THE MATERNAL PREGNANCY OUTCOMES FOLLOWING IN-UTERO EXPOSURE TO VARIED DOSES OF LAMOTRIGINE IN ALBINO RATS (RATTUS NORVEGICUS)

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Abstract: The relative teratogenicity risks of lamotrigine the mostly used antiepileptic medicines on the development fetal memory circuitry structures following *in-utero* exposure remains unclear. Though some studies have reported that the in-utero exposure to lamotrigine may perturb the normal morphogenesis of the fetal brain, there is paucity of data on its histostereological teratogenic effects on the development of the fetal prefrontal and medial temporal cortices the key components of memory circuitry system. In addition, data on the e histo-anatomical quantitative effects following exposure to varied doses and at differing gestational periods is generally lacking. The broad objective of this study was therefore to evaluate the maternal outcomes on the development of prefrontal cortex and medial temporal lobe following their in-utero exposure to varied doses and at different gestational periods in albino rats. A post-test only experimental design with control group study design was adopted in conducting the study. Animal experimentation was carried out at Small Animal Facility for Research and Innovation (SAFARI) animal house while tissue processing for histology and stereological analysis will be done in the department of human anatomy. A Sample size of 30 albino rat dams (*Rattus norvegicus*) weighing between 230±30grams were used in the study as determined by use of the resource equation for One Way Analysis of Variance method. These 30 Albino rats were divided into 2 broad groups of 3 control and 27 experimental rats. To evaluate the maternal pregnancy outcomes 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 (25mg/kg) (ii) Medium lamotrigine group (327.5mg/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 on pregnancy outcomes that forms the parametric data (inferential data) 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 expressed as mean± standard error of the mean (SEM) for all values. It was analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison tests. All results whose $P<0.05$ will be considered to be significant considered.

Keywords: Lamotrigine, Anticonvulsant, Teratogenic, Histostreology.

I. INTRODUCTION

Memory that is the most important survival component in human life entails the capacity to encode, store, consolidate and retrieve information plays a critical role in cognitive abilities of a human being and the most important in learning, survival functions. (Cowan (2014); Barrouillet *et al.,* 2011). With increasing cases of mental related disorders, it can be traced backwards from the in-utero exposure to known teratogenic chemical agents in anticonvulsant medicines like lamotrigine. Clinicians have always found it difficult to prescribe anticonvulsants medicines to epileptic expectant mothers due to their unclear safety indexes, as they are all associated wide-range of effects to fetal brain memory structures as well as other organ systems of the developing fetus (Kaplan (2004); Eroğlu *et al.,* 2008; Hill *et al.*, 2010). Compounding the intricate balance of selecting the anticonvulsant medicines by the clinicians is the fact that, they are all known to cross the placental blood barrier as a result of induced fluctuating levels of drug-metabolizing enzymes during pregnancy, coupled with their low molecular weight, (Syme *et al* 2004; Semczuk-Sikora & Semczuk, (2004); Bank et al., 2017).

Lamotrigine is the most preferred new generations medicines during pregnancy and belong to category C anticonvulsant. It is greatly preferred because of its efficacy, tolerability and minimal teratogenic effects to the developing fetus Veroniki *et al.*, 2017; Bansal *et al.,* 2018; Prakash *et al.,* 2007; Yasama *et al.,* 2016; French & Gazzola. (2011. Some studies have however established some associated neuro teratogenic effects to all anticonvulsant medicines that can affect both short-term and long-term memory structurers that are reported to manifest either in form of structural defects or behavioural abnormalities in both childhood and adulthood (Kamali *et al.,* 2020; Hill *et al.,* 2010; Marchi *et al.,* 2001). In the context, there is paucity of data on the fetal short and long-term memory circuitry structures including the prefrontal neo cortices as well as medial temporal lobe allocortical and subcortical structurers including hippocampus, entorhinal cortices, perirhinal cortices and parahippocampal cortices. Such data would be key in guiding the clinicians in making choices of the lamotrigine, what kind of doses and the most vulnerable period of its teratogenicity based on maternal outcomes.

II. MATERIALS AND METHODS

Study site/Location

All experimental procedures that included breeding of the rats, daily weighing, administration of Lamotrigine, sacrificing and measurements of the fetal parameters were carried out at the Small Animal House situated in the University of Nairobi (UON), Chiromo Campus.

Study Design

A post-test only with control group laboratory based experimental study design was adopted

Description of Albino rats used in the study

Female albino dams used in the study were of the 3rd series breed and weighed between 230±20g.They were used because of the following known scientific facts; (a)low incidence of spontaneously occurring congenital defects, (b)they have a large litter size, (d)low cost of maintaining the animals (d)a relatively short gestational span and, (v)considerable amount of the reproductive data on the rat is already available (Bailey *et al.,* 2014; Pritchett & Corning, 2016).

Acquisition and feeding of the male and female albino rats

The albino dams were purchased from a small animal house located in the University of Nairobi (UON), Chiromo campus. They 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.* They were kept in spacious polycarbonate plastic cages in the animal house as determined by (Allen *et al.,* 2016).

Sample size calculation

Sample size for the albino rats was determined by use of resource equation for group comparisons for One Way Analysis of Variance. Based on this approach, the acceptable range of degrees of freedom (DF) for the error term in an 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 of subjects per group. **(**Charan & Kantharia, 2013). Therefore, **n=20/10+1 =3.** Therefore, number of dams is **30.**

Every adult female rat is assumed to have a minimum average of six (6) fetuses per pregnancy. The expected numbers of fetuses were determined as follows 6 x 30=180 fetuses. All fetuses were weighted and three fetuses with the median weights per rat were taken for study making a total of 3 x 30= 90 fetuses Sample size: 90 fetuses from 30 albino rats were used in the study.

Mating and confirmation of pregnancy

Two male albino rats from 3rd series breed of a pure colony and sexually mature were introduced into a standard cage with four female rats overnight and males removed and returned to their separate cages. 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

Grouping of rats

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

Determination and acquisition of lamotrigine

A simple guide for conversion of human to animal dosages was used as determined by (Nair & Jacob, 2016) formula as follows; The correction factor (Km) is estimated by dividing the average body weight (kg) of species to its body surface area (m2). For example, the average human body weight is 60 kg, and the body surface area is 1.62 m2. Therefore, the Km factor for human is calculated by dividing 60 by 1.62, which is 37. The Km factor values of a rat is used to estimate the HED as: HED mg / kg = Rat dose mg / kg Animal K /Human K Eq. As the Km factor for each species is constant, the Km ratio is used to simplify calculations. Hence, Equation is modified as: HED mg / kg = Animal dose mg / kg K ratio Eq. The Km ratio values are already provided and are obtained by dividing human Km factor by animal Km factor or vice versa. Lamotrigine tablets from Vega Biotec Private Limited (Gujarat India) batch number M2017103 were obtained from a local chemist in Thika and were used to make the reconstitutions and administration was done using an oral gavage needle gauge 16.

Administration of lamotrigine

All rats in first trimester (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 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}

Ethical clearance

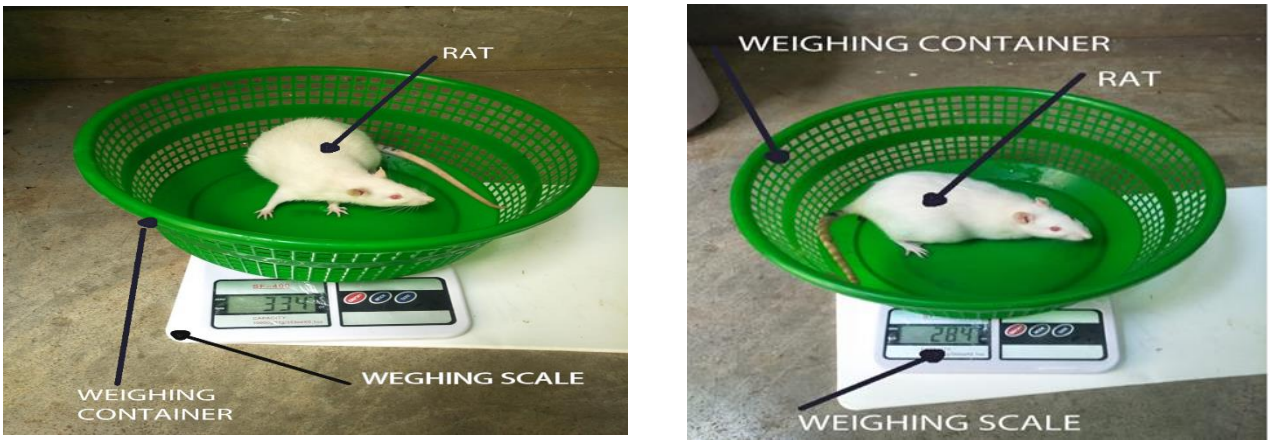
The ethical clearance was sought from University of Nairobi (UON) Animal Ethical Committee (AEC) before initiation of the study.

Statistical analysis

Data on pregnancy outcomes that forms the parametric data (inferential data) 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 expressed as mean$\pm$ standard error of the mean (SEM) for all values. It was analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison tests. All results whose $P<0.05$ will be considered to be significant considered.

The maternal pregnancy outcomes

Maternal daily weight, placenta weight and the weight of the fetuses were obtained by use of a digital weighing scale (figure 1 (A, B), 2 (A, B) number of devoured and resorbed glands (figure 3 and 4(A, B), litter size and dead fetuses were counted and recorded.



A **B**

Figure 1 (A and B): Measurements of maternal weight using a measuring container and a digital weighing scale

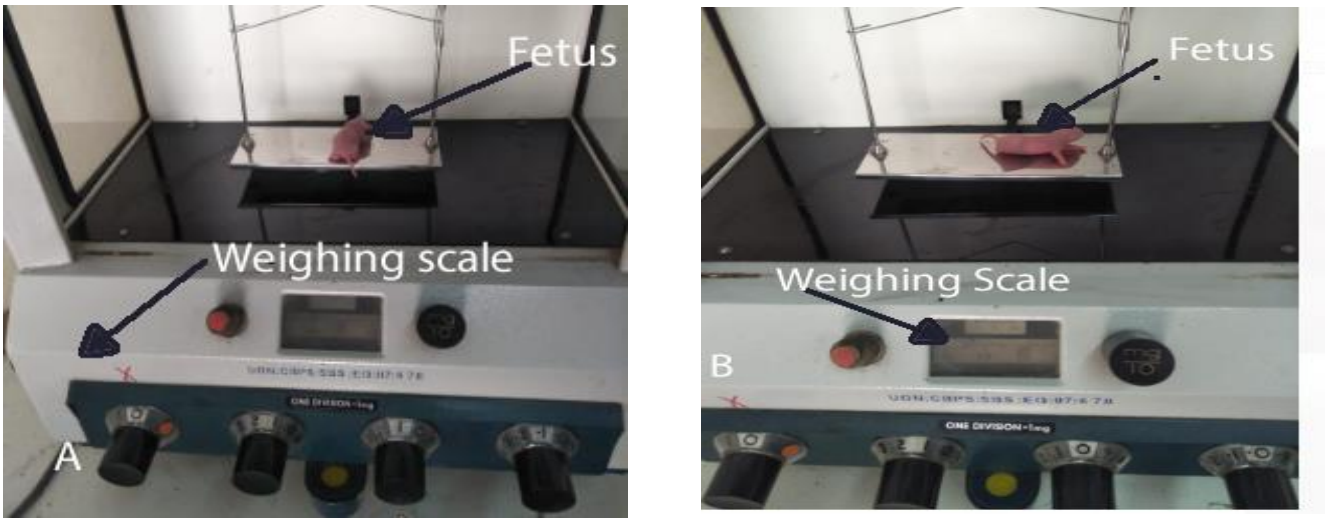
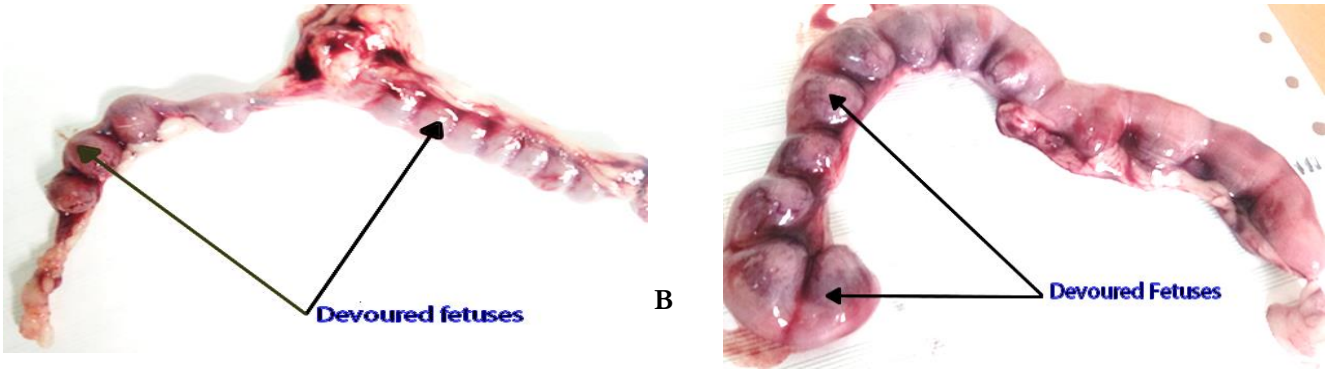


Figure 2 (A and B): Shows how fetal weight were taken using an electronic weighing scale



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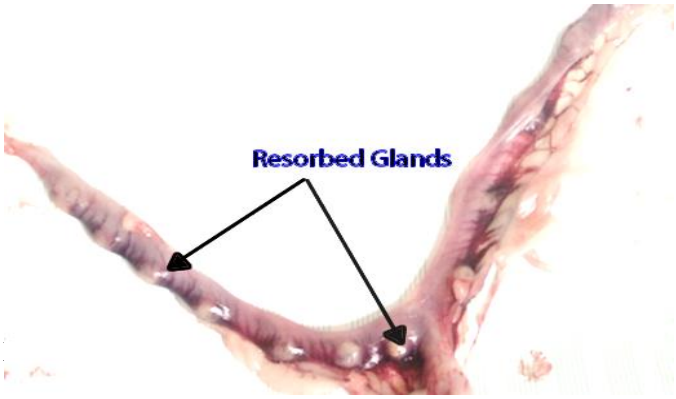
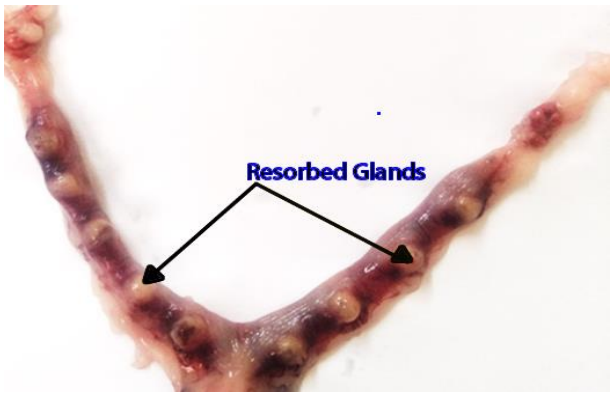
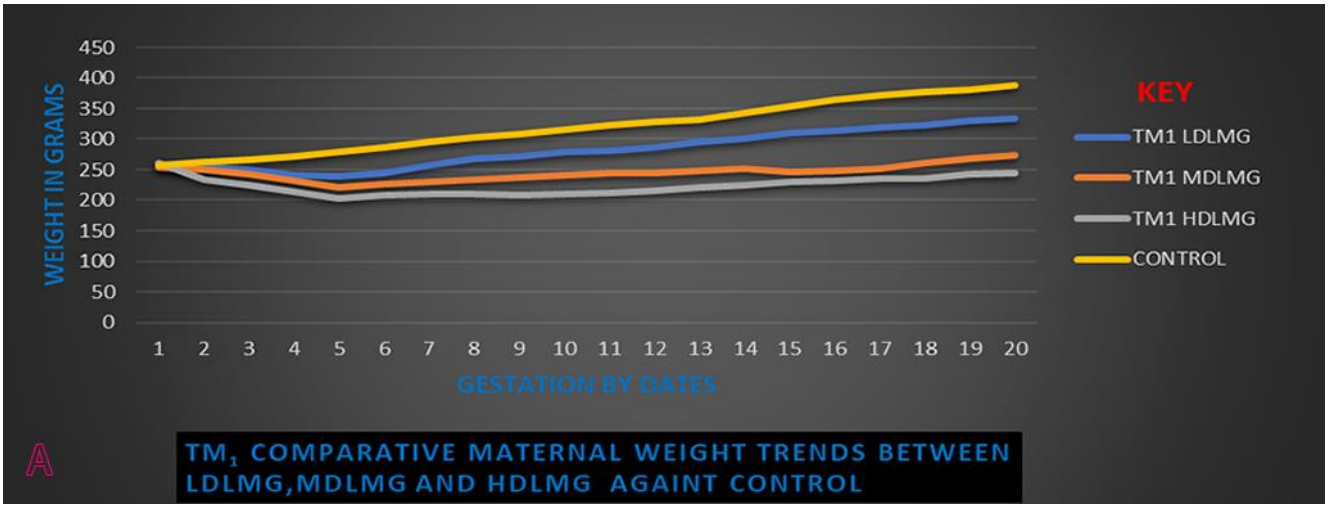
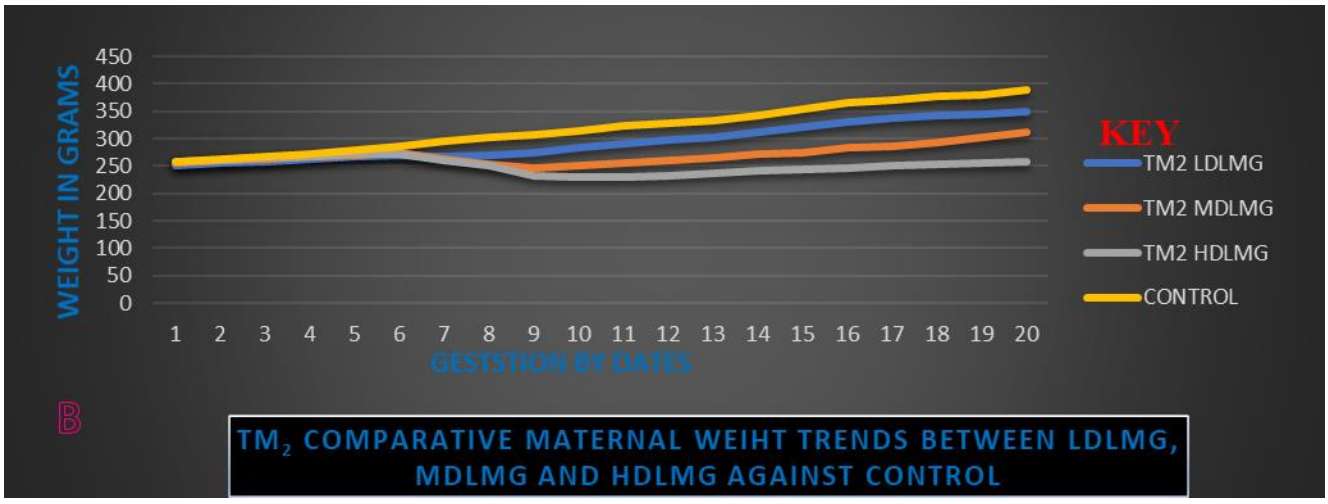


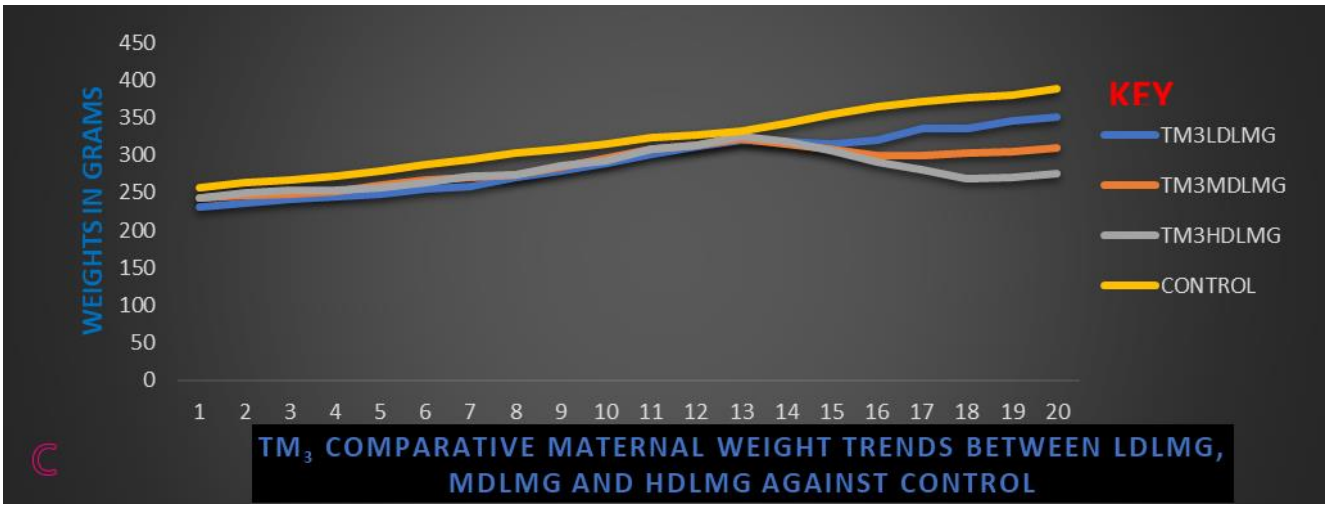


III. RESULTS

3.1 Influence of Lamotrigine on Maternal Weights Trends Throughout the Gestation Period







It can be observed from the three-line graph above (A, B and C) that there was a notable decrease in maternal weight trends in trimester one, trimester two and trimester three (TM_1, TM_2 and TM_3) experimental groups as compared against the control group throughout the gestation period (GD_1-GD_{20}). The first three to four days following lamotrigine administration in all the treatment groups were marked with significant decrease in weight more marked in high dose groups, and then a steady increase in weight gain up to GD_{20}. This phenomenon that could be attributed to the lamotrigine acclimatization factor.

3.2 Influence of Lamotrigine on litter size

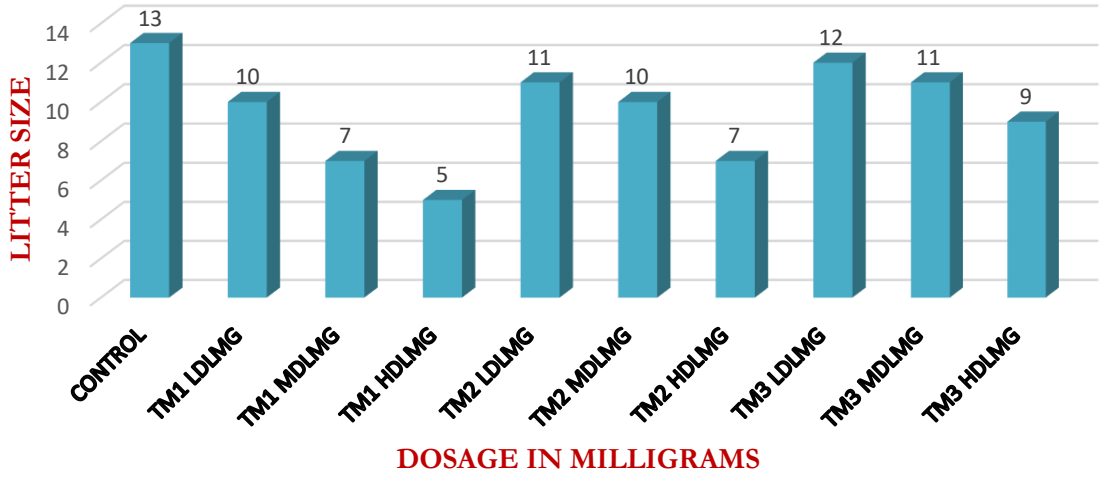


Figure 5: Comparative litter size between LDLMG, MDLMG and HDLMG at TM_1, TM_2 and TM_3 against Control

It can be observed from the bar-graph above that in trimester one, trimester two and trimester three (TM_1, TM_2 and TM_3) experimental groups, there is a marked reduction in litter size which is dose dependent. High doses have the least litter size as compared to medium and low dosages. However, the control group has the highest litter size

3.3 Influence of lamotrigine on the number of dead fetuses

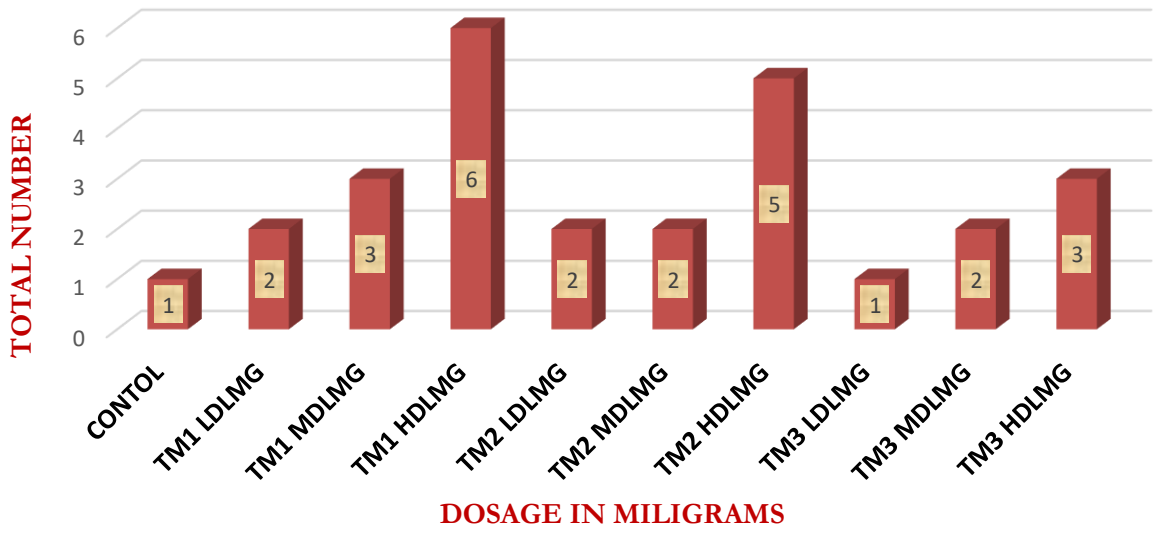


Figure 6: Comparative Dead fetuses between LDLMG, MDLMG and HDLMG in TM_1, TM_2 and TM_3 against Control

From the bar-graph above, it can be observed that the number of dead fetuses increases with increase in dosages in that they are in high dosages as compared with medium and low dosages. The same scenario is observed in the trimesters whereby they are high during trimester one followed by trimester two and finally by trimester three (TM_1, TM_2 and TM_3) experimental groups. However, the number of dead fetuses is low in control group.

3.4 Influence of Lamotrigine on the number of Devoured and Resorbed fetuses

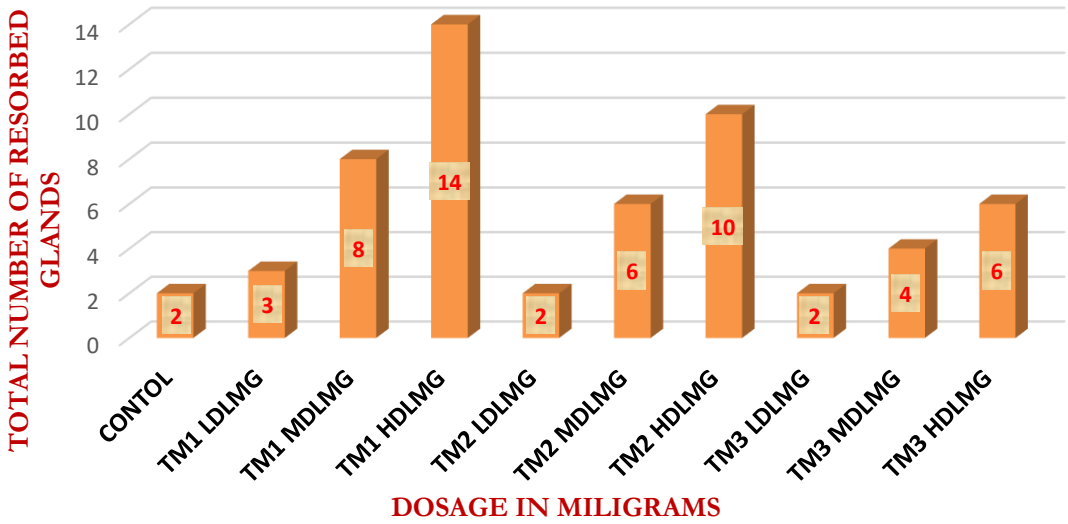


Figure 7: Comparative Number of Resorbed Glands and Devoured Fetuses in LDLMG, MDLMG and HDLMG in TM_1, TM_2 and TM_3 against Control

It is evident from the bar-graph above, that the number of devoured fetuses and resorbed glands goes high with increase in dosages. This is because in high dosages, the numbers are high as compared to medium and low dosages.

In terms of trimesters, the number of resorbed glands is highest in trimester one followed by trimester two and finally in trimester three (TM_1, TM_2 and TM_3) experimental groups as compared with the control.

3.4 Influence of Lamotrigine on the number of Devoured and Resorbed fetuses

Table 1:

Parameter	Control (C)	Low Lamotrigine Group (LLMG) (25mg/kg)			Medium Lamotrigine Group (MLMG) (327.5mg/kg)			High Lamotrigine Group (HLMG) (500mg/kg)		
Mean Maternal weight Gain(g) ± SEM	130.67±5.78	105.7 ±5.55 ^{bc*}	112.67±7.42 ^{bc*}	116.7 ±13.860	88.3± 12.1 ^{bc*}	83.33 ±5.840 ^{bc*}	81.00 ±3.512 ^{bc*}	37.7± 7.3 ^{bc*}	60.33 ±5.93 ^{bc*}	60.67 ±2.67 ^{bc*}
Mean Placenta Weight(g) ± SEM	5.61± 0.020	4.957± 0.023 ^{bc*}	6.28± 0.010 ^{bc*}	5.39 ± 0.023	4.66± 0.03 ^{b*}	5.10± 0.03 ^{bc*}	5.37± 0.01 ^{bc*}	4.27± 0.020*	4.73± 0.015*	5.24± 0.016*

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

When the analysis was done on whether or not the mean maternal weight gain (grams) has a dose and time dependent relationship (**table 1)**, it was established that when treatment was instituted in trimester one and two (TM_1 &TM_2), there was significant reduction in maternal weight gain in all the treatment groups (LLMG, MLMG, HLMG) (P=0.002). This is unlike in TM_3 where there was no significant difference for the low and medium dose groups compared with the control (P=0.21), apart from the high dose.

The mean placental weight that is usually a key indicator of maternal nutritional exchange with the fetus was also observed to have similar inverse dose response relationship with the dose of treatment and the time of exposure. It was found that the lowest placental weight recorded was in high lamotrigine treated group at TM_1 and lowest in the low carbamazepine group at TM_3 (**table 1**).

IV. DISCUSSION

In the current study, lamotrigine was observed to have effects on daily maternal weight trends, litter size, placenta weight, number of resorbed endometrial glands/ devoured fetus as well as the number of dead fetuses in a time and dose dependent manner. This id despite the fact that past literature has associated it with high level of tolerability coupled with good efficacy, and have a general belief that it is safer than the older, frontline AEDs (Hill *et al.,* 2010). Daily maternal weight trends were observed to increase steadily in control group as opposed to the experimental groups (line graphs A, B and C). These trends were observed to be dose dependent in that in high lamotrigine dosages, daily weight was observed to have a minimal increase, followed by medium dosages and finally the low dosage groups as compared to the control. Results of (Punnell, 2008) advised on further studies, since the results available on lamotrigine were not conclusive.

The mean maternal weight gain was statistically reduced (P<0.05) in treatment groups as compared with the control (table 1). These results were intendem with those reported by Elshama *et al* (2015) indicating that high dosages of carbamazepine, an anticonvulsant affects corpus luteum in the pregnant mothers' which secrets progesterone and 20-hydroxy progesterone, that maintains in-utero fetal growth and development. Sucheston *et al*., (1986) and Marli

Gerenutti *et al.,* (2008) also reported that high dose carbamazepine dose exposure during pregnancy leads to delay in growth and development of various fetal organs during embryogenesis leading to low maternal weight gain.

In the current study, the mean placenta weight demonstrated a time and dose relationship in that it was high in low dosages during the third trimester and low in high dosages during trimester one as compared with the control (Table 1) $P<0.05$. These findings were in agreement with those of Christensen *et al.* (2004) who similarly indicated that low carbamazepine dosages have no effects on weight of the placenta as well as fetus's offspring vitality. Similar effects were also recorded by Marli Gerenutti *et al* (2008), who stated that high doses of carbamazepine cause alterations initiated by a simple pharmacologic mechanism: blockage of ion channels in the heart of the growing embryo, that leads to bradycardia hemodynamic alterations, hypoxia and deoxygenation negative effects to fetal organs as well as the placenta.

In the current study, litter size was low in lamotrigine treatment groups as compared with the control group (figure 5) in time and dose dependent. Results of Baeward *et al.,* (2005) are in agreement with the current results that reported that there is an existence of a correlation between the number of corpus luteum and the number of ovulations as well as the number of embryo implantations since in each ovulation, an oocyte that can be fecundated is released and turns into a pre-embryo. Christensen *et al.*, (2004) on the other hand has assured that the administration of carbamazepine in the low dosages has no effects on the rate of pre-implantation losses hence no negative effects on the reproductive performance of a female.

The number of resorbed endometrial glands/devoured fetuses and dead fetuses were observed to be higher in lamotrigine treatment groups when it was administered in the first trimester at high dosages and lowest in control group (table1). This study concurred with the one conducted Mohammad Afshar *et al.,* (2015) who reported a statistically significant increase in resorptions in treatment groups compared with the control groups. He further observed presence of a number of external congenital malformations. Marli Gerenutti *et al* (2008) also reported that carbamazepine administration in low dosage of during rats' pregnancy period, has not occasioned significant alteration in the external measures of the morphological parameters of the fetuses, congenital malformation implantation sites.

V. CONCLUSION AND RECOMMENDATIONS

It has been observed that the use of lamotrigine, a second-generation anticonvulsant during gestational period has effects on maternal pregnancy outcomes. These effects have been shown to be time and dose dependent. Since lamotrigine continues to be prescribed widely by clinicians as the safest and first line anticonvulsant medicine, further studies in higher primates closer to human species as well as clinical trials should be carried out to rule out its safety during pregnancy.

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