k ORIGINAL RESEARCH ARTICLE

The intra-uterine effects of phenobarbital on fetal growth and development in albino rats (Rattus Norvegicus)

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ABSTRACT

The intrauterine developmental consequences of phenobarbital, when administered in varied doses, on foetal growth and development remain poorly understood. This study is therefore set to evaluate the intrauterine effects of phenobarbital at differing doses when administered at different incubation periods in albino rats. In carrying out this study, a post-test only control experimental study design was adopted, and a sample size of 30 Albino rats was used. These rats were obtained from the Small Animal Facility for Research and Innovation in the School of Biomedical Sciences at Jomo Kenyatta University of Agriculture and Technology. They were arbitrarily allocated into two large study groups of 3 control rats and 27 experimental rats. The 27 rats in the experimental group were further subdivided into three study groups of 9 rats, each with low, medium, and high phenobarbital doses. On gestation day 20, all the rats were humanely sacrificed, and three fetuses from each rat were selected. The parameters evaluated in this study included the foetal weight, bi-parietal diameters, and crown lump length. The data was collected using a structured 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 causal effects, the statistical significance was determined by using Tukey's post hoc multiple comparison tests, and all values with a p value less than 0.05 were considered to be significant. This research discovered that there was a reduction in all foetal parameters, which was statistically significant ($P<0.05$), especially during trimesters one and two. The effects of phenobarbital administered on foetal parameters depended on the time of exposure and dose administered. Phenobarbital, administered prenatally, had dose- and time-dependent effects on foetal parameters. Therefore, more studies need to be done on higher primates to ascertain their teratogenic safety in pregnancy.

Key words: Phenobarbital, stereology, anticonvulsants, teratogenic.

URL: https://ojs.jkuat.ac.ke/index.php/JAGST
ISSN 1561-7645 (online)
doi: 10.4314/jagst.v23i6.7

1.0 Introduction.

Phenobarbital, a first-line anticonvulsant, is commonly prescribed in the management of a wide range of conditions, such as insomnia, neuralgic pains, and epilepsy, among other convulsive disorders (Mwangi et al., 2023). Though all anticonvulsants are known to have teratogenic effects on the fetus phenobarbital usage during pregnancy is particularly gaining preference in the management of maternal epilepsy, pre-eclampsia, and eclampsia, among other conditions (Pennell, 2016). Phenobarbital is commonly prescribed because of its low cost, its pharmacotherapeutic effectiveness in the management of neuralgic pains, and its easy accessibility in local pharmacies (Li et al., 2019). However, the teratogenic safety of phenobarbital during pregnancy has been controversial because of its unclear teratogenic effects on the growth and development of the foetus, making it difficult to prescribe (Ashtarinezhad et al., 2015). There is limited data on its teratogenic effects when given in varied doses and at different window periods on foetal growth parameters, which include foetal weights (FWs), crown rump length (CRL), biparietal diameters (BD), and the foetal head cumference (FHC) (Kweri, C. K et al., 2023). This study aims to generate data that can help scientists carry further studies to non-human primates that have a closer genetic relationship to humans with a view to guiding clinicians in prescribing phenobarbital during pregnancy.

2.0 Material and methods

2.1 Study location

The animal experimentation, which comprised animal feeding, drug administration, maternal weights, foetal weights, foetal growth and developmental parameters, and sacrificing the mothers, was carried out in the Small Animal Facility for Research and Innovation of Jomo Kenyatta University of Agriculture and Technology (JKUAT).

2.2 Study design

A post-test-only control experimental study design was used in conducting the study.

2.3 Sample study.

A pure colony of thirty (30) female albino rats that has not bore offspring was used as the study model. The choice to use this species was based on the following known facts about albino rats: (i) they have a low prevalence of spontaneously occurring congenital malformation in their foetuses; (ii) they usually have a large litter size of between and 16; and (iii) their gestation period is relatively short compared with other experimental animals as it is 21 days.

2.4 Acquisition of the rats.

The 30 rats were obtained from the Small Animal Facility for Research and Innovation in the School of Biomedical Sciences at Jomo Kenyatta University of Agriculture and Technology (JKUAT).

2.5 Determination of sample size.

In determining the size of the sample, the resource equation (Arifin & Zahiruddin, 2017), whose formula is n=DF/k + 1, was used. In this study, n represented the total number of rat dams that

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formed my sample size. DF was the degree of freedom, while k represented the total number of subgroups. Based on this research equation, the satisfactory range of degrees of freedom (DF) was taken to be between 10 and 20. However, since a value less than ten may not yield actual significant results, and in this case, DF of 20 was taken, a total of 30 animals was obtained. This number of animals was considered adequate because a value greater than twenty has been demonstrated in previous studies to augment the cost of the research without increasing the importance of the results. To effectively evaluate the effects of phenobarbital in terms of the trimester of exposure as well as effects as per varied doses of exposure, the study model had a total of 10 sub-groups of three rats each, namely: control group, low dose TM1, low dose TM2, low dose TM3, medium dose TM1, medium dose TM2, medium dose TM3, high dose TM1, high dose TM2, and high dose TM3.Hence n =20/10 +1 = 3 (subjects per group).

Therefore 10 groups x 3 subjects per group = 30 dams.

2.6 Grouping of rats in to study groups.

The 30 rats were first randomly assigned into 2 large groups: a control group consisting of 3 rats and an experimental group consisting of 27 rats. To assess the intrauterine effect of phenobarbital when administered in varied doses, the twenty-seven rats (27) from the experimental category were subdivided into 3 groups, consisting of 9 rats in each group depending on the dosages, that is: 9 rats for the high phenobarbital group (HPBG) that received 41.5 mg/kg/bw; 9 rats for the medium phenobarbital group (MPBG) that received 19.2 mg/kg/bw; and lastly, 9 rats for the low phenobarbital group (LPBG) that received 3.1 mg/kg/bw. To further assess the intrauterine effects of phenobarbital when administered on different dates of gestation, the nine rats were further sub-divided into 3 sub-groups of 3 rats each depending on the trimester when they received the phenobarbital treatment as follows; 3 rats for trimester one that received phenobarbital from the gestational day one (GD1) all the way to gestational day 20(GD20); three rats for second trimester that started receiving phenobarbital drug from seventh day of conception GD7 all the way to20th day of gestation (GD20) and 3 rats for trimester three that started receiving phenobarbital therapy from 14th day of conception (GD14) all the way to gestational day 20(GD20) respectively.

2.7 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 2 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. Vaginal wash was done to confirm the pregnancy after 24 hours of mating and the presence of polyhedral epithelial cells on the swab was used to denote estrous changes, that marked the first day of pregnancy (GD1), (El-Sakhawy et al., 2019).

2.8 The feeding of the rats:

Standard rodent pellets were used to feed the rats acquired from Unga Feed Limited loc in Thika town that contained weight (g/100g): 20% protein, 68% starch, 5% lipid, and 4% cellulose, in

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the form of calories: 72% carbohydrates, 20% proteins, 54 mg/kg zinc, and 12% lipids, and they also received water ad libitum that was given via rat water bottle every morning at 0830 hours.

2.9 Determination of the phenobarbital doses used in the study.

Phenobarbital tablets from Hikma Pharmaceuticals in the USA, batch number NSC 9848, were bought from a government chemist in Nairobi. An uncomplicated guide for converting human doses to animal dosages (Nair et al., 2018; Nair & Jacob, 2016) was used, which declares that dose is equally related to body weight. The minimum dose of phenobarbital in humans is 30 mg/day, the medium dose is 185 mg/day, and the maximum dose is 400 mg/day. To determine the human equivalent dose (HED) for the phenobarbital, the average body weight of a human being was 60 kg. These doses were divided by 60kg to obtain HED, and 0.5 mg/kg/bw, 3.1 mg/kg/bw, and 6.7 mg/kg/bw were obtained for low, medium, and high doses, respectively.

After obtaining the HED, the animal equivalent dose (AED) was arrived at by multiplying the human equivalent dose (HED) by the Km factor, which is 6.2, which is equivalent to 3.1 mg/kg/bw for the low phenobarbital dose group, 19.2 mg/kg/bw for the medium phenobarbital dose group, and 41.5 mg/kg/bw for the high phenobarbital dose group. Since the study used low, medium, and high dosages, these dosages were arrived at by multiplying the weights of each rat with the animal equivalent dose calculated for each category, which is 3.1 mg/kg/bw, 19.2 mg/kg/bw, and 41.5 mg/kg/bw, respectively.

2.10 Reconstituting the doses.

Phenobarbital, which was obtained in the form of tablets (30 mg), was dissolved in 10 millilitres of distilled water. The dissolved phenobarbital was then administered to the rats, guided by their weights and specific dosages.

2.11 Drug administration.

All experimental animals received phenobarbital treatment, which was administered as follows: for all rats that were to receive phenobarbital treatment in trimester one (TM1), treatment was done from the first day of conception (GD1) to the 20th day of gestation (GD20); for those that were to receive the last trimester two (TM2); treatment was done from gestational day GD7 to gestational day 20(GD20) and those that were to receive the treatment in last trimester (TM3); treatment was done from gestational day 14 (GD14) to gestational day 20 (GD20).

2.12 Sacrificing the animals.

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

2.13 Statistical analysis.

The parametric data, which included foetal weight, crown lump length, head circumference, and bi-parietal diameter parameters, was collected using a structured check list. It was then inserted into the computer using an Excel spreadsheet for Windows 10. The data in the Excel

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ISSN 1561-7645 (online)
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spreadsheets was then exported to the Statistical Package for Social Scientists (SPSS) version 25 for statistical analysis. To determine the teratogenic effects of phenobarbital, parametric data was compared across and within groups, and the multivariate analysis of variance (MANOVA) was applied. To determine the causal effects, Tukey's post hoc multiple comparison tests were applied, and all P values with a P value less or equal to 0.05 were considered to be statistically significant.

2.14 The fetal pregnancy outcome Parameters.

After sacrificing the animals, foetal parameters were taken as follows: the biparietal diameters (BPD) were measured using a digital vernier calliper from left to right parietal eminence just above the ears without removing the scalp; the head circumference (HC) from the bottom of the ear all round; the crown lump length (CRL) from the snout to the base of the tail were measured using a tape measure; and the foetal weight (FW) was measured using an electronic weighing vice. The results were recorded.

3.0 Results.

The effects of phenobarbital on foetal weight (FW), crown lump length (CRL), head circumference (HC), and bi-parietal diameter (BPD). This study depicted that the means of bi-parietal diameter, foetal weights, head circumference, and crown-rump length showed an inverse dose relationship while at the same time exhibiting a direct dose-response relationship with the time of exposure. A statistically significant difference ($p<0.05$) was discovered when the drugs were given during TM1 for MPBG and HPBG. When compared with the control, those who were given the drug during the third trimester did not depict a statistically significant difference ($P > 0.05$) in all the treatment groups (LPBG, MPBG, and HPBG).

In the high phenobarbital group (HPBG), it was noted that there was a statistically significant difference in mean total foetal weight in first trimester TM1 (3.310000±0.095394) ($p<0.00$) and second trimester TM2 (4.665397±0.038921) ($p<0.00$) compared with that of the control (6.353175±0.064628). There was also a significant difference ($p<0.00$) between the mean foetal weight of the medium dose group (MPBG) when administered in trimester one (TM1) (4.034167±0.018276) compared with that of the control. At the same time, when the total foetal weight was compared with the time of exposure, it showed a higher effect during TM1, followed by M2, and lastly TM3 across all the treatment groups (LPBG, MPBG, and HPBG). For instance, when phenobarbital was administered during low-dose LPBG, TM1 (4.651204±0.086878), TM2 (5.612963±0.039847), and TM3 (6.045455±0.024052).

This study also found that there was a statistically significant difference in mean total foetal bi-parietal diameter (BPD) when phenobarbital was administered in high doses (HPBG) in trimester one (TM1) (0.526667±0.039299) and trimester two (TM2) (0.850317±0.031999) compared with that of the control (1.4552 ±1.328984) at ($p<0.00$). In the medium phenobarbital dose group (MPBG), it was noted that there was a statistically significant difference ($p<0.00$) in mean foetal bi-parietal diameters when administered in trimester one (TM1) (0.679167±0.015023) compared with that of the control. When the total foetal bi-parietal diameter was compared

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ISSN 1561-7645 (online)
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with the time of exposure, it also showed a higher effect during TM1, followed by TM2, and finally TM3 across all the treatment groups.

When phenobarbital was administered in high doses (HPBG), there was a statistically significant difference in mean foetal crown lump length CRL in trimester one (3.020000±0.035119 ($p<0.00$)) and trimester two (3.462698±0.054578) ($p=0.01$) compared with that of the control (4.347619±0.059809). In the medium phenobarbital dose group (MPBG), it was noted that there was a statistically significant difference ($p<0.00$) in mean foetal crown lump lengths when administered in trimester one (TM1) (3.270833±0.006365) compared with that of the control. Furthermore, when the crown lump length was compared with the time of exposure, it was observed that the mean foetal crown lump length had a direct response relationship to the time of exposure in that more effects were observed when administered at TM1, followed by TM2, and then the least effects at TM3.

For the head circumference, it was found that there was a statistically significant difference in mean head circumference when phenobarbital was administered in high doses (HPBG) in trimester one (TM1) (3.212857±0.089834) and trimester two (TM2) (3.452976±0.05490) compared with that of the control (4.205925±0.080717) at $p<0.00$. In the medium phenobarbital dose group (MPBG), it was noted that there was a statistically significant difference ($p<0.00$) in mean foetal head circumference when administered in trimester one (TM1) (3.212857±0.089834) compared with that of the control. Additionally, when the total head circumference was compared with the time of exposure, it showed a higher effect during TM1, followed by TM2, and finally TM3.

Generally, when the treatment was administered at a low dose, there was no statistically significant difference across all the trimesters for all the foetal parameters.

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Table 3.1 The inter group and inter group comparative means of the crown lump length, fetal body weight, bi-parietal diameter, head circumference and of LPBG, MPBG and the HPBG in (TM3, TM2 and TM1) 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	------	6.353175±0.064628a	1.4552 ±1.328984 a	4.347619±0.059809 a	4.205925±0.080717a
LD Phenobarbital group	TM1				
	TM2				
	TM3	4.651204±0.086878a			
		5.612963±0.039847a			
		6.045455±0.024052a	0.795185±0.032750a		
		1.087222±0.023498a			
		1.442424±0.010926a	3.620648±0.046997a		
		3.798519±0.049464a			
		4.248485±0.021852a	3.492593±0.107650a		
		3.557618±0.083378a			
		4.187879±0.047625a			
MD phenobarbital group	TM1				
	TM2				
	TM3	4.034167±0.018276b*			
		5.095833±0.023199a			
		5.913030±0.008881a	0.679167±0.015023b*		
		0.933333±0.016667a			
		1.325152±0.003573a	3.270833±0.006365b*		
		3.584722±0.034219a			
		4.116061±0.006970a	3.212857±0.089834b*		
		3.452976±0.054907a			
		3.994377±0.015175a			
HD phenobarbital group	TM1				
	TM2				
	TM3	3.310000±0.095394c*			
		4.665397±0.038921b*			
		5.646032±0.030656a	0.526667±0.039299c*		
		0.850317±0.031999b*			
		1.276323±0.032675a	3.020000±0.035119c*		
		3.462698±0.054578b*			
		3.847090±0.025103a	2.9250000±114564c*		
		3.282778±0.050464b*			
		3.840476±0.021162a			

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

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4.0 Discussion.

This study has found that all the foetal growth parameters, which included foetal weight, bi-parietal diameters, head circumferences, and crown-rump length, had an inverse dose-dependent relationship in that as the phenobarbital dose was increased, those parameters decreased. It further established that all those parameters had a direct time-dependent relationship in that when phenobarbital therapy was administered at TM1, TM2, and TM3, these parameters increased directly with time of exposure.

Upon administration of phenobarbital, it was perceived that the mean foetal weight significantly declined with an augmenting dose of phenobarbital among the treatment groups, specifically when administered during TM1 at a high dose as compared with the control (Table 3.1). The mean crown lump length was also found to be statistically significantly low in the high dose group when phenobarbital was dispensed during the first trimester (TM1) and second trimester (TM2), compared to that of Table 3.1. This current study's results are in agreement with those of a study done by Hamdi et al. (2016) which depicted that in the valproic acid-treated group, an anticonvulsant in the same generation as phenobarbital, there was a significant reduction in crown lump length and foetal weight in comparison to the control group. Another study also done by El-Gaafarawi and Abouel-Magd (2015) showed that upon administration of anticonvulsants like carbamazepine, which is in the same generation as phenobarbital, there was decreased crown-rump length and foetal body weight among groups exposed to carbamazepine compared to control.

This study also found that there was a statistically significant reduction in head circumference ($p<0.05$) when phenobarbital was given during the first trimester (TM1) and at a high dose compared to the control group (Table 3.1). This result is in unanimity with the results of research done earlier by Margulis et al. (2019) which showed that there was a reduction in head circumference when anticonvulsants including carbamazepine and valproic acid, which are in the same generation as phenobarbital,.

5.0 Conclusion and recommendation.

5.1 Conclusion.

In conclusion, the study established that phenobarbital administered during pregnancy has time- and dose-dependent effects on foetal weight, crown-rump length, bi-parietal diameters, crown lump lengths, and head circumference. The doses that have been established to have more teratogenic effects are the high dose of 41.5 mg/kg/bw (HPBG) and the medium dose of 19.2 mg/kg/bw (MPBG), especially when administered in the first trimester (TM1) and trimester two (TM2), for all the low, medium, and high doses. Its teratogenic effect on the developing infant when prescribed in the last trimester has no major outcomes except when dispensed in high doses. The most teratogenic dose was, however, established to be 41.5 mg/kg/bw (HPBG), while the most vulnerable gestation period for phenobarbital teratogenicity was the first trimester (TM1).

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5.2. Recommendations.

The study recommends that since phenobarbital was found to negatively influence foetal growth and development in rats, more studies need to be done on the higher primates to ascertain its safety in pregnancy in order to curb cases of congenital anomalies that may be associated with it.

6.0 Acknowledgement

6.1 Funding

None

6.2 Ethical approval

Ethical approval was sought from the JKUAT Animal Ethics Review Committee dated 10th November 2021 reference number FVM BAUEC/2021/327. Ethical conduct was observed at all times during the entire period of the study. Animals were not used for any other purpose other than that indicated in the proposal.

6.3 Conflict of interest

The authors declare no conflict of interest

7.0 References.

Arifin, W. N., & Zahiruddin, W. M. (2017). Sample size calculation in animal studies using resource equation approach. Malaysian Journal of Medical Sciences, 24(5), 101–105. https://doi.org/10.21315/mjms2017.24.5.11

Ashtarinezhad, A., Panahyab, A., Mohamadzadehasl, B., Vatanpour, H., & Shirazi, F. H. (2015). FTIR microspectroscopy reveals chemical changes in mice fetus following Phenobarbital administration. Iranian Journal of Pharmaceutical Research, 14(July 2013), 121–130. https://doi.org/10.22037/ijpr.2015.1721

El-Gaafarawi, I., & Abouel-Magd, M. (2015). Teratogenic Effect of Carbamazepine Administration in Pregnant Rats. The Egyptian Journal of Hospital Medicine, 59, 244–257. https://doi.org/10.12816/0012182

El-Sakhawy, M. A., Mohamed, D. W., & Ahmed, Y. H. (2019). Histological and immunohistochemical evaluation of the effect of tartrazine on the cerebellum, submandibular glands, and kidneys of adult male albino rats. *Environmental Science and Pollution Research*, *26*, 9574-9584

Hamdi, H., El Ghareeb, A. E. W., Kandil, A. M., Ahmed, O. M., & Yahia, R. (2016). The potential impacts of the anti-epileptic drug (Oxcarbazepine) on albino rat's neonates during lactation. Asian Journal of Pharmaceutical and Clinical Research, 9(August), 244–251.

Kweri, C. K., Kariuki, J., Mwangi, A., Kanyoni, J. M., & Macharia, P. (2023). Evaluation of embryonic teratogenic effects on fetal growth and development following prenatal exposure to different doses of levetiracetam in albino rats (rattus norvegicus). *JOURNAL OF AGRICULTURE, SCIENCE AND TECHNOLOGY*, *22*(2), 72-82.

Li, J., Yang, D., Zhao, D., Li, N., & Lin, W. (2019). Efficacy of phenobarbital and sodium valproate in treating convulsive epilepsy in rural northeast China. Seizure, 71(March), 207–213.

URL: https://ojs.jkuat.ac.ke/index.php/JAGST
ISSN 1561-7645 (online)
doi: 10.4314/jagst.v23i6.7

https://doi.org/10.1016/j.seizure.2019.06.012

Margulis, A. V., Hernandez-Diaz, S., McElrath, T., Rothman, K. J., Plana, E., Almqvist, C., D'Onofrio, B. M., & Oberg, A. S. (2019). Relation of in-utero exposure to antiepileptic drugs to pregnancy duration and size at birth. PLoS ONE, 14(8), 1–21. https://doi.org/10.1371/journal.pone.0214180

Mwangi, A. W., Kweri, J. K., Kweri, C. K., Kanyoni, J. M., Kigundu, A. M., Mwangi, E. G., & Marera, D. O. (2023). The growth and development outcomes of foetuses born of albino rats (Rattus Norvegicus) prenatally exposed to varying doses of lamotrigine. *Journal of Agriculture, Science and Technology*, *22*(3), 136-145.

Nair, A. B., & Jacob, S. (2016). A simple practice guide for dose conversion between animals and human. Journal of Basic and Clinical Pharmacy, 7(2), 27–31. https://doi.org/10.4103/0976-0105.177703

Nair, A., Morsy, M. A., & Jacob, S. (2018). Dose translation between laboratory animals and human in preclinical and clinical phases of drug development. Drug Development Research, 79(8), 373–382. https://doi.org/10.1002/ddr.21461

Pennell, P. B. (2016). Use of Antiepileptic Drugs During Pregnancy: Evolving Concepts. Neurotherapeutics, 13(4), 811–820. https://doi.org/10.1007/s13311-016-0464-0

Rai, J., & Kaushik, K. (2018). Reduction of Animal Sacrifice in Biomedical Science & Research through Alternative Design of Animal Experiments. Saudi Pharmaceutical Journal, 26(6), 896–902. https://doi.org/10.1016/j.jsps.2018.03.006

URL: https://ojs.jkuat.ac.ke/index.php/JAGST
ISSN 1561-7645 (online)
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