

Effects of In-utero Exposure to Varied Doses of Carbamazepine on Fetal Growth and Development in Albino Rats (*Rattusnorvegicus*)

Mwangi A. Wairimu^{1*}, Kweri J. Kariuki¹, Kanyoni J. Mwangi¹, Thuo Reuben¹
¹{Department of Human Anatomy, School of Medicine (SOMED), College of Health Sciences (COHES)
Jomo Kenyatta University of Agriculture and Technology (JKUAT) Kenya}
*Corresponding Author: Mwangi A. Wairimu

Abstract: Prenatal exposure to carbamazepine has been shown to interfere with the normal morphogenesis and differentiation of the various fetal organs when applied in management of various maternal conditions like bipolar disorders, trigeminal neuralgia, and seizures associated with epilepsy among others. Such perturbations on normal development of the fetal organs in-utero can help in explaining the cause of some of the structural, behavioral and functional mental disorders observed in adulthood whose cause is yet to be established. Such disorders of embryological origin are currently a major contributor to the disability-adjusted life years (DALY) in adulthood. Though, the existing literature has linked the prenatal exposure to alteration in various growth and development parameters, data on whether these effects on the fetuses are time and dose dependent is yet to be elucidated. The broad objective of this study was therefore to determine the fetal growth and development outcomes of prenatal exposure to varied doses of carbamazepine, as well as to establish whether the outcomes are also time dependent. In carrying out the study a total of 30 nulliparous female Albino rats (*Rattusnorvegicus*) weighing between 150 - 250g were assigned four groups by use of Simple random sampling. One group was used as a control and the other groups i.e. Low carbamazepine group (LCG), Medium carbamazepine group (MCG) and high carbamazepine group (HCG) were used as the treatment groups. Treatment groups were further subdivided according to trimesters as trimesters one, two and three (each n=3). Carbamazepine was administered to the treatment groups through the oral route by use of a gavage needle. The control group received food and water ad-libitum/day while the experimental groups received varied doses of carbamazepine as follows 20.7, 72.5, 124g/kg/Bw of carbamazepine/day for Low dose, medium and high doses groups respectively as well as water ad-libitum. At 20th day of gestation, all animals were euthanized and sacrificed by hysterectomy. The fetal Anthropometric body parameters such as weights, Crown-rump length, and head circumference among others were taken and recorded. The visceral morphometric and morphological growth parameters were also examined and recorded accordingly. Data was then entered in to the computer and analyzed using EPI-Info and Statistical Package for Social scientists version 24 for windows Chicago Illinois. The linear regression statistics and intra-and inter group comparisons were done using one-way analysis of variances and P-values of less than 0.05 were taken to be significant. The finding of the study showed that there was statistical significant decrease ($p=0.0001$) which was less than 0.05 significant level) in the fetal growth and development between the carbamazepine treated groups as compared with the control as illustrated by the decrease in all growth parameters including, fetal weight ($p=0.01$), crown lump length ($P=0.0001$), head circumference (0.0001), head length (0.0001), and bi-parietal diameter ($p=0.0001$). This was also collaborated in fetal viscera like the brain, liver, lungs, heart, kidney, spleens among others when their gross morphometric analysis was done between the experimental groups versus the control ($p=0.0001$) which was less than 0.05 significant level). It was also established that the effects of carbamazepine on fetal growth and development were dose and time dependent as high dose carbamazepine group fetuses treated in the first trimester shown the highest effects compared with Low dose carbamazepine treated in the third trimester ($P=0.0001$). The findings of this study sets a basis for further studies with higher primates like baboons that would lead in carbamazepine dose rationalization and its application during pregnancy for the attainment of maximum maternal benefits and reduction of fetal teratogenic effects while exposed in utero. Clinical trials should also be emphasized to come up with more literature on carbamazepine safety during pregnancy in human beings.

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I. Introduction

Though studies have shown that in-utero exposure to carbamazepine perturbs the normal morphogenesis and cyto-differentiation of fetal organs when applied in management of maternal conditions during pregnancy^{1,2}, the specific perturbation based on the overall fetal anthropometric measurements vis-a-vis the morphogenesis of specific organs has not been well elucidated. Further, whether or not the effect of carbamazepine on growth and development of the fetal viscera alongside the overall fetal growth parameters are dose and time dependant is yet to be elucidated. Some studies have linked the in-utero-teratogenic disturbances of carbamazepine with fetal genetic factors³. Other studies have linked genetic factors of the mother as well as environmental factors to argue the intra-uterine effect to interfering with fetal growth^{5,6,7}, while others studies indicates that offspring of mothers born exposed to carbamazepine during in-utero life demonstrates effects on various morphological parameters upon delivery⁴. The present study therefore aim at evaluating whether effects of carbamazepine on fetal growth and development associated with carbamazepine in-utero exposure depends on the time of carbamazepine exposure and the dose administered.

II. Material and Methods

Study site/area

30 female nulliparous Albino rats were used as dams in this study because of the following reasons; (i) They have a large litter size, (ii) Low incidence of spontaneously occurring congenital defects, (iii) a relatively short gestational span, (iv) low cost of maintaining the animals and, (v) considerable amount of the reproductive data on the rat is already available^{8,9}. The albino rats were purchased from the Safari Animal House of JKUAT and fed on a standard diet as determined by *American institute of nutrition (1977)* before starting the experiments^{10,11}. All the animals weighed between 150 and 250 grams. They were kept in spacious polycarbonate plastic cages in the animal house and received food (rodent pellets) from UNGA meals and water *ad libitum*. Acclimatization was allowed for a period of seven days.

Study Design: Laboratory based experimental study

Study Location: All experiments including breeding, handling, weighing, Carbamazepine administration and measurements of fetal parameters and visceral organs was done at the Safari Animal house in the School of biomedical Sciences of JKUAT.

Study Duration: The study was carried out from November 2018 to January 2019

Sample size calculation: The sample size was determined using the resource equation method since the standard deviation from previous studies was not available as well as the effect size^{12,13}.

The measured value 'E' which is the degree of freedom of analysis of variance (ANOVA) based on a decided sample size value ('E') should lie between 10 and 20 animals according to this equation. Therefore, a value less than 10 necessitates adding more animals which increases the chance of getting significant results while a value more than 20 has been shown to increase the cost of the study without increasing the significance of the results¹⁴.

$E = \text{Total number of animals} - \text{Total number of groups}$

Total number of groups = 10

Total number of animals = 30

$E = 30 - 10$

Since, every adult female rat is assumed to have a minimum average of six (6) fetuses per pregnancy^{9,15}, then the expected number of fetuses were determined as follows $6 \times 30 = 180$ fetuses.

All fetuses were weighted and three fetuses were identified for the study with the highest, median and lowest weights per rat making a total of $3 \times 30 = 90$ fetuses (This was hence, the sample size).

Sample size: 90 fetuses were used in the study.

Grouping of animals; once pregnancy was confirmed, animals were randomly assigned to either the control or the experimental category (i.e. 3 rats as control group and 27 rats as experimental). The 27 rats in the experimental category were further divided into three broad study groups of 3 rats each assigned :- low (LCG), Medium (MCG) and High carbamazepin group (HCG) each of the broad subgroups of the LCG, MCG and HCG were further subdivided into first (TM1), second(TM2) and third(TM3) trimesters comprising of 3 rats each.

Determination of the cabermazepin doses for the experiment; A simple guide for conversion of animal dosages from human dosages was applied¹⁶, which states that, dose is equally related to body weight although it is not the lone factor which influences the scaling for dose calculation. 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 various animal species is used to estimate the HED as:

HED mg / kg = Animal 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 2 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.

Calculation and administration of the doses: The maximum carbamazepine dose in humans is 1200mg/kg, medium dose is 700mg/kg and minimum dose is 200mg/kg.¹⁷ A 200mg carbamazepine tablet obtained from Novartis pharma batch number TL787 was used to make the reconstitutions. Carbamazepine tablets were diluted in 5% DMSO

- All trimester ones (TM₁) animals:- (LCG,MCG,HCG) categories received cabermazepin from gestation day GD1-GD20
- All trimester twos (TM₂) animals:- (LCG,MCG,HCG) categories received cabermazepin doses from gestation day GD7-GD20
- All trimester three (TM₃) animals:- (LCG,MCG,HCG) categories received cabermazepin doses from gestation day GD14-GD20

Determination of the critical dose of cabermazepin

Animal groupings was done as follows; in each of the groups (LCG, MCG, HCG), the 9 dams were randomly sub divided in three sub-groups the Trimester 1(TM₁) = 3dams, Trimester 2 (TM₂) = 3dams and Trimester 3 TM₃=3 dams

NB> the gestation period of a rat is 21 days, therefore trimester one was between gestational day GD1 to GD7, while trimester 2 was between GD7-GD14 and third trimester GD14-20.

Determination of fetal growth parameters

All fetal and organ weights were determined by use digital weighing scale on the first day of delivery, while crown-rump length, measured from the snout to either right or left side of the anus) was taken by use of a Vernier caliper. Head length and bi-parietal diameter were also measured by use of a Vernier calipers while head circumference was determined by use of a thread and placed in a ruler. All measurements were recorded.

Statistical analysis

Data was analyzed using Epi- Info SPSS version 24.0 (SPSS Inc., Chicago, IL).The results were expressed as mean ± standard error of the mean (SEM) for all values. One-way Analysis of Variance ANOVA followed by Tukey's post hoc multiple comparison tests was done and results were expressed as mean ± standard error of the mean (SEM) for all values. The results were considered to be significant at P<0.05.

Ethical Approval

Author hereby affirms that the experimental protocol was approved by the Jomo Kenyatta University of Agriculture and Technology Animal ethical Committee (JKUAT AEC). The animals were only used once. They were all sacrificed using humane end points at the end of the study¹⁸.The protocol followed to the later the Guidelines for Care and Use of Laboratory Animals in Biomedical Research¹⁹

III. Results

Analysis of the external morphological parameters of the fetuses

Fetal growth and development was reflected through the measurements of the following parameters; Weight of the fetuses, Head circumference, Crown rump length (CRL), Occipital-frontal length(head length), and bi-parietal diameter (BD) on day 20 of gestation period (G20). This showed how the three carbamazepine dose levels (Low, medium, high) in the three trimesters (TM1, TM2 and TM3) compared with the control. Data was analyzed using SPSS and Excel statistical software and was expressed as mean \pm standard error (SEM). To determine the significance, one way analysis of variance with Tukey post hoc test was used, and 5% significance level ($\alpha = 0.05$) was assumed. The results were considered to be significant whenever the probability value was less than 0.05 ($p < 0.05$). The results were presented as below;

Measurements of fetal parameters

Table 1: Intragroup comparison of fetal weight, crown-rump length, head circumference, bi-parietal diameter and head length in control against (LCG, MCG and HCG) in the first Trimester (TM1)

Parameter	Control	Low	Medium	High	F (3,8)	P-value
Fetal Weight	6.73 \pm 0.026a	6.42 \pm 0.007b	6.31 \pm 0.046 b	5.42 \pm 0.02 c	395.49	0.0001*
Crown lump length	4.723 \pm 0.030a	4.123 \pm 0.009b	3.865 \pm 0.044c	3.4 \pm 0.023d	346.4	0.0001*
Head circumference	3.89 \pm 0.010a	3.22 \pm 0.025b	2.95 \pm 0.018c	2.35 \pm 0.013d	1287.4	0.0001*
Bi-parietal diameter	0.7155 \pm 0.018a	0.659 \pm 0.00073b	0.6296 \pm 0.00325c	0.579 \pm 0.002d	746.9	0.0001*
Head length	1.34 \pm 0.002a	1.304 \pm 0.00082b	1.28 \pm 0.0008c	1.252 \pm 0.001d	3675.6	0.0001*

The means, followed by the same letter in a row are not statistically different at ($P < 0.05$) using one way ANOVA with Tukey test on post-hoc t-tests. * indicates significance ($p < 0.05$).

Fetal weight in the control group (6.73 \pm 0.026) was found to be significantly higher than that in the low dose group (6.42 \pm 0.007b), medium (6.31 \pm 0.046) and high dose (5.42 \pm 0.02), $F(3, 8) = 395.49$, $p = 0.0001$. The weight in low dose and the medium dose was not statistically different but was found to be lower in the high dose group.

The effect of the dosage on Crown lump length of the fetus was statistically significant. The Crown lump length under the control group (4.723 \pm 0.030) was found to be significantly higher followed by the low dose group (4.123 \pm 0.009), followed by the medium dose group (3.865 \pm 0.044) and lowest in the high dose group (3.4 \pm 0.023). This was indicated by a significant p-value, $p = 0.0001$ which was less than 0.05 significance level. The head circumference under the control group (3.89 \pm 0.010) was found to be significantly higher followed by the low dose group (3.22 \pm 0.025), followed by the medium dose group (2.95 \pm 0.018c) and lowest in the high dose group (2.35 \pm 0.013). This was indicated by a significant p-value, $p = 0.0001$ which was less than 0.05 significance level. Again, bi-parietal diameter under the control group (0.7155 \pm 0.018) was found to be significantly higher followed by the low dose group (0.659 \pm 0.00073), followed by the medium dose group (0.6296 \pm 0.00325) and lowest in the high dose group (0.579 \pm 0.002). This was indicated by a significant p-value, $p = 0.0001$ which was less than 0.05 significance level. Head length, Brain weight, medium and high). The parameters in low group, the medium and the high were all found to be significantly different from each other as indicated by a significant p-value (less than 0.05).

Table 2: Intragroup comparison of fetal weight, crown-rump length, head circumference, bi-parietal diameter and head length in control against (LCG, MCG and HCG) in the second Trimester (TM2)

Parameter	Control	Low	Medium	High	F (3,8)	p-value
Fetal Weight	6.73 \pm 0.026a	6.57 \pm 0.011b	6.42 \pm 0.018c	5.92 \pm 0.0035d	431.9	0.0001*
Crown rump length	4.723 \pm 0.030a	4.45 \pm 0.009b	4.15 \pm 0.0029c	3.85 \pm 0.0024d	555.20	0.0001*
Head circumference	3.89 \pm 0.010a	3.453 \pm 0.029b	3.22 \pm 0.019c	3.04 \pm 0.021d	312.4	0.0001*
Bi-parietal diameter	0.716 \pm 0.0018a	0.686 \pm 0.0008b	0.655 \pm 0.0005c	0.635 \pm 0.0011d	971.1	0.0001*
Head length	1.34 \pm 0.0002a	1.32 \pm 0.0008b	1.31 \pm 0.0004b	1.30 \pm 0.0069c	25.1	0.0001*

The means, followed by the same letter in a row are not statistically different at ($P < 0.05$) using one way ANOVA with Tukey test on post-hoc t-tests. * indicates significance ($p < 0.05$).

From the results in table 2, Fetal weight in the control group (6.73±0.026) was found to be significantly different (higher) than the low dose group (6.57±0.011), the medium dose group (6.42±0.018) and the high dose group (5.92±0.0035), F (3,8) = 431.9, p=0.0001. The results for the post hoc test also revealed that the fetal weight in the three dose groups was also significantly different from each other. Crown rump length in the control group (4.723±0.030) was found to be significantly different (higher) than the low dose group (4.45±0.009), the medium dose group (4.15±0.0029) and the high dose group (3.85±0.0024), F (3, 8) = 555.20, p=0.0001. The results for the post hoc test also revealed that Crown rump length in the three dose groups was also significantly different from each other.

Head circumference in the control group (3.89±0.010) was found to be significantly different (higher) than the low dose group (3.453±0.029), the medium dose group (3.22±0.019) and the high dose group (3.04±0.021), F (3, 8) =312.4, p=0.0001. The results for the post hoc test also revealed that head circumference in the three dose groups was also significantly different from each other.

Bi-parietal diameter in the control group (0.716±0.0018) was found to be significantly different (higher) than the low dose group (0.686±0.0008), the medium dose group (0.655±0.0005) and the high dose group (0.635±0.0011), F (3, 8) =971.1, p=0.0001. The results for the post hoc test also revealed that Bi-parietal diameter in the three dose groups was also significantly different from each other.

Head length in the control group (1.34±0.0002) was found to be significantly different (higher) than the low dose group (1.32±0.0008), the medium dose group (1.31±0.0004) and the high dose group (1.30±0.0069), F (3, 8) =25.1, p=0.0001. The results for the post hoc test also revealed that Head length in the medium and the low dose group were not significantly different while the medium and the high, were again not significantly different from each other.

Table 3; intragroup comparison of fetal weight, crown-rump length, head circumference, bi-parietal diameter and head length in control against (LCG, MCG and HCG) in the third Trimester (TM3)

Parameter	Control	Low	Medium	High	F (3,8)	p-value
Fetal Weight	6.73±0.026a	6.66±0.0168a	6.533±0.004b	6.21±0.010c	197.8	0.0001*
Crown rump length	4.723±0.030a	4.55±0.027b	4.44±0.028b	4.34±0.011b	32.9	0.0001*
Head circumference	3.89±0.0105a	3.57±0.037b	3.52±0.015b	3.41±0.021b	77.7	0.0001*
Bi-parietal diameter	0.716±0.0018a	0.692±0.0026b	0.69±0.004b	0.68±0.0028b	30.21	0.0001*
Head length	1.34±0.002a	0.297±0.0033b	0.216±0.008c	0.1200±0.012d	624.3	0.0001*

The means, followed by the same letter in a row are not statistically different at (P<0.05) using one way ANOVA with Tukey test on post-hoc t-tests. * indicates significance (p<0.05).

From the results in Table 3, Fetal weight in the control group (6.73±0.026) was found to be significantly different (higher) than the low dose group (6.66±0.0168), the medium dose group (6.533±0.004) and the high dose group (6.21±0.010), F (3,8) = 197.8, p=0.0001. The results for the post hoc test also revealed that the fetal weight in the three dose groups was also significantly different from each other.

Crown rump length in the control group was found to be significantly different (higher) than the low dose group, the medium dose group and the high dose group, F (3, 8) = 32.9, p=0.0001. The results for the post hoc test also revealed that Crown rump length in the three dose groups was also significantly different from each other.

Head circumference in the control group (3.89±0.0105) was found to be significantly different (higher) than the low dose group (3.57±0.037), the medium dose group (3.52±0.015) and the high dose group (3.41±0.021), F (3, 8) =77.7, p=0.0001. The results for the post hoc test also revealed that head circumference in the three dose groups was also significantly different from each other.

Bi-parietal diameter in the control group (0.716±0.0018) was found to be significantly different (higher) than the low dose group (0.692±0.0026), the medium dose group (0.69±0.004) and the high dose group (0.68±0.0028), F (3, 8) =30.21, p=0.0001. The results for the post hoc test also revealed that Bio-parietal diameter in the three dose groups was not significantly different from each other

Intergroup analysis was also performed per dose levels in the three trimesters. In this study, the three dose levels are compared in all the three trimesters and the control. The results per dose level are as shown below;

Table 4: Intergroup comparison of fetal weight, crown-rump length, head circumference, bi-parietal diameter and head length in control against LCG in the Trimester 1, 2, and 3 (TM1, TM2, and TM3)

Parameter	Control	T1	T2	T3	F (3,8)	P-value
Fetal Weight	6.73±0.026a	6.42±0.007b	6.57±0.011c	6.66±0.0168a	61.98	0.0001*
Crown lump length	4.723±0.030a	4.123±0.009b	4.45±0.009c	4.55±0.027c	138.52	0.0001*
Head circumference	3.89±0.010a	3.22±0.025b	3.453±0.029c	3.57±0.037c	103.63	0.0001*
Bi-parietal diameter	0.7155±0.018a	0.659±0.00073b	0.686±0.0008c	0.692±0.0026c	196.65	0.0001*
Head length	1.34±0.002a	1.304±0.00082b	1.32±0.0008c	0.297±0.0033d	84103.6	0.0001*

The means, followed by the same letter in a row are not statistically different at ($P < 0.05$) using one way ANOVA with Tukey test on post-hoc t-tests. * indicates significance ($p < 0.05$).

Fetal weight in the control group (6.73±0.026) was found to be significantly higher than that in the trimester one low dose group (6.42±0.007), trimester two low dose group (6.57±0.011) and trimester three low dose group (6.66±0.02), $F(3, 8) = 61.98$, $p = 0.0001$. The weight in low dose trimester one, low dose trimester two and low dose trimester three was statistically different, but no statistical difference was between control and trimester three group.

The effect of the low dosage on Crown lump length of the fetus was statistically significant. The Crown lump length under the control group (4.723±0.030) was found to be significantly higher followed by the low dose trimester three group (4.55±0.027), followed by the low dose trimester two group (4.45±0.009) and lowest in the trimester one low dose group (4.123±0.009). This was indicated by a significant p-value, $p = 0.0001$ which was less than 0.05 significance level. No statistical difference was observed between trimester two and three groups, but it was found to be lower in trimester one. The head circumference under the control group (3.89±0.010) was found to be significantly higher followed by the low dose trimester three group (3.57±0.037), followed by the low dose trimester two group (3.22±0.018) and lowest in the trimester one low dose group (3.22±0.025). This was indicated by a significant p-value, $p = 0.0001$ which was less than 0.05 significance level. No statistical difference was observed between trimester two and three low dose groups but was lower in trimester one. Again, bi-parietal diameter under the control group (0.7155±0.018) was found to be significantly higher followed by the low dose trimester three group (0.692±0.0026), followed by the low dose trimester two group (0.686±0.0008) and lowest in the low dose trimester one group (0.659±0.00073). This was indicated by a significant p-value, $p = 0.0001$ which was less than 0.05 significance level. No statistical difference was observed between trimester two and three low dose groups but was lower in trimester one. The results for the post hoc test also revealed that head length in the three dose groups was not significantly different from each other.

The parameters in low carbamazepine group in all trimesters were all found to be significantly different from each other as indicated by a significant p-value (less than 0.05).

Table 5: Intergroup comparison of fetal weight, crown-rump length, head circumference, bi-parietal diameter and head length in control against MCG in the Trimester 1, 2, and 3 (TM1, TM2, and TM3)

Parameter	Control	T1	T2	T3	F (3,8)	P-value
Fetal Weight	6.73±0.026a	6.31±0.046 b	6.42±0.018ab	6.53±0.004c	40.97	0.0001*
Crown lump length	4.723±0.030a	3.865±0.044b	4.15±0.0029c	4.44±0.028d	148.3	0.0001*
Head circumference	3.89±0.010a	2.95±0.018b	3.22±0.019c	3.52±0.015d	638.40	0.0001*
Bio-parietal diameter	0.7155±0.018a	0.6296±0.00325b	0.655±0.0005c	0.69±0.004d	203.56	0.0001*
Head length	1.34±0.002a	1.28±0.0008b	1.31±0.0004c	0.216±0.008d	18844.21	0.0001*

The means, followed by the same letter in a row are not statistically different at ($P < 0.05$) using one way ANOVA with Tukey test on post-hoc t-tests. * indicates significance ($p < 0.05$).

From the results in Table 5, Fetal weight in the control group (6.73±0.026) was found to be significantly different (higher) than trimester one medium dose group (6.31±0.046), trimester two medium dose group (6.42±0.018) and trimester three medium dose group (6.53±0.004) F (3,8) = 40.97, p=0.0001. The results for the post hoc test also revealed that the fetal weight of medium groups in trimester one and two was not statistically different but was higher in trimester three.

Crown rump length in the control group (4.723±0.030) was found to be significantly different (higher) than the medium dose trimester one group (3.865±0.044), trimester two medium dose (4.15±0.0029) and medium dose trimester three group (4.44±0.028), F (3, 8) = 148.3, p=0.0001. The results for the post hoc test also revealed that Crown rump length in the three dose groups was also significantly different from each other.

Head circumference in the control group (3.89±0.0105) was found to be significantly different (higher) than the medium dose trimester one group (2.95±0.018), the medium dose trimester two group (3.22±0.019) and the trimester three dose group (3.52±0.015d), F (3, 8) =638.40, p=0.0001. The results for the post hoc test also revealed that head circumference in the three dose groups was also significantly different from each other.

Bi-parietal diameter in the control group (0.716±0.0018) was found to be significantly different (higher) than the medium dose trimester one group (0.6296±0.00325), the medium dose trimester two group (0.655±0.0005) and the trimester three medium dose group (0.69±0.004), F (3, 8) =203.56, p=0.0001. The results for the post hoc test also revealed that Bi-parietal diameter in the three dose groups was significantly different from each other

Head-length in the control group (1.34±0.002) was found to be significantly different (higher) than the medium dose trimester one group (1.28±0.0008), the medium dose trimester two group (1.31±0.0004c) and the trimester three medium dose group (0.216±0.008), F (3, 8) =18844.21, p=0.0001. The results for the post hoc test also revealed that head-length in the three dose groups was significantly different from each other

Table 6: Intergroup comparison of fetal weight, crown-rump length, head circumference, bi-parietal diameter and head length in control against HCG in the F Trimester 1, 2, and 3 (TM1, TM2, and TM3)

Parameter	Control	T1	T2	T3	F (3,8)	P-value
Fetal Weight	6.73±0.026a	5.42±0.02 b	5.92±0.0035c	6.21±0.010d	996.45	0.0001*
Crown lump length	4.723±0.030a	3.4±0.023b	3.85±0.0024c	4.34±0.011d	867.27	0.0001*
Head circumference	3.89±0.010a	2.35±0.013b	3.04±0.021c	3.41±0.021d	1476.28	0.0001*
Bi-parietal diameter	0.7155±0.018a	0.579±0.002b	0.635±0.0011c	0.68±0.0028d	911.74	0.0001*
Head length	1.34±0.002a	1.252±0.001b	1.30±0.0069c	0.1200±0.012d	7675.22	0.0001*

Notes: The means, followed by the same letter in a row are not statistically different at (P<0.05) using one way ANOVA with Tukey test on post-hoc t-tests. * indicates significance (p<0.05).

From the results in Table 6, Fetal weight in the control group (6.73±0.026) was found to be significantly different (higher) than trimester one high dose group (5.42±0.02), trimester two high dose group (5.92±0.0035c) and trimester three high dose group (6.21±0.010) F (3,8) = 996.45, p=0.0001. The results for the post hoc test also revealed that the fetal weight all groups was statistically different.

Crown rump length in the control group (4.723±0.030) was found to be significantly different (higher) than the high dose trimester one group (3.4±0.023b), high dose trimester two (3.85±0.0024) and high dose trimester three group (4.34±0.011), F (3, 8) = 148.3, p=0.0001. The results for the post hoc test also revealed that Crown rump length in the three dose groups was also significantly different from each other.

Head circumference in the control group (3.89±0.0105) was found to be significantly different (higher) than the high dose trimester one group (2.35±0.013), the high dose trimester two group (3.04±0.021) and the trimester three dose group (3.41±0.021), F (3, 8) =1476.28, p=0.0001. The results for the post hoc test also revealed that head circumference in the three dose groups was also significantly different from each other.

Bi-parietal diameter in the control group (0.716±0.0018) was found to be significantly different (higher) than the high dose trimester one group (0.579±0.002), the high dose trimester two group (0.635±0.0011) and the trimester three high dose group (0.68±0.0028), F (3, 8) =911.74, p=0.0001. The results for the post hoc test also revealed that Bi-parietal diameter in the three dose groups was significantly different from each other

Head-length in the control group (1.34±0.002) was found to be significantly different (higher) than the high dose trimester one group (1.252±0.001), the high dose trimester two group (1.30±0.0069) and the trimester three medium dose group (0.1200±0.012), F (3, 8) =7675.22, p=0.0001. The results for the post hoc test also revealed that head-length in the three dose groups was significantly different from each other

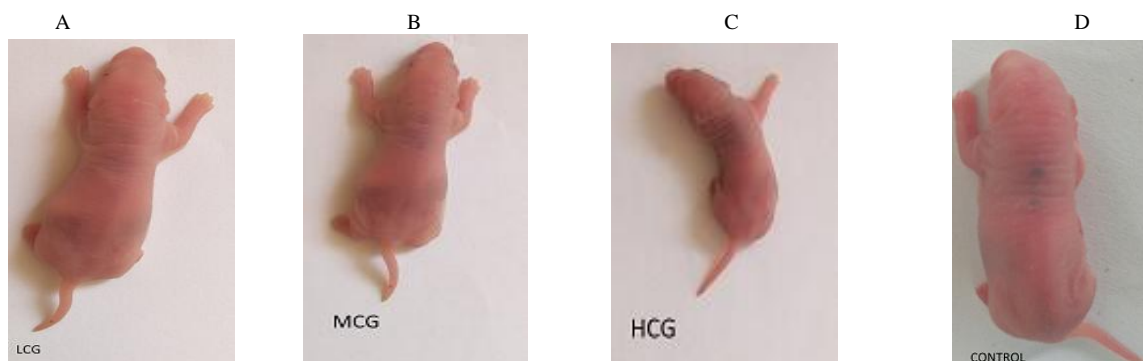


Fig 1:A photograph showing growth retardation in HCG, G and LAG fetuses compared with the control(C) at gestation day 20(GD₂₀)

From the photograph 1 above:carbamazepine treated groups have a small size for age (LCG, MCG, HCG) as compared with the control. It can be observed that intrauterine fetal exposure to Carbamazepine lead to intra-uterine growth retardation (IUGR) of the fetuses which is dose dependent. This IUGR across the carbamazepine treated groups was found to have a significant relationship with the recorded fetal growth parameters including; fetal weight, Crown-Lump length (CRL), Bi-parietal Diameters (BD), and Head Lengths (HL).

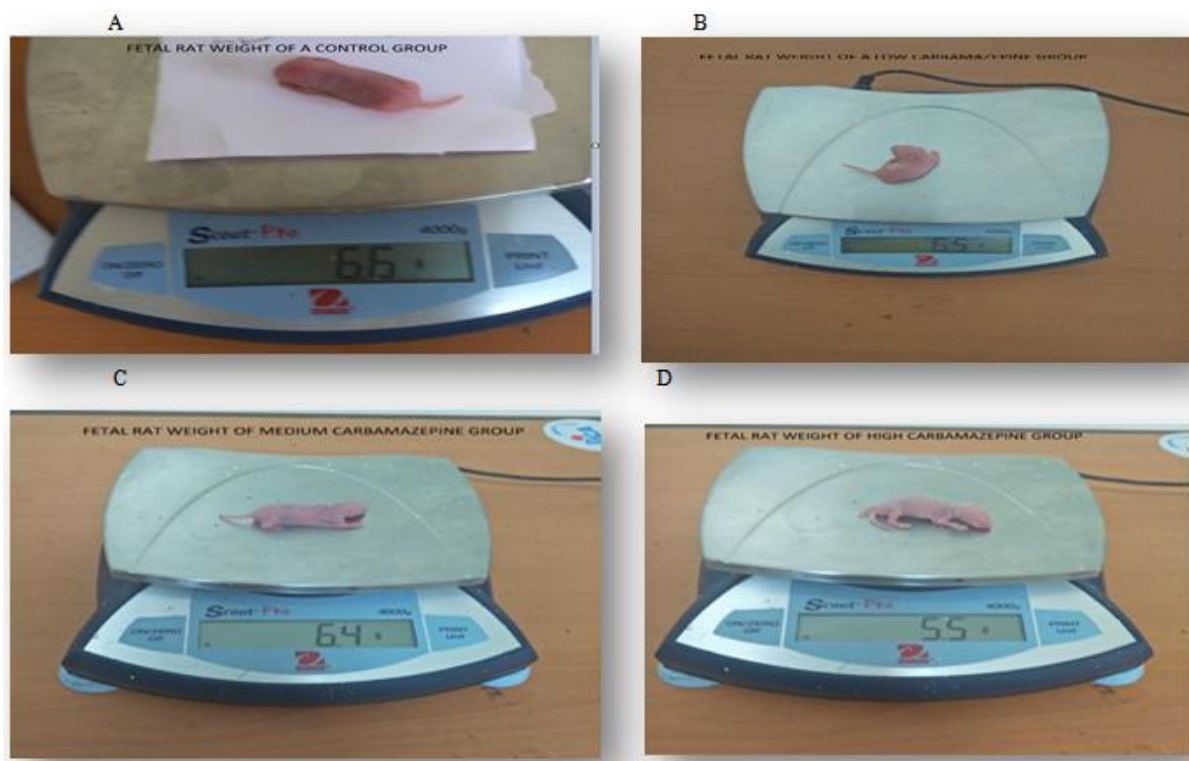


Fig 2: A photograph showing measurements of fetal weights in HCG, MCG and LCG as compared with the control(C) at gestation day 20(GD₂₀)

NB/The measurements taken using (Scout pro model SPU4001 S/N B519923500 from Uhaus Corporation, USA)

From the photograph 2 above:Carbamazepine treated groups (LCG, MCG, and HCG) have reduced body weight as compared with the control. Intrauterine fetal exposure to Carbamazepine lead to reduction in fetal body weights that is observed to decrease as the doses increases.

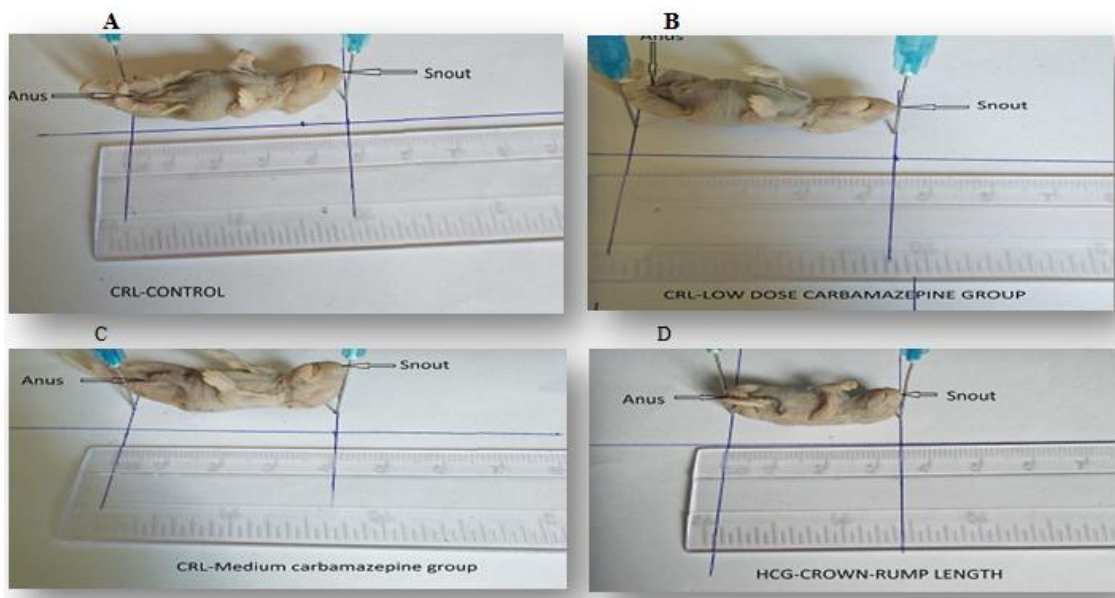


Fig 3: photographs showing measurements of Crown-lump length in HCG, MCG and LCG fetuses compared with the control(C) at gestation day 20(GD₂₀)
 NB; Measurements taken by use of a ruler; CRL measured from the snout (tip of the nose) to the side of the anus- root of tail)

From the photograph 3 above:carbamazepine treated groups (LCG, MCG, and HCG) have reduced crown-rump length ascompared with the control. The reduction in crown-rump length upon administration of carbamazepine is also observed to increases as the dosage of carbamazepine increases.

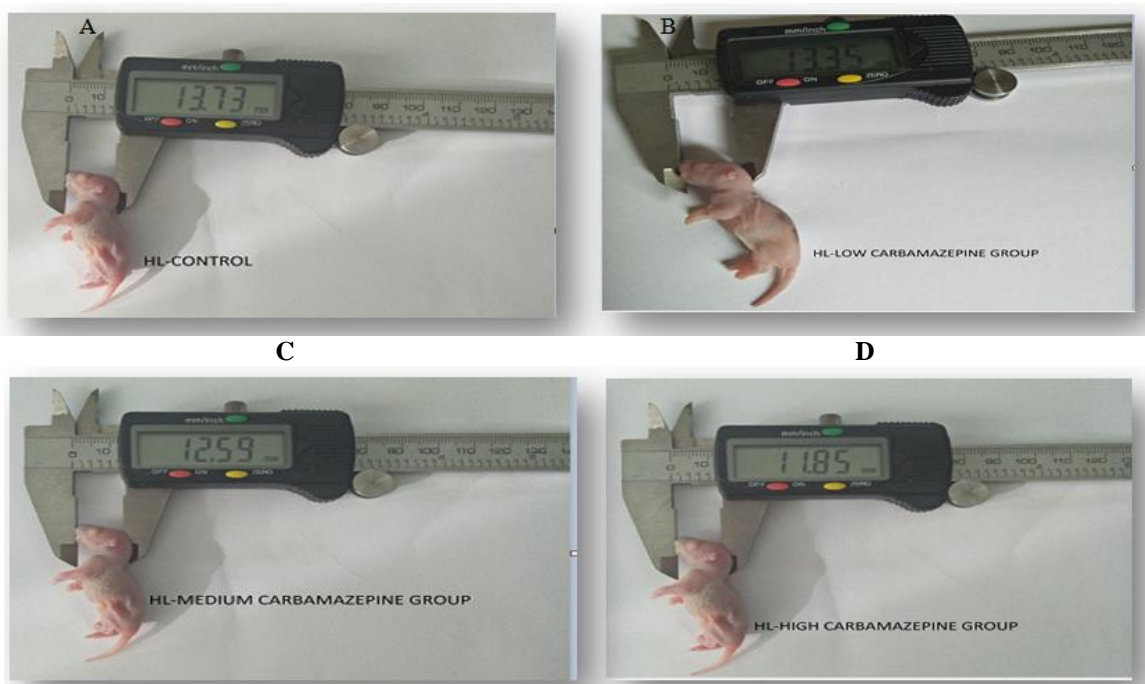


Fig 4: photographs showing measurement of Head lengths of fetal rats in HCG, MCG and LCG as compared with the control(C) at gestation day 20(GD₂₀)
 NB/Measurements taken using a digital Vernier caliper; Head-length measured from back of the skull to the extremity of the nose)
 Model; Hercules from sealing products

From the photograph 4above: carbamazepine treated groups (LCG, MCG, and HCG) have reduced head-length compared with the control. The reduction in Head-length upon administration of carbamazepine is observed to increases as the dosage of carbamazepine increases.

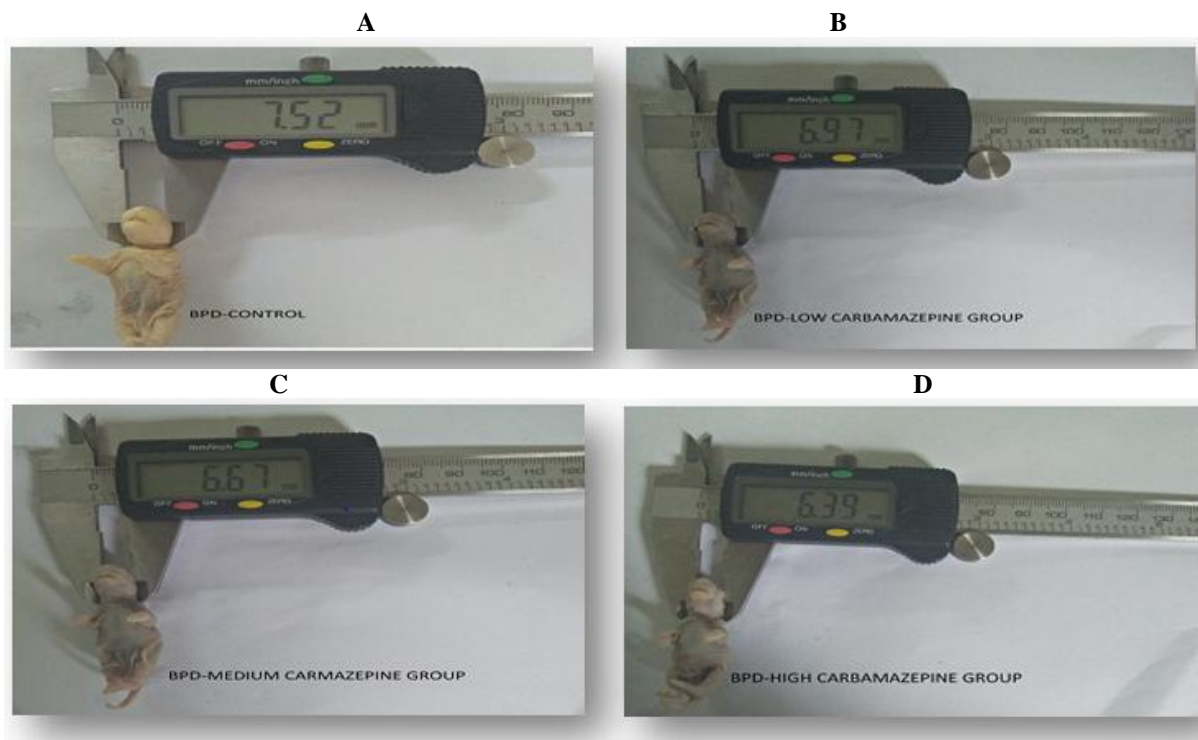


Fig 5: A photograph showing measurements of bi-parietal diameter of fetal rats in HCG, MCG and LCG as compared with the control(C) at gestation day 20(GD₂₀) NB/Measurements taken using a digital Vernier caliper; Head-length measured from one side of the R.ear to the L.ear) Model; Hercules from sealing products

From the photograph 5above: carbamazepine treated groups (LCG, MCG, and HCG) have reduced Bi-parietal diameter as compared with the control. The reduction in Bi-parietal diameter upon administration of carbamazepine is observed to increases as the dosage of carbamazepine increases

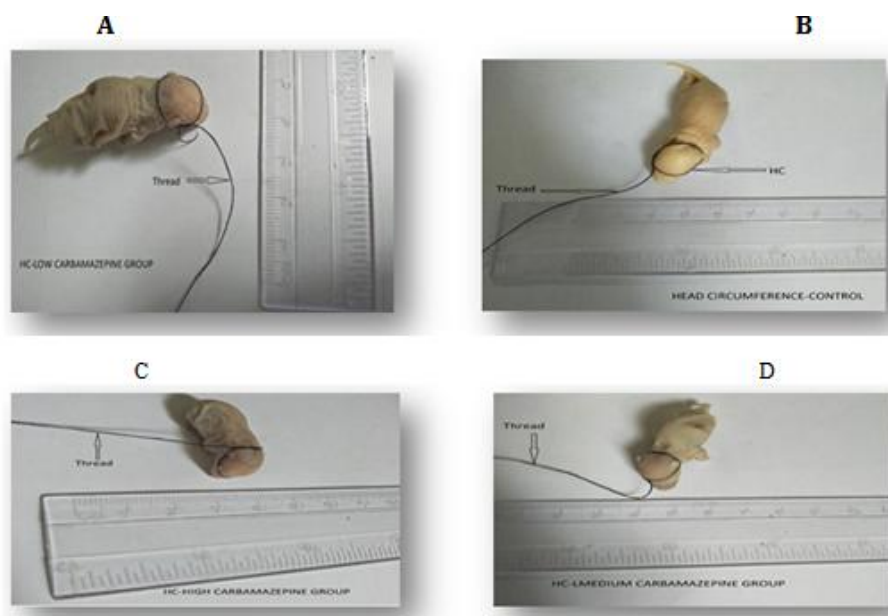


Fig 6: A photograph showing measurements of head circumference of fetal rats in HCG, MCG and LCG as compared with the control(C) at gestation day 20(GD₂₀) NB/Measurements taken using a thread and aruler

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Measurements of Head-circumference were as well observed to reduce in carbamazepine treatment groups as compared with the control with the lowest measurements recorded in high carbamazepine group and the highest recorded in the control group.

Table 7; Comparison between Mean \pm SD of different organs weight of fetal rats in the first day of delivery in control with LCG, MCG and HCG in the Trimester 1, 2, and 3 (TM1, TM2, and TM3)

GROUPS	PERIOD OF CARBAMAZEPINE TREATMENT	MEAN BRAIN WEIGHT \pm SE	MEAN HEART WEIGHT \pm SE	MEAN LIVER WEIGHT \pm SE	MEAN LUNGS WEIGHT \pm SE	MEAN KIDNEY WEIGHTS \pm SE	MEAN PANCREAS WEIGHT \pm SE
Control (C)		0.39 \pm 0.01	0.260 \pm 0.06	0.450 \pm 0.05	0.390 \pm 0.07	0.290 \pm 0.08	0.174 \pm 0.02
Low dose carbamazepine group (LCG)	Trimester 1 (TM1)	0.30 \pm 0.01*	0.221 \pm 0.12	0.420 \pm 0.07*	0.350 \pm 0.16*	0.265 \pm 0.06*	0.170 \pm 0.01*
	Trimester 2 (TM2)	0.35 \pm 0.01*	0.230 \pm 0.92*	0.430 \pm 0.05*	0.360 \pm 0.71*	0.273 \pm 1.21*	0.171 \pm 0.12*
	Trimester 3 (TM3)	0.39 \pm 0.01	0.241 \pm 0.87*	0.440 \pm 0.03*	0.387 \pm 0.03*	0.281 \pm 0.27*	0.173 \pm 0.01*
Medium dose carbamazepine group (MCG)	Trimester 1 (TM1)	0.22 \pm 0.01*	0.213 \pm 0.01*	0.340 \pm 0.05*	0.183 \pm 0.05*	0.20 \pm 0.001*	0.164 \pm 0.17*
	Trimester 2 (TM2)	0.30 \pm 0.00*	0.201 \pm 0.21*	0.350 \pm 0.15*	0.321 \pm 0.21*	0.230 \pm 0.03*	0.167 \pm 0.32*
	Trimester 3 (TM3)	0.37 \pm 0.01	0.187 \pm 0.09*	0.360 \pm 0.07*	0.340 \pm 0.13*	0.252 \pm 0.41*	0.169 \pm 0.22*
High dose carbamazepine group (HCG)	Trimester 1 (TM1)	0.12 \pm 0.02*	0.120 \pm 0.04*	0.330 \pm 0.04*	0.100 \pm 0.01*	0.120 \pm 0.04*	0.151 \pm 0.14*
	Trimester 2 (TM2)	0.25 \pm 0.01*	0.141 \pm 0.08*	0.340 \pm 0.81*	0.130 \pm 0.02*	0.141 \pm 0.32*	0.159 \pm 0.23*
	Trimester 3 (TM3)	0.33 \pm 0.00*	0.160 \pm 0.22*	0.350 \pm 1.02*	0.151 \pm 0.31*	0.183 \pm 0.05	0.160 \pm 0.41*

* shows the figures that have a statistically significant difference compared with the controls $p < 0.05$
S.E=Standard Error

From the table 7 above, fetal weight of the organs including; Brain, liver, lungs, heart, kidney, heart and spleen, demonstrated a significant decrease in weight in the carbamazepine treated groups when they were compared with the control group ($p=0.001$, with P value being less than 0.05 significance level). The highest decrease in weight was recorded in in trimester one high dosage groups, while the highest weights were recorded in control groups.

There was a statistical significant difference between mean brain weight of the carbamazepine treated groups in trimesters one and two, and trimester three HCG ($p=0.001$) as compared with the control (P value than 0.05 significance level). Trimester three (LCG and MCG) had no statistical significance difference as compared with the control ($p= 0.999$, $p=0.059$) respectively and no statistical difference was noted when trimester three (LCG and MCG) were compared ($p= 0.69$).Statistical significant decrease of heart weight was recorded in carbamazepine groups when compared with the control group ($p=0.001$, with P value being less than 0.05), but no statistical significance difference was noted when the heart weight of the low carbamazepine dose of trimester one was compared with the control. Mean liver weight was statistically lowest in trimester one high dose (0.330 \pm 0.04) and highest in control group (0.450 \pm 0.05). Similarly lungsweight was observed to have a significance decrease with the increase of the doses of carbamazepine with the HCG-subgroup treated at trimester one(TM1) recording the lowest mean lungs weight (0.120 \pm 0.04) while the control recorded the highest liver weight gain (0.290 \pm 0.08).Mean kidney weight recorded was highest in control (0.290 \pm 0.08) and lowest in trimester one high carbamazepine group (0.120 \pm 0.04).It can also be observed lowest mean pancreas weight recorded was (0.151 \pm 0.14) in the HCG-subgroup treated at trimester one(TM1) while the control recorded the highest pancreas mean weight of(0.174 \pm 0.02).Mean fetal weights of the liver, lungs, kidney and pancreas in the carbamazepine groups had statistical significance difference as compared with the control ($p= 0.001$, with P value being less than 0.05

IV. Discussion

In the present study, there was alteration of different growth and development parameters like fetal birth weight (table fig 2), different organ weights (table 7), crown lump length (Fig 3), head circumference (fig 6) among others as have been reported in other studies that have been done before^{20,21}. Carbamazepine was observed to cause decrease in all the anthropometric measurements taken and organ weights, indicating a negative growth and development index to the fetuses. Highest decrease in measured parameters (95%) was recorded in fetuses whose mothers were exposed to carbamazepine during the first trimester (TM1) while high dosages were administered (table 4), while 5% was observed in trimester one low dose and the other trimesters. This decrease in growth parameters measured was observed to be dependent on the time and the dose of carbamazepine administered.

Body weight and crown-rump length

Reductions of fetal body weight and crown rump length was significantly observed in all CBZ treated groups as compared with control group (table 1-6 above), with $p=0.0001$ which was less than 0.05 significance level. The decrease in body weight and crown-rump length was observed to increase as the carbamazepine dosage increased and was more pronounced in the trimester one high carbamazepine group (table 1, fig 2 and fig 3 above). Similar classical effects of reduction in growth parameters associated with high carbamazepine dosages was described by **Said Elshama et al (2015)**, who stated that high carbamazepine dosages affects corpus luteum that plays an important role in reproductive performance as it secretes progesterone and 20-hydroxy progesterone that maintains fetal growth leading to severe growth retardation²². In the present study, growth retardation was also demonstrated by small size for age observed in carbamazepine treatment groups at day 20 of gestation period (fig 1 A,B,C,D). This is attributed by the fact that intrauterine fetal exposure to carbamazepine lead to intra-uterine growth retardation (IUGR) which was significantly severe in high carbamazepine dose group to have a significant strong relationship with the recorded fetal growth parameters including; fetal weight (fig 2), Crown-Lump length-CRL (fig 3), that showed reduction in these parameters as the dose of carbamazepine increased. Similarly, **Numa f et al (1983)** and **Al-asmakhet et al (2007)** reported that corpus luteum is the main source of progesterone that plays a major psychological role during fetal organs growth and development^{23,24}. **Ei-Gaafarawi et al (2015)** in his study as well reported that growth retardation was manifested by reduction in weight and length of the fetus during the first day of delivery was attributed by teratogenic effects of high dosages of carbamazepine on ossification centers of long bones. He further reported that anti-proliferative effects of high carbamazepine dosages increases the mitotic index and causes persistent block of the boundary between metaphase and anaphase stages of cell cycle². These explanations support the results of the present work since the fetal weight and crown-rump length indicated a significant decrease as the carbamazepine dosages increased as compared with the control (Table 1-6 above, $p=0.001$, which is less than 0.05 significance level). In addition, the study further confirms that the decrease in the fetal growth parameters is high when the high dosages are administered during the first trimester.

Head circumference, head length and Bi-parietal diameter

In the current study, head circumference, head length (and bi-parietal diameter) were found to reduce significantly with increase in the dosage of carbamazepine (table 1-6, $p=0.001$, fig 4, fig 5 and fig 6). This reduction in these fetal head measurements were also supported by **Marli Gerenutti et al (2008)**, who noted carbamazepine is associated with malformations and delays in growth and development. He concluded that these alterations are 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 reoxygenation.³ He further reported there was no evident of modifications of physical fetal parameters like antero-posterior and latero-lateral measures of the skull, antero posterior and latero-lateral measures of the thorax. This information contradicts the current study, since these measurements were significantly reduced depending on the dosages (table 1-6, $p=0.001$ which was less than 0.05 significance level). **McLean and McDonald et al (1986)** reported that the anticonvulsant activity of Carbamazepine is through blockage of voltage dependent Na^+ and K^+ channels resulting in limited sustained high frequency repetitive firing of mammalian central neurons, and interaction with excitatory glutamate receptors. However in higher doses, it can have negative effects to the fetal body that presents with reduced fetal parameters of the skull and the brain similar to fetal hydration syndrome²⁵. These results are supported by the current study and in addition confirms that these effects are time and dose dependent. (table 4, fig 6).

Weight of other fetal body organs

The current study showed significant decrease in various organs weight including the brain, liver, lungs, heart, kidney and pancreas on the first day of delivery in carbamazepine treatment groups (table 7, $p < 0.05$ significance level) as compared with the control. This is in line with **Marli Gerenutti et al (2008)** and **Said Elshama et al (2015)**²², whose reports associated carbamazepine exposure with delay in growth and development of various fetal organs. **Suchestonet al. (1986)** went further and reported on measurements of the limbs and concluded that decrease in fetal organs and especially limbs could be attributed by reduction of length and width of ossified regions of humerus and femur associated with high carbamazepine dosages²⁵. This information is supported by the current study, but contrasted by **Marli et al. (2008)** who reported that low doses of carbamazepine leads to increase of cartilage in the ends of long bones³. **Elumaet al. (1984)** who indicated that the decrease in organ weight of carbamazepine are associated with different doses concurs with the present study that have proved that decrease of fetal organs weight depends on dose administered and in addition, time of carbamazepine administration

V. Conclusion and recommendations

It has been observed that the use of CBZ during gestational period is associated with decrease in fetal growth parameters that includes body weight, head circumference, crown-lump length, head circumference and head length as well as other fetal body organs like brain, liver, lungs, kidney, heart and pancreas. The decrease in fetal growth and development parameters were observed to be dose and time dependent. Since carbamazepine continues to be prescribed in management of various maternal conditions, further studies with higher primates like baboons that would lead in carbamazepin dose rationalization and its application during pregnancy for the attainment of maximum maternal benefits and reduction of fetal teratogenic effects while exposed in utero. Clinical trials should also be emphasized to come up with more literature on carbamazepine safety during pregnancy in human beings.

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Conflict of interest: The authors declare that they have no competing interests

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