HISTOSTEREOLOGICAL TERATOGENIC EFFECTS OF GENTAMICIN ON FETAL KIDNEYS IN ALBINO RATS (RATTUS *NORVEGICUS*)

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Histostereological Teratogenic Effects of Gentamicin on the Fetal Kidneys in Albino Rats (Rattus *Norvegicus*)

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

I wish to dedicate this thesis to my wife Risper Angelyne and my children Nobel and Naureen for their moral support and perseverance during this thesis development process.

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LIST OF ABBREVIATION & ACRYONS

ANOVA	Analysis of variance
CRL	Crown Lump Length
C	Control
CE	Coefficient of error
CG	Control group
COHES	Collage of Health Sciences
CRL	Crown rump length
GFR	Glomerular filtration rate
GM	Gentamicin
GD	Gestation day
gms	grams
hESCs	Human embryonic stem cells
HDGG	High dose gentamicin group
H & E	Hematoxylin and Eosin
HP	Hematoxylin phloxin
JKUAT	Jomo Kenyatta University of Agriculture and Technology
kg/bwt	kilogram per bodyweight

LD_{50}	Lethal dose
LDGG	Low dose gentamicin group
MAF	Modified aldehyde fucshin
m-RNA	Messanger Ribo-nucleic acid
MET	Mesenchymal to epithelial transformation
MDGG	Medium dose gentamicin group
Mgs	milligrams
MM	Metanephric mesenchyme
r-RNA	Ribosamal Ribo-nucleic acid
t-RNA	Transfer Ribo-nucleic acid
S_1	Segment one
S_2	Segment two
S_3	Segment three
SEM	Standard error of the mean
SAFARI	Small Animal Facility for Research and Innovation
SPSS	Statistical package for social sciences
TM	Trimester
TM_1	Trimester one

TM_2	Trimester two
TM_3	Trimester three
UB	Ureteric bud
WIM	Water immersion method

DEFINITION OF TERMS

Histomophological The use of microscope to study the cellular structures, distribution, cellular patterning and interconnecting stromal tissues in the extracellular matrices.

Histostereology Is the study of microscopic anatomy of the cell and tissue in three dimensional quantification of two dimension cross section to extract quantitative information

Histo-morphometry This is a three-dimensional measurement of microscopic structures important to obtain reliable quantitative data that enables calculation of volumes and volume ratio, the area of samples, the number of particles per unit volume, particle size, unit volume, length and weight.

ABSTRACT

The nephroteratogenic effects of gentamicin on developing fetal kidneys when prenatally exposed has been a subject of controversy with some studies showing that gentamicin is teratogenic while others showing it is not. At the same time information on whether or not the teratogenic effects of gentamicin are time and dose dependent is generally lacking. This current study therefore aimed to evaluate the histomophological and steriological effects of gentamicin when prenatally exposed in varying doses and at different window periods. The study was carried out in SAFARI animal house of JKUAT and a static-group controlled-experimental study design was adopted. A total of 30 Albino rats dams weighing between 200-250grams (aged 11/2 months) from a pure colony were used in the study. These 30 dams were randomly assigned in to two broad study groups of 3 rats in control and 27 rats in experimental. To evaluate whether the gentamicin nephro-teratogenecity is dose dependant, the 27 rats in the experimental group were further subdivided into three study groups (of 9 rats each) of Low [LDGG-19g/kg/bwt], medium [MDGG-28g/kg/bw], and High gentamicin dose group [HDGG-37g/kg/bw]. To further evaluate whether gentamicin nephro-teratogenicity is time dependant, the 9 rats in each of the three dose groups of low, medium and high gentamicin were further sub-divided into three sub groups according to the time of exposure as follows, 3 rats for TM_1, 3 rats for TM_2, and 3 rats for TM_3. All rats received standard rodent diet and water adlibitum while those rats in experimental category received gentamicin as per their study groups. All rats were humanly sacrificed at GD_{20} and then 3 fetuses with the lowest, median and highest weight from each mother selected and their kidneys harvested, weighed and processed for histo-morphological and stereological analysis. Data was collected using structured coded tally sheets, entered and stored in excel and analysed by SPSS version 23.0. One-way Analysis of Variance (ANOVA), followed by Tukey's post hoc multiple comparison tests were done and results expressed as mean ± standard error of the mean (SEM) for all values. All results whose comparatives P - value was less than 0.05 were considered to be statistically significant. This study established that there was no significant reduction in the mean maternal weight gain across doses and trimesters ($P>0.05$) but there was reduction in all fetal growth parameters in all the gentamicin treated groups especially in 1st and 2nd trimester at LDGG, MDGG and HDGG ($P<0.05$). Histologically it was clearly demonstrated by the marked increase in thicknesses of all cortical and medullary layers of the fetal kidneys in treatment group especially in 1st and 2nd trimester across all doses. Steriologically when a comparative mean kidney length was done across the three trimesters TM_1, TM_2 and TM_3, it was depicted that at TM_1 the mean fetal kidney length was highest in the HDGG group at 0.12±0.0121 followed by MDGG at 0.216±0.008 and LDGG at 0.297±0.0033. This was found to be statistically higher as compared with the control ($p<0.05$) at 0.395±0.005. This teratogenicity was also observed to be dose dependant with the high gentamicin doses of 37mgs/Kg/Bwt per day being the most critical gentamicin teratogenic dose. The study recommends that gentamicin usage during pregnancy should be eluded unless established that the benefit to the mother outweighs the anticipated teratogenic outcomes to the fetus. Further studies need to be carried in higher primates.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Gentamicin, an aminoglycoside broad spectrum antibiotic, is widely used in treatment of both gram positive and negative bacterial infections. It has molecular weight of 477.6g/mole with four major components (Cl, C1a, C2 and C2a) and other minor components including A, A1, A2, A3, C2b, A4, B, B1 and X2 (Georgina *et al.*, 2015). Due to its low molecular weight, it crosses the placenta to reach the fetal circulation and eventually the fetal kidneys where it exacts its teratogenic effects (Samiee-zafarghandy and Anker, 2013). Despite numerous studies demonstrating gentamicin fetal kidney teratogenicity, there is paucity limited data on the histostereological changes on the developing fetal kidney upon exposure to gentamicin at varied doses and at different window periods which forms the basis of this study.

The use of gentamicin during pregnancy has remained a subject of controversy to-date with some authors showing that gentamicin use during pregnancy causes nephrotoxicity in the fetus while others showing that is has some pertinent advantage in treatment of maternal conditions that may harm the fetus (Pacifici and Marchini, 2017). Exposure to gentamicin in-utero has been shown not to have any effect on the litter size of Albino rats (Razieh *et al.*, 2019). However, the birth weight has been shown to be low (Devkota *et al*, 2016and Pacifici and Marchini, 2017). Inasmuch as this is known about gentamicin and the fetus, there are no documented histostereological effects of gentamicin on the fetal kidney (Samiee-zafarghandy and Anker, 2013). Moreover it is still controversial if gentamicin accumulates in the renal cortex where it can induce renal morphological changes and an overall syndrome that may be similar in humans and Albino rats (Askenazi *et al.*, 2016).

In-utero nephrotoxicity arising from gentamicin is thought to occur through its ability to bind weakly to plasma proteins which allows it to be freely cleared into the glomerular filtrate as the major route of excretion leading to high tubular concentrations (Hennig and Staatz, 2017). Serum half-life of gentamicin is inversely related to glomerular filtration rate (GFR), furthermore, the drug intensely binds to specific apical brush border receptors which leads to high accumulation of the drug within the kidney tubules thus tubular epithelial cell cytotoxicity with subsequent tubular damage (Girardi *et al.*, 2015 and Hamdi *et al.*, 2018). However the particular representation of the pathophysiological and molecular apparatuses underlying gentamicin nephrotoxicity at the organism all the way to molecular levels has been generally found in animal and cellular experimental models (Bashandy *et al.*, 2016).

Nephrogenesis begins from the 10^{th} day of intra-uterine life and carries on throughout the gestational period, thus gentamicin administration may impact on its development (Mcwilliam *et al.*, 2017). Studies have shown that in-utero exposure to gentamicin results in reduced fetal kidney size with a reduction in fetal weight as well (Pacifici and Marchini, 2017). Despite the reduction in fetal kidney weight upon in-utero exposure to gentamicin, experimental studies in animals have shown no weight nor morphological differences between the right and the left kidneys (Seely, 2017). Histologically the drug can cause changes in the appearance of the glomeruli showing enlarged, congested glomeruli and mesangial cells proliferation (Devkota *et al.*, 2016). Gentamicin is believed to cause the proximal convoluted tubules to become cystic and dilated. Gentamicin can cause infiltration of inflammatory cells and congestion of blood vessels in the kidneys (Glerin and Volenddin, 2016).

1.2 Problem statement

Structural abnormalities of the kidney have been on the rise recently (Sutherland *et al.,* 2011), and they have been shown to significantly contribute to the global burden of renal failure which had a global case mortality of 10 million people in the year 2017 (Wenhimet *et al*, 2017). Despite this rising burden of structural abnormalities of the kidney, specific causes have not been well documented (De-Rechter *et al*., 2017) and it has been postulated that inadvertent in-utero exposure to nephrotoxic drugs among them being aminoglycoside antibiotics could be a cause (Chilwant and Muglikar, 2016). Gentamicin, a common aminoglycoside has been used for long in the treatment of various infections in different populations (Dontabhaktuni *et al.,* 2016). This includes pregnant women albeit lots of controversy on its renal teratogenic profile (Karger, 2017). It is in this context that this study was anchored where it sought to access the teratogenic histostereological effects of gentamicin on the kidney in Albino rats.

1.3 Justification

To curb the rising cases of renal conditions of unknown origin, the teratogenicity resulting from the pre-natal exposure to drugs that interfere with kidney development like gentamicin cannot be over emphasized. Hence accessibility to information on the in-utero teratogenic disruptions from antibiotics like gentamicin may present a vital conjecturer in some of the structural changes inaugurated in the developing fetal kidneys. This will consequently update causes of some of the renal and kidney conditions detected in the adulthood in recent times and whose origins are yet to be known. Nonetheless there is scarcity of data on the histoquantitative teratogenic effects of gentamicin when it is prenatally exposed in varied doses and at different window periods. The findings of the study will also help in resolving contravesy of use or non-use of gentamicin during pregnancy.

1.4 Significance of the study

Data obtained from this study would be helpful in setting a platform for directing clinicians and future researches on the use of gentamicin during pregnancy. It will also be used to consider the maternal benefits for usage while providing safety to the fetus from the related teratogenic effects on the developing fetal kidney. In addition, the morphological features observed in the histological sections of a developing fetal kidney as a result of in-utero exposure to different doses of gentamicin at different window periods would help in excluding some of the causes of increasing adult kidney conditions like polycystic kidney disease, hydro-nephrosis, renal agenesis among others which are on the increase worldwide. Lastly, the data obtained from this study will also be useful in redirecting future teratogenic studies on gentamicin as well as in readdressing the modification of the treatment procedures in terms of dosages and the appropriate gestational periods and when to apply it in management in some conditions during pregnancy following findings from non-human primates.

1.5 Objectives

1.5.1 Broad objective

To evaluate the histostereological effects of in-utero exposure to varied doses of gentamicin on the development of the fetal kidneys in albino rats (Rattus *norvegicus*).

1.5.2 Specific objectives

1. To establish the teratogenic pregnancy outcomes of the pre-natal exposure to varied doses of gentamicin.
2. To evaluate the histomophological changes that occur in the developing fetal kidneys upon pre-natal exposure to varied doses of gentamicin.

3. To evaluate the histosteriological changes that occur on the developing fetal kidneys upon pre-natal exposure to varied doses of gentamicin.
4. To establish the most vulnerable period of gentamicin teratogenicity to the developing fetal kidneys in-utero
5. To establish the most critical dose of gentamicin teratogenicity on the developing fetal kidneys.

1.6 Hypothesis (H_0)

In-utero exposure to varied doses of gentamicin do not impair fetal kidney development neither does gentamicin teratogenic effects of developing fetal kidneys has any relationship to dose nor time of exposure.

CHAPTER TWO

LITERATURE REVIEW

2.1 Gentamicin classification, structure and mode of action

Aminoglycosides are antibiotics that are widely used in treatment of bacterial infections by preventing bacterial from producing toxins or inhibit their multiplication by altering protein synthesis (Georgina *et al.,* 2015). Regardless of these some strains of bacteria might be found to be resistant to some aminoglycosides (Bashandy *et al.,* 2016). In such situations gentamicin is used because of its wide coverage. Gentamicin is mainly used for the management of infections caused by gram-negative bacteria (Krause *et al.*, 2016). However the use of gentamicin is reported to cause nephrotoxicity when given especially during fetal periods (Dontabhaktuni *et al.,* 2016).

Aminoglycosides demonstrates bactericidal action against many gram-negative aerobic and facultative anaerobic bacilli mainly dependent on its concentration, but is has little or no effect against gram-negative anaerobes and most of gram-positive bacteria (Chilwant and Muglikar, 2012). Gentamicin requires short contact time, and is very effective against vulnerable bacterial populations that multiply quickly (Negrette-Guzmán *et al.,* 2013). These events are facilitated by a primary mode of action by hindering protein synthesis via the supplemental mechanisms which are designed for some particular agents. Hindrance of protein production is enabled by gentamicin energy-dependent, occasionally permanent attachment, to the cytosol via membrane-related ribosomes of the bacterial (Samiee-zafarghandy and Anker, 2013). This antibiotic crosses bacterial cell walls in gram-negative bacteria and cell membranes, through energy dependent transport (Fuchs *et al.,* 2016).

In addition explicit steps involved in protein synthesis, may vary among specific aminoglycoside agents, as can their affinity and degree of attachment (Hennig and Staatz, 2017). Precisely, binding impairs proofreading of translated part, resulting to

misinterpretation of the messenger RNA, initial termination, or both, and so leads to erroneousness of the transformed protein product (Royal College of Physicians of Ireland, 2016). Subsets of abnormal proteins that are combined into the bacterial cell membrane may then account to changes in its permeability and then to further facilitation of gentamicin transport (Mahmoud, 2018). Inhibition of translocation of ribosomes for instance movement of the peptidyl-tRNA from the P- to the A-site and vice versa can be witnessed in Aminoglycosides (Georgina *et al.,* 2015).

2.2. The teratogenic induction mechanism of gentamicin on fetal tissues

Teratogenic effects of aminoglycosides on embryogenesis of fetal tissues has been demonstrated through various studies (Samiee-zafarghandy *et al.,* 2013). which clearly demonstrated that during controlled cell differentiation of the neural and hepatic fate, significant cell death is noted through the activation of caspase flow (Randjelovi *et al.,* 2017). Cellular toxicity of gentamicin has been reported in animal models (Fuchs *et al.,* 2016). In humans, therapeutic doses of gentamicin have demonstrated to cause nephrotoxicity in neonates whose mothers have been treated with this antibiotic (Ahn *et al.,* 2012 and Humes *et al.,* 1988).

High therapeutic doses of gentamicin show widespread inflammation and necrosis of proximal kidney tubular cells (Seely, 2017 and Isoherranen *et al*., 2000). Although aminoglycosides cross maternal placental blood barrier, the effect of maternal use of these antibiotics on early embryonic development and disruption of the fetal kidney histo-architecture if any is still not well known. Since gentamicin crosses the maternal placental blood barrier, it may result to adverse effects on the developing organs of the fetus (Mcwilliam *et al.,* 2017 and Salice *et al.,* 2001). Therefore it is important to understand the effect of routine use aminoglycosides on proliferation and their differentiation towards neural and hepatic fate keeping in mind that, this will facilitate the understanding of other side effects of these gentamicin in early human embryogenesis (Quiros *et al.,* 2011).

2.3. Comparative fetal kidney morphogenesis process between humans and rats

The comparative fetal kidney morphogenesis in humans and rats demonstrates many similarities in terms of morphogenic structures across their entire gestational periods despite the differences in their species and gestational ages (Sutherland *et al.*, 2011b). During early development, all mammals share a common chronological and structural process which arises from the tri-lamina germ disc. The mesoderm which is one of the embryonic germ layers, has the paraxial, intermediate and the lateral plate mesoderm (Solhaug *et al.*, 2020). All the three stages of kidney development which are:- the pronephros, mesonephros, and metanephros or the adult kidney occur in the intermediate mesoderm (Norman *et al*., 2019). The pronephros and mesonephros are transitional structures in mammals which disintegrates allowing the metanephric kidney to develop into the functional adult kidney (Isoherranen and Lavy, 2000).

The pronephros and its duct is the very initial stage of kidney development and appears around gestational day 22 in humans and gestational day 11 in the rat (Little and Combes, 2019). The pronephros is nonfunctional but important because as the pronephros degenerates the caudal segment of the pronephric duct remains and ultimately becomes the Wolffian duct which induces the mesonephros to develop (Rosenblum *et al*., 2017). The mesonephric nephrons are formed but later regress while the Wolffian duct elongates caudally to join the urogenital sinus (Rosenblum *et al*., 2017). The urogenital sinus eventually differentiates into the urinary bladder. During mesonephric stage, a diverticulum off the Wolffian duct becomes the ureteric bud (Phillips *et al.*, 2016). While the mesonephros disappears, the metanephric kidney starts to develop by having an outgrowth and branching of the ureteric bud into the metanephric mesenchyme initiating nephrogenesis process (Sutherland *et al.*, 2011). As the ureteric bud contacts the metanephric mesenchyme it continues to branch, each branch representing a future nephron.

Furthermore, the ureteric bud has an important function in controlling kidney development as well as determining the numbers of nephrons per kidney. This ureteric bud also donates to the formation of the collecting ducts, renal pelvis, and ureters (Rosenblum *et al*., 2017). Nephrogenesis commences in the fetus and is completed in humans before birth but continues after delivery in the rat up to the 10^{th} postnatal day (Solhaug *et al.*, 2020 and Yaris *et al.*, 2004). The formation of nephrons involves tightly controlled genetic conduits which result in the transformation of the metanephric mesenchyme to epithelial lined structures which further undergoes configurational structural changes to form the nephrons. This entire process is referred to as the mesenchymal epithelial transformation. (El-Kotby *et al*., 2020)

2.4. Comparative morphological features of fetal kidneys between humans and rats

The comparative gross characteristics of albino rat and those of humans show major similarities. The kidneys are paired bean-shaped organs lying retroperitoneal beside the posterior body wall on each side of the vertebral column (Yoldas and Dayan, 2014). They are not attached to the body wall, but are held loosely by adipose tissue. The right kidney is slightly bigger and located more ventral in both rat and human. The anterior pole of the right kidney is usually at the level of the 12th rib and that of the left kidney is marginally lower (Seely, 2017). There is a sex difference, the male kidney being consistently heavier and larger throughout life (Seely, 2017and Adrian *et al*., 2004). The kidney is dorsoventrally flattened with a wide convex lateral and a short concave on the medial border. The concavity of the hilum where blood vessels and the ureter enter the kidney (El-bestawy *et al.,* 2017 and Gilbert *et al.,* 1986). Two main layers are the cortex and medulla, can be visualized without the aid of a lens if the kidney is bisected in both rat and human. The cortex trails the shape of the lateral convex border, and the medulla is like a broad pyramid with its convex base close-fitting against the concave surface of the cortex (Gilbert, 2014).

Nephrons are the basic functional units of the kidney held together by a gentle, richly vascular connective tissue and covered in a thin connective tissue capsule. Anterior surface

is covered by a peritoneum (Rosenblum *et al*., 2017). Every nephron is a tubule with a broadened end, Bowman's capsule, surrounded by a tuft of blood capillaries and the glomerulus. The tubule has a proximal and distal convoluted portions with a straight section, Henle's loop, crammed between (Little and Combes, 2019). The nephron is architecturally similar to that of man, as is the blood-vessel network (Yoldas and Dayan, 2014and Salice *et al.*, 2001). The outer zone of the cortex is dominantly made up of glomeruli, bowman's capsule and convoluted tubes. The straight tubules of Henle's loops gives a rayed appearance to the inner zone of the cortex. The medulla has a striated appearance, composed primarily of straight collecting tubes uniting toward the papilla (Singroha *et al.*, 2013). The granular cells in the walls of the afferent glomerular arteries can be easily demonstrated in the rat and humans (Sutherland *et al.,* 2011a). There is a sex variance in the comparative number of Bowman's capsules that have parietal linings of cuboidal fairly than squamous cells (Ali and Abood, 2019).

2.5. Histo-morphological effects of aminoglycosides on the developing fetal kidneys

Aminoglycosides results to tubular cell necrosis, which is basically limited to the proximal convoluted tubule and pars recta of the fetal kidneys (Luyckx *et al*., 2005). The initial lesion is observed by an electron microscope, where an upsurge in the size and number of secondary lysosomes (Luo *et al.,* 2016). Lysosomal alterations due to gentamicin administration does not necessarily contribute directly to renal cell necrosis and organ failure. However, these morphologic alterations are explicit but not limited to aminoglycoside antibiotic toxicity and can occur in other tissues by altering focal cell injury driven by a variety of toxins (Darmstadt *et al.,* 2008). Succeeding lysosomal injury, damage to renal proximal tubule cells continues, as emulated by mitochondrial inflammation and consequential loss of brush-border membranes in fetal kidneys (Pacifici and Marchini, 2017). Cell injury to the proximal tubule compares reasonably well with the degeneration excretory function of the renal system. The pattern of extensive involvement of first and the second segments of proximal tubules in gentamicin injury contrasts with the markedly

greater contribution of the third segment sections in heavy-metal nephrotoxicity (Dontabhaktuni *et al.,* 2016).

The restoration of proximal tubule cells is initiated during the stage of patchy necrosis. The comparatively undifferentiated, immature, regenerating cells subsequently begin to regain its normal height and structure (Luyckx *et al*., 2005). Local interstitial inflammatory infiltrates seen in the cortex during this stage and can become more conspicuous with time (Liu *et al.,* 2014). Ultimately, the largest areas of the affected kidney regain normal architecture and function, but residual scars having collapsed, atrophied tubules can grow in the cortex (Luyckx *et al*., 2005). Aminoglycoside toxicity to the kidney is occasioned by various renal functional changes. The renal excretory failure with near termination of effective glomerular filtration rate is but the final appearance of this clinical syndrome (Mahmoud, 2018). The initial renal expression of aminoglycoside toxicity is enzyme dependent (Samiee-zafarghandy and Anker, 2013).

Within 24 hours after a single therapeutic dose of gentamicin, the urinary excretion of a variety of brush border membrane enzymes raises then slowly rises as therapy continues (Mallie *et al.,* 1988). Continous urinary excretion for lysosomal enzymes can also be witnessed as risk factors in aminoglycoside nephrotoxicity which include: a). Dose and duration of drug treatment b). Recent aminoglycoside treatment c). Preexisting renal inadequacy d). Preexisting hepatic disease e). Magnesium depletion Proximal tubular transport processes also worsen during aminoglycoside toxicity (Luo *et al.,* 2016). This variations results in glycosuria, tubular proteinuria, aminoaciduria, and transport defects constant with a Fanconi-like syndrome (Chilwant and Muglikar, 2012). These discriminatory effects on the renal handling of intracellular cations reflects a specific action of aminoglycosides. This actions includeand alteration of plasma membrane transport of, or permeability to, these specific ions (Samiee-Zafarghandy and Anker, 2013).

2.6. The histo-steriological effects of aminoglycosides class of antibiotics on developing fetal kidneys

Some earlier histo-stereological studies have shown that quantitative effects to the fetal kidney upon use of gentamicin which displays varying results, illustrating the kind of injuries stimulated to the developing fetal kidneys (Ali and Abood, 2019). Other studies demonstrated significant reduction in numerical densities of nephrons in the cortex and medullar resulting to dilatation of proximal convoluted tubules upon administration of gentamicin (Hejazi *et al.,* 2018).

Another study on comparative histo-quantitative effects of amikacin, tobramicine and neomicine medicines which have similar mode of action like gentamicin exhibited variable outcomes on the developing fetal kidneys following in-utero exposure (Pessoa *et al.,* 2009 and Hedaiaty *et al.,* 2016). These effects includes noticeable cellular necrosis and death that leads to reduction in the total number of nephrons (Negrette-Guzmán *et al.,* 2013). A comparative study on quantitative effects of amikacin and tobramicin on fetal kidney demonstrated reduced mean values as regards to body weight, crown lump length and bi-parietal diameter in treatment groups as compared with the control (Samiee-zafarghandy and Anker, 2013 and Georgina *et al.,* 2015).

2.7. The patterns of fetal kidney teratogenicity in terms of dose and time of exposure

Earlier studies done on other aminoglycoside antibiotics with the same effects as gentamicin demonstrates that fetal kidney teratogenic effects upon in-utero administration depends on the time of exposure (Pacifici *et al*., 2017). Also aminoglycoside antibiotics has a potential to affect fetal development throughout the gestation period. The effects of in-utero exposure to tobramicin, has shown that the pattern of exposure in causing these fetal organs like the kidney anomalies varies, with most of the drugs resulting to structural malformations in the first trimester, a period that relates to the embryonic phase during which major organs

develops (Fresenius *et al*. (2013). Hence gentamicin is bound to cause more teratogenic effects to fetal tissues when given in multiple doses and over a prolonged period of time during fetal periods (Negrette-Guzmán *et al.,* 2013).

Other previous studies went further and indicated that aminoglycoside drugs issued as monotherapy are relatively safe as compared with polytherapy, with some of the antibiotic drugs having more teratogenic effects as compared with others (Georgina *et al*, 2015and Samiee-zafarghandy and Anker, 2013and Mingeot-Leclercq and Tulkens, 1999) stated that monotherapy doubles the risk of malformations while polytherapy triples it. Patil *et al.,* (2010) reported that the risk of major congenital malformations is influenced not only by type of antibiotic drug, but also by dose and other variables, which should be taken into account in the management of bacterial infections in women of childbearing potential. Similarly, a study to find out the aminoglycosides associated with high risk of teratogenicity, a study by Dontabhaktuni *et al.,* (2016), indicated that older medicines such as streptomycin and neomicine are associated with a higher risk of major malformations than newer aminoglycosides such as gentamicin and amikacin (Patil *et al.,* 2010).

2.8 Histoquantitative changes occurring in the developing fetal kidneys of treatment groups with varying doses of gentamicin.

The histological study of kidney after exposure of aminoglycosides demonstrates an abnormal a pattern similar to the one that can be seen in the proximal tubules after nephrotoxicants such as gentamicin have been used (Singroha *et al.,* 2013 and Amin *et al.,* 2004). Cellular damage and kidney dysfunction occur due to gentamicin metabolites that are excreted from kidney, the toxic effects of several chemical substances by one or more common pathogenic mechanism can cause nephrotoxicity (Alestestm *et al.,* 2016). The cytotoxic form of gentamicin involved formation of free radicals. Moreover, it increases renal lipid peroxidation, which may explain the nephrotoxicity (Saleh *et al.,* 2016).

2.9 The most vulnerable period and dose of gentamicin teratogenicity on the developing fetal kidneys.

Nephrogenesis usually begins during the second trimester (day 10) in rats, where it progresses through to 10 days post-natal. The kidneys then attains morphological and functional maturity in about 11 days post-natal when the total number of nephrons are seen (Mahmoud, 2018). So far there is little known about the most vulnerable period, since no sufficient data to support the most vulnerable period. Initial doses of gentamicin is normally calculated from body weight (3.5 to 7 mg/kg/dose) or from body surface area (60-70 mg/m2/dose) (Tiengryga and Ihemins, 2013). In the practice of medicine the maximum total daily dose that can be prescribed is 100mg in two divided doses while doses above this are considered to be very toxic and teratogenic. (Bello and Chika, 2009) The optimum peak serum levels of gentamicin is generally considered to be 8 to 12mg/L 30 minutes after finishing an intravenous administration. Hence nephrotoxicity can results from a small but significant proportion of the prescribed dose of gentamicin being retained in the epithelial cells of the proximal tubules (Samiee-zafarghandy and Anker, 2013).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was carried out at the Small Animal Facility for Research and Innovation (SAFARI) animal house base at Jomo Kenyatta University of Agriculture and Technology (JKUAT) Juja, human anatomy department and histology laboratories, in the College of Health Sciences (COHES) of JKUAT.

3.2 Study design in carrying out the experiment.

A laboratory static-group controlled-experimental study design was adopted.

3.3 Study sample or subject:

A total of 30 nulliparous albino rats dams weighing between 200-250grams of the species Rattus *norvegicus* were used as the animal experimental model in this study. The rats were obtained from a pure colony due to the following facts (a) They have low incidence of naturally occurring congenital defects, (b) have a short gestational span, (c) relatively large litter size, (d) low maintenance cost of the animals and, (e) and considerable amount of the reproductive data on the rat is already available (Chernoff, 1977). Albino rats have red eyes and white fur in appearance (Pritchett and Corning 2016). They become sexually mature at about 4-5 weeks in females and at 45-48 postnatal dates in males. They live for an average of average 3 years (Pallav Sengupta, 2013). The gestation period in females that is estimated to be between 21 to 23 days during which the fetuses are viable, with 3 trimesters, trimester one being the first 7 days after conception, second trimester from day 7-14 and third trimester from day 14 to day 21. The usual litter size is 6 to 12 pups. Adult female weighs (250 to 400 grams

and male rats weighs (350 to 600 grams), and male rats are usually larger than females in appearance (Pallav Sengupta, 2013). In this study, fifteen males were used since every two females were allocated one male.

3.4 Sampling method

The sample size was determined in the following levels

3.4.1. First level and acquiring the experimental dams

In this level, resource equation method was applied where 30 dams were acquired (Arifin *et al.*, 2017). The Resource equation formula which is E= is the Total number of Animals- the Total number of groups (E=TA-TG). The measured value 'E' which signifies the degree of freedom for analysis of variance (ANOVA) based on a projected sample size value ('E') should lie within 10 and 20 animals according to this equation. Therefore, a figure less than 10 necessitates addition of more animals which increases the probability of getting significant results while a value more than 20 has been proved to increase the cost of the study without necessarily increasing the significance of the results in the study (Charan and Kantharia, 2013).

E= The Total number of Animals-The Total number of groups. Total number of groups is=10 whereas the total number of animal is = 30. E therefore is 30-10 which is 20

3.4.2. Second level: acquiring the fetuses to be used in the study

In the level, all the fetuses per each dam were weighed and organized in an ordinal sequence as per their weights. Objectively, three fetuses from each rat that had the highest, median and lowest weights were selected for stereology and histo-morphological evaluation by eliminating biasness, making a total sample size of 90 fetuses (i.e. 3 fetuses from each of

the 30 study equals 90 fetuses). The remaining fetuses were preserved in Zenker's solution for future use in case of any mis-ups.

3.5 Grouping of dams

The 30 dams used in the study were randomly assigned to either three rats as the control and 27 in the experimental category. To evaluate whether the gentamicin nephro-teratogenecity is dose dependant, the 27 rats in the experimental group were further subdivided into three study groups (of 9 rats each) of Low [LDGG], medium [MDGG], and High gentamicin dose group [HDGG]. To further evaluate whether gentamicin nephro-teratogenicity is time dependant, the 9 rats in each of the three dose groups were further sub-divided into three sub groups according to the time of exposure as follows 3 rats for TM_1, 3 rats for TM_2, and 3 rats for TM_3 (Figure 3.1).

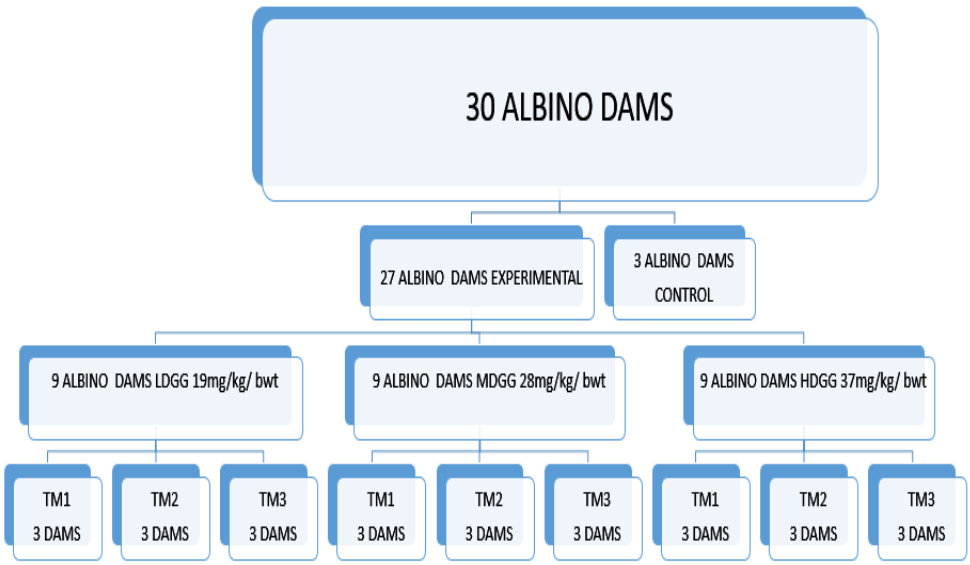

Figure 3. 1: Grouping of animals in both control and experimental groups according to doses of exposure as LDGG, MDGG and HDGG and according to trimester of exposure as TM_1, TM_2 and TM_3.

3.6 Selection criteria

3.6.1 Inclusion criteria

i) The healthy rats
ii) Rats that conceived
iii) All live fetuses

3.6.2 Exclusion criteria

a) All animals with negative pregnancy after several overnight exposures to male rats
b) All animals that fell sick in the process of gentamicin administration
c) All fetuses of sick mothers

3.7 Feeding of rats

All rats were fed on a standard rodent pellets from Unga feeds limited located in Thika, and water ad libitum. Feeding was done every morning at 0830 hrs. Acclimatization was allowed for a period of seven days. The animals in the control and in the experimental categories were fed as follows:-

(i) The control sub group received standard diet as determined by the academy of nutrition and dietetics containing by 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 water ad libitum for the whole of the gestation period day 1-20. The mothers were then sacrificed on 20^{th} day of gestation

(ii) The experimental groups: The animals in the experimental were similarly fed on standard rodent pallets as above in the control and water ad-libitum but in addition the received gentamicin injections based on their doses of low, medium and High (LDGG, MDGG, HDGG) as well as according to the trimester of exposure (TM_1,

TM_2 and TM_3) as follows: All rats received a standard diet, water ad-libitum and a constant daily injection of gentamicin 5mg/kg bwt (TM_1), 10mg/kg bwt (TM_2) and 20mg/kg/ bwt for (TM_3) .The 9 rats in trimester one (TM_1) received the gentamicin injection from day one (GD_1) today 20 (GD_{20})and those in trimester two (TM_2) from gestational day 7 (GD_7) all trough gestation day 20 (GD_{20}), while those in trimester three (TM_3) received the injection from gestational day 14 (GD_{14}) all through to-gestational day 20 (GD_{20}) i.e. the last day of gestation.

3.8 Handling of Rats

The rats were handled investigator and a trained research assistant for the purpose of obtaining daily weights between 0800 am and 0830hrs (figure 3.2). All procedures were performed according to the guidelines for care of laboratory animals by the National Institute of Animal Research (Gomez *et al.*, 2010).

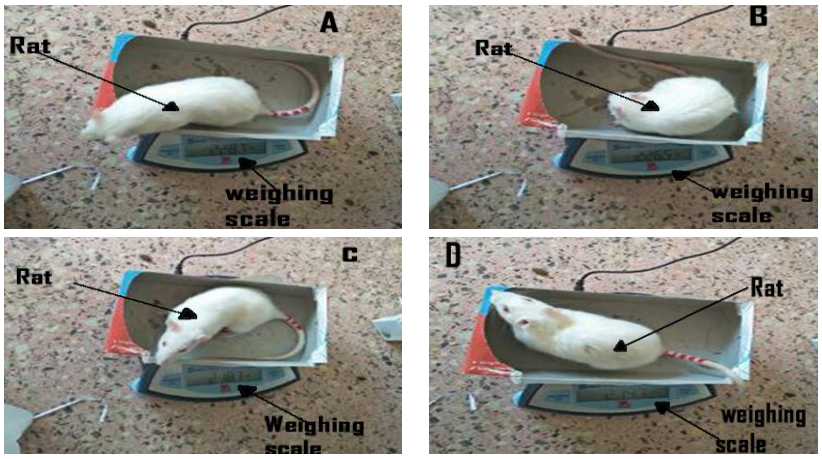

Figure 3. 2: Shows how weighing of albino rats (taken using Scout pro model SPU4001) in (a) Control rat (b) LDGG (c) MDGG (d) HDGG)

3.9 Breeding of animals

One sexually mature male albino rat from (from postnatal dates 50 and above) and obtained from a pure colony was introduced into a standard cage with two female rats at 1430HRS (+/- 30 minutes) and after 24 hours, the males were removed and returned to their separate cages.

3.10 Pregnancy determination

Pregnancy determination was done by taking vaginal smears from the mated dams. Presence of spermatozoon on the smear when observed under the microscope was a confirmed coitus while presence of numerous cornified, polyhedral epithelial cells and neutrophils (Hamid and Zakaria 2013) on the microscope slide confirmed the 1st day of pregnancy (GD_1).

a) Materials used in determination of pregnancy

i) Cotton tipped swab
ii) 0.85%phosphate buffered saline
iii) Microscope slides
iv) Ethanol (95%)
v) Absolute alcohol
vi) 10mls blunt tipped disposable pipettes
vii) Giemsa stain

b) Determination of pregnancy

1. The animal was restrained with a gauze holder against the body
2. 1ml of saline was introduced into the vaginal cavity using a blunt tipped disposable pipette (vaginal wash)
3. Cotton tipped swab moistened with phosphate buffered saline was then gently inserted into the vaginal cavity
4. The swab was slightly rolled before withdrawing

5. The moist swab was withdrawn and rolled onto a clean glass microscope slide
6. The specimen was then spray fixed using 95% ethanol
7. The slides were subsequently air dried and others by dipping in 100% alcohol
8. The slide was then stained with giemsa stain
9. Observations of the slide followed, and was done under the BP Olympus microscope

c) Observations

Large polyhedral, cornified epithelial cells, many neutrophils on the smear and scattered epithelial cells in majority of the rats was observed on a light microscope slide. This served as an indicator that fertilization had taken place and this was counted as the first day of pregnancy (gestation day one). Those that never tested positive for pregnancy were put back with males for more attempts after which they were excluded from the study if they still tested negative. 99% of the rats tested positive for the pregnancy.

3.11 Determination, calculation and administration of the doses

A lethal dose of gentamicin is 100 mgs/kg/bwt has been shown to be the LD_{50} for adult rats (i.e. the dose that would cause disturbance to the development of the kidney to 50% of the animal population (Anroop *et al.,* 2016 and Hiroshi K. *et al.,* 2001**).** This dose was taken as the reference dose. [$N=LD_{50}$]

The dose that could cause kidney mal-development to the fetus was determined by applying the surface area versus the volume ratio of the fetuses to mother was established to be 1:5 respectively by performing intra-cardiac puncture procedure. Given the molecular nature of gentamicin and the physiological counter current flow of maternal and fetal blood, a blood gentamicin concentration equilibrium between the two was achieved at any given time

3.11.1 Determination of the gentamicin doses in rats

The weight in grams of the gentamicin for the reference dose (LD_{50}) of 100mgs/kg/bwt for an average weight dam of 225g was calculated as:-

Weight of rat= 225g

Lethal dose LD_{50}= 100mgs/kg/BWT

Therefore: 1 rat =200g x $\frac{200mgs}{1000}$ = 40g gentamicin

Therefore:

Then: the animals that will be receiving

(i) The low gentamicin dose group (LDGG) received half N= Min Human dose x Km (6.2) = 3 x 6.2 = 18.6mg/kg/ bwt = 19mgs/kg/ bwt

(ii) The Medium gentamicin dose group (MDGG) Avarage Human dose x Km (6.2) = 4.5 x 6.2= 28mgs/kg/ bwt

This dose was taken to be the N dose for this experiment

(iii) The High gentamicin dose group (HDGG) High Human dose x Km (6.2)= 6 x 6.2= 37mg/kg/bwt

3.12 Determination of the critical teratogenic dose and critical period of gentamicin on the fetal kidneys

The most critical teratogenic dose and the most vulnerable period of gentamicin injections were determined as follows**: -**

Animal groupings was done as described in (figure1) and administration of gentamicin injections in each of the groups was done as follows:

In each of the groups (LDGG, MDGG, HDGG), the 9 dams were randomly sub divided in three sub-groups the Trimester 1 (TM_1) = 3dams, Trimester 2 (TM_2) = 3dams and trimester 3 TM_3=3 dams. The gestation period of a rat is 21 days, therefore trimester one was between gestational day GD_1 to GD_7, while trimester 2 was between GD_7-GD_{14} and third trimester GD_{14}- GD_{20}. To determine the vulnerable periods of gentamicin teratogenicity on the fetal kidney, it was administered as follows: -

- All trimester ones (TM_1) rats: - (LDGG,MDGG,HDGG) categories received Gentamicin injections from gestation day GD_1-GD_{20}
- All trimester twos (TM_2) rats: - (LDGG, MDGG,HDGG) categories received Gentamicin injections doses from gestation day GD_7-GD_{20}
- All trimester three (TM_3) rats: - (LDGG, MDGG,HCG) categories received Gentamicin injections doses from gestation day GD_{14}- GD_{20}

3.13 Humane sacrificing the pregnant albino rats, harvesting the fetuses and fetal kidneys

3.13.1 Humane sacrificing of the pregnant albino rats

All rats were humanly sacrificed on the 20^{th} day by use of concentrated carbon dioxide

3.13.2 Materials required for the humane sacrificing of rats

i) The pregnant rat GD_{20}
ii) Carbon dioxide
iii) Cotton gauze or cotton wool
iv) Bell or dissector jar
v) Physiological saline 0.85% concentration
vi) Mounting board
vii) Mounting pins
viii) A pair of scissors
ix) A pair of forceps (toothed)
x) Scalpel blade
xi) Scalpel blade handle
xii) Fixatives- 10% Formaldehyde and 5% Zenker's solution for light microscopy
xiii) Drip set 2 in number
xiv) Hypodermic needle gauge 20
xv) Gloves (surgical)
xvi) Magnifying glass
xvii) Ruler
xviii) Electronic weighing machine
xix) Specimen collection bottle

3.13.3 Procedure for anaesthetizing and perfusing the rats

1. Concentrated carbon dioxide was introduced into a bell jar

2. A tight fitting lid was then put into the bell jar

3. The pregnant rat was then put into the bell jar

4. The rat was waited for 10-15 minutes to be anaesthetized

5. The rat was removed from the bell jar and mounted onto the board using mounting pins with dorsal side on the board (figure 3.3)

6. Using a pair of scissors and forceps the rat was cut through the ventral medial side from the symphysis pubis to the sternal angle of the thoracic cage

7. The perfusion needle was inserted to the left ventricle of the heart while connected to the perfusion set containing 400mls of normal saline

8. The blood was cleared from the rat with physiological saline (200mls of 0.85mol/ltr) through the left ventricle of the heart.

9. After sufficiently clearing the saline drip was removed (the needle then left in position of the heart and the fixative formaldehyde was introduced.

10. The firmness of the tail was checked as a sign of effective fixation of the rats

11. The drip was disconnected and the perfusion needle removed from the heart

12. It was immersed it in a container with fresh fixative to continue fixation for 12 hours

13. The fetuses were then harvested by opening the abdomen along the linear Alba and the uterine horns opened along the anti-mesomentrial boarders, (figure 3.3).

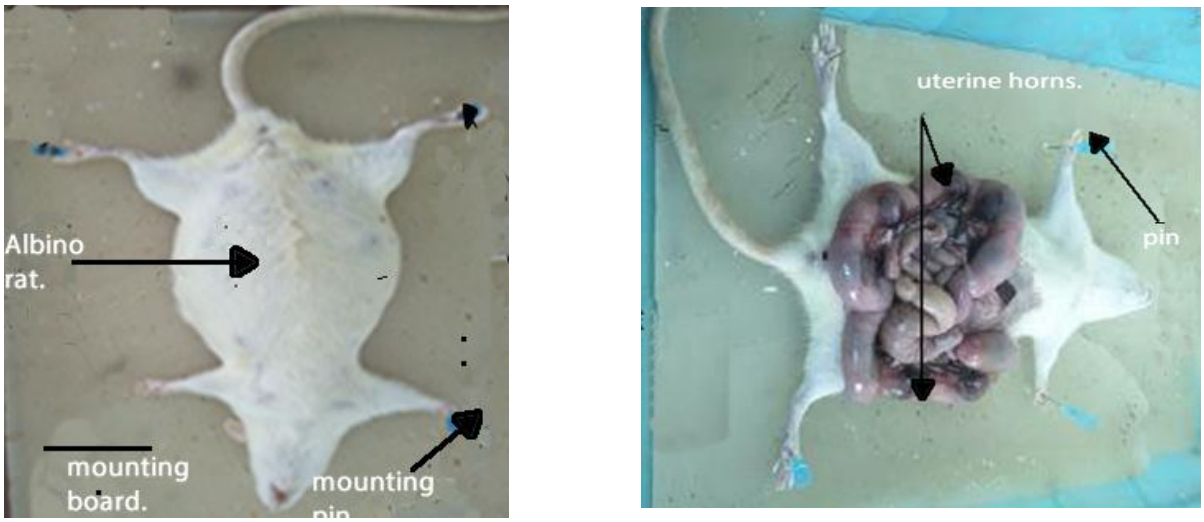

Figure 3. 3: Showing (a) pregnant rat mounted on a dissection board, (b) Uterine horns with fetuses after opening the abdomen.

3.13.4 Harvesting of fetuses

In all cases, the pregnant rats were sacrificed by either inhaled carbon dioxide between 0930HRS and 1100HRS at gestational day 21. This was done to prevent the mothers from devouring any damaged or congenitally deformed fetus. Twenty minutes after anesthesia with concentrated carbon dioxide, the abdominal wall of the mother was opened from the xiphisternal joint to the symphysis pubis along the linear alba and the full extent of both uterine horns exposed promptly.

Before opening either horn, fetal positions within the horns, as well as the number of live and dead fetuses, as was indicated by their movement following a gentle prodding with a probe was recorded (total litter size).

The uterine horns were excised along the anti-mesometrial border to expose the fetuses, embryonic membranes and placentas. They were gently removed in totality from the uterus utilizing the blunt end of a pair of forceps. An incision along the dorsal surface of the

membranes revealed the fetuses, then each fetus and its placenta were removed and weighed and the general fetal morphology examined and recorded immediately. Fetus size were determined by measuring the anal-nasal length. External examination was done before and after fixation in 10% formaldehyde solution.

For each litter in each rat, three kidneys from three fetuses with the lowest, median and highest weights were resected for both histological and morphometric analysis. One was processed for light microscopy, and one for stereology, and another one kept in paraplast as security to avoid loss of information in case of technical problem that could arise when processing and sectioning.

3.13.5 Procedure followed in harvesting of fetuses

i) The uterine horns were excised along the anti-mesometrial borders using a pair of scissors
ii) The number of present fetuses and the resorbed sites counted and recorded
iii) The fetuses were removed to continue being fixed in situ for 6 hours with the same fixative used during perfusion fixation
iv) The CRL, Bi-parietal diameters, head-lengths for each fetus were taken by use of a vernier caliper to assess the effects of gentamicin on overall fetal development
v) Other congenital anomalies were assessed and recoded
vi) The fetal weights in grams were taken with electronic weighing balance and recorded (figure 3.4)
vii) Other fetal growth parameters including fetal lengths, crown-ramp lengths and bi-parietal diameters were taken and recorded

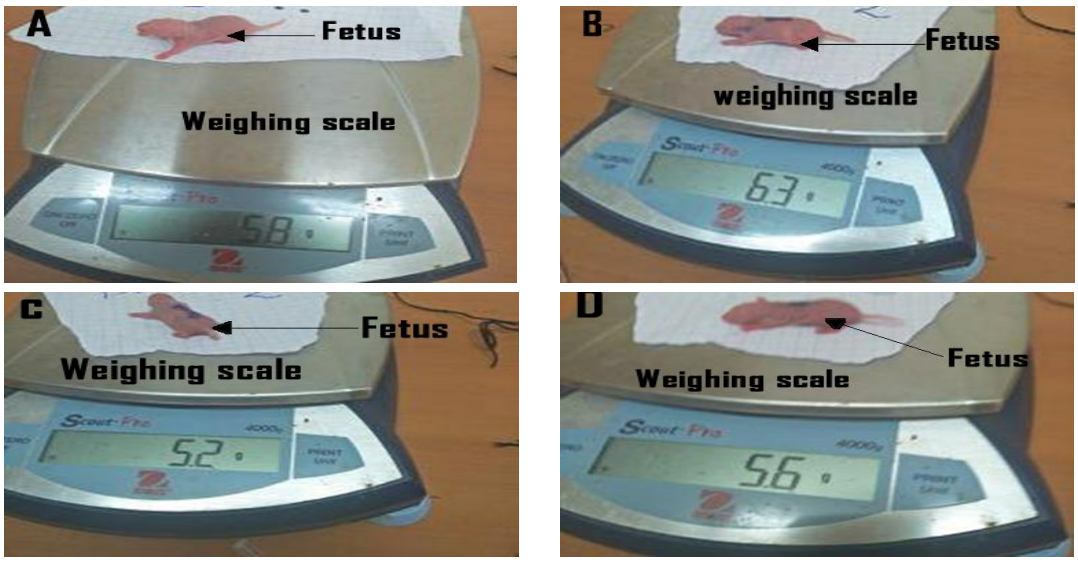

Figure 3. 4: Shows how the fetal weights were taken in (a) LDGG, (b) Control, (c) HDGG, (d) MDGG, (Using scout pro model SPU4001 S/N B519923500 from Uhaus Corporation, USA).

3.13.6 Harvesting of the fetal kidneys

Fetal kidneys were harvested using the following procedure:-

a. Fetus were mounted onto the board using mounting pins (dorsal side facing the board)
b. Using a pair of scissors and forceps the abdominal muscle layers were dissected at the middle to expose the abdominal viscera of the fetus.
c. Using a magnifying glass the whole fetal kidneys was identified and removed.
d. To avoid damaging the fetal kidneys the parietal peritoneum of the posterior abdominal was be opened in the center along the vertebral column retracted carefully since the kidneys lies retroperitoneal.
e. The entire kidneys were excised at the level of the renal pelvis.

f. The kidneys were then be immersed in the preferred fixative (Glutaraldehyde or Zenker's solution) to enable perfusion to proceed with processing either for light or histostreology for 12 hours.

3.14 Processing for light microscopy

3.14.1 Materials used for staining

i) The specimens (the fetal kidneys)
ii) Zenkers solution (1 litre)

-Mercuric chloride 5gms

-Potassium dichromate 2.5g

-Sodium sulphate 1gram

-Distilled water

-Acetic acid 5mls

iii) DPX moutant
iv) Glass slides and cover slips
v) Masson trichrome
vi) Hematoxylin and eosin
vii) Glass staining square jars
viii) Paraffin wax
ix) Microtome knives
x) Rotary microtome (American Optical CO)
xi) Heater and water bath container
xii) Specimen bottles
xiii) Slide holders
xiv) Distilled water
xv) Formaldehyd40%concentration
xvi) Xylene
xvii) Isopropyl alcohol
xviii) Van Grisons stain

xix) Glass ware for preparing dilutions
xx) Wood blocs
xxi) Beakers
xxii) Egg albumin
xxiii) Dropper
xxiv) Cedar wood oil
xxv) Toluidine solution

3.14.2 Procedure that were used for processing the fetal kidney specimens for light and stereo microscopy

1. The fetal kidneys were fixed in the Boiun's (Zenkers' solution) for 24 hours
2. They were dehydrated in an ascending concentration of alcohol (50%, 60%, 70%, 80%, 90%, 95% and 100% (absolute) each for one hour.
3. They were cleared by immersion with cedar wood oil for 12 hours.
4. They were then infiltrated with paraplast wax for 12 hours at 56^0c
5. The kidney tissue was then orientated in the longitudinal axis
6. They were then embedded in paraffin wax on the wooden blocs
7. Excess wax was trimmed-off till the entire length of the fetal kidney tissue is exposed
8. 5µm thick longitudinal sections were cut from head to tail regions with Leitz© sledge rotary microtome
9. The cut sections were floated in water at 37^0 to spread the tissue
10. The sections were stuck onto glass slides using egg albumin, applied as thin film with a micro-dropper.
11. The slides were then dried in an oven at 37^0 for 24 hours
12. Blinding was done by coding all the slides by the research assistance in absence of the researcher
13. They were stained with different stains including: -Hematoxylin and Eosin (H&E), Hematoxylin Phloxin stain (HP) and Modified aldehyde fucshin Stain (MAF) based on the cellular structures that needed to be studied.

3.15 Stereological analysis

3.15.1 Estimation of reference total fetal kidney volume using Archimedes principle-Water/fluid displacement method.

After the removal of the entire fetal kidney from both the control and experimental groups, the total kidney volumes were determined using the water/fluid displacement method the (Archimedes' volume). This was obtained by inserting the whole kidney tissue into graduated beakers containing normal saline, and the amount of fluid displacement upward was measured.

The normal saline displaced by the kidney represented the actual kidney volume that were used as the reference volumes when determining the calvarieli stereological total kidney volumes and volume densities.

3.15.2 Determination of actual stereological total fetal kidney volume, volume density, Cortical and medullary layers using cavarieli point counting method.

The stereological total kidney volume and the estimation of the volume densities of both the cortical and medullary layers of the kidney structurers was determined by using the cavalieri method of point counting (Cruz-Orive, 1999).

Through the following procedure:-

Preparation of kidney cavalieri sections (5µ thick)

Selection of the spacing for the point probe

The point probe was tossed randomly onto each section

The points that hit the region of interest was counted using stepnizer stereology tool

All sections were processed keeping a tally of counts per section

Calculation of the volume

Between twenty to twenty five sections of 5µm thickness were, sampled from each longitudinal kidney section, obtained by systematic uniform random sampling. Using the microscope's stage Vernier, images were viewed at magnification of X 10. The volume was obtained by fully sectioning the kidney into a series of cuts which was the product of the sum of the cut areas (starting with the first to the last section). The sum of points that hit the structure were estimated (the area of the structure 'i'). Point counting was done using the stepanizer software as determined by Bolender and Weibel (1973) (figure 3.5)

The digital images of the kidney tissues were captured using stereological sampling rules with the same magnification, and saved in the jpeg (joint photograph expert group) file format. The picture height was ensured that it match the height of the computer monitor, both defined in pixels. All images captured both for the control and experimental groups were organized appropriately and saved in one folder.

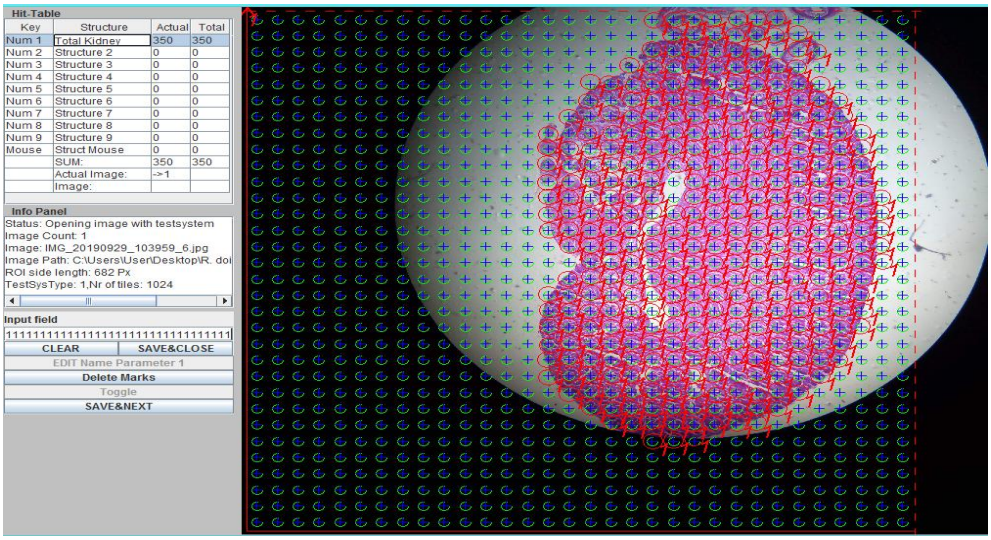

Figure 3. 5: Showing a section image from a control slide of fetal kidney superimposed on an equidistant point counting grid. H&E x40.

Point counting using the cavalieri principle was employed to estimate the total fetal kidney volume using the formula:

$$_{Est}V = \frac{\sum^{m}_{i-1} P.\ a/\ p.\ t}{M^2}$$

Where: $_{est}V$= was the estimation of the volume of the kidney,

$\sum^{m}_{i-1}P$-= was the sum of the number of points landing within the various

Components of the fetal kidney profiles from the first (i) to the last I

a/p= was the area associated with each point,

t = was the distance between sections and

M= was represent the magnification (Welniak–Kaminska *et al.*, 2019)

On each sampled section, five fields were selected in a systematic random manner on the newscast computer screen projected by the stereology new cast microscope. A transparent test system on the grid was then superimposed on the images projected on the computer screen on the cortical and medullary structurers and points hitting these areas counted at a final magnification of x40. Then, estimates of their volume density, (Vv) of the in the reference space were obtained using the formula:

EstVv = P (part)/P (ref),

Where **P (part)** and **P (ref)** were the number of test points falling in all structure profiles and in the reference space, respectively (Keller *et al.,* 2018)

3.15.3 Correction for kidney tissue shrinkage

The following method was applied to quantify the percentage kidney tissue shrinkage caused by fixation and histological procedures. This was done by considering both the initial kidney volume by Archimedes displacement method and the stereological calvarieli method which was calculated as follows (Alarifi *et al.,* 2012)

Shrinkage = Volume before-Volume

Volume before

3.16 Photography (materials and procedure)

1. Digital camera (32 megapixels)

2. BP Olympus microscope

3. Histological glass slide

b) Procedure followed in taking photomicrographs

Histological slides were mounted on the stage of the microscope

Focus was enhanced by adjusting the microscope

The field was magnified appropriately

Photographs of the regions were taken as they were viewed best under the focus of the microscope and transferred to a laptop

The photographs were uploaded in Adobe fireworks programme for labelling

3.17 Statistical analysis

The histomophological data that entailed the qualitative data was collected using photomicrographs at different magnifications using a digital camera (32 megapixels), then exported to Adobe fireworks for qualitative analysis.

Histostereological data plus data on pregnancy outcomes was collected using structured checklists and stereological data sheets. It was then stored and coded in excel spreadsheets version 23 and then exported for analysis to SPSS programme windows version 23 for analysis (Chicago Illinois).

The data was statistically tested using one-way analysis of variance (ANOVA) , followed by Tukey's post hoc multiple comparison tests and results expressed as mean ± standard error of the mean (SEM) for all values. All results whose $P \leq 0.05$ were considered to be statistically significant.

3.18 Ethical consideration

All procedures were performed with approval from the JKUAT Institutional Ethics Review Committee.

CHAPTER FOUR

RESULTS

4.1 Maternal and fetal pregnancy outcomes

4.1.1 Influence of gentamicin on mean daily maternal weight trends from GD_1 to GD_{21}.

The comparative maternal weight in the entire gestational period- (GD_1 to GD_{20}) between the control and the treatment groups following prenatal exposure to varied doses of gentamicin showed no reduction on the daily mean weight gains between the gentamicin treatment groups as compared to the control. The daily weight gains in the treatment groups were not significantly lower ($p>0.05$) compared with the control as shown in table 4.1. It was notable that the mean maternal weight gain in all the experimental groups LDGG, MDGG, and the HGG when treated at trimester 1 (TM_1) and trimester two (TM_2) they depicted no direct dose response relationship in that the higher the dose the lower the mean maternal weight gain. When the treatment was done in trimester three (TM_3) there was no statistical significant ($p>0.05$) differences between the gentamicin treated groups and the control.

When the analysis was done on whether or not the mean maternal weight gain has a time dependent relationship, it was established that when treatment was instituted in trimester one and two (TM_1 andTM_2), there was no significant ($p>0.05$) reduction in maternal weight gain in all the treatment groups (LDGG, MDGG, HDGG) same as in TM_3 where there was no significant difference ($P>0.05$) for the low and medium dose groups compared with the controls.

Table 4.1: Comparative mean maternal weight gain trends for the gentamicin treated groups LDGG, MDGG, and HGG against control group at TM_1, TM_2 and TM_3

Parameter	Trimester	Control	LDGG	MDGG	HDGG	F-Statistic	P-value
Maternal weight gain	TM1	277.5±27.89a	276.5±25.74a	279.8±0.27.04a	278.4±26.08a	0.058	0.982
	TM_2	277.5±27.89a	279.9±28.7a	284.5±26.9a	277.6±25.9a	0.299	0.826
	TM_3	277.5±27.89a	276.4±26.8a	286.3±28.2a	289.8±27.1a	1.212	0.311

Notes: The means, followed by the same letter in a row are not statistically different at (P>005) using one way ANOVA with Tukey test on post-hoc t-tests.

Table 4.2: Comparative maternal weight gain trends for the gentamicin treated groups among TM_1, TM_2 and TM_3 against the Control, LDGG, MDGG, and HDGG groups.

Parameter	Trimester	Control	TM_1	TM_2	TM_3	F-Statistic	P-value
Dosage	LDGG	277.5±27.89a	276.5±25.74a	279.87±28.65a	276.4±26.83a	0.074	0.974
	MDGG	277.5±27.89a	279.8±27.04a	284.5±26.91a	286.3±28.22a	0.464	0.709
	HDGG	277.5±27.89a	278.4±25.38a	277.6±25.88a	289.8±27.13a	1.077	0.364

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.

4.1.2 The mean litter size, placental weights, resorbed endometrial glands and the percentage embryolethalities

The comparative mean litter sizes between the gentamicin treated groups against the control depicted an inverse dose response relationship in that as the dose increased the mean litter size reduced particularly when treatment was done in trimester one and trimester two (TM_1, and TM_2) and this was found to be statistically significant ($p \leq 0.05$) as compared with the

control. The mean litter size was lowest in the HDGG treated at TM_1 with 4.01 followed by MDGG at 6.02±1.53 and lastly LDGG at 9.04±1.151, while that of the control was 13.34±0.881 (table 4.3).

The mean placental weight that is usually a key indicator of the maternal nutritional exchange with the fetus was also observed to have similar inverse dose response relationship with the dose of treatment and the time of exposure. It was found that the lowest placental weight recorded was in the high gentamicin treated group when treated at TM_1 and lowest in the low gentamicin group at TM_3 (table 4.3).

The number of the resorbed endometrial gland were also seen to directly vary with the dose of exposure as well as with the time of exposure in that with increasing doses of gentamicin exposure, there was a corresponding significant increase ($p \geq 0.05$) in number of endometrial glands resorptions particularly in TM_1 and TM_2 across all the gentamicin treated groups as compared with the control. At $_{TM3}$ the mean number of the resorbed endometrial glands only had a significant difference ($P<0.05$) in the high gentamicin treated group (HDGG) when compared with the control (table 4.3).

On the percentage embryo-lethality, it was observed that the comparative mean number of dead fetuses in utero increased with gentamicin dose and the time of exposure. When gentamicin was administered at TM_1 the mean embryo-lethality in HDGG was at 1.67±0.882 followed by MDGG at 0.67±0.67 and lastly at LDGG (figure4.2), it was statistically higher ($p<0.05$) when compared with the control. When gentamicin treatment was instituted at TM_2, the embryo-lethality was 1.33±0.667 for the HDGG, which was statistically higher ($p<0.05$) than the control, while MDGG and LDGG embryo-lethality at TM_2 were not statistically different ($p>0.05$). When treatment was done at TM_3 the percentage embryo-lethality did not show statistically significant difference ($p>0.05$) with the control (table 4.3).

Table 4.2: The comparative mean litter size, placenta weight, resorbed endometrial glands and percentage embryo-lethality in LDGG, MDGG and HDGG with time of exposure (TM_1, TM_2 and TM_3) against the control group (CG).

Study groups	Period of gentamicin treatment	Mean litter size ± SEM	Mean placenta weights± SEM	Mean resorbed endometrial glands± SEM	Mean embryo lethality± SEM
Control group	-none-	13.34±0.881a	5.58±0.021a	0.671±0.67a	0.332±0.333a
Low dose gentamicin group (LDGG, 19mg/kg)	TM1	9.04±1.15a*	3. 53±0.049c*	0.672±0.67a	0.333±0.333a
	TM2	10.33±0.667a*	4.0±0.049b*	0.673±0.667a	0.330±0.333a
	TM3	11.000±0.577a	4.40±0.050ac	0.331±0.333a	0.331±0.333a
Medium dose gentamicin group (MDGG, 28mg/kg)	TM1	6.02±1.52b*	3.633±0.034b*	1.332±1.33a*	0.671±0.67a*
	TM2	9.03±0.577b*	4.03±0.034b*	1.1±0.577a*	0.331±0.333a
	TM3	10.01±0.577a	4.133±0.0612c	0.667±0.333a	0.332±0.333a
High dose gentamicin group (HGG, 37mg/kg)	TM1	4.00±0.00c*	3.23±0.018b*	2.7±1.53b*	1.670±0.882a*
	TM2	6.04±0.567b*	3.64±0.0200c*	3.0±0.000b*	1.332±0.667a*
	TM3	7.67±0.333b*	3.96±0.030d*	0.700±0.6506a*	1.001±0.577a*

Key: *The mean values in a column that have the same letter are not statistically different at ($P>0.05$) using one way ANOVA with Tukey test on post-hoc t-tests. Any value that has an steric star (*) indicates that they depicted both intra and inter group statistical significance differences ($p<0.05$)*

4.1.3 Influence of gentamicin on congenital anomalies

A total of 12 fetuses among 280 fetuses were found with one type or having a combination of a number of congenital anomalies. The most common types of anomalies observed were the renal system abnormalities. These abnormalities were found to be concentrated in the high and medium gentamicin groups (MDGG and HDGG). This was particularly so when

Gentamicin was administered in the first and the second trimesters (TM_1, TM_2). The distribution and the types of anomalies observed are shown in table 4.4 below.

Table 4.4: Showing the types of congenital anomalies observed, their numbers and their distribution LDGG, MDGG & HDGG against the control.

	Distribution of fetuses with congenital abnormalities across the study groups				
Types of congenital abnormalities	**Control**	**Low gentamicin group (19mg/kg)**	**Medium Gentamicin group (28mg/kg)**	**High Gentamicin group (37mg/kg)**	**Total number of fetuses**
Spina bifida	0	0	0	2	2
Hypospadias	0	0	0	1	1
Renal dysplasia	0	0	2	3	5
Anencephaly	0	0	0	1	1
Microcephaly	0	0	0	0	0
Meromelia	0	0	0	0	0
Syndactyly	0	0	0	0	0
Oligodactyly	0	0	0	0	0
Microphthalmia	0	0	0	0	0
Aphalagia	0	0	0	0	0
Total	0	0	2	8	12

4.1.4 Correlation analysis on maternal pregnancy outcomes

To establish the intra and inter-groups level of association, the strength of association and the direction of the association, the intra and the inter group comparisons using Pearson liner correlations, p values that were 0.00 were done. The correlation value of 0.05 and above indicated a strong linear relationship between variables while the values that ranged between 0.3 and 0.05 indicated a moderate linear relationship between the various parameters cross

tabulated, whereas a value below 0.3 indicated a weak relationship. The intra group evaluation of the various maternal pregnancy outcomes cross tabulated showed a strong association (0.3-0.5) at TM_1 and TM_2 in all the experimental groups compared with the control while at TM_3 they depicted a weak relationship (values of <0.3)when compared with the control (table 4.5)

Table 4.5: Intra and inter-group correlational comparisons on the various maternal pregnancy outcomes for the LDGG, MDGG and HDGG at TM_1, TM_2 and TM_3 against the control

		Weight gain (gms)	Initial weight (gms)	Terminal weight (gms)	Placenta weight (gms)	Number of resorbed glands	litter size	Dead fetuses	Congenital abnormalities
Weight gain (gms)	r	1							
	p								
Initial weight (gms)	r	-.284	1						
	p	.084							
Terminal weight (gms)	r	.845*	.268	1					
	p	.0002	.100.17						
Placenta weight (gms)	r	.841*	-.126	.668*	1				
	p	.0001	.0.15002	.0001					
Number of resorbed glands	r	-.0.1559	.00.147	-.0.1437	-.669	1			
	p	.0001	.723	.0012	.0001				
litter size	r	.792	-.132	.721	.894	-.667	1		
	p	.0001	.447	.0001	.0001	.0001			
Dead fetuses	r	-.0.151*	.00.151	-.490**	-.40.251**	.522**	-.478**	1	
	p	.011	.665	.012	.046	.053	.068		
Congenital abnormalities	r	-.335	.061	-.252	-.387*	.459**	-.430**	.281*	1
	p	.0643	.686	.147	.026	.002	.004	.031	

NB: r is the Pearson's correlation coefficient, P is the p-value, * and ** indicate significant difference i.e. $p<0.05$

4.1.5 Influence of gentamicin on fetal body weight, CRL, head circumference and bi-parietal diameter.

It was observed that the mean fetal weights, the mean crown rump lengths, head circumference and the bi-parietal diameters depicted an inverse dose response relationship while at the same time depicting a direct dose response relationship with the time of exposure. This was particularly observed when the treatment was done in TM_1 and TM_2 across all the gentamicin treated groups (LDGG, MDGG and HDGG)

The treatment at TM_3 did not show statisticaly significant difference ($P>0.05$) across the three experimental groups as well as when compared with the control in all the four fetal parameters (table 4.6).

Table 4.6: The intra and inter group comparative means of the fetal body weight, head circumference, CRL and bi-parietal diameter of LDGG, MDGG and the HDGG in (TM_1, TM_2 and TM_3) against the control (C).

Study groups	Period of gentamicin treatment	Mean fetal body weight ± SEM	Mean head circumference ± SEM	Mean CRL ± SEM	Mean bi-parietal diameter ± SEM
Control group	----------	6.73±0.026a	3.89±0.010a	4.723±0.030a	0.7155±0.018a
Low dose gentamicin group (LDGG, 19mg/kg)	($_{TM1}$)	6.42±0.007b*	3.22±0.025b*	4.123±0.009b	0.659±0.00073b*
	($_{TM2}$)	6.57±0.011c*	3.453±0.02*	4.5±0.009b*	0.686±0.0008a*
	($_{TM3}$)	6.66±0.0168a	3.57±0.037b	4.55±0.027b	0.692±0.0026b
Medium dose gentamicin group (MDGG, 28mg/kg)	($_{TM1}$)	6.31±0.046b*	2.95±0.018c*	3.865±0.044*	0.6296±0.0033*
	($_{TM2}$)	6.42±0.018a*	3.22±0.019c*	4.15±0.0029*	0.655±0.0005b*
	($_{TM3}$)	6.53±0.004c	3.52±0.015b	4.44±0.028b	0.69±0.004d
High dose gentamicin group (HDGG, 37mg/kg)	($_{TM1}$)	5.42±0.02 b*	2.35±0.013d*	3.4±0.023d*	0.579±0.002b*
	($_{TM2}$)	5.92±0.00b*	3.04±0.021d*	3.85±0.0024*	0.635±0.0011a*
	($_{TM3}$)	6.21±0.010d*	3.41±0.021b*	4.34±0.011b	0.68±0.0028d

The means, followed by the same letter in a column 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)

4.2 Level 2: The histomorphological findings on the developing fetal kidneys

In decribing the histomorphological findings on the effects of prenatal exposure to varied doses of gentamicin, the following paramentres were compared in the treatment groups (LDGG, MDGG and HDGG) againt the controland looking at changes in the renal cortex size, renal medullary size, Bowmans capsule size, glomerulus size and the proximal convoluted tubule size.

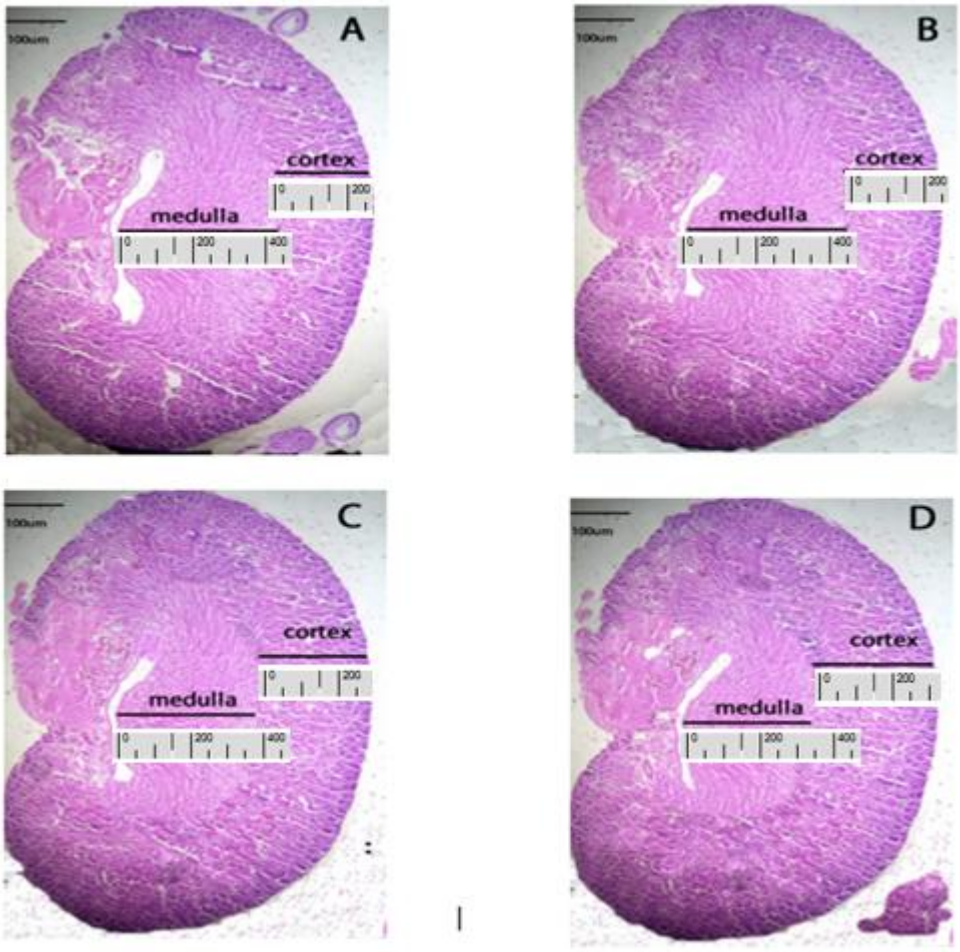

Figure 4. 1: Shows how the medullaly and cortical thickness increased in the Gentamicin treated groups against the control at TM_1 in A- control, B- LDGG, C- MDGG and D- HDGG at H & E TM X 40.

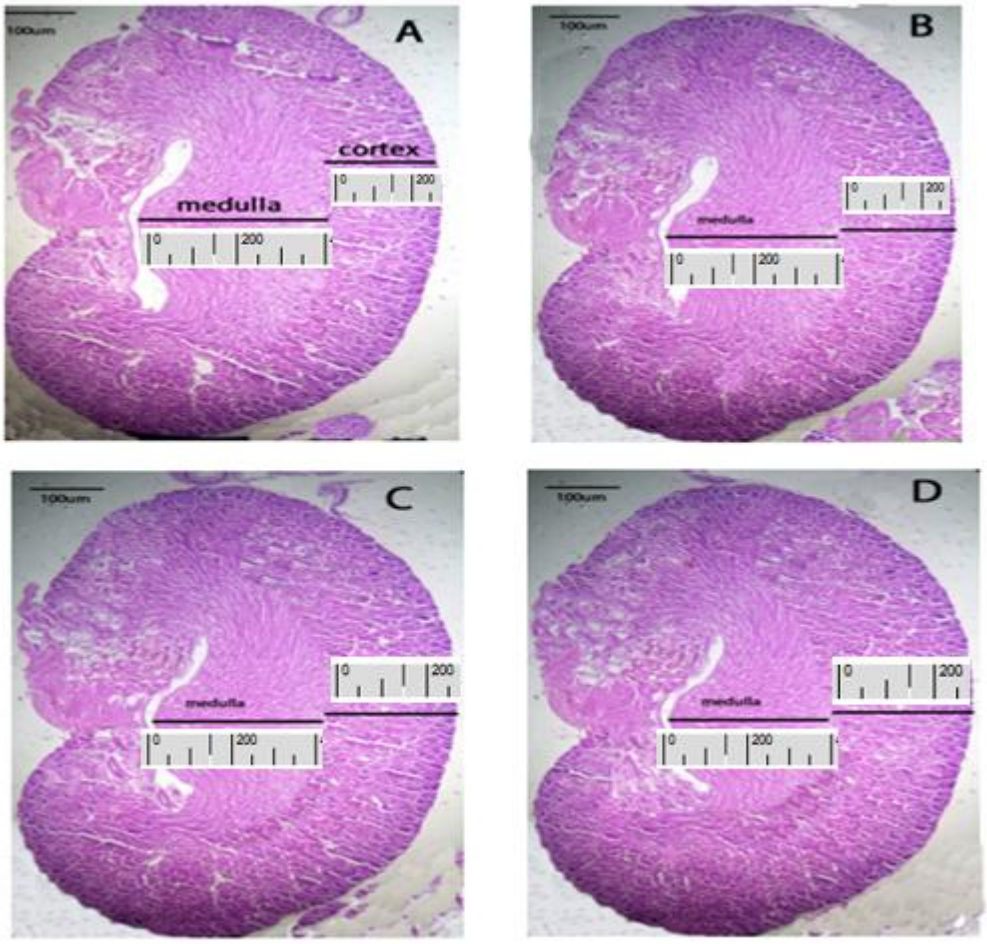

Figure 4. 2: Shows how the medullaly and cortical thickness increased in the gentamicin treated groups against the control at TM_2 in A- control, B- LDGG, C- MDGG and D- HDGG at H & E TM X 40.

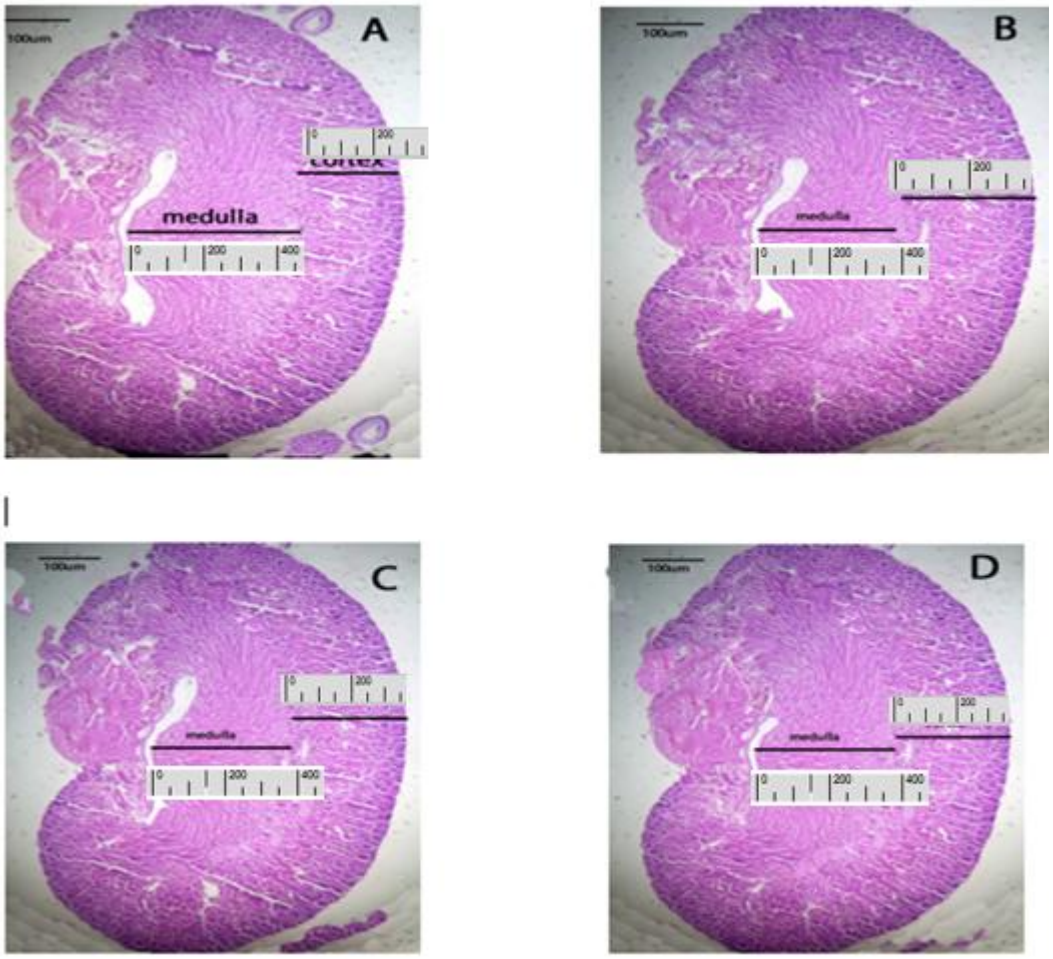

Figure 4. 3: Shows how the medullaly and cortical thickness increased in the Gentamicin treated groups against the control at TM_3 in A- control, B- LDGG, C- MDGG and D- HDGG at H & E TM X 40.

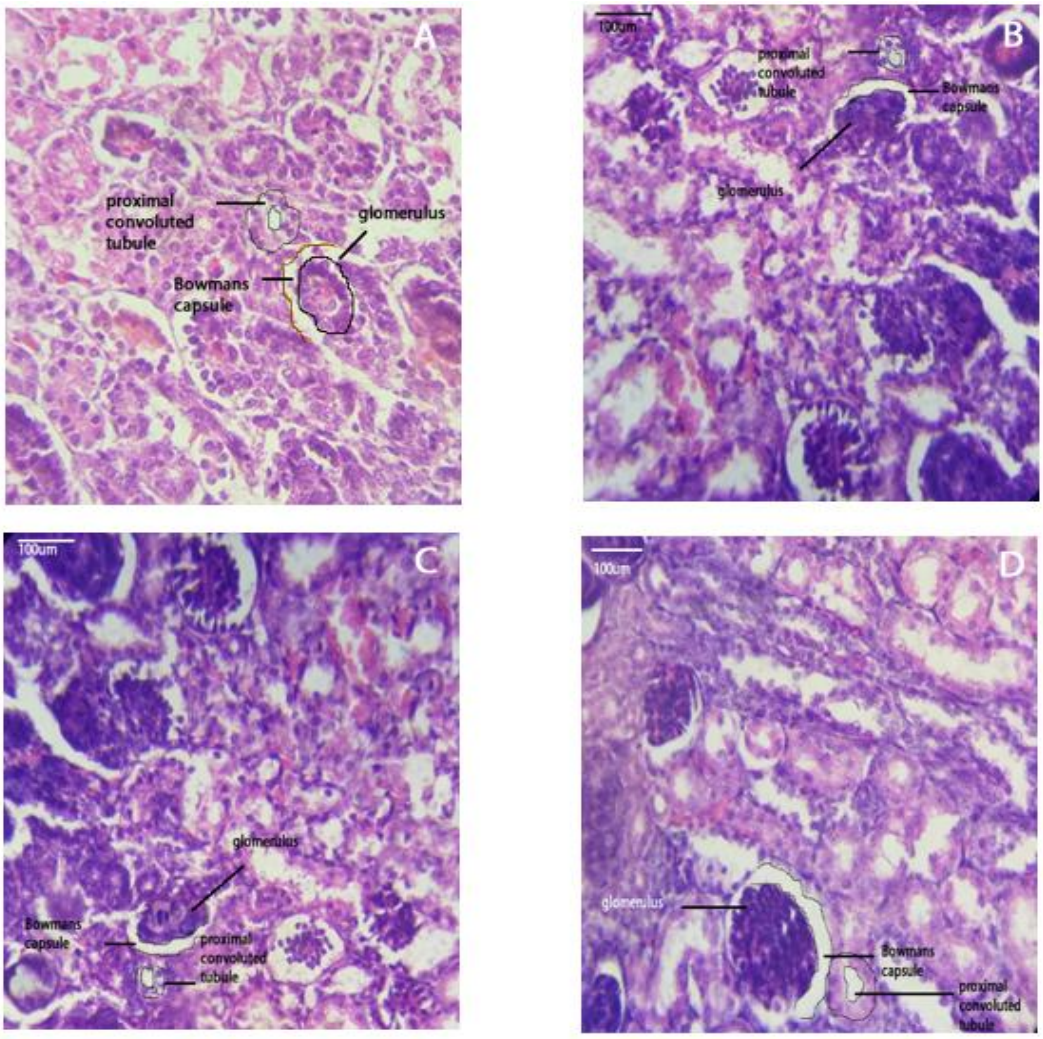

Figure 4. 4: Showing the widening of the Bowman's capsule, Glomeruli and the proximal tubule lumen in gentamicine treated groups' against A-Control, B- LDGG,C- MDGG and D- HDGG during TM_1 seen at H & E, TM X 40.

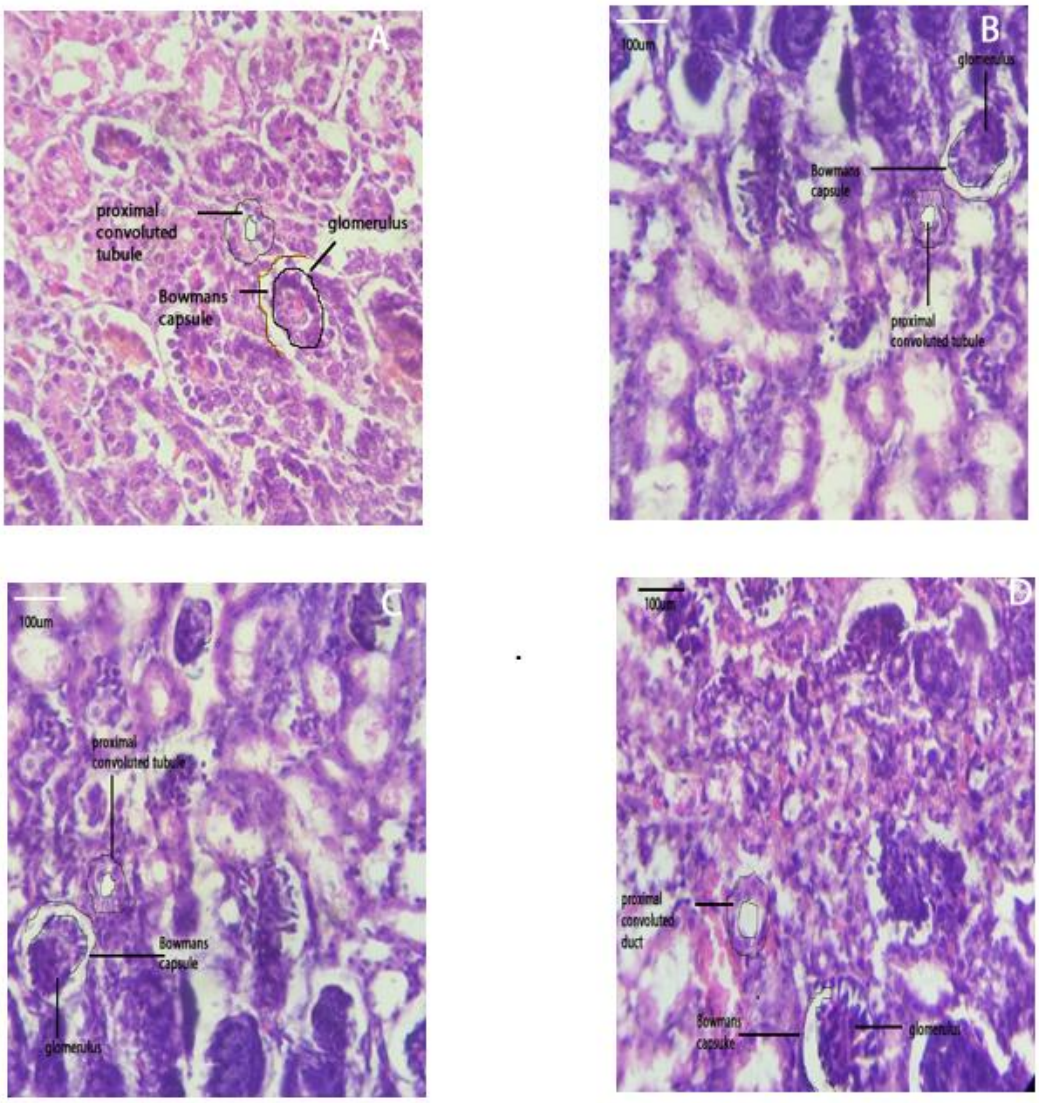

Figure 4. 5: Showing the widening of the Bowman's capsule, Glomeruli and the proximal tubule lumen in gentamicine treated groups' against A- Control, B- LDGG, C- MDGG and D- HDGG during TM_2 seen at H & E, TM X 40.

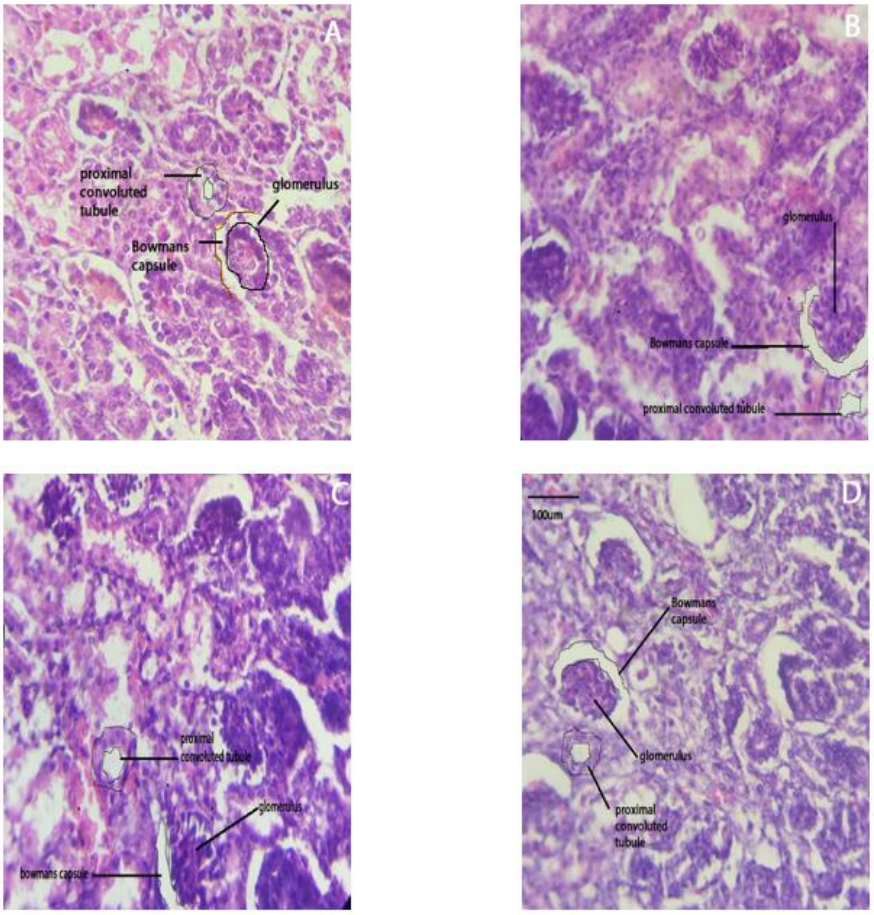

Figure 4. 6: Showing the widening of the Bowman's capsule, Glomeruli and the proximal tubule lumen in gentamicine treated groups' against A- Control, B- LDGG, C- MDGG and D- HDGG during TM_3 seen at H & E, TM X 40.

4.3: Stereological Findings

The stereological parameters evaluated and reported in this study included morphometric measurements on total fetal kidneys weights, total kidney volume by use of both water immersion method (WIM) and calvarieli method of point counting, volume densities of both cortex and medulla of the fetal kidney structures.

4.3.1 The gross fetal kidney size and weight

The intra-group and inter-group comparison of the fetal kidney showed significance difference between gentamycin treated groups when compared to that of the control. For instance, it was observed that when gentamicin treatment was done at TM_1, the mean total kidney weight was found the lowest in Control group at 0.395±0.005gms followed by MDGG at 0.22±0.008 and LDGG at 0.297±0.0033. When gentamicin was administered in TM_2, the mean totals of the fetal kidney weight was found to be 0.246±0.0071 in HDGG at followed by MDGG at 0.301±0.001, then LDGG at 0.345±0.0058 as compared with control ($p<0.05$). When treatment was done at TM_3, the mean kidney weight for the HDGG group was 0.39±0.0028, followed by MDGG at 0.370±0.008 and LDGG at 0.364±0.0051. This values were found to be statistically significant ($p<0.05$) when the comparisons were done within and across groups and when compared with the control.

For the kidney length and width a similar scenario was observed, for instance, when a comparative mean kidney length was done across the three trimesters TM_1, TM_2 and TM_3, it was depicted that at TM_1 the mean fetal kidney length was high in HDGG group at 0.12±0.0121 followed by MDGG at 0.216±0.008 and LDGG at 0.297±0.0032. This was found to be statistically lower as compared with the control ($p<0.05$) at 0.395±0.005.At TM_2, kidney length was found to be high in HDGG at 0.285±0.1433 followed by MDGG at 0.2000±0.0001, then LDGG at 0.247±0.0032. This was not statistically different as compared with control group at 0.295±0.005 ($p>0.05$). While at TM_3, the mean kidney length was high in HDGG group at 0.287±0.0017, followed by MDGG at 0.31±0.0023 and LDGG at 0.305±0.135. The kidney width were also seen to follow the same treads (table 4.7).

Table 4.7: Showing a comparative means fetal Kidney weight, Kidney length, and width for LDGG, MDGG and the HGG treated at TM_1, TM_2 and TM_3 against the control.

Study groups	Period of gentamicin treatment	Mean kidney weight ± SEM	Mean kidney length ± SEM	Mean kidney width ± SEM
Control group	----------	0.395±0.005a	0.295±0.005a	0.197±0.003a
Low dose gentamicin group (LDGG, 19mg/kg)	Trimester one ($_{TM1}$)	0.297±0.0033b*	0.297±0.0033b*	0.197±0.0033b*
	Trimester two ($_{TM2}$)	0.345±0.0058b*	0.247±0.0032a*	0.1711±0.0066b*
	Trimester three ($_{TM3}$)	0.394±0.0051a	0.305±0.135b	0.19±0.009a
Medium dose gentamicin group (MDGG, 28mg/kg)	Trimester one ($_{TM1}$)	0.216±0.008c*	0.22±0.008c*	0.116±0.0079c*
	Trimester two ($_{TM2}$)	0.301±0.001c*	0.2000±0.0001a*	0.123±0.012c*
	Trimester three ($_{TM3}$)	0.370±0.008a*	0.31±0.0023b*	0.273±0.009a*
High dose gentamicin group (HDGG, 37mg/kg)	Trimester one ($_{TM1}$)	0.12±0.0121d*	0.32±0.012d*	0.193±0.007d*
	Trimester two ($_{TM2}$)	0.246±0.0071d*	0.285±0.1433a*	0.148±0.0024d*
	Trimester three ($_{TM3}$)	0.33±0.0028b*	0.287±0.0017a*	0.12±0.00696b*

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

4.3.2 The influence of gentamicin on the total fetal kidney volume and volume densities

The calculated mean total fetal kidney volume as determined by use of water displacement method (WIM) and calculated by the calvarieri method was found to depict a direct dose response relationship in that when the dose of exposure to gentamicin increased, the mean total Kidney volume had a corresponding increase in total kidney volume and vice versa, (table 4.8). On the other hand, when the total kidney volume was compared with the time of exposure, it depicted a direct response relationship to the time of exposure in the when gentamicin treatment was administered at different trimesters one, two and three (TM_1, TM_2 TM_3), the kidney volumes increased directly with the time of exposure. For instance when

the gentamicin treatment was done at TM_1 the total kidney volume was highest in the HDGG at (0.232±0.001), followed by MDGG at (0.222±0.001) and lastly LDGG at (0.211±0.002). All the intra and intergroup comparisons were also found to be statistically significant ($p<0.05$) when compared with the control group (table 4.8). The kidney cortex and medulla volume densities were also seen to follow the same treads.

Table 4.8: A Comparative reference, calculated and percentage shrinkage on total mean fetal kidney volume using (WIM) and cavarieli method in the LDGG, MDGG and the HDGG treated at TM_1, TM_2 and TM_3 against the control.

Study groups	Period of gentamicin treatment	Mean total fetal kidney volume (WIM) ± SEM	Mean total fetal kidney volume (Calvarieli method) ± SEM	Mean shrinkage ± SEM	Mean kidney cortical volume density ± SEM	Mean kidney medulla volume density ± SEM
Control group	----------	0.248±0.00a	0.244±0.001a	0.017±0.001a	0.073±0.00a	0.171±0.0a
Low dose Gentamicin group (LDGG, 19mg/kg)	TM1	0.233±0.00b*	0.231±0.00b*	0.010.15±0.05a	0.070±0.00*	0.162±0.001b*
	TM2	0.239±0.001b*	0.2305±0.002b*	0.016±0.001a	0.071±0.00a*	0.162±0.001b*
	TM3	0.247±0.002a	0.243±0.001a	0.244±0.0005a	0.073±0.000a	0.171±0.001a
Medium dose Gentamicin group (MDGG, 28mg/kg)	TM1	0.232±0.001c*	0.232±0.001c*	0.013±0.001a	0.069±0.000b*	0.161±0.001b*
	TM2	0.238±0.001c*	0.233±0.002c*	0.019±0.006a	0.070±0.001b*	0.162±0.001b*
	TM3	0.242±0.000b*	0.239±0.001c*	0.244±0.006a	0.072±0.000b*	0.170±0.001b*
High dose Gentamicin group (HGG, 37mg/kg)	TM1	0.222±0.001c*	0.220±0.001c*	0.009±0.003a	0.066±0.002c*	0.054±0.001c*
	TM2	0.233±0.002c*	0.230±0.002c*	0.244±0.004a	0.069±0.000c*	0.054±0.001c*
	TM3	0.242±0.002b*	0.236±0.001c*	0.244±0.004a	0.071±0.000c*	0.167±0.001c*

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

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

5.1.1 Influence of gentamicin on the maternal and fetal pregnancy outcomes

The study findings on the maternal and fetal pregnancy outcomes showed reduction in the mean litter sizes, placenta weights, fetal weight, bi-parietal diameters, head lengths, head circumferences, crown-rump length and kidney weight, length and width among the gentamicin groups when compared to those of the control. Subsequently, there was increase in the percentage embroylethalities and the mean number of resorbed endometrial glands on the experimental groups and these concur with the Yaris *et al.,* (2004) and Gilbert *et al*., (1990) that found increased chances of spontaneous abortions as well as intra-uterine growth retardations of the fetuses following administration of gentamicin.

These findings depicted dose and time dependent relationship in that when high and medium doses of gentamicin (37mgs/kg/bwt and 28gms/kg/bwt respectively) was administered particularly during TM_1 and TM_2, there was significant difference ($P<0.05$) between the experimental group and the control. This present study also demonstrated that high gentamicin dosages administered in trimester one and trimester two leads to increased rate congenital defects such. These anomalies were found to be significantly higher $p<0.05$ in the high (HDGG) and medium gentamicin groups (MDGG) when compared with the control These findings are in tandem with a study that was done by Georgina and Peter, 2015and Samiee-zafarghandy and Anker, 2013) which demonstrated that the number of congenital malformations were related to high dose aminoglycoside administration.

In addition, this study found out that there was no significance difference on the pregnancy outcome parameters between the low gentamicin treatment groups and the control. This was

also reported by Bank *at al.,* (2017) who found that the administration of low aminoglycoside doses during pregnancy does not result into significant alteration in the external developmental measures in the morphological parameters of the fetuses. In a different study that was done by Salehnia *et al,* (2013) established that high doses of streptomycine which is in the same class with Gentamicin had adverse effects on corpus luteum of female rats that plays an important role in reproduction as it progesterone and 20-hydroxy progesterone that maintains in-utero fetal growth and development.

The mechanism of gentamicin interference to the overall fetal development *in-utero* could be associated with anti-proliferative inhibitory effects of gentamicin in the fetal tissues that suppresses the mitotic index and causes persistent block of the boundary between metaphase and anaphase stages of cells, leading to growth retardation manifested by reduction in weight and length of the fetus including mal-development of associated fetal viscera as well as the developing renal system that includes the kidney (El- gaafarawi *et al.,* 2015). This is collaborated by a study by González-Orozco *et al*., (2019) and Naderifar *et al.,* (2015) reported that corpi luteum is the main source of progesterone that plays a major role during fetal organs growth and development and gentamicin interferes with its role.

These perturbations on the stated parameters were found to directly impact negatively to the fetal kidney development includingand significant increase in mean fetal gross kidney weights, total kidney volumes, kidney sizes, reduction in the kidney medulla and cortical thickness, as well as in all the histo-stereological parameters as was also observed in a study by Ali and Abood, (2019) who observed that the cortical and medullary thickness were increasing with increase in the dosage.

5.1.2 Influence of gentamicin on the histomophology of the developing fetal kidney

The findings on the teratogenic effects of gentamicin on the histomorphological layers showed increase in thicknesses of both cortical and medullary layers of the fetal kidney.

These findings agree with Agarwal, *et a.l,* (2013), Ahmed, (2017) and Berghuis *et a.l,* (2017) who observed that cortical layers of the developing kidney can be enhanced following the inflammatory effects of aminoglycosides on the kidney. In addition, this study observed that the disruption to the cortical layer thickness was variably seen to differ based on the dose of gentamicin as well as the time of exposure in the cortical thickness was dependent on cell distribution and it was seen to increase appreciably among all the gentamicin treated groups (LDGG,MDGG, HDGG) when treatment was administered across the trimesters apart from $_{TM3}$ where it was established that there was no marked difference in both the cortical and medullary layer thicknesses as well as in cellular densities between the experimental groups and control. These findings are in line with a study by Gedzelman and Meador, (2012) who also reported reduction in all cortical layers of the kidney.

Similarly, Badawy *et al*., (2019) reported that tobramycin, an aminoglycoside, showed a highly significant increase in kidney weight, alteration of the cortex and medullary cellular layers, vacuolated neurophils and massive cell degeneration with cavity formation in the kidney tissue. This could be attributed to two factors including a decrease in proliferation of kidney cells and induction of cell death in kidneys of fetuses treated with high doses of gentamicin.

A study by Abd *et al.,* (2015) reported that cell necrosis detected in different zones of both cortex and medullar causes widening and irregularity of the cortex as well as in the widening of the medulla. This could have been the case in the present study.

The current study also observed that in all the experimental groups gentamicin administered in the first and second trimesters (TM_1 and TM_2) resulted in significant dilatation of the proximal tubules. These findings were in tandem with a study by Sah *et al.*, (2013) on histomophological effects of tobramycine on fetal kidney that demonstrated dilation of the proximal tubule and poor cortical differentiation.

5.1.3 Influence of gentamicin on the histoquatitative parameters of the developing fetal kidney

This study established that the fetal kidneys of experimental groups had poorly defined cortex, medulla and renal pelvis when compared against the control group. Similarly, the mean fetal kidney volume was slightly higher and the variances in size and volumes were found to depict an inverse relationship with the dose administered. Furthermoreand the reduction in total kidney volume was seen to depict a direct correlation with the time of exposure. The reduction in kidney volumes were directly related toth the time of exposure in that when gentamicin treatment was done at TM_1 the total kidney volume was highest in the HDGG at (0.242±0.001), followed by MDGG at (0.237±0.001) and lastly LDGG at (0.230±0.002). These findings are in line with a study by Berghuis *et al.*, (2017) and Bath and Scharfman, (2013) who observed that the kidney volumes were highest in high dose treatment groups as compared to the control. The findings on the kidney length and width were also seen to depict a similar finding on dose and time response relationship with the doses of exposure and with time of exposure. For instance, when a comparative mean kidney length was done across the three trimesters TM_1, TM_2 and TM_3, it was depicted that at TM_1 the mean fetal kidney length was highest in the HDGG.

Similarly, when the intra and the intergroup fetal kidney weights comparisons were done for the experimental groups, there was a marked intra-group and inter-group variances in the total gross weights and kidney sizes based on the dose of exposure and the time of exposure. For instance, it was observed that when gentamicin treatment was done at TM_1, the mean total kidney weight was highest in HDGG at 0.242±0.012gms followed by MDGG at 0.236±0.008gms and LDGG at 0.227±0.0033gms. These values were found to be statistically significant, $p<0.05$, when the comparisons were done within and across groups and when compared with the control. The results of the present study also are supported by studies done by Erisgin et al., (2019) and Sah et al., (2013) who reported that cortex

alterations and the destructive changes especially of proximal convoluted tubule during fetal kidney development (day 11- day 19) are dose and time dependent.

5.2 Conclusion

In conclusion, the study established that gentamicin does not influence maternal weight regardless of the duration of exposure as well as the amount gentamicin administered. Conversely, gentamicin administered in pregnancy was shown to have a dose and time dependent influence on litter size and fetal birth weight where it was obsereved that the litter size and feta birth weight reduced with an increase in gentamicin dose as well as the duration of exposure to gentamicin. On the other hand, in-utero exposure to gentamicin results in widening of the Bowmans' capsule as well as the proximal convoluted tubule and this demonstrated a direct linear relationship with regards to gentamicin dosage and duration of exposure. Similarly, the cortical and medullary thickness were found to have a dose and time dependent linear relationship as well. In-utero administration of gentamicin has a dose and time dependent influence on the kidney volume that demonstrates a direct and linear relationship.

5.3 Recommendations

The study recommends that.

a). The use Gentamicin during pregnancy should be avoided as it has been shown to be teratogenic to the developing fetal kidney particularly in trimester TM_1 and TM_2 by seeking appropriate alternatives that are safer to the fetus.

b). Should expectant mothers be on chronic use of gentamicin and the drug cannot be withdrawn because of associated withdrawal side effects to the mother, the doses should be adjusted to the minimal effective dosages that would confer the maximum maternal benefits and reduce the teratogenic risks to the developing fetal kidney.

c). Health care workers including clinicians, nurses, midwives and others, need to be educated on how they will need to be educating women of reproductive age and are on chronic usage of gentamicin of its teratogenicity during pregnancy on the need for early planning of their pregnancies for effective introduction of alternatives medicines, to enable them avoid use of gentamicin during pregnancy.

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APPENDICES

Appendix 1: 1st Publication

IOSR Journal of Pharmacy and Biological Sciences (*IOSR-JPBS) e-ISSN: 2278-3008, p-ISSN: 2319-7676. Volume 14, Issue 5 Ser. II* (*Sep – Oct 2019), PP 49-54 www.Iosrjournals.Org* DOI: 10.9790/3008-1405024954 www.iosrjournals.org 49 | Page

Effects of Prenatal Exposure to Varying Doses of Gentamicin on Fetal Weights in Albino Rats (RattusNorvegicus). S.M Asena1, J.K Kweri2, R. Thuo3, G. Kibe4 1 (Department of Human Anatomy, College of Health Sciences, Jomo Kenyatta University of Agriculture and Technology, Kenya) 32 (Department of Human Anatomy, College of Health Sciences, Jomo Kenyatta University of Agriculture and Technology, Kenya) 3 (Department of Surgery, College of Health Sciences, Jomo Kenyatta University of Agriculture and Technology, Kenya) 4 (Department of Human Anatomy College of Health Sciences, Jomo Kenyatta University of Agriculture and Technology, Kenya) **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 gentamicin 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 followsand trimester 1 study group TM_1 that got gentamicin from gestational day 1 to birth, trimester two study group (TM_2) day 8-day to birth and trimester three study group TM_3 (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 gentamicin respectively and water ad libitum once daily at 9:00am. Gentamicin 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 (TM_1) 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 gentamicin during pregnancy leads to low birth weight. The reduction in birth weight is time and dose dependent hence more emphasis on cautionary use of gentamicin 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. ---------- Date of Submission: 19-09-2019 Date of Acceptance: 07-10-2019 ----------

Appendix II: Data Capture Sheets

DATA CAPTURE SHEET FOR EXPECTANT ALBINO RATS

ALBINO RAT IDENTITY……

INITIAL WEIGHT……DOSE CALCULATION……

……

DATE	WEIGHT IN GRAMS	GENTAMICIN DOSE	GENERAL CONDITION OF RAT

DATA CAPTURE SHEET FOR THE ALBINO FETUSES

ALBINO RAT IDENTITY (**MOTHER**)……..

DATE OF HARVESTING…….FIXATIVE USED……..

TOTAL NO. OF FETUSES…….

TOTAL NO. OF RESORPTIONS……

TOTAL NUMBER OF FETUSES WITH CONGENITAL MALFORMATIONS…….

NO. OF DEAD FETUSES…….

	F1	F2	F3	F4	FT	F6	F7	F8	F9	F10	F11	F12
GROSS APPEARANCE												
FETAL WT (g)												
FETAL CROWN RUMP LENGTH (CM)												
OBVIOUS CONGENITAL ABNORMALITIES OF THE FETUS												
RESORPTIONS/ DEVOURED FETUSES												
PLACENTA WEIIGHT												
HEAD CIRCUMFERENCE												
BI-PARIETAL DIAMETER												
KIDNEY												
GROSS APPEARANCE												

CONGENITAL ANOMALIES OF THE KIDNEY												
KIDNEY WT (g)												
TOTAL KIDNEY VOLUME												
VOLUME DENSITY OF KIDNEY CORTEX												
VOLUME DENSITY OF DENSITY MEDULLA												

Appendix III: Ethical Approval Certificate

JOMO KENYATTA UNIVERSITY
OF
AGRICULTURE AND TECHNOLOGY
P. O. Box 62000-00200 Nairobi, Kenya Tel 0675870225 OR Extn 3209
Institutional Ethics Review Committee

April 19th, 2018 **REF: JKU/2/4/896A**

Shadrack Asena Mugami
Department of Human Anatomy.

Dear Mr. Mugami,

RE: HISTOSTEREOLOGICAL STUDY ON THE EFFECTS OF IN-UTERO EXPOSURE TO VARIED DOSES OF GENTAMICINE ON THE DEVELOPMENT OF THE FETAL KIDNEYS IN ALBINO RATS (RATTUS NORVEGICUS)

The JKUAT Institutional Ethics Review Committee has reviewed your responses to issues raised regarding your application to conduct the above mentioned study with you as the Principal Investigator.

The is to inform you that the IERC has approved your protocol. The approval period is from April 19th 2018 to April 19th 2019 and is subject to compliance with the following requirements:

a) Only approved documents (informed consent, study instruments, study protocol, etc.) will be used.
b) All changes (amendments, deviations, violations, etc.) must be submitted for review and approval by the JKUAT IERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the IERC immediately.
d) Any changes, anticipated or otherwise that may increase the risks to or affect the welfare of study participants and others or affect the integrity of the study must be reported immediately.
e) Should you require an extension of the approval period, kindly submit a request for extension 60 days prior to the expiry of the current approval period and attach supporting documentation.
f) Clearance for export of data or specimens must be obtained from the JKUAT IERC as well as the relevant government agencies for each consignment for export.
g) The IERC requires a copy of the final report for record to reduce chances for duplication of similar studies.

Should you require clarification, kindly contact the JKUAT IERC Secretariat.

Yours Sincerely,

DR. PATRICK MBINDYO
SECRETARY, IERC

J.K.U.A.T
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Setting Trends in Higher Education, Research and Innovation