ORIGINAL RESEARCH ARTICLE

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

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ABSTRACT

The histoqualitative teratogenic effects of lamotrigine, a second-line anticonvulsant medicine, on the developing fetal brain structures when exposed *in utero* in a time- and dose-dependent manner remain unclear. On the other hand, lamotrigine is currently being widely prescribed as a first-line medicine in the management of maternal conditions like epileptic seizures and bipolar disorders, among others. The preferential use of lamotrigine is attributed to the considerations of its efficacy, tolerability, and minimal teratogenic effects on fetal organs like the brain, among others, though with insufficient supportive data. The aim of this study was therefore to evaluate the histo-quantitative effects of lamotrigine on the developing fetal brain structures when exposed *in utero* at varying dosages during different trimesters. The study adopted a post-test only experimental study design where a sample size of 30 sexually mature albino rat dams of the species (*Rattus norvegicus)* weighing between 250 $\pm$ 30 grams was used. The rats were divided into two broad groups: 3 control rats and 27 dosage rats. The data collected was coded in Excel spreadsheets and analyzed in SPSS. Results were expressed as the mean $\pm$ standard error of the mean (SEM), and values with a $P < 0.05$ were considered to be significant. Study findings depicted a reduction in brain weights, length, width, volumes, and volume densities of cortical and subcortical layers in a dose- and time-dependent manner. High lamotrigine dosages, especially during the first and second trimesters, were observed to be associated with significant mean reductions in the brain weights, length, width, volumes, and volume densities of the developing fetal brain structures. Therefore, further studies with higher primates closer to the human species as well as clinical trials are recommended to rule out the safety index of lamotrigine during pregnancy.

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

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1.0 Introduction

Lamotrigine is a broad-spectrum, second-generation anticonvulsant medicine that is licensed for use worldwide in the first-line management of conditions like maternal epileptic seizures and bipolar disorders, among others, during pregnancy (Hill *et al.,* 2010). Past literature has shown that older-generation anticonvulsant medicines are associated with an increased risk of birth defects in the offspring, a major concern for all women with conditions that require the use of anticonvulsant medicine and are of childbearing potential (Muna *et al.,* 2019).

Currently, lamotrigine is considered safer because of its efficacy, tolerability, and minimal teratogenic effects on developing foetuses (Wlodarczyk *et al.,* 2012). Some study results, however, are not conclusive, with some indicating that there are no strong indications that the use of lamotrigine during pregnancy has quantitative teratogenic effects on the developing foetal brain (Vajda *et al*., 2014), while others report major effects and malformations to the developing foetal brain and nervous system in general (Nie *et al.,* 2016). In this context, it is worthwhile to have data on the effects of lamotrigine on quantitative foetal brain parameters in a time- and dose-dependent manner to guide this controversy, enhance maximum benefits to the mothers, and minimize teratogenic effects on the developing foetuses.

2.0 Materials and methods

2.1 Study location/ setting

All experimental procedures were carried out in the animal facility situated at the University of Nairobi (UON), Chiromo Campus, while tissue processing and stereological procedures were carried out in human anatomy laboratories based at the Jomo Kenyatta University of Agriculture and Technology (JKUAT), Juja main campus.

2.2 Study design

A posttest-only control experimental study design was adopted in this study.

2.3 Acquisition

30 sexually mature female albino dams weighing between 250 $\pm$ 30g were obtained from the Institute of Primates based in Nairobi County

2.4 Description of Albino rats

Albino rats were used in this study due to the following known scientific facts: (i) they are resistant to various ailments; (ii) they have a calm temperament; (iii) they are easy to handle; (iv) they have large litter sizes; (v) they require low maintenance costs; (vi) they have a low incidence of spontaneously occurring congenital defects; (vii) they have a relatively short gestational span, and (viii) a considerable amount of the reproductive data on the rat is already available (Beermann *et al.,* 2004).

2.5 Sample size determination

The sample size was calculated by using the resource equation for the one-way analysis ofVariance (ANOVA) is as follows: n = DF/k + 1, where DF is the total number of subjects, k is

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the number of groups, and n is the number of subjects per group. The acceptable range of degrees of freedom (DF) for the error term in the analysis of variance (ANOVA) was taken to be between 10 and 20. Therefore, K = 10, n = 20/10 + 1 = 3, (10 groups x 3 rats) = 30 rats. Since a female albino rat has a normal litter size of between 3 and 16 foetuses by using the simple random sampling method, three foetuses were chosen from each of the 30 rats to make a total sample size of 90 foetuses (Charan & Kantharia, 2013).

2.6 Grouping of rats

After confirmation of pregnancy, the rats were assigned into two broad study categories: 3 rats in the control group and 27 rats in the dosage group (Figure 1).

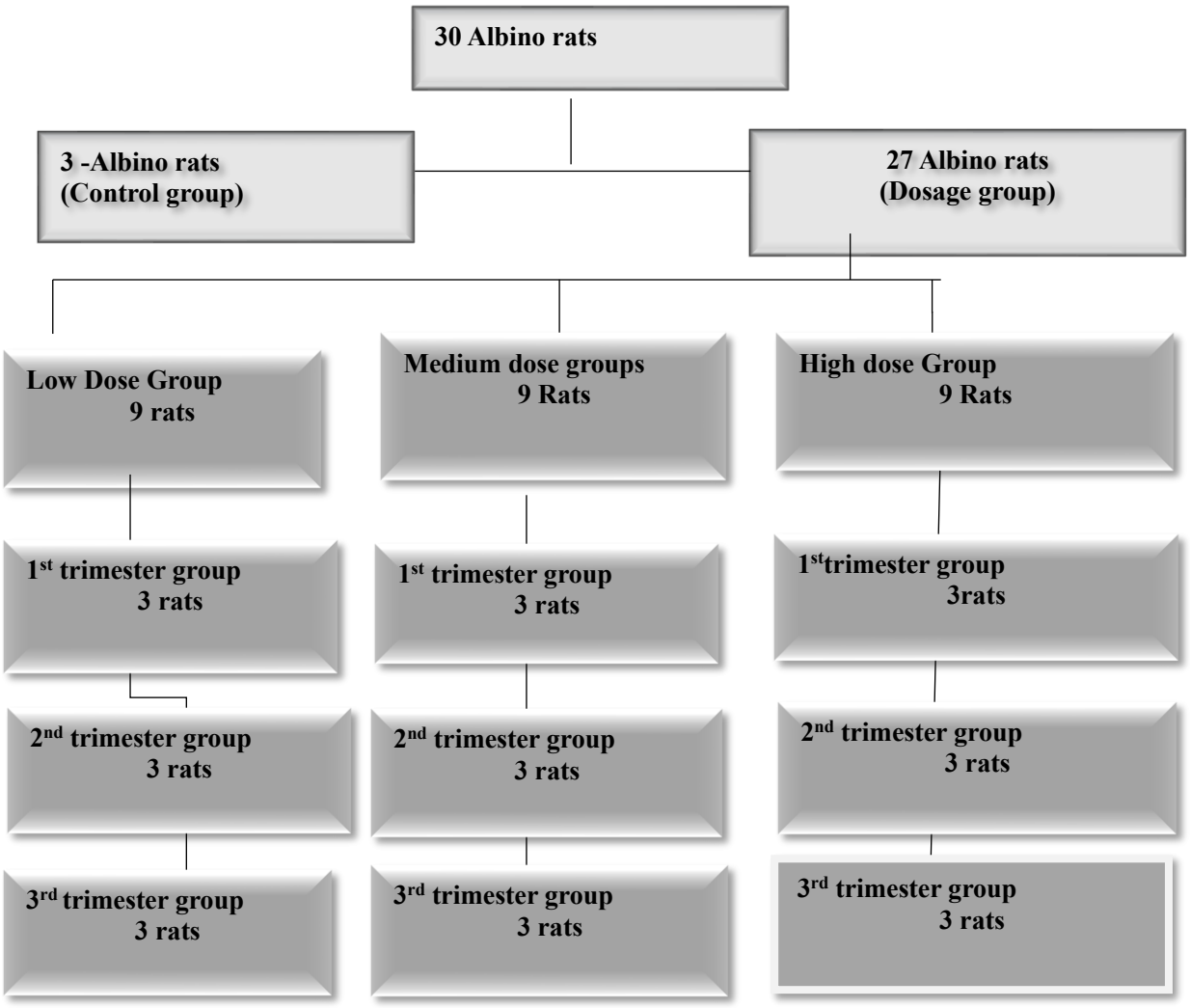

Figure 1: Showing the grouping of the 30 dams in both the control and dosage groups according to doses of exposure as low, medium and high dose lamotrigine groups (LLMTG, MLMTG and HLMTG) and according to trimester of exposure as trimester one, two and three (TM_1, TM_2 and TM_3).

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2.7 Mating and confirmation of pregnancy

The mating process was done by introducing two sexually mature males of the albino rat breed into a standard polycarbonate cage with four female rats overnight, after which the males were removed and returned to their separate cages the following morning (Dikshit & Taskar 1959). Confirmation of pregnancy was done by taking a vaginal swab from the mated rats, smearing it on a slide, and observing them under the microscope for the presence of spermatozoa and changes in epithelial cells (Shedrack *et al.,* 2006).

2.8 Feeding of the albino rats

All rats were fed a standard diet that included rodent pellets from UNGA Meals Limited (Nairobi) and water ad libitum. They were put in the polycarbonate plastic cages fitted with a wire mesh for the rats to access them (Kanyoni *et al*., 2023; *Kweri et al.,* 2023).

2.9 Acquisition of lamotrigine and determination of lamotrigine dosages

Lamotrigine tablets from Vega Biotec Private Limited (Gujarat, India) with 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 of 16. Dosages were determined by using a simple guide for conversion of animal dosages from human dosage as determined by Nair and Jacob (2016). The low lamotrigine dosage group was given 52 mg/kg, the medium lamotrigine dosage group received 24 mg/kg, and the low lamotrigine dosage group received 3 mg/kg.

2.10 Humane sacrificing of the pregnant albino rats and harvesting of fetuses

All rats were humanely sacrificed on day 20, just before delivery, to avoid devouring any foetus. This was done by using concentrated carbon dioxide soaked in cotton wool and put in a bell jar. The anterior abdominal wall was opened along the midline from the xyphoid process to the symphysis pubis, and foetuses were resected from the anti-mesomentrial border (Figure 2).

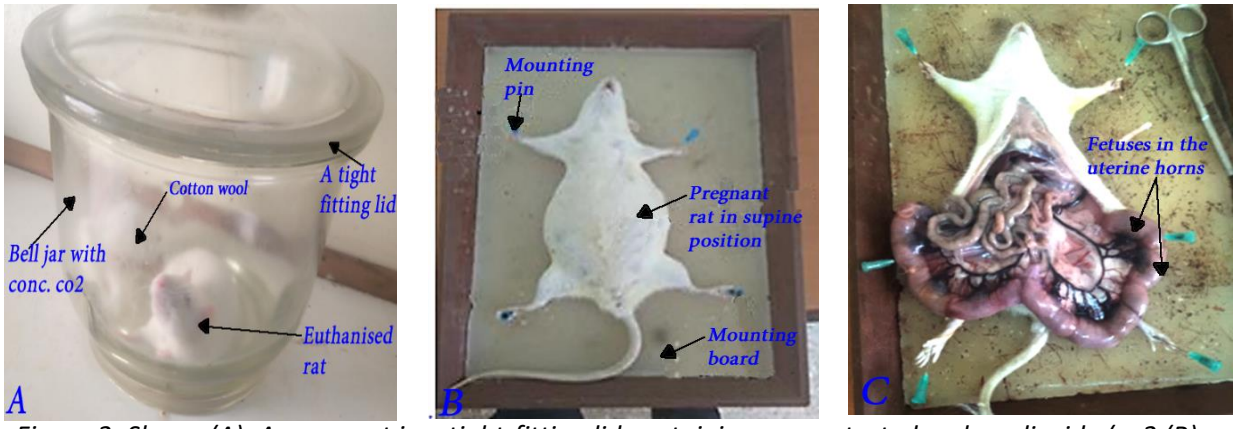

Figure 2: Shows (A); A pregnant in a tight-fitting lid containing concentrated carbon dioxide (co2 (B); pregnant rat mounted on a board before opening the anterior abdominal wall (C); rat whose anterior wall abdominal wall has been opened, portraying fetuses in the uterine horns

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2.11 Procedure for harvesting the fetal brains

After the foetuses were removed from the maternal uterine horns, they were euthanized by using concentrated carbon dioxide. The following procedure was followed to harvest their brains: (i) foetuses were mounted onto the dissection board using mounting pins on the dorsal side facing the board; (ii) using a pair of scissors and forceps, lateral bonders were placed along the lower margin, and the skull cap was removed. (iii) Using a magnifying glass, the whole foetal brain was identified, (iv) To avoid damaging the foetal brain, the meninges were opened along the superior sagittal sinus and retracted carefully since the brain lies within the meninges. (iv) the entire brain was excised at the level of the 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, while brain weights were taken using a digital weighing scale. (vii) The brains were immersed in the formaldehyde to proceed with processing, either for light microscopy or histostereological processing.

2.12 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 tissues were oriented 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 a Leitz sledge rotary microtome; (ix) the cut sections were floated in water at 370 °C to spread the tissue; (x) the sections were stuck onto glass slides using egg albumin, applied as a thin film with a microdropper; (xi) the slides were further dried in an oven at 37 °C for 24 hours; (xii) blinding was done by coding all the slides by the research assistant in the absence of the researcher; (xiii)They were stained with different Hematoxylin and Eosin (H&E), based on the cellular structures that needed to be studied and observed under the light microscope.

2.13 Determination of fetal total brain volumes

Foetal brain volumes were determined by using two methods: the initial Archimedes displacement method and the terminal Cavalieri point counting method.

2.13.1 Determination of total brain volumes using Archimedes (displacement method)

The initial Archimedes volumes were taken immediately after harvesting the brains as follows (Hughes, 2005): (i) Normal saline was put in a graduated beaker to a certain level; (ii) the initial readings were taken; (iii) the harvested brains were immersed in the beaker; (iv) normal saline was displaced after emersion of the brains and (v) the displaced saline displaced denoted the initial volume of the brains.

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2.13.2 Determination of total brain volume by use of Cavalieri point counting method

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 a magnification of X10 using the microscope's stage vernier. Digital images were captured using a 36-megapixel Swift camera and uploaded to the Swift software on the computer screen, then superimposed in a STEPanizer tool for point counting. A guard area was set to be consistent throughout the entire experiment. 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.

A summary of steps that were followed in the calculation of total brain volume using the Cavalieri point counting method is as follows: (i) Brain sections of (5µ) mm thickness were prepared; (ii) the spacing for the point probe was selected; (iii) in each section, a point probe was tossed randomly, and (iv) all points that hit the region of interest were counted, keeping a tally of counts per section. The Cavalieri formula provided below was used to calculate the volume. (Wairimu *et al.,* 2019; Welniak–Kaminska *et al*., 2019**)**

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

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

2.14 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. The Cavalieri method of tissue processing was used to obtain brain volume after sectioning, and shrinkage was calculated as per the following formula (Tran *et al.,* 2015);

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

Where;

i. Volume before: Archimedes volume
ii. Volume after- Cavalieri volume

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2.15 Determination of volume densities of the brain structurers

To calculate volume densities, cavalieri method of point counting was used as follows (Gundersen *et al*., 1988);

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

Where;

i. Est Vv -Estimated volume density
ii. P (part)- All points that fell in the area of interest

2.16 Data collection and statistical analysis

Data on quantitative stereological outcomes that included mean foetal brain measurements (mean brain weight, brain length and brain width), as well an mean brain volumes and volume densities forms the parametric data. This data was collected using structured checklists, stored and coded in Excel spreadsheets Windows 10 (version 2013). It was further exported for analysis to the SPSS programme for Windows version 25 (Chicago, Illinois). Data was analysed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple comparison tests, and was expressed as mean $\pm$ standard error of the mean (SEM) for all values. All results whose $p < 0.05$ were considered to be statistically significant. Results were presented in form of tables.

3. 0 Results

3.1 Effects of lamotrigine on the fetal brain measurements

The histostereological brain measurements includes; mean foetal brain weights, brain length, brain width, brain volumes, and volume densities. These parameters were compared in the low dose lamotrigine group (LLMTG), medium dose lamotrigine group (MLMTG), and high dose lamotrigine group (HLMTG) during varying trimesters, i.e., the first trimester (TM_1), second trimester (TM_2), and third trimester (TM_3) as follows;

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Table 1: The comparative means for fetal brain weight, brain length, and brain width in low, medium and high lamotrigine groups (LLMTG, MLMTG And The HLMTG), treated at trimesters one, two and three (TM_1, TM_2 and TM_3) against the control group.

Study groups	The time of exposure to Lamotrigine treatment	Mean Lamotrigine Brain weight(g) ± SEM	Mean Lamotrigine brain length(mm) ± SEM	Mean Lamotrigine brain width(mm) ± SEM
Control	----------	1.255±0.004^{a}	1.576±0.006^{a}	1.321±0.004^{a}
Low dose Lamotrigine group (3mg/kg)	Trimester one (TM1) Trimester two (TM2) Trimester three (TM3)	1.027±0.032*^{b} 1.075±0.001*^{c} 1.182±0.009^{a}	1.224±0.018*^{b} 1.250±0.003 ^{a} 1.277±0.003^{a}	1.031±0.455*^{b} 1.080±0.003*^{c} 1.094±0.002^{a}
Medium dose Lamotrigine group (24mg/kg)	Trimester one (TM1) Trimester two (TM2) Trimester three (TM3)	0.744±0.006*^{d} 1.042±0.006*^{e} 1.176±0.009*^{f}	1.130±0.007*^{b} 1.240±0.004*^{c} 1.274±0.006^{a}	0.944±0.009*^{b} 1.066±0.001*^{d} 1.084±0.000^{a}
High dose Lamotrigine group (52 mg/ kg)	Trimester one (TM1) Trimester two (TM2) Trimester three (TM3)	0.845±0.016*^{g} 0.931±0.004*^{h} 1.130±0.009*^{i}	1.041±0.009*^{d} 1.149±0.007*^{e} 1.237±0.006*^{f}	0.885±0.020*^{e} 0.960±0.008*^{f} 1.051±0.003*^{g}

Key: All mean values that bear (*) as a superscript indicates that they depict a statistical significance difference ($p < 0.05$) when compared with the control, while the means, followed by the same letter in a column are not statistically different at ($p < 0.05$) using One Way ANOVA with Tukey post-hoc t-test

It can be observed that the control group had the highest means of foetal brain weight, length, and width (1.255 ± 0.004, 1.576 ± 0.006, and 1.321 ± 0.004, respectively). The mean brain weight (BWT) depicted a statistically significant difference ($p < 0.05$) when treatments were administered during the first trimester (TM_1) ($F(6, 14) = 125.374$, $p < 0.001$), the second trimester (TM_2) ($F(6, 14) = 847.724$, $P < 0.001$), and the third trimester (TM_3) ($F(6, 14) = 204.541$, $p = 0.001$). High dosage groups across the trimesters were however associated with the lowest mean foetal brain weight, followed by medium dosage groups, and lastly low dose groups had the highest mean foetal brain weight. All dosage groups were statistically different from the control groups ($p < 0.05$) except for the low dose trimester 3 group ($p = 0.982$) (Table 1).

The comparative stereological results of mean brain length (BL) depicted no statistically significant difference when treatments were administered during the first trimester (TM_1) ($F(6, 14) = 461.946$, $p < 0.001$), the second trimester (TM_2) ($F(6, 14) = 1101.655$, $p < 0.001$), and the third trimester (TM_3) ($F(6, 14) = 814.643$, $p < 0.001$). The Tukey post hoc results in both intergroup and intragroup groups showed that the mean foetal brain weight (BW) depicted a direct response relationship with the time of exposure in that, it was observed to be lower when treatments were administered during the first trimester (TM_1), followed by trimester

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two (TM2), and lastly, they were lowest when treatments were instituted during the third trimester (TM_3). It was also noted that the low and medium lamotrigine dosage groups were not statistically different from the control (p = 0.321 and p = 1.000, respectively) (table 1).

When mean brain width in millimeters was compared by use one way ANOVA, it was observed that at TM_1, HLAMTG dosage was at 0.885±0.020 followed by MLAMTG at 0.944 ± 0.009, 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 by MLAMTG at 1.066 ± 0.001 and LLAMTG at 1.277 0.003. This was similarly statistically different as compared with the control group at 1.255 ± 0.004 (P <0.001). At TM_3, the mean brain width was lowest for HLAMTG at 1.1500 ± 0.0034/1.0513 ± 0.0030, followed by MLAMTG at 1.084 ± 0.001, and lastly LLAMTG at 1.031 ± 0.455. Similarly, there was a statistically significant difference as compared with the control group at 1.255 ± 0.004 (P = 0.003). The low and medium dosage groups during the third trimester were not statistically different from the control group (p = 0.784, p= 1.001) respectively **(**Table 1).

Table 2: The comparative means for fetal brain initial brain volume, terminal fetal brain volume, mean shrinkage, mean fetal brain volumes and mean fetal brain volume densities in low, medium and high lamotrigine groups (LLMTG, MLMTG and the HLMTG) treated at trimester one, two and three (TM_1, TM_2 and TM_3) against the control group.

	Study groups	Mean (LAMTG) total initial brain volume (IBV) (mm^3) ± SE)	(LAMTG) Mean terminal/Cavalieri brain volume (TBV) (mm^3) ± SE)	(LAMTG) Mean brain tissue shrinkage (BTSHR) (mm^3) ± SE)	(LAMTG) Mean cortical brain volume density (CVBD) (mm^3) ± SE)	(LAMTG) Mean sub-cortical l brain volume density (CVBD) (mm^3) ± SE)
None	Control	0.317±0.003^{a}	0.314±0.000^{a}	0.004±0.003 ^{a}	0.219.000^{a}	0.094±0.000^{a}
Trimester One (TM_1)	LLAMTG	0.269±0.003*^{b}	0.264±0.005*^{b}	0.005±0.003^{a}	0.185±0.001*^{b}	0.079±0.000*^{b}
	MLAMTG	0.247±0.003*^{c}	0.243±0.003*^{c}	0.006±0.003^{a}	0.170±0.004*^{c}	0.074±0.002*^{c}
	HLAMTG	0.229±0.003*^{d}	0.222±0.006*^{d}	0.007±0.002^{a}	0.155±0.002*^{d}	0.067±0.001*^{d}
Trimester Two (TM_2)	LLAMTG	0.285±0.001*^{b}	0.278±0.003*^{e}	0.007±0.001^{a}	0.195±0.002*^{e}	0.083±0.001*^{e}
	MLAMTG	0.257±0.004*^{e}	0.251±0.004*^{f}	0.006±0.003^{a}	0.176±0.003*^{f}	0.075±0.001*^{f}
	HLAMTG	0.237±0.003*^{f}	0.228±0.003*^{g}	0.009±0.001^{a}	0.159±0.002*^{g}	0.068±0.001*^{g}
Trimester Three (TM_3)	LLAMTG	0.294±0.002^{a}	0.290±0.004^{a}	0.004±0.002^{a}	0.203±0.003^{a}	0.087±0.001^{a}
	MLAMTG	0.266±0.009*^{g}	0.246±0.000^{a}	0.020±0.010^{a}	0.171±0.004*^{h}	0.074±0.000^{a}
	HLAMTG	0.246±0.001*^{h}	0.233±0.001*^{h}	0.013±0.001^{a}	0.268±0.003*^{i}	0.069±0.000*^{h}

Key: All mean values that bear (*) as a superscript indicates that they depict a statistical significance difference (p < 0.05) when compared with the control, while the means, followed by the same letter in a column are not statistically different at (p <0.05) using One Way ANOVA with Tukey post-hoc t-test

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The comparative foetal brain volumes (the initial reference Archimedes displacement volume and the calculated mean Cavalieri volume through the 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 treatment was administered at different trimesters (TM_1, TM_2, and TM_3), the brain volumes decreased directly with the time of exposure. All treatment groups showed a statistically significant difference when they were compared with the control group ($p< 0.003$). It was further established that the mean shrinkage was not significant across the treatment groups as well as during different trimesters ($p < 0.001$), (Table 2).

The mean cortical and subcortical volume densities in treatment groups were similarly 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) ($p = 0.004$). Higher dosages (HLAMTG) were similarly associated with low mean cortical and subcortical volume densities, followed by medium dosages (MLAMTG), and lastly at low dosages (LLAMTG, $p = 0.011$, table 2).

4.0 Discussion

The current study results have established that both intragroup and intergroup comparative mean analyses of foetal brain measurements (mean foetal brain weights, mean foetal brain lengths, and mean foetal brain widths) in the lamotrigine treatment groups depicted a statistically significant reduction as compared with the control ($p < 0.05$). The mean reduction in these foetal brain measurements has been shown to have an inverse relationship with the trimesters of exposure in that they were lowest when treatment was administered at trimester one (TM_1) and trimester two (TM_2). However, the reduction was lowest when lamotrigine was administered during the third trimester (TM_3) (Table 1).

For instance, when intragroup and intergroup mean foetal brain weights comparisons were done using one way ANOVA, between the lamotrigine dosage groups and the control, the means were statistically significantly low in the high and medium lamotrigine groups ($P < 0.001$), while the low dose lamotrigine groups had no statistically significant difference ($P > 0.05$) when they were compared with the control. The low mean foetal brain weight was more marked when lamotrigine was administered during the first and second trimesters (table 1). The results of the present study are in tandem with findings from studies by Badawy *et al.,* 2019, whose study results showed a highly significant decrease in the brain weight of foetuses that received gabapentin, a second-generation anticonvulsant medicine in the same class with lamotrigine. Study results by Veroniki *et al. (*2017*) are* also in agreement with the current study results since they reported teratogenic effects of a number of anticonvulsant medicines on the developing foetal nervous system.

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The current study findings upon computation of one way analysis of variance (ANOVA) on means of foetal brain length as well as brain width, their means were similarly observed to depict a time and dose response relationship. High and medium lamotrigine dosage groups were associated with low mean foetal brain length and mean foetal brain width, which were statistically different from the control group ($p < 0.013$). Low dose groups, however, had no statistically significant difference ($p > 0.05$) when they were compared with the control, especially when lamotrigine was administered during the third trimester (Table 1). Previous study results by Wlodarczyk *et al. (*2012) similarly reported that upon administration of phenytoin and phenobarbital *in-utero*, there was a marked reduction in foetal brain length in the dosage groups as compared with the control groups. The results of the current study are also in tandem with findings from a study by Wairimu *et al., (*2019*),* whose results showed that upon administration of carbamazepine, an anticonvulsant medicine, it statistically led to a reduction in brain length and width.

Upon computation of one-way analysis of variances (ANOVA) for the means of total fetal brain volume and volume densities of the cortical and subcortical fetal brain structurers, the results established that they had a time and dose dependency. High and medium dosage groups had lower means of total fetal brain volume and volume densities, especially when lamotrigine was administered during the first and second trimesters (TM_1 and TM_2). Low lamotrigine dosage groups however, especially during the third trimester, were not statistically different ($P > 0.05$) when they were compared with the control group. Previous study results by Kellogg and Meador (2017) showed that anticonvulsants like phenobarbital and phenytoin are associated with effects on foetal brain tissue volume and volume densities.

5.0 Conclusion and recommendations

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

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6.2 Ethical consideration and clearance

The animals used in the study and the 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 the initiation of the study (approval letter; ref: FVM BAUEC/2021/323).

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6.3 Conflict of interest

The authors declare no conflict of interest.

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