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Effects of Prenatally Exposed Phenytoin Varying Doses on Fetal Growth of Albino Rats

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Abstract: *Phenytoin is an antiepileptic drug with no sedative effects widely used in treatment of convulsive disorders. However, its use in pregnancy has been associated with various fetal malformations and impairment of fetal growth.Many factors have been attributed to these effects including phenytoin capacity to cause hypoxia in embryonic tissue, interference with maternal folate metabolism, maternal hemodynamic alterations, its inhibition on potassium channels among others,these consequently affect various developing fetus structures and organs that be associated with currently common postnatally problems of unknown cause such as growth retardation, congenital rickets ,congenital heart diseases, hypertension among others. This study aims toinvestigate the effects of prenatally used varying doses of phenytoin(low, medium, high)during different gestational periods (trimester1, 2, 3) on albino rat fetal growth. Pregnant albino rats were divided into four groups; group 1(control) and group 2,3,4 (experimental).The control group receive normal standard diet while the Experimental(group 2,3,4 which are low, medium and high phenytoin group respectively. These three groups of 9 albino rats each received daily phenytoin drug. Group 2-low phenytoin group received 30 mg/kg of phenytoin, group 3-medium phenytoingroup received 60mg/kg of phenytoin and group 4 -high phenytoin group received 120mg/kg of phenytoin. Animals in each group 2,3,4 were randomly assigned to trimesters of 3 animals each(trimester 1,2 and 3).Trimester 1 animals received phenytoin from day 1-20 of gestation, trimester 2 animals received phenytoin from day 7-20 of gestation and trimester 3 animals received phenytoin from day 14-20 of gestation .All the pregnant rats were humanly sacrificed on the 20th day of gestation and the fetuses removed and the following measurements taken and recorded before they were euthanized; fetal weights, crown rump length, head circumference, head length and bi-parietal diameter . The fetal e was remarkably reduced in the high dose group particularly the first trimester compared to the control group reflected by reduced fetal weights, crown rump length, head circumference, head length and bi-parietal diameter. Phenytoin use during pregnancy affect fetal growth parameters (fetal weights, crown rump length, head circumference, head length and bi-parietal diameter differently depending and the dose and time of exposure as seen by remarkably reduced fetal growth parameters in the high dose group fetuses as well as trimester 1 group compared to low dose group fetuses and trimester 3 respectively, more clinical trials need to be conducted for proper and more accurate dose adjustment of phenytoin during pregnancy.*

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I. Introduction

Fetal growth is a continuous process from the time of fertilization and it is marked by increasing body weight and complexion of the body structures throughout the gestational period(1–5)(3). Conversely, there are many factors that have been shown to interfere with this in utero process categorized into maternal, fetal , placental among others(6–12)(13). In addition, studies done in relation to prenatal exposure to the antiepileptic drugs such as phenytoin may influence the fatal outcome(13–20).However, relationship between the phenytoin dose, time of exposure and the fetal effects is not clear.

Phenytoin, an anticonvulsive and antiarrhythmic agent commonly known as Dilantin and abbreviated PTH is one of the most widely prescribed drug in treatment of convulsive disorders like seizures and epilepsy across all the age groups and even during pregnancy(21–23)**.** Phenytoin do not have the sedative effects that other antiepileptic drugs have when it is being used in treatment of seizures(19) **.** Clinical reports have recorded higher rate of congenital malformations in children born to epileptic mothers who use phenytoin during pregnancy by 2-3 times compared to children born to normal mothers(19). Some of the fetal effects include fetal hyadantoin syndrome , fetal clefts, limb defects among others(10). Report by mullers – kupper in 1963 of a three and a half years old male child with microcephally and cleft palate and a positive history on prenatal

exposure to phenytoin that was used by the mother during pregnancy although the relationship between time, dose and the effects was not clear.

II. Material And Methods

This experimental study was carried out using pregnant female albino rats in Small Animal Facility for Research and Innovation (SAFARI) and the study was approved by the committee for ethical approval for animal use in Jomo Kenyatta University of Agriculture and Technology (JKUAT)from January 2018 to January 2019. A total 30pregnant female albino rats weighing between 150-250g were used in this study.

Study Design: Laboratory Experimental study
Study Location: Small Animal Facility for Research and Innovation (SAFARI)Department of Biomedical Sciences in Jomo Kenyatta University of Agriculture and Technology (JKUAT)
Study Duration: August 2018 to January 2019.
Sample size: 30 pregnant albino rats were used.
Sample size calculation: Resource equation method was used to determine the sample(1). E which is the value measured in the degree of free of analysis of variance based on sample size value decided. According to this equation, it should be between 10 and 20 animals. So a value that is below 10 means more animals should be added in order for the results to be significant and a value beyond 20 do not increase significance of the results but the cost
E=Total number of animals-Total number of groups
Total number of groups=10
Total number of animals=30 (this is the desired total number of animals for the study)
E=30-10
E=20 which falls between 10 and 20, therefore 30 is an adequate and representative sample size for this study
Fetuses used to measure growth parameters; all live fetuses delivered by the pregnant rats were used in recording the fetal growth parameters for equal representation and to avoid biasness
Phenytoin dose determination;The weight in milligrams of the phenytoin for the reference dose(LD_{50}) of 300mg/kg/BW for an average weight dam of 200g was calculated as:-

Weight of rat= 200g
Lethal dose LD50= 300mg/kg/BW

Therefore: 1 rat =200g x $\frac{300mg}{1000}$ = 60mg phenytoin

Therefore:
Then: the animals that will be receiving
(i) The Medium phenytoin dose group (MPG)= 60mg/kg/bw
This dose was taken to be the N dose for this experiment
(ii) The low phenytoin dose group (LPG) received half N= ½ x N=1/2 x 60 = 30mgs/kg/bw
(iii) The High phenytoin dose group (HPG) received twice N= 2 x N= 2x60= 120mg/kg/bw
Dilutions
All the volumes were administered in a standard volume of 3 mls (the standard allowable daily oral volume of a rat per day).

Animal Grouping and period of phenytoin administration: Animals used in this experiment were grouped as follows;
Group 1(N= 3 rats) – Did not receive phenytoin drug
Group 2 (N=9 rats) – Each received 30mg/kg of phenytoin daily
Group 3 (N=9 rats)–Each received 60mg/kg of phenytoin daily
Group 4 (N= 9 rats)- Each received 120mg/kg of phenytoin daily

Each of the group 2,3 and 4 were further divided into 3 other subgroups each: Trimester 1, Trimester 2, Trimester 3
All Trimester 1 - Received phenytoin from day 1-20 of gestational period
All Trimester 2- Received phenytoin from day 7-20 of gestational period
All Trimester 3- Received phenytoin from day 14-20 of gestational period

Inclusion criteria:
1. Pregnant albino rats weighing 150-250g and all live fetuses delivered by the these rats
Exclusion criteria:
1. Female albino rats which did not get pregnant and dead delivered fetuses (10)

Statistical analysis

The study examined the effects of *phenytoin*on fetus growth and development recorded in different growth parameters upon administration of *phenytoin*.

The data was analyzed using SPSS version 24 and Excel statistical software and was expressed as mean ± standard error (SEM). The study compared how the three dose levels (Low, medium and high) and control in different gestational periods (T1, T2 and T3) affected the different growth parameters. These parameters were: Fetal weight, Crown Lump length, Head Circumference, Head length and Bi-parietal diameter. To determine the significance, one way analysis of variance with Tukey post hoc test was used and 5% significance level (α = 0.05) was assumed. The results were considered to be significant whenever the probability value is less than 0.05 ($p<0.05$). The results are presented below.

Ethical Approval

The experiments performed in the course of this study were ethically approved by Jomo Kenyatta University of Agriculture and Technology Animal Ethical Committee (JKUAT AEC). All the protocols were observed according to the guidelines for care and use of laboratory animals in biomedical research.

III. Results

This study evaluated fetal growth effects of varying doses of phenytoin administered during different gestational periods and manifested through intra and intergroup comparison of these parameters ;fetal weight, crown rump length, head circumference, head length and bi-parietal diameters .

Intragroup comparisons of fetal weights showed statistical significant difference between the control and LPG, MPG and HPG. $P<0.05$. On the other hand , intragroup comparisons of crown rump length and fetal heart weights showed statistical significant difference between the control group and LPG, MPG. $P<0.05$.However , the intergroup comparison showed no statistical significance difference between the control group and LPG in trimester three.

Table 1: Mean of all variables between the control against various Phenytoin groups – LPG, MPG, and HPG at T1

Parameter	Group 1 (Control)	Group 2 (LPG)	Group 3 (MPG)	Group 4 (HPG)	F	P-value
fetal weights	6.4±0.064a	4.67±0.09b	4.06±0.027c	3.34±0.086d	323.2	0.000*
crown rump length	4.36±0.059a	3.63±0.048b	3.28±0.003c	3.03±0.030d	199.5	0.000*
head length	2±0.032a	1.37±0.034b	1.19±0.041c	1.08±0.025c	153.6	0.000*
head circumference	4.19±0.094a	3.47±0.094b	3.23±0.051bc	2.94±0.081c	42.6	0.000*
Bi parietal diameter	1.46±0.031a	0.802±0.031b	0.700±0.013b	0.56±0.03c	212.2	0.000*

*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 group 1-control (6.4±0.064) was found to be significantly higher than that in group 2-LPG (4.67±0.09), group 3-MPG (4.06±0.027) and group 4-HPG (3.34±0.086), F =323.2, p = < 0.0001.
The effect of the dosage on Crown lump length of the fetus was statistically significant. The **Crown lump**

length under the group 1- control (4.36±0.059) was found to be significantly higher compared to group 2-LPG (3.63±0.048), followed by group 3-MPG (3.28±0.003) and lowest in group 4-HPG (3.03±0.030). This was indicated by a significant p-value, $p<0.0001$ which was less than 0.05 significance level.

Head lengthof the group 1- control (2±0.032) was found to be significantly higher compared to group 2-LPG (1.37±0.034), followed by group 3-MPG (1.19±0.041) and lowest in group 4-HPG (1.08±0.025). This was indicated by a significant p-value, $p<0.0001$ which was less than 0.05 significance level.

Head circumference under the control group (4.19±0.094) was found to be significantly higher followed by the LPG (3.47±0.094), followed by the MPG (3.23±0.051) and lowest in the HPG (2.94±0.081). This was indicated by a significant p-value, p<0.0001 which was less than 0.05 significance level.

Bi-parietal diameter under the control group (1.46±0.031) was found to be significantly higher followed by the LPG (0.802±0.031), followed by the medium dose group (0.700±0.013) and lowest in the high dose group (0.56±0.03). This was indicated by a significant p-value, p<0.0001 which was less than 0.05 significance level.

Table 2: Mean of all variables between the control against various Phenytoin groups – LPG, MPG, and HPG at T2

Parameter	Group 1 Control	Group 2 (LPG)	Group 3 (MPG)	Group 4 (HPG)	F	P-value
fetal weights	6.36±0.06a	5.62±0.041b	5.12±0.029c	4.68±0.038d	255.9	0.000*
crown rump length	4.36±0.059a	3.81±0.050b	3.604±0.031bc	3.48±0.053c	61.8	0.000*
head circumference	4.19±0.094a	3.56±0.061b	3.42±0.058b	3.26±.0.075b	30.3	0.000*
head length	2±0.032a	1.56±0.025b	1.51±0.026b	1.47±0.045b	60.8	0.000*
Bi-parietal diameter	1.46±0.031a	1.09±0.024b	0.95±0.019c	0.867±0.031c	96.2	0.000*

*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 (6.36±0.06) was found to be significantly different (higher) than the low phenytoin group-LPG (5.62±0.041), the medium phenytoin group-MPG (5.12±0.029) and the high phenytoin group-HPG (4.68±0.038), F = 255.9, 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 (4.36±0.059) was found to be significantly different (higher) than the low phenytoin group-LPG (3.81±0.050), the medium phenytoin group-MPG (3.604±0.031bc) and the high phenytoin group-HPG (3.48±0.053), F = 61.8, p=<0.0001. The results for the post hoc test also revealed that Crown rump length in group 2 and 3, 3 and 4 were not significantly different from each other but there was significance different between group 2- low phenytoin group and group 4 –high phenytoin group.

Head circumference in the control group (4.19±0.094) was found to be significantly different (higher) than the low dose group (3.56±0.061), the medium dose group (3.42±0.058) and the high dose group (3.26±.0.075), F =30.3, p=<0.0001. The results for the post hoc test also revealed that head circumference in the three dose groups was not significantly different from each other.

Head length in the control group (2±0.032) was found to be significantly different (higher) than the low dose group (1.56±0.025), the medium dose group (1.51±0.026) and the high dose group (1.47±0.045), F =60.8, p=<0.0001. The results for the post hoc test also revealed that Head length in the three groups (LPG,MPG,HPG) were not significantly different from each other.

Bi-parietal diameter in the control group (1.46±0.031) was found to be significantly different (higher) than the low dose group (1.09±0.024b), the medium dose group (0.95±0.019c) and the high dose group (0.867±0.031c), F (3, 8) =96.2, p=<0.0001. The results for the post hoc test also revealed that Bi-parietal diameter in the group 2-LPG was also significantly different from group 3(MPG) and 4(HPG). However, there was no significant difference in bi-parietal diameter between group 3(MPG) and group 4(HPG).

Table 3: Mean of all variables between the control against various Phenytoin groups – LPG, MPG, and HPG at T2

Parameter	Control	Low	Medium	High	F	P-value
fetal weights	6.36±0.06a	5.62±0.041b	5.12±0.029c	4.68±0.038d	255.9	0.000*
crown rump length	4.36±0.059a	3.81±0.050b	3.604±0.031bc	3.48±0.053c	61.8	0.000*
head circumference	4.19±0.094a	3.56±0.061b	3.42±0.058b	3.26±.0.075b	30.3	0.000*
head length	2±0.032a	1.56±0.025b	1.51±0.026b	1.47±0.045b	60.8	0.000*

Bi-parietal diameter	1.46±0.031a	1.09±0.024b	0.95±0.019c	0.867±0.031c	96.2	0.000*

*+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 (6.36±0.064) was found to be significantly different (higher) than the low phenytoin group-LPG (6.04±0.0233), the medium phenytoin group-MPG (5.12±0.029) and the high phenytoin group-HPG (5.63±0.040), F = 163.7, p=<0.0001. The results for the post hoc test also revealed that the fetal weight in the three phenytoin groups was also significantly different from each other.

Crown rump length in the control group (4.36±0.059) was found to be significantly different (higher) than the low phenytoin group-LPG (4.24±0.022), the medium phenytoin group-MPG (4.13±0.006) and the high phenytoin group-HPG (3.86±0.028), F = 37.1, p=<0.0001. The results for the post hoc test also revealed that Crown rump length in group 2 and 3 were not significantly different from each other but there was significance different between group 2- low phenytoin group and group 4 –high phenytoin group as well as between group 3 and group 4.

Head circumference in the control group (4.19±0.09) was found to be significantly different (higher) than the low dose group (4.19±0.036), the medium dose group (4±0.014) and the high dose group (3.86±0.033), F =8.63, p=<0.007. The results for the post hoc test also revealed that head circumference between group 2 and 3 and group 3 and 4 was not significantly different from each other. However there was significance difference between head circumference of low and high phenytoin group.

Head length in the control group (2±0.0324) was found to be significantly different (higher) than the low dose group (2.04±0.017), the medium dose group (1.93±0.0076) and the high dose group (1.81±0.019), F =23.9, p=<0.0001. The results for the post hoc test also revealed that Head length in groups 2 and 3 (LPG, MPG) were not significantly different from each other. However, there was significance difference between head length of the low, medium phenytoin group with that of the high phenytoin group

Bi-parietal diameter in the control group (1.46±0.031a) was found to be significantly different (higher) than the low dose group (1.45±0.012a), the medium dose group (1.34±0.005b) and the high dose group (1.29±0.028b), F =15.1, p=<0.001. The results for the post hoc test also revealed that Bi-parietal diameter in the group 2-LPG was also significantly different from group 3(MPG) and 4(HPG). However, there was no significant difference in bi-parietal diameter between group 3(MPG) and group 4(HPG).

IV. Discussion

The results in this experimental study shows that phenytoin influence fetal growth differently depending on the dose and the time of exposure as indicated by varrying intra and intergroup statistical findings on the fetal weights, crown rump length, head circumfrence, head length and bi-parietal diameter. The growth parameters of the fetuses obtained from the control group were statistical different (higher) than the experimental groups (LPG,MPG,HPG) through the gestational period(trimester 1, trimester 2 and trimester 3). In a study that was conducted by Waltman and the group in 2003 on fetal effects of phenytoin showed a variety of manifestations such as fetal hyadantoin syndrome, facial clefting, heart malformations, limb deformities and still births explained by phenytoin alteration of vitamin D metabolism through inhibition of calcium absorption during pregnancy which inturn lead to low level of calcium in the fetus(24). From the clinical studies , it is well known that calcium is important in growth of the the fetus particularly the growth of bones and by the fact that this study found reduced crown rump length, head circumfrence, head length and bi-parietal diameter of the experimental group indicates slow growth of bones due to low calcium levels. Higher fetal rats weights of the group that did not take phenytoin (control) compaired to the fetuses of the rats that used phenytoin during pregnancy were significantly different as shown in Table 1,2 and 3 above can be explained by the findings in the previous studies that phenytoin has the capacity to cause hypoxia in the embryonic tissue due to low heart rate of the embryo and this is even increased by hemodynamic alterations of the mother such as arryhythmia, hypotention which reduce blood flow through the placenta(10).Nutrients ,oxygen included are very essential in growth and development of various tissues and organs of the embryo, therefore embryonic bradycardia reduce nutrient supply to the tissues hence poor development manifested clinically as low fetal weight with or without malformations. The same effects are also attributed to the hemodynamic alterations of the mother that result in reduced placental blood flow hence reduced nutrients to the embryo by the fact that the placenta is the main site through which nutrients diffuse to the developing embryo. Antiepileptic drugs(AED's) phenytoin being one of them were earlier postulated to disturb embryonic and fetal developing structures by interfering with moternal

folate metabolism which leads to folate deficiency in the mother(9). Maternal folate deficiency is one of the predisposing factors to fetal malformations particularly the neurotube defects and these supports this study findings of the different(reduced) fetal crown rump length, head circumfrence, head length and bi-parietal diameter of the experimental group from the control group.

V. Conclusion

In utero exposure to phenytoin affect the fetal growth differently depending on the dose and time exposure, marked fetal growth parameters reduced in fetuses whose mothers received high dose (120mg/kg) phenytoin and those who received phenytoin during the first trimester (T 1)of gestational period.

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