at (0.220±0.001), followed by MCG at (0.231±0.001) and lastly LCG at (0.232±0.002). All the intra and intergroup comparisons were also found to be statistically significant (P=0.001) when compared with the control group (**table 2)**

Table 2: A Comparative reference, calculated and percentage shrinkage on total mean fetal brain volume using (WIM) and cavalieri method in the LCG, MCG and the HCG treated at TM_1, TM_2 and TM_3 against the control.

Study groups	Period of CBZ treatment	Mean total fetal brain volume (WIM) (mm^3) ± SEM	Mean total fetal brain volume (Cavalieri method) (mm^3) ± SEM	Mean shrinkage (mm^3) ± SEM	Mean cortical volume density(mm^3) ± SEM	Mean sub-cortical volume density(mm^3) ± SEM
Control group	----------	0.248±0.002	0.244±0.001	0.017±0.001	0.073±0.000	0.171±0.001
Low dose carbamazepine group (LCG, 20.7mg/kg)	TM1	0.233±0.002 ^{b*}	0.232±0.001 ^{b*}	0.015±0.005 ^{bc}	0.070±0.000 ^{b*}	0.162±0.001^{bc*}
	TM2	0.239±0.001 ^{b*}	0.235±0.002 ^{b*}	0.016±0.001 ^{bc}	0.071±0.000	0.162±0.001
	TM3	0.247±0.002	0.243±0.001 ^{b*}	0.244±0.005 ^{bc}	0.073±0.000	0.171±0.001

Medium dose carbamazepine group (MCG, 72.3mg/kg)	TM1	0.232±0.001 ^{b*}	0.231±0.001 ^{b*}	0.013±0.001 ^{bc}	0.069±0.000 ^{b*}	0.161±0.001 ^{bc*}
	TM2	0.238±0.001 ^{b*}	0.233±0.002 ^{b*}	0.019±0.006 ^{bc}	0.070±0.001 ^{b*}	0.162±0.001 ^{bc*}
	TM3	0.242±0.000 ^{c*}	0.239±0.001 ^{b*}	0.244±0.006 ^{bc}	0.072±0.000 ^{b*}	0.170±0.001 ^{bc*}
High dose carbamazepine group(HCG, 124mg/kg)	TM1	0.222±0.001 ^{b*}	0.220±0.001 ^{bc*}	0.009±0.003 ^{bc}	0.066±0.002 ^{b*}	0.154±0.001 ^{bc*}
	TM2	0.233±0.002 ^{b*}	0.230±0.002 ^{bc*}	0.244±0.004 ^{bc}	0.069±0.000 ^{b*}	0.154±0.001 ^{bc*}
	TM3	0.242±0.002 ^{c*}	0.236±0.001 ^{bc*}	0.244±0.004 ^{bc}	0.071±0.000 ^{b*}	0.167±0.001 ^{bc*}

Key: All value that bear (*) as a superscript indicates that they depicts a statistical significance differences

($p<0.05$) when compared with the control. Values with (^{b}) &$^{(c)}$ superscripts have a statistical significance

difference ($p<0.05$) in the intragroup and intergroup comparisons respectively using one way ANOVA with Turkey post-hoc t-tests

V. DISCUSSION

This study established that the fetal brains from the carbamazepine treated groups had their gross appearances being relatively small in size with poorly defines lobes, sulci and gyri when compared with the control group. Similarly, the calculated mean total fetal brain volume was also smaller and the variances in size and volumes were found to depict an inverse relationship with the dose administered (table 1). Further, the reduction in total brain volume and the volume densities of both the cortical and the subcortical layers of both the cerebral and the cerebellar cortices were seen to depict a direct correlation with the time of exposure in that when carbamazepine treatment was administered at trimesters one TM_1, two TM_2 the effects were not as pronounced as in the first two trimesters.

The reduction in brain volumes were seen to decrease directly with the time of exposure in that when carbamazepine treatment was done at TM_1 the mean total brain volume (in mls) was lowest in the HCG at (0.222±0.001), followed by MCG at (0.232±0.001) and lastly LCG at (0.233±0.002), (table 1). These findings were in line with a study by (Berghuis *et al*, 2017) and another one by (Bath & Scharfman, 2013).

The findings on the brain length and width were also seen to depict a similar scenario on dose and time response relationship with the doses of exposure and with time of exposure, (table 1). For instance, when a comparative mean brain length (in mm) was done across the three trimesters TM_1, TM_2 and TM_3, it was depicted that at TM_1 the mean fetal brain length was lowest in at HCG group at 1.02±0.012 followed by MCG at 1.12±0.008 and LCG at 1.197±0.0033. This was found to be statistically lower as compared with the control (P=0.003) at 1.295±0.00. At TM_2, brain length (in mm) was found to be lowest in HCG at 1.285±0.1433 followed by MCG at 1.2000±0.000, then LCG at 1.247±0.0033. This was not statistically different as compared with control group at 1.295±0.005 (P=0.64). At TM_3, the mean brain length (in mm) was lowest at HCG group at 1.287±0.0018, followed by MCG at 1.31±0.0024 and LCG at 1.304±0.136, (table 1).

Similarly, when the intra and the intergroup fetal brain weights comparisons were done for the experimental groups, there was a marked intra-group and inter-group variances in the total gross weights and brain sizes based on the dose of exposure and the time of exposure. For instance, it was observed that when carbamazepine treatment was done at TM_1, the mean total brain weight (in grams) was found the lowest in at HCG group at 0.12±0.012gms followed by MCG at 0.216±0.008 and LCG at 0.297±0.0033. When carbamazepine was administered in TM_2, the mean totals of the fetal brain weight (in grams) was found to be 0.247±0.0071gms in HCG at followed by MCG at 0.300±0.000, then LCG at 0.347±0.0059 as compared with control (P=0.010).

When treatment was done at TM_3, the mean brain weight (in grams) for the HCG group was 0.33±0.0028, followed by MCG at 0.370±0.008 and LCG at 0.394±0.0051. These values were found to be statistically significant (P=0.001) when the comparisons were done within and across groups and when compared with the control. The results of the present study are also in tandem with findings from studies by Erisgin *et al.,* (2019, Afshar *et al*., (2009) and Sah *et al.,* (2013) who reported that cortical alterations and the destructive changes especially of pyramidal cortical layer during fetal brain development (day 14 to day 19) are dose and time dependent.

VI. Conclusion And Recommendations

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.

The study recommends that;

1.The use carbamazepine during pregnancy should be avoided by all means as it has been shown to be teratogenic 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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