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The Histostereological Teratogenic Effects of In-Utero Exposure to Varied Doses of Lamotrigine in Albino Rats (Rattus Norvegicus)

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ABSTRACT: The in-utero exposure to lamotrigine, a second line anticonvulsant medicine that is currently being prescribed as first line medicine in management of maternal conditions like epileptic seizures, depression associated with bi-polar disorders among others, has been surrounded by controversies on its histoquatitative teratogenic effects to the developing fetal brain. Though several studies have considered lamotrigine safer because of its efficacy, tolerability and minimal teratogenic effects to the developing fetus, others have advocated for further studies on its quantitative effects to the developing fetal brain in a dose and time related manner. The current study is therefore of paramount importance to enhance maximum benefit to the expectant women and minimal teratogenic effects to the developing fetal brain. The broad objective of this study was therefore to evaluate the histostereological effects following in-utero exposure to varied doses of lamotrigine when administered at different gestational periods in albino rats.

Materials and methods: This study adopted a post-test only control experimental study design. The animal experimentation and measurement of the various fetal brain parameters was carried out in the animal research facility located in the University of Nairobi, Chiromo campus. Tissue processing and stereological procedures were carried out in Human Anatomy laboratories based in Jomokenyatta University of Agriculture and Technology (JKUAT), Juja main Campus.

Sample size determination: A sample size of 30 sexually mature ((6weeks old) albino rat dams of species (Rattus norvegicus) weighing between 250±30grams were used in the study as determined by use of the resource equation for One Way Analysis of Variance method (ANOVA). The 30 Albino rats were divided into 2 broad groups of 3 control and 27 experimental rats. To evaluate the quantitative stereological effects of lamotrigine on differing doses, the 27 rats in the experimental group were further subdivided into three study groups of 9 rats as follows; (i) Low lamotrigine group (25 mg/kg) (ii) Medium lamotrigine group (235.7 mg/kg) and (iii) High lamotrigine group of (500mg/kg). To further evaluate the comparative effects of lamotrigine on differing gestation periods, the 9 rats in each of the three dose categories were further be 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 in each study group

Data Analysis-The quantitative stereological effects on fetal brain that included the mean brain weight, mean brain length, mean brain width, mean brain volume and mean brain volume densities formed the parametric data that was collected using structured checklists then stored and coded in excel spreadsheets windows 10, version 2013. It was further exported for analysis in SPSS for windows version 25 (Chicago Illinois). Statistical analysis was done using one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple comparison tests. Results were expressed as mean±

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standard error of the mean_(SEM) and all values whose P<0.05 was considered to be significant.
Study findings-Study findings from the current study have shown that lamotrigine has a direct dose effects and inverse gestational effects to the developing fetal brain when exposed in-utero. Higher doses of lamotrigine (HLMTG), have been shown to have the highest statistical significance effects (P<0.05) when compared with the control as compared to medium and low dosages ((MLMTG and (LLMTG)). Similarly, these quantitative stereological effects have been observed to be higher when lamotrigine was administered during the first trimester (TM_1), followed by second trimester (TM_2) and were less when lamotrigine was administered during the third trimester, (TM_3),
Conclusion- Though lamotrigine is considered as the first line medicine in management of maternal conditions during pregnancy, high dosages and especially during first gestation of pregnancy should be avoided, since they have been shown to have adverse reduction in quantitative measures to the developing fetal brain. Further studies with higher primates closer to human species as well as clinical trials are therefore recommended to rule out the safety index of lamotrigine during pregnancy.

KEYWORDS: Stereology, Lamotrigine, Anticonvulsant, Teratogenic

I. INTRODUCTION

Lamotrigine is a broad-spectrum second-generation anticonvulsant medicine that is licensed for use worldwide as first line in management of maternal conditions like epileptic seizures, bipolar disorders among others during pregnancy (Hill et al.,2010). Though all older generation anticonvulsant medicines are associated with increased risk of birth defects to the offspring, a major concern for all women with conditions that require use of anticonvulsant medicine and are of childbearing potential (Muna et al., 2019), lamotrigine has been considered to be safer because of its efficacy, tolerability and minimal teratogenic effects to the developing fetuses (Wlodarczyk et al., 2012). However, many study results are not conclusive with some indicating that there are no strong indications that the use of LMT during pregnancy has quantitative teratogenic affects to the developing fetal brain (Beernaert's et al., 2009, Vadja et al.,2014,) while others report of major effects and malformations to the developing fetal brain and nervous system in general (Marchi & Tognola., (2001), Nie et al., 2016). In this context, it is worthwhile to have data on effects of lamotrigine on quantitative fetal brain parameters in a time and dose dependent manner, to guide on this controversy, enhance maximum benefits to the mothers and minimal teratogenic effects to the developing fetuses

II. MATERIALS AND METHODS

Study Location/ Setting: All experimental procedures that included breeding, mating, daily weighing, feeding, administration of lamotrigine, humane sacrificing of the rats, harvesting of fetuses and harvesting of fetal brains were carried out at the animal facility situated in the University of Nairobi (UON), Chiromo Campus. Tissue processing and stereological procedures were carried out in Human Anatomy laboratories based in Jomokenyatta University of Agriculture and Technology (JKUAT), Juja main Campus.

Study Design: A posttest-only with control experimental study design was adopted where 30 female albino rats were randomly assigned to either control or experimental group.

Acquisition and description of Albino rats: Sexually mature female albino dams (6wks) of pure breed (4th series breed) weighing between 250±30g were obtained from the department of biomedical science, Chiromo campus. They were used in the study due to following known scientific facts; (i) Resistant to various ailments (ii)calm temperament (iii) Are easy to handle (i)Large litter size, (ii)low maintenance cost (iii)low incidence of spontaneously occurring congenital defects, (iv)Relatively short gestational span and, (v)considerable amount of the reproductive data on the rat is already available (Bryda, (2013). Bailey et al., 2014; Pritchett & Corning, 2016). Sexually mature male rats of the same family of albino rats (8wks) were used for mating purposes. Rats were kept in spacious polycarbonate plastic cages as determined by (Kuramoto et al., 2012, Allen et al., 2016).

Sample Size Determination: Sample size was determined by use of resource equation for group comparisons using One-Way Analysis of Variance (ANOVA). Based on this approach, the acceptable range of degrees of freedom (DF) for the analysis of variance (ANOVA) is between 10 to 20. The formula is n = DF/k + 1, where DF = total number of subjects, k = number of groups, and n = number

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of subjects per group. (Charan & Kantharia, 2013). **n**=20/10+1 =3. Therefore, number of dams is **30.** Every adult female rat is assumed to have a minimum average of six (3) fetuses per pregnancy. The expected number of fetuses were determined as follows 3 x 30=90 fetuses. All fetuses were obtained by use of simple convenient sampling method

Grouping of rats in the study: After confirmation of pregnancy, the rats were assigned into two broad study categories 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 (LLMTG), Medium (MLMTG) and High lamotrigine group (HLMTG). To determine whether the effects of lamotrigine are time dependant, each of the subgroups of the LLMTG, MLMTG and HLMTG 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

Mating and confirmation of pregnancy: The mating process was done by introducing two sexually mature males of albino rat breed were introduced into a standard polycarbonate cage with four female rats overnight, after which males were removed and returned to their separate cages the following morning, Slonaker, (1918), Dikshit & Taskar (1959). Confirmation of pregnancy was done by taking a vaginal swab from the mated rats and smearing it on a slide and observing them under the microscope for presence of spermatozoon and changes in epithelial cells Shedrack et al., 2006). The rats that did not conceive were given more chances with the males for mating again till they conceived.

Feeding of the albino rats: All rats were fed on a standard diet as determined by American institute of nutrition (2011) that included rodent pellets from UNGA meals limited (Nairobi), and water adlibitum. Food and water were administered through a mesh in a standard polycarbonate cage (fig 2.1).

Fig 2.1: Showing how feeding of the rats was done by use of rodent pellets) from a wire-mesh at the polycarbonate plastic cages) and water ad-libitum (using the plastic bottles).

Acquisition of lamotrigine and Determination of Lamotrigine dosages: Lamotrigine tablets from Vega Biotec Private Limited (Gujarat India) batch number M2017103 were obtained from a government chemist in Nairobi, Kenya. They were reconstituted using distilled water and administered using an oral gavage needle gauge 16. Dosages were determined by use of a simple guide for conversion of animal dosages from human dosage as determined by (Nair & Jacob, 2016) 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. Administration of lamotrigine: All rats in trimester one (TM_1) group in the Low, Medium and High dose categories received lamotrigine from gestation day GD_1-GD_{20} while the rats in second trimester (TM_2) group in

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Low, Medium and High dose categories received lamotrigine from gestation day GD_7-GD_{20}. Rats in third trimester (TM_3) group in Low, Medium and High dose categories received lamotrigine from gestation day GD_{14}-GD_{20}

Humane sacrificing of the pregnant albino rats and harvesting of fetuses: All rats were humanly sacrificed on day 20th just before delivery to avoid devouring any devoured fetus, by use of concentrated carbon dioxide soaked in a cotton wool and put in a bell-jar. (Figure 2.2 and figure 2.3)

Figure 2.2: Showing how A; Pregnant rats were euthanized by use of concentrated carbon dioxide put in a bell jar B: How the pregnant rat was mounted on a board using pins for fetal harvesting of fetuses.

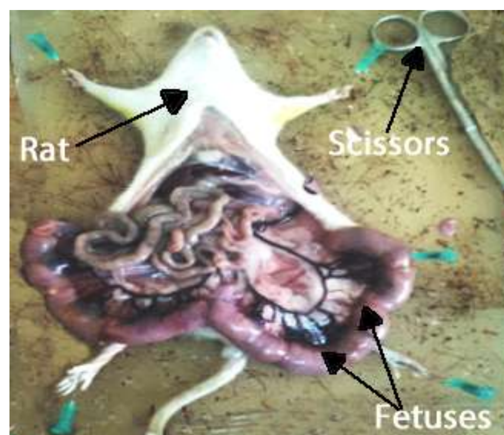

Figure 2.3: Showing how anterior abdominal wall of the pregnant rat was incised along the linear alba to expose the fetuses.

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 length and width were measured against a calibrated ruler (fig 2.4) wand brain weights were taken by use of a digital weighing scale (fig 2.5) (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 560 c, (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 370 to spread the tissue, (x)The sections were stuck onto glass slides using egg albumin, applied as thin film with a microdropper, (xi)The slides were further dried in an oven at 37degrees 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.

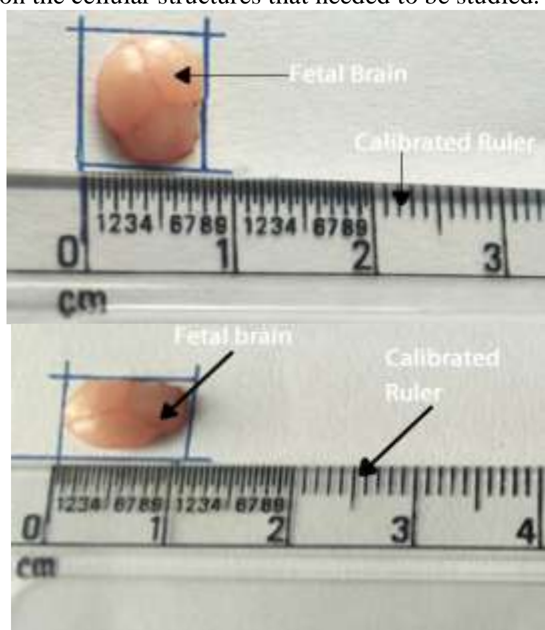

Figure 2.4: A; Showing how brain length was measured against a calibrated ruler and B; how brain width of the fetal brains were taken

Figure 2.5: Showing how fetal brain weight was taken using a weighing scale from Oertring company-Japan model

Histostereological Analysis

Histostereological analysis was done by calculating the brain volume initially by use of Archimedes Principal (displacement method) and by Cavalieri Principal

Determination of total brain volume using Archimedes principle (Displacement method)

Fetal brain volumes were determined by immersing them in graduated beakers containing normal saline by use Archimedes' principle, and the amount of fluid displaced was measured to represent the initial brain volume

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

- Cavalieri brain sections of (5µ) 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 random start was used to select twenty sections of 5µm thickness from each longitudinal section of a brain. The entire brain slice was viewed at magnification of X10, 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 (Fig 2.6)

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

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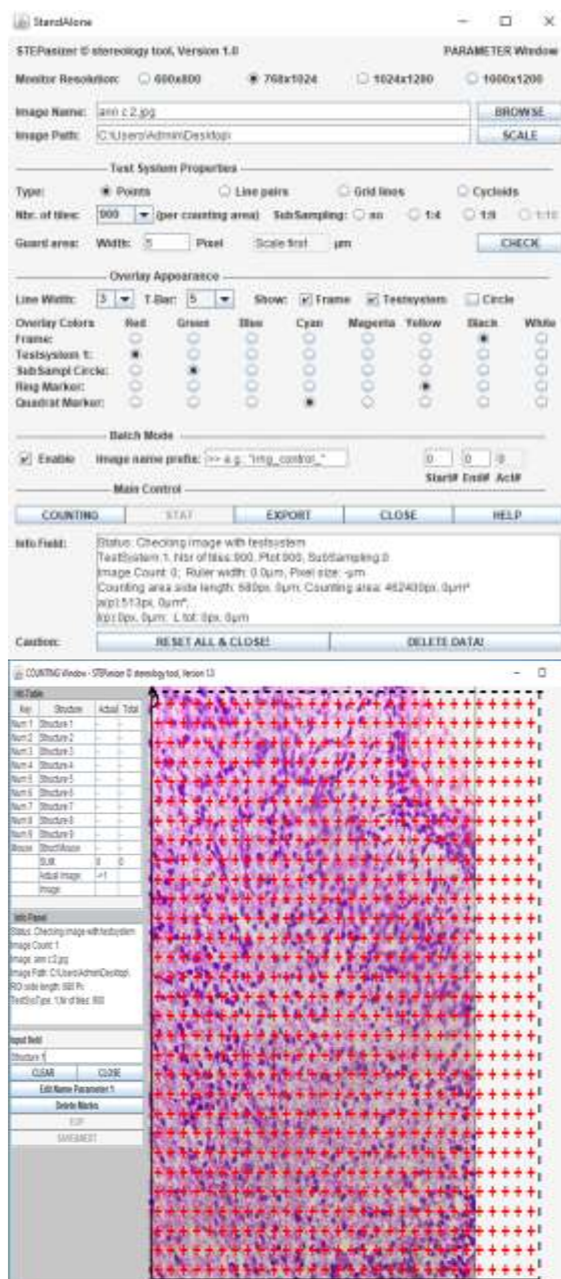

Figure 2.6 A STEPnizer stereology tool with an equidistant point grid with a stand-alone window with various parameters and a brain slice image superimposed in the counting frame

All the fields of the prefrontal and medial temporal lobe 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.

The following formula was used to calculate the total brain volume;

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

Where;

- $\hat{V}$ = Is the volume
- A_p is the Area associated with a point
- **m':** Is the section evaluation interval
- **t bar:** Is the mean section cut thickness
- p_i Are the points counted on the grid

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;

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

Where;

Volume before: Archimedes volume

Volume after- Cavalieri volume

Determination of volume densities of prefrontal cortex and medial temporal lobe using cavalieri method of point counting

In determining the volume densities of the prefrontal cortex and medial temporal lobe declarative 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;

$$\text{Est } V_v = \frac{P\ (\text{Part})}{P\ (\text{Ref}),}$$

Where;

Est Vv -Estimated volume density

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

Ethical consideration and clearance

Animals used in the study and procedures carried out were in accordance with the guidelines of the National Institutes of Health Animal Care and the animal research. Approvals were sought and given 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/2021/323)

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Data collection and statistical analysis: Data on quantitative stereological outcomes that includes; mean brain weight, mean brain length, mean brain width and mean brain volumes formed parametric data. It was collected using structured checklists, stored and coded in excel spreadsheets windows 10, version 2013. It was then be exported for analysis to SPSS programme for windows version 25 for analysis (Chicago Illinois). Data was analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison tests and was expressed as mean± standard error of the mean_(SEM) for all values. All results whose P<0.05 was considered to be statistically significant.

III. RESULTS

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

Upon comparative gross appearance of the fetal brain in the control group versus those in the lamotrigine treated groups, it was observed that the fetal brains from the experimental groups looked relatively small in size when compared with the control group. The intra and intergroup comparisons of mean fetal brain weights, mean brain width, mean brain length, fetal volumes and volume densities depicted a marked variances based on the dose and time of exposure.

Comparative histostereological parameters evaluated are presented in terms of the morphometric measurements on total fetal brains weights, brain length and width, total brain volume by use of both initial Archimedes method {water immersion method (WIM)}, cavalieri method of point counting, volume densities of both cortical and sub-cortical layers of the fetal brain structures. All these parameters were compared along the varying lamotrigine doses (Low-25 mg/kg, Medium-235.7mg/kg, High-500mg/kg) respectively against the period of exposure in TM1, TM2 and TM3 as follows.

Intragroup and intergroup comparative mean analysis of fetal brain weights and lengths in lamotrigine treatment groups were marked variances in the total gross weights and brain lengths based on the dose of exposure and the time of exposure. When lamotrigine treatment was done at $TM_{1,}$ the mean total brain weight in (grams) and brain length in (mm) was found to be lowest in high treatment groups (HLAMTG) group, followed by medium treatment group (MLAMTG) and lastly by low lamotrigine group (LLAMTG). When all the dosages were compared with the control group, there was a notable statistical significance difference(P=0.001).

When lamotrigine and levetiracetam treatments were administered in TM_2 and TM_3 the mean values of the fetal brain weight and fetal brain lengths were found to be statistically significant (P=0.001) when the comparisons were done within and across the groups and when compared with the control group. Mean total brain weight and length were both found to be highest when administration of treatments was done in low dosages, followed by medium dosages and were found to be lowest in lowest in high dosage groups **(table 3.1).**

When mean brain width in (millimeters) was compared, a similar scenario was observed. Mean brain width at TM_1 HLAMTG dosage was at 0.8847±0.0203 followed by MLAMTG at 0.9439±0.0093 and LLAMTG at 1.0311±0.4546. This was found to be statistically lower as compared with the control group at 1.2545±0.004 (P=0.001). At $TM_{2,}$ brain width was found to be lowest in HLAMTG at 0.9598±0.0080 followed MLAMTG at 1.0661±0.0008 then LLAMTG at 1.2769±0.0032. This was similarly statistically different as compared with control group at 1.2545±0.004 (P=0.001). At $TM_{3,}$ the mean brain width was lowest HLAMTG at 1.1500±0.0034/1.0513±0.0030, followed by MLAMTG at 1.0838±0.0004 and lastly LLAMTG at 1.0311±0.4546. Similarly, there was statistically different as compared with control group at 1.2545±0.004 (P=0.001) **(table 3.1).**

Table 3.1: Showing a comparative means fetal brain weight, brain length, and width for LLMTG, MLMTG and the HLMTG treated at TM_1, TM_2 and TM_3 against the control.

Study groups	The time of exposure to LAMTG treatment	Mean LAMTG Brain weight(g) ± SEM	Mean LAMTG brain length(mm) ± SEM	Mean LAMTG brain width(mm) ± SEM
Control group	---------------	1.2545±0.0004	1.5762±0.0057	1.3207±0.0041

Low dose LAMT group (25 mg/kg)	Trimester one (TM1) Trimester two (TM2) Trimester three (TM3)	1.0069±0.0319* 1.0752±0.0005* 1.0821±0.0089*	1.2236±0.0176* 1.2499±0.0033* 1.2772±0.0029*	1.0311±0.4546* 1.0795±0.0027* 1.0937±0.0015*
Medium dose LAMT group (235.7mg/kg)	Trimester one (TM1) Trimester two (TM2) Trimester three (TM3)	0.9436±0.0061* 1.0416±0.0064* 1.0760±0.0091*	1.1303±0.0065* 1.2397±0.0036* 1.2741±0.0057*	0.9439±0.0093* 1.0661±0.0008* 1.0838±0.0004*
High dose LAMT group (500Mmg/ kg)	Trimester one (TM1) Trimester two (TM2) Trimester three (TM3)	0.8450±0.0159* 0.9509±0.0040* 1.0304±0.0093*	1.0411±0.0088* 1.1486±0.0072* 1.2366±0.0058*	0.8847±0.0203* 0.9598±0.0080* 1.0513±0.0030*

Key: All value that bear (*) as a superscript indicates that they depict a statistical significance difference (p<0.05)when compared with the control in the intragroup and intergroup comparisons respectively using one way ANOVA with Turkey post-hoc t-tests

Influence of lamotrigine on the total fetal brain volume

The comparative fetal brain volumes (initial reference Archimedes displacement volume and calculated mean cavalieri volume through point counting method) were found to depict an inverse dose response relationship in that when the dose of exposure to lamotrigine was increased, the mean total brain volume had a corresponding decrease and vice versa. 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 that when lamotrigine and levetiracetam treatment were administered at different trimesters (TM_1, TM_2 TM_3), the brain volumes decreased directly with the time of exposure. All treatment groups depicted a statistically significant difference when they were compared with the control group (($P \leq 0.05$). When reference Archimedes volume was compared with the cavalieri volume method, total shrinkage was not statistically significant ($P \geq 0.05$).

The comparative mean cortical and subcortical volume densities in treatment groups were observed to be statistically higher when treatments were instituted at trimester three (TM_3), followed by trimester two (TM_2) and finally at trimester one (TM_1) (P=0.001). Higher dosages (HLAMG) were similarly associated with low mean cortical and subcortical volume densities, followed by medium dosages (MLAMG) and lastly at low dosages (LLAMG, P=0.001, **table 3.2**).

Table 3.2: A Comparative reference, calculated and percentage shrinkage on total mean fetal brain Volume using (WIM) and cavalieri method in the LLAMG, MLAMG and the HLAMG treated at TM_1, TM_2 and TM_3 against the control.

	The time of exposure to LEV/LAMT treatment	Mean total LAMTG fetal brain volume (WIM) (mm^3) ± SEM	Mean total LAMTG fetal brain volume (Cavalieri method) (mm^3) ± SEM	Mean LAMTG cortical volume density((mm^3) ± SEM	Mean LAMTG sub-cortical volume density((mm^3) ± SEM

Control group	------------	0.3171±0.0034	0.3134±0.0003	0.0042±0.0000	0.0128±0.0001
Low dose LAMT group (25 mg/kg)	TM1 TM2 TM3	0.2406±0.0027* 0.2447±0.0007* 0.2534±0.0026*	0.2405±0.0027* 0.2445±0.0007* 0.2532±0.0026*	0.0032±0.0000* 0.0032±0.0000* 0.0034±0.0000*	0.0096±0.0000* 0.0098±0.0000* 0.0101±0.0001*
Medium dose LAMT group (235.7mg/kg)	TM1 TM2 TM3	0.2330±0.0004* 0.2342±0.0007* 0.2458±0.0004*	0.2328±0.0006* 0.2340±0.0007* 0.2455±0.0039*	0.0031±0.0000* 0.0031±0.0000* 0.0033±0.0000*	0.0082±0.0000* 0.0094±0.0000* 0.0981±0.0001*
High dose LAMT group (500Mmg/kg)	TM1 TM2 TM3	0.2051±0.0126* 0.2277±0.0039* 0.2328±0.0007*	0.2049±0.0004* 0.2275±0.0039* 0.2327±0.0007*	0.0027±0.0000* 0.0030±0.0000* 0.0031±0.0000	0.0100±0.0005* 0.0091±0.0002* 0.0093±0.0000*

Key: All value that bear (*) as a superscript indicates that they depict a statistical significance difference ($p<0.05$)
when compared with the control in the intragroup and intergroup comparisons respectively using one way ANOVA with Turkey post-hoc t-tests

IV. DISCUSSION

The current study has established that upon administration of lamotrigine, there is a significant reduction in mean fetal brain weight, length and width, as well as mean brain volume and volume densities of both the cortical and the subcortical layers the fetal brain. The reduction in the said parameters have been shown to have an inverse relationship with the gestation period of exposure in that they were lowest when treatment was administered at trimesters one (TM_1) and trimester two (TM_2). However, the reduction was minimal when the medicines were administered during the third trimester TM_3). Upon administration of various dosages, a direct dose response relationship was depicted in that at higher lamotrigine dosages (HLAMG-500 mg/kg), more effects were observed, followed by medium lamotrigine dosages (MLEVG 325.7 mg/kg) and lastly by low dosage group LLAMTG-25 mg/kg (table 3.1 and 3.2).

When intra and intergroup mean fetal brain weights comparisons were done in the current study, the lamotrigine experimental groups mean weights were observed to vary based on the dose and time of exposure. For instance, it was observed that when lamotrigine treatment was done at $TM_{1,}$ the mean total brain weight (in grams) was found the lowest in at HLMTG group at 0.8450±0.0159gms followed by MLMTG at 0.9436±0.0061 and LLMTG at 1.0069±0.0319. When carbamazepine was administered in $TM_{2,}$ the mean totals of the fetal brain weight (in grams) was found to be 0.9505±0.0040gms in HCG at followed by MCG at 0.0416±0.0064, then LCG at 1.752±0.0005 as compared with control at 1.2545± 0.0004(P=0.010). When treatment was done at $TM_{3,}$ the mean brain weight (in grams) for the HCG group was 1.0304±0.0093, followed by MCG at 1.0760±0.0091 and LCG at 1.0821±0.0089. These values were found to be statistically different (P=0.001) when the comparisons were done within and across groups and when compared with the control **(table 3.1).** The results of the present study are also in tandem with findings from studies by Badaway et al., whose study results showed a highly significant decrease in the brain weight of fetuses who received gabapentin, a second-generation anticonvulsant medicine in the same class with lamotrigine. Study results by Veroniki et

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al., 2017 also in agreement with the current study results since they reported on the effects on anticonvulsant medicine to the developing fetal nervous system.

The findings on the mean brain length were also seen to depict a similar scenario on dose and time response relationship. 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 observed that at TM_1 the mean fetal brain length was lowest at HCG group at 1.0411±0.0088 followed by MCG at 1.1303±0.0065 and LCG at 1.2236±0.0176. This was found to be statistically lower as compared with the control (P=0.003) at 1.5762±0.0057. At $TM_{2,}$ brain length (in mm) was found to be lowest in HCG at 1.1486±0.0072 followed by MCG at 1.2397±0.0036, then LCG at 1.2499±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 1.2366±0.0058, followed by MCG at 1.2741±0.0057 and LCG at 1.2772±0.0029, **(table 3.1).** Study results by Wlodarczyk et al., 2012, similarly reported that upon administration of phenytoin and phenobarbital, there was marked reduction in fetal brain length in treatment groups as compared with the control groups.

Mean brain width at TM_1 HLAMTG dosage was at 0.8847±0.0203 followed by MLAMTG at 0.9439±0.0093 and LLAMTG at 1.0311±0.4546. This was found to be statistically lower as compared with the control group at 1.3207±0.0041 (P=0.001). At $TM_{2,}$ brain width was found to be lowest in HLAMTG at 0.9598±0.0080 followed MLAMTG at 1.0661±0.0008 then LLAMTG at 1.0795±0.0027. This was similarly statistically different as compared with control group at 1.3207±0.0041 (P=0.001). At $TM_{3,}$ the mean brain width was lowest HLAMTG at 1.0513±0.0030, followed by MLAMTG at 1.0838±0.0004 and lastly LLAMTG at 1.0937±0.0015. Similarly, there was statistically different as compared with control group at 1.3207±0.0041 (P=0.001) **(table 3.1).** The results of the current study are also in tandem with findings from studies by Wairimu et al., 2019 whose results showed that upon administration of carbamazepine, an anticonvulsant medicine, it statistically led to reduction in brain length and width

The current study results have depicted a reduction in mean brain volumes and volume densities in lamotrigine treatment groups in a time and dose related manner. When lamotrigine treatment was done at TM_1 the total mean fetal brain volume and volume densities (in mls) were lowest in the HLMTG, followed by MLAMTG and lastly LLAMTG **(table 3.2).** Study results by Kellogg, M., & Meador, K. J. (2017) showed that anticonvulsant like phenobarbital and phenytoin are associated with effects on fetal brain tissue volume and volume densities

V. CONCLUSION AND RECOMMENDATIONS

The study has established that use of lamotrigine has effects on histostereological fetal brain parameters including mean fetal brain weight, length and width, as well as mean brain volume and volume densities of both the cortical and the subcortical layers the fetal brain when administered in utero that are time and dose dependent. Since lamotrigine continues to be prescribed widely by clinicians as the first line anticonvulsant medicine in management of maternal conditions, further histostereological studies in higher primates closer to human species as well as clinical trials should be carried out to rule out safety of lamotrigine during pregnancy.

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