ORIGINAL RESEARCH ARTICLE

Comparative histoquatitative effects of lamotrigine and levetiracetam on fetal memory circuitry structurers in albino rats

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Abstract

Memory, a key component of the human brain, entails the cognitive ability to encode, store, and retrieve information, that is fundamental for survival. Memory is stored in different circuitry structurers in the brain. On the other hand, Lamotrigine (LAM) and levetiracetam (LEV) are anticonvulsant medicines currently being prescribed as first line in the management of maternal conditions like unipolar depression, acute bi-polar depression, schizophrenia, fibromyalgia, among others. Use of these two medicines is guided by past literature on their efficacy and torrelability. Despite their current use, data on their comparative histoquatitative outcomes on fetal memory circuitry structurers upon their maternal exposure remains equivocal, hence the basis of this study. Data is aimed at establishing which among the two medicines is safer when used prenatally in management of maternal conditions. A post-test-only with control experimental design was adopted in the study. 30 sexually mature female albino rats 250+30grams were used as experimental models. They were categorized as 3 rats for the control group and 27 rats for both LAM and LEV treatment groups. Collected data was coded in excel spreadsheets, and analysis was done using SPSS version 25. The findings of the study were expressed as mean+ standard error of mean (SEM). Values with $P<0.05$ were considered to have a statistical significantly difference. The comparative findings of the study delineated statistically significant reduction in means of total fetal brain volume, as well as the volume densities of memory circuitry structurers that includes; prefrontal cortex, entorhinal cortex, hippocampal gyrus, subiculum and dentate gyrus for both lamotrigine and levetiracetam. The mean reduction was observed to depict a time and dose dependency, with the most reduction being more marked in high dosages administered during the first trimester. Lamotrigine was associated with deleterious effects than levetiracetam. The study recommends further studies with animals close to human species.

Keywords: Memory, Levetiracetam, Lamotrigine, Effects, Histoquatitative, Anticonvulsants.

1.0 Introduction

Lamotrigine and levetiracetam are 2nd generation anticonvulsant medicines being prescribed currently as 1st line in management of maternal conditions like primary generalized tonic-clonic seizures, partial seizures, bipolar I disorder maintenance, Lennox-Gastaut syndrome,

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among others (Cerulli *et al.,* 2023, Mari *et al.,* 2022). Their prescription is guided by past literature that draws a picture of them as being safer than 1st generation anticonvulsants (Vajda *et al.,* 2014, Ozyurek *et al.,* 2010). However, past literature have associated them with cognitive neuropsychiatric disorders like loss of memory among others, but advocated for further studies since data is inconclusive (Sarangi *et al.,* 2016, Labiner *et al.,* 2009). Mode of neuro-teratogenicity for both LAM and LEV is due to closure of their metabolites through the maternal placenta barrier owing their low molecular weight of After their closure, they accumulate in neuropsychiatric developing structurers including those concerned with memory, hindering their development processes (Costa, B., & Vale, N. (2023), Hernández-Díaz and Levin, 2014). There is paucity of data on which among the two medicines is safer, at which dosage level and during which trimester when they are exposed prenatally, hence the basis of this study. Data obtained will form the basis for further studies on higher primates close to humans for clear guidance on their use.

2.0 Materials and methods

2.1 Study location

Implementation of the study was done at Chiromo Campus in the University of Nairobi. Tissue processing however was carried out in the department of Human Anatomy, in JKUAT main campus.

2.2 Study design

The study adopted a post-test only with control experimental design

2.3 Acquisition of albino rats model

The female albino rat models were purchased from the department of biomedical science, Chiromo campus at the university of Nairobi.

2.4 Why use female albino rats model

Female albino rat model was used in this study because of the following scientific facts; (i) they are not prone to suffer from various illnesses (ii) they are often peaceful (iii) they are not difficult to handle (iv) their litter size is usually large (v) they are not expensive to maintain (vi) they are rarely associated with congenital malformations, (vii), they usually have a short gestation period (viii) their data on reproduction is readily available, Wairimu *et al.,* 2023.

2.5 Determination of sample size

30 sexually mature female albino rat dams were used, guided by resource equation method for One-way Analysis of Variance (ANOVA), Althubaiti, (2022).

2.6 Grouping of female albino rats' model

Female albino rats were divided into two main groups of 3 rats in the control group and 27 in the treatment groups respectively. To establish whether the teratogenic outcomes of lamotrigine and levetiracetam were dependant on the dose administered, the treatment category of 27 rats was further clustered into three smaller groups of 9 rats of low treatment

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groups, medium treatment group, and high treatment groups. Further to establish whether the teratogenic effects of the two medicines were dependant on the time of drugs administration, the 3 dose categories were further subdivided according to the 3 trimester into three subgroups of 3 rats for trimesters I, II and III.

2.7 Mating and pregnancy confirmation

Breeding was done by introducing 1 male albino rat which was sexually mature and from the 3rd series breed of a pure colony, in a cage with two sexually mature female albino rats. Mating was allowed for 1200-hours of dark and 1200-hours of light cycle (Segut *et al.,* 2024). The once that had not conceived after pregnancy test was done, were allowed to undergo one other extra attempt, after which they were considered unfertile and hence replaced accordingly.

2.8 Feeding of the rats

Rodent pellets and water *adlibitum* were administered as guidelines for care of laboratory animals, Nyaga *et al.,* 2023. Folate supplementation was also done.

2.9 Administration of lamotrigine and levetiracetam

Low, medium and high dosages of both medicines were reconstituted in distilled water and given orally using gauge 16 of gavage needle as per the groupings of trimesters I, II & III. Dosages were calculated by use of a conversion formula of human dosages to the rat dosages Wairimu *et al.,* 2023, Kweri *et al.,* 2023, Nair *et al.,* 2016.

2.10 Humane sacrificing of pregnant rats

Humane sacrificing of the rats was done on the last day before delivery. This was done by use of concentrated carbon dioxide soaked in cotton wool, and put inside a bell jar.

2.11 Humane harvesting of the fetuses

Fetus were harvested from the anti-mesometrial border of the placentas after gently prodding them. 3 fetuses were selected from each rat by use of systematic uniform random sampling and also humanly sacrificed by use of concentrated carbodioxide.

2.12 Humane harvesting of fetal brains

The 3 fetuses were also humanely sacrificed using concentrated carbon dioxide soaked in a cotton wool. Their brains were harvested by scooping them below the meninges. Their initial volumes were taken and recorded. They were further processed for light microscopy and stereology

2.14 Data collection and statistical analysis

Excel spreadsheets were used to code the data; after which it was analysed using statistical package for social sciences (SPSS) version 25. Study findings were expressed as mean± standard error of the mean (SEM). Values whose P value was less than 0.05, were reported to be have a statistically significantly difference.

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3.0 Results

3.1 Comparative Histoquatitative Effects of Lamotrigine and Levetiracetam on mean Total Fetal Brain Volume

The current study established a statically significant reduction in the mean total brain volume in both LAM and LEV treatment groups (*P*<.05) when comparison was done with the control ([F (18,38] =423.412, *P*=.003) and (F [18,38] =324.653, *P*=.001) respectively. The mean reduction in both medicines was observed to time dependent, with high dosages being associated with more reduction, followed by medium dosages and lastly low dosages. Subsequently, the mean reduction was observed to be time dependent, with the first trimester having the most reduced means, followed by second trimester. The third trimester was however associated with the least reduced means. Upon comparison of the overall effects in terms of mean reduction in the brain volume, it was further observed that lamotrigine had more effects as compared with the levetiracetam in all study groups (table 1).

Table 1: The Comparative ANOVA Results on how Lamotrigine and Levetiracetam Influenced the Total Fetal Brain Volume

The study groups	Study groups and dosage levels.	The time of exposure to treatment	The comparative means of initial (Archimedes volume, terminal Cavalieri volume for various study groups	
			Mean initial Archimedes brain volume (mm^3) ± SD)	Mean terminal Cavalieri brain volume (mm^3) ± SD)
Control.	Control (C) no treatment	None.	0.31±0.03	0.314±0.01
Levetiracetam treatment groups	Low dosage group (103mg/kg/bw)	Trimester one	0.281±0.06*	0.274±0.07*
		Trimester two	0.289±0.07	0.288±0.07
		Trimester three	0.301±0.01	0.297±0.06
	Medium dosage group (207mg/kg/bw)	Trimester one	0.258±0.04*	0.256±0.01*
		Trimester two	0.271±0.07*	0.264±0.03*
		Trimester three	0.281±0.03	0.290±0.06*
	High dosage group (310 mg/kg/bw)	Trimester one	0.246±0.07*	0.238±0.03*
		Trimester two	0.248±0.03*	0.241±0.02*
		Trimester three	0.261±0.04*	0.256±0.03*
Lamotrigine treatment groups	Low dosage group (3mg/kg/bw)	Trimester two	0.269±0.04*	0.264±0.07*
		Trimester one	0.278±0.06	0.278±0.05
		Trimester two	0.294±0.07	0.290±0.06
	Medium dosage group (24mg/kg/bw)	Trimester one	0.239±0.04*	0.251±0.02*
		Trimester two	0.261±0.07*	0.245±0.07*
		Trimester three	0.274±0.03	0.280±0.03*
	High dosage group (52mg/kg/bw)	Trimester one	0.239±0.04*	0.229±0.07*
		Trimester two	0.237±0.06*	0.239±0.05*
		Trimester three	0.249±0.02*	0.233±0.04*

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Overall comparison by ANOVA [F, P values]	F (18,38) =423.412 P=0.003	F (18,38) =324.653 P=0.001

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

3.2 Comparative histoquatitative effects of lamotrigine and levetiracetam on the volume density of the prefrontal cortical histological layers

The current study established a statistical significant reduction in the volume density of all the prefrontal cortical histological layers as follows; (I) (ML) (18,38) =322.463, P=0.011) (II)outer granular layer (OGL) (F (18,38) =365.635, P=.001), (III) outer pyramidal layer (OPL) (F (18,38) =251.009, P=.001), (IV)inner granular layer (IGL) (F (18,38) =317.717, P=.011) (V)inner pyramidal layer (IPL) (F (18,38) =125.321, P=.013), and (VI) multiform layer (MTL) (F (18,38) =252.212, P=.001). The mean reduction depicted a time dependency, with high dosages having more reduction in means, as opposed to medium and low dosages. In addition, the mean reduction was observed to be time dependent, with the first trimester having more reduced means than second and third trimesters. The lamotrigine groups had more reduced means than levetiracetam groups (table 2).

Table 2: The Comparative ANOVA Results on how Lamotrigine and Levetiracetam Influenced the Volume Density of the Prefrontal Cortical histological layers

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

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	High dosage group (52mg/kg/bw)	TM1 TM2 TM3	0.007±0.04* 0.008±0.07* 0.009±0.03*	0.005±0.02* 0.006±0.07* 0.006±0.03*	0.004±0.06* 0.005±0.07* 0.005±0.06*	0.004±0.04* 0.006±0.03* 0.006±0.07*	0.001±0.02* 0.002±0.07* 0.003±0.04*	0.001±0.01* 0.002±0.03* 0.002±0.06*
Overall comparison by ANOVA [F,P values]			F (18,38) =269.322 P=0.001	F (18,38) =311.328 P=0.012	F (18,38) =532.603 P=0.001	F (18,38) =381.262 P=0.011	F (18,38) =562.342 P=0.003	F (18,38) =558.332 P=0.001

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

3.3 Comparative histoquatitative effects of how lamotrigine and levetiracetam influenced the volume density of the entorhinal cortical histological layers

The current study observed a significant deleterious mean reductions of the volume density of all the histological layers of the entorhinal cortex at various dosage levels and across all the three trimesters as follows; (*P*<.05) as follows; (I) molecular layer (ML) (*F* (18,38) =269.322, *P*=.001), (II) stratum sterale layer (SSL), (*F* (18,38) =311.328, *P*=.012), (III) external principal striatum layer (EPSL), (*F* (18,38) =532.603, *P*=.001), (IV) lamina dissecat layer (LDL), (*F* 18,38) = 381.262, *P*=.011), (V) internal principal striatum layer (IPSL) (*F* (18,38) =562.342, *P*=.011), and (VI) multiform layer (MTL) (*F* (18,38) =558.33, *P*=.011). The mean reduction of the entorhinal cortical histological layers delineated a time dependency, with high dosages being associated with more reduction in means, followed by medium dosages and lastly low dosages. It was further observed that the mean reduction was time dependent, with the first trimester having more reduced means than second and third trimesters. The lamotrigine groups had more reduced means than levetiracetam groups (Table 3).

Table 3: The Comparative ANOVA Results on how Lamotrigine and Levetiracetam Influenced the Volume Density of the Entorhinal Cortical Histological layers

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

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LAM	Low dosage group (3mg/kg/bw)	TM1	0.009±0.07*	0.007±0.03*	0.005±0.01*	0.006±0.02*	0.003±0.04*	0.004±0.07*
		TM2	0.010±0.03	0.008±0.02	0.006±0.04*	0.008±0.06*	0.005±0.07*	0.004±0.03
		TM3	0.014±0.04	0.009±0.03	0.007±0.03	0.008±0.04	0.005±0.03	0.004±0.04
	Medium dosage group (24mg/kg/bw)	TM1	0.009±0.04*	0.006±0.03*	0.004±0.04*	0.006±0.04*	0.003±0.03*	0.002±0.04*
		TM2	0.010±0.03*	0.007±0.05*	0.005±0.03*	0.007±0.03*	0.004±0.05*	0.003±0.03*
		TM3	0.011±0.07	0.008±0.03	0.006±0.01*	0.006±0.07*	0.004±0.03*	0.003±0.01*
	High dosage group (52mg/kg/bw)	TM1	0.007±0.04*	0.005±0.02*	0.004±0.06*	0.004±0.04*	0.001±0.02*	0.001±0.01*
		TM2	0.008±0.07*	0.006±0.07*	0.005±0.07*	0.006±0.03*	0.002±0.07*	0.002±0.03*
		TM3	0.009±0.03*	0.006±0.03*	0.005±0.06*	0.006±0.07*	0.003±0.04*	0.002±0.06*
[F,P values]			F (18,38) =269.322 P=0.001	F (18,38) =311.328 P=0.012	F (18,38) =532.603 P=0.001	F (18,38) =381.262 P=0.011	F (18,38) =562.342 P=0.003	F (18,38) =558.332 P=0.001

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

3.4 Comparative histoquatitative effects of lamotrigine and levetiracetam on the volume density of subiculum, presubiculum and parasubiculum

The current study established a mean reduction in volume densities of the key cellular components, the nerve fibre bundles forming the inputs and output loops to the subiculum, presubiculum and the parasubiculum in both lamotrigine and levetiracetam treatment groups on the follows; (a) ssubiculum (SUB) (F (18,38) =321.371, P=.001), (b) presubiculum (PrS) (F (18,38) =461.576, P=.006) and (c) parasubiculum (PaS) (F (18,38) =576.434, P=.011). These deleterious mean reduction was observed to occur in a dose and time dependent manner. At trimester three (TM_3) there was no much noticeable differential effects as were observed in both trimesters two(TM_2) and trimester one (TM_1). High dosage groups on the other hand were associated with more reduction in means, followed by medium dosage groups and lastly low dosage group. Upon comparison of the overall mean reduction in volume density of subiculum, presubiculum and parasubiculum, it was observed that lamotrigine had more effects as compared with the levetiracetam in all study groups (Table 4).

Table 4: The Comparative ANOVA Results on how Lamotrigine and Levetiracetam Influenced the Volume Density of Subiculum, Presubiculum and Parasubiculum

The study groups	Study groups and dosage levels.	The time of exposure to treatment	The comparative mean volume density of subiculum, presubiculum and parasubiculum for various study groups		
			Mean subiculum (mm^3) ± SD)	Mean presubiculum (mm^3) ± SD)	Mean parasubiculum (mm^3) ± SD)
Control.	Control (C) (no treatment)	None.	0.010±0.07	0.014±0.03	0.005±0.03

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Levetiracetam treatment groups	Low levetiracetam group (LLEVG)-(103mg/kg/bw)	Trimester one Trimester two Trimester three	0.008±0.04* 0.009±0.03 0.010±0.06	0.012±0.07* 0.013±0.03 0.014±0.07	0.004±0.01* 0.004±0.07 0.005±0.01
	Medium dosage group (207mg/kg/bw)	Trimester one Trimester two Trimester three	0.008±0.01* 0.008±0.07* 0.008±0.07	0.011±0.02* 0.012±0.08* 0.013±0.03*	0.004±0.01* 0.004±0.04* 0.005±0.04
	High dosage group (310 mg/kg/bw)	Trimester one Trimester two Trimester three	0.007±0.03* 0.007±0.04* 0.008±0.03*	0.011±0.05* 0.011±0.03* 0.011±0.03*	0.004±0.03* 0.004±0.01* 0.004±0.05*
Lamotrigine treatment groups	Low dosage group (3mg/kg/bw)	Trimester one Trimester two Trimester three	0.008±0.01* 0.009±0.04 0.008±0.04	0.012±0.06* 0.012±0.03 0.012±0.03	0.004±0.01* 0.004±0.04 0.004±0.04
	Medium dosage group (24mg/kg/bw)	Trimester one Trimester two Trimester three	0.007±0.04* 0.008±0.03* 0.008±0.07	0.011±0.03* 0.012±0.05* 0.012±0.03	0.003±0.04* 0.004±0.03* 0.004±0.01
	High dosage group (52mg/kg/bw)	Trimester one Trimester two Trimester three	0.007±0.01* 0.007±0.07* 0.007±0.07*	0.010±0.02* 0.010±0.03* 0.011±0.03*	0.003±0.06* 0.004±0.06* 0.004±0.06*
Overall comparison by ANOVA [F, P values]			F (18,38) =321.371 P=0.001	F (18,38) =461.576 P=0.006	F (18,38) =576.434 P=0.011

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

3.5 Comparative histoquatitative effects of the lamotrigine and levetiracetam on the volume density of hippocampal gyrus histological layers

The current study indicates an overall statically significant reduction in volume densities of all the histological layers of the hippocampal gyrus as follows; [a] stratum alveus layer (SAL) (F (18,38) =522.426, *P*=.001), [b] stratum oriens layer (SOL) (F (18,38) =675.321, *P*=.012), [c] stratum pyramidale layer (SPL) (F (18,38) =443.429, *P*=.001), [d]stratum radiatum layer (SRL) (F (18,38) =372.335, *P*=.013), [e] stratum lacunosum layer (SLL) (F (18,38) =652.344, *P*=.001), The mean reduction of these volume densities was observed to be highest in high treatment groups as compared to medium and low dosage groups. Comparably, the mean reduction was observed to be highest when the two medicines were administered during the first trimester, followed by the second trimester and lastly during the third trimester. Lamotrigine treatment group was observed to have lower means as compared to those of levetiracetam throughout the gestation period and across the three trimesters (table 5).

Table 5: The Comparative ANOVA Results on How Lamotrigine and Levetiracetam Influenced the Volume Density of the Hippocampal Gyrus

Study groups and dosage levels.	The comparative volume density of stratum aureus, stratum oriens, stratum pyramidale, stratum radiatum and stratum lacunosum for various study groups

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

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

3.6 Comparative histoquatitative effects of the lamotrigine and levetiracetam on the volume density of the dentate gyrus and the amygdaloid nuclei

The results of this study depicted a statistical significant reduction in volume densities of both dentate gyri an amygdaloid nucleus in a dose and time related manner in both lamotrigine and levetiracetam treatment groups as follows; [a] amygdaloid nucleus (AN) (F (18,38) = 962.447, P=.011), [b] dentate gyrus (DG) (F (18,38) =885.355, P=.013. The mean reduction of these volume densities was observed to be highest in high treatment groups as compared to medium and low dosage groups. Comparably, the mean reduction was observed to be highest when the two medicines were administered during the first trimester, followed by the second trimester and lastly during the third trimester. Lamotrigine treatment group was observed to have lower means as compared to those of levetiracetam throughout the gestation period and across the three trimesters, (table 6).

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Table 6: The Comparative ANOVA Table on How Lamotrigine and Levetiracetam Influenced the Volume Density of the Dentate Gyrus and the Amygdaloid Nucleus

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

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

4.0 Discussion

The current study results have established a mean decrease in total brain volume in a dose and time dependent manner, for both lamotrigine and levetiracetam groups as compared with the control. In both LAM and LEV treatment groups, decrease in mean total fetal brain volume was observed to be highest in high dosage groups when treatments were administered during the first trimesters. The means of the total fetal brain volume in both medium and low dosage groups when the medicines were administered during the second and the third trimesters were however observed not to be much reduced. It was further noted that lamotrigine treated groups had more deleterious effects as compared with levetiracetam treated groups across all trimesters as well as treatment groups (table 1). The current study results are in agreement with those of Badawy *et al.,* 2019., that similarly established a significant disorganization of cerebral and cerebellar cortical histological layers as a result of neurodegenerative processes,

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apoptosis of neuronal cells as well as altered ultrastructure after administration of gabapentin, an anticonvulsant medicine in the same class with both LAM nd LEV. This resulted in decrease in fetal brain weight as well as the total brain volume. The current study results however contradict those of Erisgin *et.al.,* 2017, who demonstrated that upon prenatal exposure to oxcarbazepine (OXC) and gabapentin (GBP) which are second-generation anticonvulsants just like LAM and LEV at different gestational periods, there was no effect to the neuronal tissue even during organogenesis. The results could have been influenced by the small sample size in their study.

The current study results have further established that there was a significant mean reduction in the volume density of the prefrontal and entorhinal cortical histological layers, in a dose and time dependent manner for both LAM and LEV treatment groups as comparison was made with the control. The decrease was observed to be highest in both LAM and LEV treatment groups, when treatments were instilled during the first trimester, followed by second trimester and lastly by third trimester. In terms of dosages, it was established that low dosage groups had less effects as opposed to medium and high dosage groups. Similarly, it was evidenced that across all trimesters as well as treatment groups that LAM treatment groups had more detrimental effects as compared with LEV treatment groups (table 2 & 3). The current study results are intendem with those of Ikonomidou *et.al.,* 2010, whose results demonstrated that upon exposure to vigabatrin and valproate with similar mode of action as LAM and LEV, they induced cortical and hippocampal dysplasia resulting in a defect in neuronal migration defect and ultimately neuronal death.

The current study results have established a mean reduction in volume density of Hippocampal gyrus, dentate gyrus, amygdaloid nucleus, subiculum, presubiculum and parasubiculum histological layers in in a dose and time dependent manner, for both lamotrigine and levetiracetam groups as compared with the control. In both LAM and LEV treatment groups, decrease in mean total fetal brain volume was observed to be highest in high dosage groups when treatments were administered during the first trimesters. However, the means of the total fetal brain volume in both medium and low dosage groups when the medicines were administered during the second and the third trimesters were not much reduced. It was further noted that lamotrigine treated groups had more deleterious effects as compared with levetiracetam treated groups across all trimesters as well as treatment groups (table 4, 5 &6). These results concur with those of González-Maciel *et. al.,* 2020, whose study findings upon prenatal exposure to oxcarbazepine during organogenesis was observed to alter the histo-cytoarchitecture of the hippocampal gyrus, dentate and amygdaloid nucleus by inducing neuronal apoptosis with resultant reduction in its volume density.

5.0 Conclusion / recommendations

Prenatal exposure to both lamotrigine and levetiracetam results in histoquatitative effects to the developing fetal memory circuitry structurers in a time and dose dependent manner. More detrimental effects are associated with lamotrigine as compared to levetiracetam. Further studies on animal species close to humans are recommended focusing on findings of this study.

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6.0 Recommendation

6.1 Conflicting interests

There was no conflicting interest.

6.2 Ethical approval

The study approval was sought from an ethical committee based at the faculty of veterinary medicine, department of veterinary Anatomy and Physiology in the University of Nairobi (REF: FVM BAUEC/2021/323).

6.3 Funding

None

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