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Effects of Prenatal Exposure to Varying Doses of Gentamicine on Fetal Weights in Albino Rats (*RattusNorvegicus)*.

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Abstract:*Prenatal exposure to gentamicin has been shown to perturb the normal development of a wide range of fetal organs including the kidneys, ear, lungs the nervous system among others. Though gentamicin is classified under class D medicine it's still being used in treatment of some maternal bacterial infections in case of multidrug resistant trains or due to lack of other antibiotic options. Though data exists on its teratogenic effects on the developing fetal organs there is paucity of data on its teratogenic effects on fetal outcomes following prenatal exposure to varied doses and when exposed at different window periods. The broad objective of this study was therefore to determine its teratogenic effects on fetal birth weight when prenatally administered at varied doses and at different gestational periods. To determine this teratogenic effects on the fetal weight outcomes a sample size of 30 female nulliparous albino rats (dams) weighing between 200 to 250 g were used in the study. This sample size of 30 females dams were randomly grouped into two broad study groups of 3 control and 27 experimental. The 27 experimental rats were further randomly assigned in to Low dose gentamicin group(19mg/kg/bwt)composed of nine rats (LGG =9), medium dose gentamicin group (28mgs/kg/bwt) (MGG = 9) and high dose gentamycin group(37mg/kg/bwt) (HGG = 9). To determine the teratogenic effects of different doses when exposed at different window periods the 9 rats in each of the experimental groups of the low, medium and high dose groups were further assigned into three study subgroups per trimester as follows; trimester 1 study group TM1 that got gentamicine from gestational day 1 to birth, trimester two study group (TM2) day 8-day to birth and trimester three study group TM3 (day 15 to birth). The control group received rodent pellets from Unga limited (Kenya)and water ad-libitum while the experimental category received LGG 19mg/kg bwt, MGG 28mg/kg bwt and HGG 37mg/kg bwt of gentamicine respectively and water ad libitum once daily at 9:00am. Gentamicine was administered through intramuscular injection. The expectant rats were sacrificed on the 21st day of gestation upon euthanasia with carbon dioxide and the abdomen was opened to expose the uterine horns. The fetuses were harvested then weighed using Scout pro model® from Japan, serial no.B519923500 and recorded in grams. Data was then entered in excel sheet and exported to SPSS version 23 for analysis. The intra and inter group comparative quantitative data was statistically tested using one way analysis of variance (ANOVA) and p-values of less than 0.05 were taken to be significant. The finding of the study elucidated that the teratogenic effects of gentamicin on the fetal weights are dose and time dependent where the fetal weights are the lowest when gentamicin is administered at high dose and at trimester one (TM1) which showed a statistically significant dose and time-dependent decrease in fetal birth weight in treatment groups LGG (p=0.0001), MGG (p=0.0001) and HGG (p=0.003). In conclusion, the present study revealed that use of gentamicine during pregnancy leads to low birth weight.The reduction in birth weight is time and dose dependent hence more emphasis on cautionary use of gentamicine during pregnancy should be done to mothers. The results of this study creates a foundation for more studies with animals closer to humans should be used as well as encourage more clinical studies that would lead to dose moderation of gentamicin to guarantee greater benefits to the mother when administered in pregnancy and also promote safety to the growing fetus.*

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I. Introduction

Gentamicin an antibiotic under the class of aminoglycosides is occasionally used by expectant mothers for treatment of some bacterial infections[1]. Studies have indicated that when administered prenatally, it is able to cross the placental maternal barrier, accumulating in fetal kidneys, other organs and even in the amniotic fluid[2,3]. However its interaction with fetal tissues has been shown to disrupt the fetal kidney and other organs development[2,4,5] . This effects of gentamicin on fetal kidneys as well as other organs are due to fact that the active gentamicin molecule is able to penetrate the placental maternal barrier hence interacting with fetal nephrogenic progenitor mesenchyme and other organs during fetal period[6–8]. Consequently it interferes with cellular differentiation and maturation of fetal kidneys and other organs[6,9]. However it has shown to disturb the normal morphogenesis of fetal structures like the kidney, ear among others during embryonic and fetal periods[10]. Regardless of this, gentamicin is still being used by expectant mothers and entire population[3,10]. Therefore, the teratogenic effects of gentamicin on fetal organs and its interference with the process of organogenesis causes the overall changes on fetal weights. Worldwide use of gentamicine is on the rise due to increase in bacterial infections, this is in close relation with increase in congenital anomalies and renal disorders which will eventually increase disease burden in many nations[7]

Though the functional and biochemical effects of gentamicin has been documented there is scarcity of data on, if the effects are time or dose dependent, therefore this study was carried out to observe if the effects are dose or time dependent by use of expectant albino rats as the experimental model.

II. Material and Methods

Mature and healthy female nulliparous Albino rats (*Rattusnorvegicus*) weighing between 200 and 250 grams were used. In this study animals *were a*cclimatized to the experimental cages for a period of one week before treatment. All the animals were kept in spacious plastic cages with shredded papers in the animal house and received rodent pellets from UNGA feeds and distilled water *ad libitum.*

Study Design: Laboratory based experimental study

Study Location:All experiments including breeding, handling, weighing and gentamicin administration was done at the Safari Animal house in the School of biomedical Sciences of Jomo Kenyatta University of Agriculture and Technology (JKUAT).

Study Duration: The study was carried out from September to December, 2018.

Sample size: 90 fetuses were used in the study.

Sample size calculation: To determine the sample size, resource equation was used[11]. The value measured 'E' which is the degree of freedom of analysis of variance (ANOVA) based on a distinct 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 probability of getting significant results while a value more than 20 has been indicated to increase the cost of the study without increasing the significance of the results.

E=Total number of animals-Total number of groups
Total number of groups=10
Total number of animals=30
E=30-10
Since every adult female rat has an average of six (6) fetuses per pregnancy making a total of **180 rats (6x 30**). All fetuses were weighted and three fetuses with the least, median and highest weights per rat were taken for study making a total of **90 rats (3x30).**

Grouping of animals: Once pregnancy was confirmed from vaginal smears by observation of large cornified cells, numerous neutrophils and scattered epithelial cells under light microscope. 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 study groups of 9 rats each assigned: low gentamicine group (LGG), Medium gentamicin group (MGG) and High gentamicine group (HGG). Each of the nine rats in the three study groups were further sub-grouped into 3 rats as per trimesters: first (TM1), second (TM2) and third (TM3) trimesters subgroups.

Determination of the gentamicin doses for the experiment

A simple guide for conversion to animal dosages from human dosages was applied[11,12] which states that, dose is equally related to body weight although it is not the only 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 Human Equivalent Dose (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 gentamicin dose in adult humans is 6mg/kg, medium dose is 4.5mg/kg and minimum dose is 3mg/kg[13]).Avial of gentamicin 80mg (2mls) Manufactured by Jiangsu PengyaoPharmarmaceutical Co., Ltd. batch number. 170826 was administered to the experimental group. All trimester ones (TM1) rats :-(LGG, MGG, HGG) categories were injected with gentamicin from gestation day1 to delivery. All trimester twos (TM2) rats :-(LGG, MGG, HGG) categories received gentamicin doses from gestation day 8 to delivery. All trimester three (TM3) rats :-(LGG, MGG, HGG) categories received gentamicin doses from gestation day 15 to delivery.

How the dose of gentamicin in milliliters (mls) was determined and administered. (80mgs = 2mls)

 All trimester one (TM1) animals :- (LGG, MGG, HGG) categories were given gentamicin doses from gestation day one to gestational day 21 (delivery).

 All trimester two (TM2) animals :- (LGG, MGG, HGG) categories were given gentamicin doses from gestation day 8 to gestational day 21 (delivery).

 All trimester three (TM3) animals :- (LGG, MGG, HGG) categories were given gentamicin doses from gestation day 15 to gestational day 21 (delivery).

Determination of fetal weights.

The expectant mothers were sacrificed on day 21 of gestation and the fetal weights were determined using a digital weighing scale, (Scout pro model® from Japan, serial no.B519923500) as shown in figure 1 below. The machine was calibrated to zero before measuring each fetus,

Statistical analysis

Data was expressed and analyzed using SPSS version 23.0 (SPSS Inc., Chicago, IL). One-way Analysis of Variance (ANOVA) followed by Tukey post hoc study for multiple comparison tests was done and results were expressed as mean ± standard error of mean (SEM) for all values.The results were considered to be significant at $p<0.05$.

Ethical clearance

Ethical clearance was sought from JKUAT Animal Ethicsl Committee (AEC) before initiation of the study

Statistical analysis of fetal weight.

The study sought to examine the effect of gentamicineon fetal rat weights. The drug was administered to a control group and a treatment group. The treatment group was divided into low dose gentamicin group (LGG), medium dose gentamicin group (MGG) and high dose gentamicin group (HGG). The groups were further categorized in three namely trimester T1, T2 and T3. The data obtained was analyzed using SPSS and Excel statistical software and results expressed as mean ± standard error (SEM).

In this study the three dose levels of gentamicin including, low, medium and high dose study groups were compared with the control against the varied periods of exposure trimester 1 (day 1 to birth), trimester 2 (day 8 to birth) and trimester 3 (day 15 to birth).

The study compared how the three dose levels (Low, medium and high) and control in the three trimesters (T1, T2 and T3), affected fetal weight. 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 were presented below.

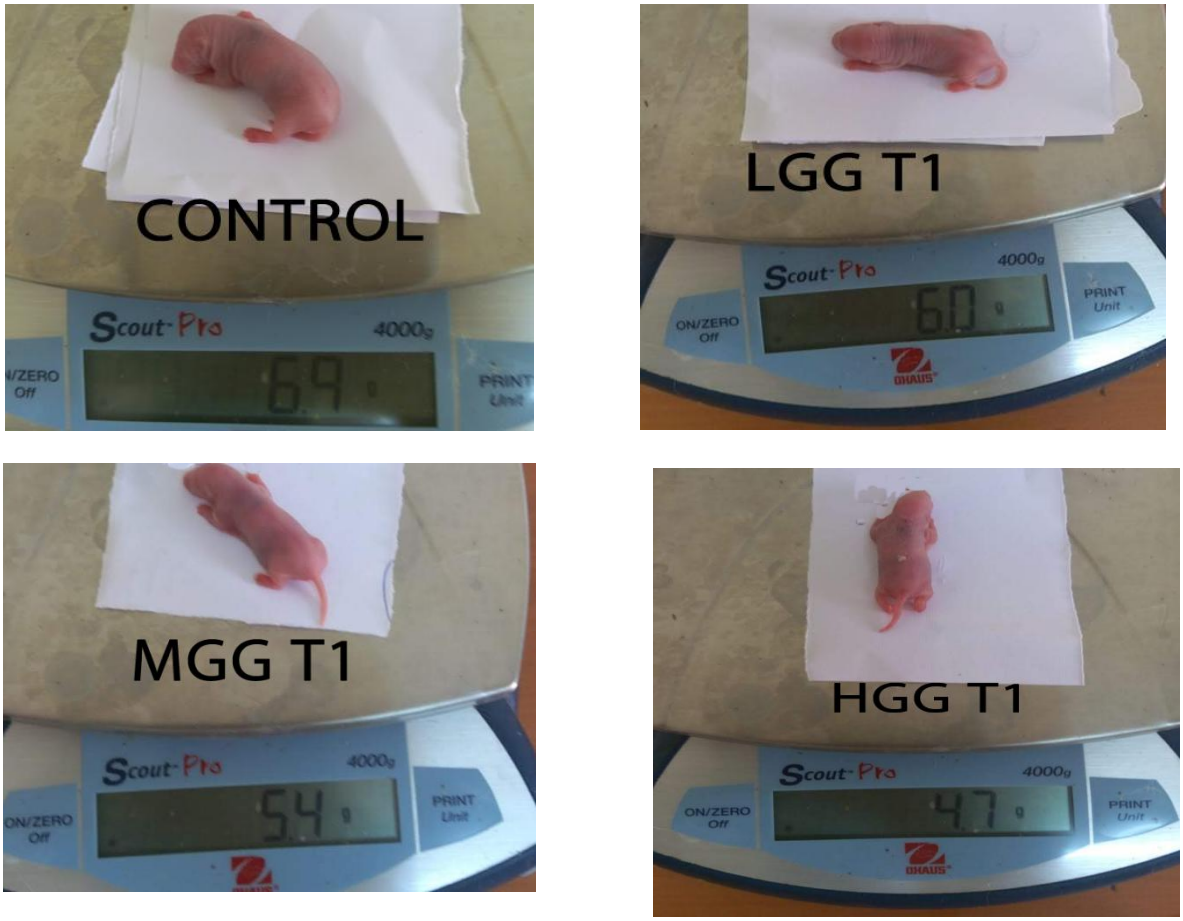

Figure 1: showing varied fetal weights of control, LGG (low gentamicin group), MGG (medium gentamicin group) and HGG (high gentamicine group)The figure above shows the varied weights of fetuses after giving varied doses of gentamicin to pregnant mothers during the first trimester. The control group fetus showing the highest weight in comparison with the other treatment group fetuses. However the high dose group fetuses had the least weight which demonstrates that gentamicin has the potential of interfering with the normal morphogenesis of fetal rats and affecting the overall weight of the fetus.

III. Results

The analysis has been performed dose wise and trimester wise as shown below.

Comparison of means in the different dose groups

Table 1: Comparison of fetal weight in the control, low, medium and high dosage groups in T1, T2 and T3

Variable	C	LGG	MGG	HGG	F-value	p-value
T1	5.65 ± 0.059a	5.42 ± 0.012b	5.46 ± 0.030b	5.08 ± 0.044c	34.447	0.0001
T2	5.65± 0.59a	5.57 ± 0.023a	5.59 ± 0.069a	4.98 ± 0.06b	31.914	0.0001
T3	5.65 ± 0.05a	5.64 ± 0.06a	5.37 ± 0.11ab	5.14 ± 0.04b	11.610	0.003

*NB: 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).*

The results in Table 1 indicated that in Trimester one (T1), fetal weight in the control group (5.65±0.059) was found to be significantly higher than that in the LGG group (5.42 ± 0.012), MGG (5.46 ± 0.030) and HGG (5.08 ± 0.044), F (3, 8) = 34.447, p = 0.0001. The weight in low dose and the medium dose was not statistically different from each other.

In Trimester two (T2), fetal weight in the HGG group (4.98 ± 0.06) was found to be significantly different (lower) from that in the control group (5.65±0.059), LGG group (5.57 ± 0.023) and MGG (5.59 ± 0.069); F (3, 8) = 31.914, p = 0.0001. Nevertheless the LGG, control and MGG group were not statistically significantly different from each other.

Finally, in Trimester 3, the results depicts that fetal weight in the control group (5.65±0.059) was found to be significantly higher than that in the HGG group (5.14 ± 0.04); F (3, 8) = 11.610, p = 0.003. Conversely, fetal weight in LGG (5.64 ± 0.06) and MGG (5.37 ± 0.11) were not significantly different from the control as shown by Tukey post hoc results.

Comparison of means in the different Trimester groups

This section examines significant differences of fetal weight among different trimester groups in LGG, MGG and HGG. The results are presented in Table 2.

Table 2: Comparison of fetal weight among different trimester groups in LGG, MGG and HGG

Variable	C	T1	T2	T3	F-value	p-value
LGG	5.65±0.059a	5.42±0.012b	5.57±0.023ab	5.65±0.059a	6.537	0.015
MGG	5.65 ±0.059a	5.46±0.030a	5.59±0.069a	5.37±0.114a	2.927	0.100
HGG	5.65±0.059a	5.08±0.044b	4.98±0.06b	5.14±0.036b	34.679	0.0001

*NB: 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).*

The results in Table 2 indicated that in the LGG group, fetal weight in the control group (5.65±0.059) was found to be significantly higher than that in the T1 group (5.42 ± 0.012); F (3, 8) = 6.537, p = 0.015. However, fetal weight in the LGG group was not significantly different from that of T2 (5.57±0.023) and T3 (5.65±0.059). This means that the time the drug in low dose was administered had an effect on fetal weight especially if administered during the first trimester of pregnancy.

In the MGG group, fetal weight in the control group (5.65±0.059), T1 group (5.46±0.030), T2 group (5.59±0.069) and T3 group (5.37±0.114) were found to be statistically insignificant; F (3, 8) = 2.927, p = 0.100. This means that the time of MGG administration did not significantly affect fetal weight.

Finally in the HGG group, the control group (5.65±0.059) was found to be statistically and significantly different from that in T1 group (5.08±0.044), T2 group (4.98±0.06) and T3 group (5.14±0.036); F (3, 8) = 34.679, p = 0.0001. However, The T1, T2 and T3 groups were not significantly different. This meant that that the time of HGG administration significantly affect fetal weight.

IV. Discussion

Gentamicin is classified as pregnancy category D drug which shows that it should not be administered during pregnancy[14,15,16].In this study, prenatal exposure to gentamicin has demonstrated to have an effect on fetal birth weights when administered during pregnancy. In this study, gentamicin administration at high, medium and low dosesestablished to have an effect on fetal weight as exhibited by reduced fetal birth weights(figure 1). Most of the effects were observed in fetuses that were exposed to gentamicin in the first and the second trimesters as shown in Table 1 and 2 above.Gentamicin has been shown to exert injurious effects that lead to weight reduction through multiple ways with influence on organs causing abnormal cell organization, apoptosis, as well as genetic expression.[1,17,18].

In the current study, on comparing the mean fetal birth weights at gestational day 21, the fetal birth weight was found to be significantly reduced in the high(p=0.0001),and low (p=0.015) gentamicin groups while in the medium group there was no statistically significant difference (p=0.1). This concurs with a study which was done by Tga T. *et al* (2013), where prenatal exposure to gentamicin was found to cause intrauterine growth retardation[4,19]. On the other hand, this contradicts with a study which was done by Sammie *et al* (2013)whoassociated teratogenic effects of gentamicin with birth weight and found that gentamycin had no effects on fetal weights. The study further demonstrated that single daily dosing was safe in pregnancy hence recommended for use during this period. In another study done by Gerald G et al (2006), it was found that, there is sufficient evidence to use gentamicine during pregnancy[20].At the same time the fetal body weights as shown in figure 1 above were found to be time dependent i.e. T1 (p=0.0001), T2 (p=0.0001) and T3 (p=0.003).

V. Conclusion

Prenatal gentamicin exposure is associated with reduced fetal birth weight which is a clear indicator of the injurious effects on fetal growth and developmental depending on the dose, duration and developmental stage of the embryo at exposure.

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Ethical Approval

I hereby affirm the experimental protocol that was approved by the Jomo Kenyatta University of Agriculture and Technology Animal ethical Committee (JKUAT AEC) was used. The animals were only used once. They were all sacrificed using humane end points at the end of the study.

References

[1]. Alarifi S, Al-doaiss A, Alkahtani S, Al-farraj SA, Al-eissa MS, Al-dahmash B, et al. Blood chemical changes and renal histological alterations induced by gentamicin in rats. Saudi J Biol Sci [Internet]. 2012;19(1):103–10. Available from: http://dx.doi.org/10.1016/j.sjbs.2011.11.002
[2]. Locksmith GJ, Chin A, Vu T, Shattuck KE, Hankins GD V. High Compared With Standard Gentamicin Dosing for Chorioamnionitis : A Comparison of Maternal and Fetal Serum Drug Levels. 2005;105(3):473–9.
[3]. Theiler R. NIH Public Access. 2017;(October 2008):0–9.
[4]. Liu P, Feng Y, Dong C, Yang D, Li B, Chen X, et al. Administration of BMSCs with Muscone in Rats with Gentamicin-Induced AKI Improves Their Therapeutic Efficacy. 2014;9(5).
[5]. Edition F, Pregnancy I. Medication Guidelines For Obstetrics and Gynaecology In Pregnancy and Lactation. 2016;2(November).
[6]. Samiee-zafarghandy S, Anker JN Van Den. Nephrotoxic effects of aminoglycosides on the developing kidney. 2013;2(2):1–7.
[7]. Cui J, Bai X, Sun X, Cai G, Hong Q, Ding R, et al. Rapamycin protects against gentamicin-induced acute kidney injury via autophagy in mini-pig models. Nat Publ Gr [Internet]. 2015;(December 2014):1–17. Available from: http://dx.doi.org/10.1038/srep11256
[8]. Virani S, Bhatt S, Saini M, Kk S. ALOE VERA ATTENUATES GENTAMICIN-INDUCED NEPHROTOXICITY IN WISTAR ALBINO RATS : HISTOPATHOLOGICAL AND BIOCHEMICAL CHANGES. 2016;9(1).
[9]. Mouedden M El, Laurent G, Tulkens PM, Che- AA, Mouedden EL, Al ET. Gentamicin-Induced Apoptosis in Renal Cell Lines and Embryonic Rat Fibroblasts. 2000;239:229–39.
[10]. Ahn J, You SJ, Lee Y, Oh S, Ahn S, Kim S, et al. Hypoxia-Inducible Factor Activation Protects the Kidney from Gentamicin-Induced Acute Injury. 2012;7(11):1–9.
[11]. A WN, Z WM. Sample Size Calculation in Animal Studies Using Resource Equation Approach. 2017;24(5):101–5.
[12]. Reagan-shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. Dose Transl from Anim to Hum Stud. 2007;659–61.
[13]. Article R. A simple practice guide for dose conversion between animals and human. 2016;27–31.
[14]. Patil AS, Sheng JS, Dotters-katz SK, Schmoll MS, Onslow ML. Principles of Anti-infective Dosing in Pregnancy. Clin Ther [Internet]. 2016;38(9):2006–15. Available from: http://dx.doi.org/10.1016/j.clinthera.2016.08.005
[15]. Fresenius Kabi USA. Lake Zurich IL 60047. Fresenius Kabi USA Lake Zurich 60047. 2013;
[16]. Practice C. American Gastroenterological Association Institute Technical Review on the Use of Gastrointestinal Medications in Pregnancy. 2006;283–311.
[17]. Mahmoud O. The effect of Lcarnitine on gentamicin-induced nephrotoxicity and associated anemia in adult male albino rats . 2018;(December).
[18]. Mallie J, Coulon G, Billerey C, Faucourt A. In utero aminoglycosides-induced nephrotoxicity in rat neonates. 1988;33:36–44.
[19]. Tga T, Information P. Prescribing medicines in pregnancy database 3. http:/www.tga.gov.au/hp/medicines-pregnancy.htm. 2013;(April 2012):1–41.
[20]. Nahum GG. What Is and Is Not Known About Teratogenic and Toxic Risks. 2006;107(5).

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