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The Intra-Uterine Teratogenic Effects Of Phenytoin On Fetal Growth And Development In Albino Rats (Rattus Norvegicus)

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Abstract:
The intrauterine developmental effects of phenytoin when administered in varying doses on fetal growth and development remains poorly understood. This study therefore, aims to evaluate the intrauterine effects of varied doses of phenytoin when administered at different gestational period in albino rats. In carrying out this study, post-test only control experimental study design with control was adopted and a sample size of 30 Albino rats were used. These rats were obtained from the Small Animal Facility for Research and Innovation in the school of biomedical sciences of Jomo Kenyatta University of Agriculture and Technology. They were randomly assigned into two broad study groups of 3 control rats and 27 experimental rats. The 27 rats in the experimental group were further subdivided in to three study groups of 9 rats each that is high, medium and low phenytoin doses. At gestation day 20, all the rats were humanely sacrificed and 3 fetuses from each rat were selected. The parameters evaluated in this study included the bi-parietal diameters, fetal weight and crown lump length, and. The data was collected using a structured a checklist, then entered into the computer using an excel spreadsheet, the data was then exported to the Statistical Package for the Social Scientist (SPSS). To determine the effects, the statistical significance was determined by use of Turkey's post hoc multiple comparison tests and all values whose P<0.05 were considered to be significant. The finding of the study shown that there was statistical significant reduction (P<0.05) in fetal weight and bi-parietal diameter and crown lump length especially during the first and second trimester. Phenytoin administered prenatally had a dose and time dependent influence on fetal parameters. Therefore, more studies need to be done on higher primates to ascertain its teratogenic safety in pregnancy.

Keywords: *Albino rats, Bi-parietal diameter, crown lump length,fetal weight, Teratogenic, phenytoin*

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I. Introduction

Phenytoin is an anticonvulsant (Patocka et al., 2020) considered first-line therapy for some types of seizures despite the risk of teratogenicity (Etemad et al., 2012). It is commonly used to treat tonic-clonic and partial seizures, complex partial seizures and also status epilepticus (Kim et al., 2020). (Gupta *et al* 2021), and eclampsia (Moussa et al., 2015). Phenytoin is commonly prescribed because of its low cost, its cost effectiveness and also it is easily available (Kalorin et al., 2008). Though all anticonvulsants are known to have teratogenic effects on the developing fetus (Moussa et al., 2015), Phenytoin is commonly prescribed anticonvulsant (used to manage some conditions in pregnancy like eclampsia, epilepsy (Properties & Methods, 2015). However, the teratogenic safety of phenytoin during pregnancy has been controversial because of its unclear teratogenic effects on growth and development of fetus, making it difficult to prescribe (Azarbayjani, 2001). There is limited data on its teratogenic effects when administered in varied doses and at different window periods on fetal growth parameters including bi-parietal diameters (BD), fetal weights (FWs) and crown lump length(CRL). This study aims to generate data that can help scientist carry further studies to non-human primates that have a closer genetic relationship to humans with a view to guiding the clinicians in prescribing phenytoin during pregnancy.

II. Material And Methods

Study area: The animal experimentation that included animal feeding, drug administration, maternal weights, fetal weights, the fetal growth, and developmental parameters and sacrificing the mothers was carried out in the Small Animal Facility for Research and Innovation (SAFARI) of Jomo Kenyatta University of Agriculture and Technology (JKUAT).

Study Design: In conducting the study a post-test-only control experimental study design was adopted.

Study sample: A pure colony of 30 nulliparous Albino rat dams of the *Rattus norvegicus* species were used as the study model. The choose to use this species was based on the following known facts on albino rats;(i) they have Low prevalence of spontaneously occurring congenital malformation in their fetuses, (ii) they usually have large litter size of between 1-16, (iii) Their gestation period is relatively short compared with other experimental animals as it is 21 days. *(*Ferreira *et al.,* 2019*)*

Rats acquisition: The 30 albino rat were obtained from the Small Animal Facility for Research and Innovation (SAFARI) in the school of biomedical sciences of Jomo Kenyatta University of Agriculture and Technology (JKUAT).

Sample size determination: In determining the sample size, the resource equation by Arifin & Zahiruddin, 2017, whose formula is n=DF/k + 1 was used where in this study: - **n** represented the total number of rat dams that formed my sample size. **DF** was the degree of freedom while **k** represented the total number of subgroups. Based on this research equation, the acceptable range of degrees of freedom (DF) was taken to be between 10 to 20. However, since a value less than ten may not yield actual significant results and in this case DF of 20 was taken therefore a total number of 30 animals was obtained. This number of animals was considered adequate because, a value of more than 20 has been shown in previous studies to increase the cost of the study without increasing the significance of the results. To effectively evaluate the effects of phenytoin in terms of the trimester of exposure as well as effects as per varied doses of exposure, the study model had therefore a total of 10 sub-groups of three rats each namely: - Control group, Low dose TM_1, Low dose TM_2, Low dose TM_3, Medium dose TM_1, Medium dose TM_2, Medium dose TM_3 and High dose TM_1, High dose TM_2 and High dose TM_3.
Hence **n** =20/10 +1 = 3 (subjects per group).
Therefore 10 groups x 3 subjects per group = **30 dams.**

Grouping of rats: The 30 rats were first randomly assigned into two broad study groups of 3 rats (control) and 27 rats (experimental). To evaluate the intrauterine effect of phenytoin when administered in varied doses, the 27 rats in the experimental group were subdivided in to three broad study groups of 9 rats each according doses as follows: - ; 9 rats for the high phenytoin group (HPTG)- that received 124 mg/kg/bw; 9 rats for the medium phenytoin group (MPTG) that received 62mg/kg/bw and lastly 9 rats for the low phenytoin group (LPTG) that received 31mg/kg/bw.. To further evaluate the intrauterine effects of phenytoin when administered on differing gestation periods, the 9 rats in each of the three dose groups, the nine rats were further sub-divided into three sub-groups of 3 rats each according to the trimester when they received the phenytoin treatment as follows; 3 rats for trimester one that received phenytoin treatment from the gestational day one (GD_1) all the way to gestational day 20(GD_{20}); three rats for trimester two that started receiving phenytoin treatment from gestational day 7 GD_7 all the way to gestational day 20(GD_{20}), and 3 rats for trimester three that started receiving phenytoin treatment from gestational day 14 (GD_{14}) all the way to gestational day 20(GD_{20}) respectively.

Mating of the rats and determination of their pregnancy: The mating process was done by introducing one male albino rat from third series breed of a pure colony in to the standard cage mating cages with two female rats at 1530 hours (+/-30 minutes). Then the male rats were removed the following morning at 0930 hours (+/-30 minutes) and returned to their separate cage. The confirmation of pregnancy was done by taking vaginal wash from the mated rats after 24 hours, the presence of polyhedral epithelial cells on the swab was used to denote estrous changes, that marked the first day of gestation (GD_1), (Telendo *et al.,* 2019)

The feeding of the rats: All rats were fed on standard rodent pellets obtained from Unga feed Limited situated in Thika town that contained weight (g/100g): - 68% starch, 4% cellulose, 5% lipid (corn oil) and 20% protein) and by calories: - 20% proteins, 72% carbohydrates,12% lipids, and 54mg/kg zinc and they also received water *ad libitum* that was given via rat water bottle every morning at 0830 hours as outlined by (Curfs *et al*. (2011),

Phenytoin doses determination: Phenytoin tablets obtained from Aurobindo Pharma - Milpharm Ltd in india batch number AUST R 321937 bought from government chemist in Nairobi. A simple guide for converting animal dosages from human dosages by (A. Nair et al., 2018),Nair & Jacob, (2016) was applied, which states that dose is equally related to body weight. The minimum dose of Phenytoin in human is 300 mg/day, the medium dose is 600 mg/day, and the maximum dose is 1200 mg/day. To determine human equivalent dose (HED) for the Phenytoin, average body weight of a **human being that** is 60 kg was used. These doses were

divided by 60kg to obtain HED and 5 mg/kg/bw, 10 mg/kg/bw and 20 mg/kg/bw were obtained for low, medium and dose respectively.

After obtaining the human equivalent dose HED, animal equivalent dose (AED) was arrived at by multiplying human equivalent dose (HED) by Km factor which is 6.2 which is equivalent to 31mg/kg/bw for the low phenytoin dose group, 62mg/kg/bw for the medium Phenytoin dose group and 124mg/kg/bw for high phenytoin dose. Since the study used low, medium and high dosages, these dosages were arrived at by multiplying the weights of each rats with animal equivalent dose calculated for each category, that is 31mg/kg/bw, 62mg/kg/bw and 124mg/kg/bw respectively.

Reconstituting the doses: Phenytoin which was obtained in form of tablet (100mg) were dissolved in 10 millimeters of distilled water. The dissolved phenytoin was then administered to the rats guided by their weights and specific dosage.

Drug administration: all experimental animals received phenytoin treatment and the phenytoin treatment was administered as follows:- For all rats that were to receive phenytoin treatment in trimester one ($TM_{1);}$ treatment was done from gestational day GD_1 all through to gestational day 20(GD_{20}) while those that were to receive the treatment in trimester two ($TM_{2);}$ treatment was done from gestational day GD_7 all through to gestational day 20(GD_{20}) and those that were to receive the treatment in trimester three ($TM_{3);}$ treatment was done from gestational day GD_{14} all through to gestational day 20(GD_{20})

Sacrificing the animals: All the pregnant rats were humanely sacrificed on the gestation day 20th between 0900 hours and 1100 hours by use of concentrated carbon dioxide. The sacrificing of the rats on day 20th was to prevent the mothers from devouring any malformed offspring (Rai & Kaushik, 2018).

Statistical analysis: The fetal growth parametric data that included Bi-parietal Diameter (BPD) Head Circumference (HC), Crown Lump Length (CRL), Fetal Weight (FW) was collected using a structured a check list. It was then entered into the computer using an excel spreadsheet for windows version 10, this data in the excel spreadsheets was then exported to the Statistical Package for the Social Scientist (SPSS) version 25 for statistical analysis. To determine the teratogenic effects of phenytoin, the analysis of variance (ANOVA) was applied. To determine the causal and interaction effects Turkey's post hoc multiple comparison tests was applied and all values whose $P<0.05$ were considered to be statistically significant.

The fetal pregnancy outcome Parameters.

Bi-parietal Diameter (BPD) Head Circumference (HC), Crown Lump Length (CRL), Fetal Weight (FW) were recorded.

III. Results

The effects of phenytoin on bi-parietal diameter (BPD), fetal weight (FW) and crown lump length (CRL).

This study found out that mean fetal weights, mean bi-parietal diameter (BPD), fetal weight (FW) and crown lump length (CRL) depicted an inverse dose relationship at the same time depicting a direct relationship with the time of exposure. A statistical significant difference ($p<0.05$) was observed when the drugs were given during TM_1 and TM_2 for HPTG for the mean bi-parietal diameter (BPD), fetal weight (FW) and crown lump length (CRL). However, there was no statistical significance difference when the drug was administered in trimester three (TM_3) at high doses for all the three parameters when comparison was made with the control. When the drug was also administered in medium dose and low doses, it did not show statistical significant difference ($P>0.05$) across all the trimesters when comparison was made with the control also for the mean bi-parietal diameter (BPD), fetal weight (FW) and crown lump length (CRL).

Table 3.1 The intra and inter group comparative means of the fetal body weight, CRL and bi-parietal diameter of LPBG, MPBG and the HPBG in (TM_1, TM_2 and TM_3) against the control (C).

Study groups	Time of exposure	Mean fetal weight (g)	Mean fetal BPD (cm)	Mean fetal CRL (cm)	Mean head circumference (cm)
Control	------	6.4643±.1022	1.559±.05508	4.464±.1028	4.291±.1636
LD phenytoin group	TM_1	4.7701±.1547	.916±.06407	3.728±.0865	3.571±.1668
	TM_2	5.7201±.0769	1.193±.04945	3.911±.0842	3.696±.0390
	TM_3	6.1393±.0500	1.548±.01347	4.331±.0373	4.285±.0701

MD phenytoin group	TM_1 TM_2 TM_3	3.9706±.1543 5.1604±.0761 6.0381±.0504	.791±.0073 1.055±.0306 1.433±.0037	3.390±.0292 3.699±.0611 4.228±.0206	3.325±.0981 3.520±.1063 4.109±.0340
HD phenytoin group	TM_1 TM_2 TM_3	3.432±.157* 4.788±.073* 5.767±.046	.671±.034* .961±.048* 1.353±.012	3.116±.076* 3.585±.099* 4.068±.139	3.082±.135* 3.355±.126* 3.958±.063

*Key: The means followed 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).*

IV. Discussion

This study has found out that all the fetal growth parameters that included bi-parietal diameters, fetal weight, crown-rump length had a direct time dependent relationship in that when phenytoin treatment was administered at TM_1, TM_2 and TM_3, these parameters increased directly with time of exposure. It further established that all those parameters had an inverse dose dependent relationship in that as the phenytoin dose was increased, those parameters decreased.

When phenytoin was administered, it was observed that the mean fetal weight significantly low with increasing dose of phenytoin among the treatment groups particularly when given during $TM_{1\ and}$ TM_2 at high dose (3.432±.157*4.788±.073*) as compared with the control (6.4643±.1022) (p < .05.) (Table 3.1). Mean crown lump length mean was also found to be statistically significantly reduced in high dose group when phenytoin was administered during trimester one ($TM_{1)}$ and two ($TM_{2)}$ (3.116±.076*3.585±.099*) respectively compared to that of the control (4.464±.1028) (Table 3.1). This current study results are also in agreement with those of a study done by (Ritchie et al., 2019) who found out that when phenytoin was administered, there was reduced birth weight and head circumference in rats administered with it. Another study also done by (Hamdi *et al*., 2016b) which showed that in valproic acid treated group, an anticonvulsant which has the same mode of action as phenytoin, there was significant reduction in crown lump length and fetal weight in comparison to the control group. This could be attributed by interference of the medicines on the maternal nutritional status hence reduced fetal parameters

V. Conclusion

In conclusion, the study established that, phenytoin administered during pregnancy have a dose and time dependent influence on fetal weight, bi-parietal diameters and crown-rump length. The doses that have been established to have more teratogenic effects are high dose of 124mg mg/kg/bw (HPTG) especially when administered during first trimester (**TM_1**) and second trimester (**TM_2**), medium and high doses. The most teratogenic dose was however established to be 124mg mg/kg/bw (HPTG) while most vulnerable gestation period for phenytoin teratogenicity was the first trimester (**TM_1**).

VI. Recommendations.

The study recommends that;

Phenytoin was found to negatively influence fetal growth and development in rats hence more studies need to be done on the higher primates to ascertain its safety in pregnancy in order to curb cases of congenital anomalies which may be associated with it.

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