HISTOSTEREOLOGICAL NEPHROTERATOGENIC EFECTS OF ENALAPRIL ON THE FETAL KIDNEYS IN ALBINO RATS (*RATTUS NORVEGICUS*)

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Histostereological nephroteratogenic effects of enalapril 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

This is a dedication to my parents, my spouse Jimmy and our queens; Reychell and Emmah who provided me with moral support throughout this study period.

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LIST OF ABBREVIATIONS AND ACRONYMS

AHD	Antihypertensive drug
ACEis	Angiotensin converting enzyme inhibitors
ANOVA	Analysis of variance
et al	Latin phrase which means "and others"
BD	Bi-parietal Diameter
BW	Body weight
C	Control
CNS	Central nervous system
COHES	College of Health Sciences
CRL	Crown rump length
GD	Gestational dates
HDEG	High dose Enalapril group
HED	Human Equivalent Dose
IUGR	Intrauterine growth retardation
JK**UAT**	Jomo Kenyatta University of Agriculture and Technology
Keq	Constant equilibrium -is a characteristic numerical value
KG	Kilogram
LDCG	Low Dose Enalapril group
Ltr	Liter
MDEG	Medium dose Enalapril group
MG	Milligrams
NIH	National Institutes of Health
SAFARI	Small Animal Facility for Research and Innovation
SEM	Standard Error of the Mean
SPSS	Statistical Package of Social Scientist
TM_1	Trimester one
TM_2	Trimester two
TM_3	Trimester three

DEFINITION OF TERMS

Histomophology The use of histology to study the cellular morphology, cell distribution, cellular densities and connecting stromal tissues using a microscope.

Histostereology 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.

Morphometry Is the process of measuring the sizes of an organ including the external shape and dimensions using numerical measuring gadget

Embryolethalities Spontaneous abortion, or stillbirth, due to adverse drug or chemical toxic effects while the embryo is in the mother's womb.

Perfusion The passage of blood, a blood substitute, or other fluid through the blood vessels or other natural channels in an organ or tissue

ABSTRACT

The existing literature has shown that enalapril, a widely prescribed antihypertensive due to its monotherapeutic success and its cost effectiveness, is nephroteratogenic when applied *in-utero*. However, data on its teratogenic histostereological effects on the developing fetal kidneys is generally lacking. At the same time data on whether or not its teratogenic effects on the fetal kidneys are time and dose dependent is also scarce. In carrying out the study, a static-group controlled-experimental study design was adopted where a pure breed of 30 sexually mature albino rat dams weighing between 150-250g were used. These 30 dams were assigned into two study groups of 3 control and 27 experimental. To evaluate the most critical period, the experimental group was subdivided into three study groups of 9 rats each of Low, medium and high Dose Enalapril Groups namely; [LDEG-0.5mg/kg/bw], [MDEG-1mg/kg/Bw], and [HDEG-2mg/kg/bw] respectively. To evaluate the most critical period, 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 trimester one(TM_1),3rats for trimester two (TM_2,) and 3 rats for trimester three (TM_3). All rats were fed with standard rodent pellets from Unga feeds and water *ad-libitum,* while those in the experimental group in addition received enalapril treatment as per their groups of study. All rats were humanely sacrificed at GD_{20} then 3 fetuses with the lowest, median and highest weight from each rats selected and their kidneys harvested, weighed and processed for histo-morphological and stereological analysis. Data was collected using tally sheets, analyzed using SPSS version 23.0 (SPSS Inc., Chicago, IL). 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 $p<0.05$ were considered to be statistically significant. Findings were presented in form of bar charts and tables. This study elucidated that prenatal exposure to enalapril results to varied teratogenic outcomes including increased embryo-lethality, increased intrauterine fetal resorptions, fetal congenital abnormalities, and reduced mean litter sizes; significant reduction in gross kidney volumes, reduction in cortical layer thickness, widened capsular space with glomerular hypertrophy. In conclusion, when enalapril is administered in the doses of between 1mg/kg/BW and 2mg/kg/BW during pregnancy are teratogenic to the developing fetal kidney particularly during second and third trimesters (TM_2) and (TM_3) respectively. Teratogenic effects in trimester one (TM_1) occurred only in high doses. The most vulnerable window period and critical dose for enalapril teratogenicity was established to be TM_2 at 2mg/kg/BW respectively. The study recommends that maternal enalapril should be avoided particularly in TM_2, TM_3 and in TM_1 in doses of 2mg/kg/BW and alternative antihypertensive sought. Further studies are recommended in higher non-human primates like monkeys and gorillas.

CHAPTER ONE
INTRODUCTION

1.1 Description of enalapril.

Enalapril, an angiotensin- converting enzyme inhibitors sold under the brand name Vasotec, Renitec, Enacard among other names, remains the first drug of choice in the treatment of hypertension (Oh *et al.,* 2016) alongside being nephro protective, ameliorates diabetic nephropathy and improves cardiac conditions outcomes. ACE inhibitors act on the renin-angiotensin-aldosterone and inhibits the formation of angiotensin II and consequently the downstream effects through the angiotensin II type 1 (AT1) receptor, (vasoconstriction, cell growth, sodium and water retention, sympathetic activation) and the angiotensin II type 2 (AT2) receptor.(Li *et al.,*2014) Angiotensin converting enzyme (ACE). Its chemical formula is $C_{20}H_{28}N_2O_5$ with a molecular weight of 376.4467g/ and is available both in tablet and solution form (Verbeeck *et al.*,2017)

Enalapril is a pro-drug that is rapidly hydrolyzed in the liver to the active metabolite enalaprilat that is later excreted by the kidneys. The peak serum concentration of this active metabolite form (enalaprilat) occurs 3–4 hours after an oral dose. The effective half-life for accumulation of enalaprilat following multiple doses of enalapril maleate is 11 hours, while the duration of its effect is 12–14 hours (Díez, 2017). A steady-state serum concentration is achieved by the fourth day of administration. The disposition of enalapril and enalaprilat in patients with renal insufficiency is similar to that in patients with normal renal function until the glomerular filtration rate is 30 mL/min or less (Arafat *et al.*,2005)

1.2 The mmechanism of Enarapril teratogenesis on developing fetal kidneys

When enalapril and its principal metabolite (enalaprilat) accumulates in maternal blood, they create a negative osmotic gradient between the maternal and fetal tissue. This negative osmotic gradient coupled with low molecular weight of 376.4467g/mol enables them to readily cross the maternal placental barrier accumulating in the fetal tissues causing morphogenetic disturbances to the differentiating tissues of nephrogenic cord in the fetal tissues that would result in the definitive fetal kidneys (Reisenberger *et al.,* 1996)

These morphogenetic in-utero disturbances occasioned by the in utero-exposure to enalapril on the developing kidney tissues are believed to be caused by their mode of action on the renin-angiotensin system that disturb the fine balance of growth factors that are essential for renal development leading to reduced nephron endowment **.** As such, these intrauterine disturbances to the developing fetal kidney may cause a permanent morphogenetic and eventual structural damages to the kidneys increasing the possibilities of associated fetal and neonatal morbidity and death (Yosypiv *et al.*,2020)

1.3 The comparative patterns of kidney morphogenesis between rats and humans

The comparative patterns of fetal kidney development between rats and human depict similar correlational morphogenetic stages of development in that, the primordial kidneys that entail the pronephros, metanephros and mesonephros are derived from the intermediate mesoderm, one of the embryonic germ layers (Seely,2017). The morphogenetic process of kidney development becomes conspicuous in the second trimester in human and this corresponds to day 10 of gestation in rats and progresses through to additional 10 days post-natally (Sengupta,2013).Kidneys then attain morphological and functional maturity in about 11 days post-natal when the total numbers of nephrons are seen (Seely,2017). In both human and in rats the pronephros and mesonephros are transient structures that regress there after allowing the metanephros or metanephric kidney to differentiate into the adult kidney (Frazier,2017)

In both rats and humans, the pronephros along with its duct is the first stage of kidney development and appears approximately around gestational day twenty two (GD_{22}) in humans and gestational day eleven (GD_{11}) in the rat. The nonfunctional caudal portion of the pronephric duct remains and eventually becomes the Wolffian duct. As the mesonephros regresses, the metanephric kidney develops by the outgrowth and branching of the ureteric bud (UB) into the metanephric mesenchyme (MM) initiating nephrogenesis or the formation of nephrons (McMahon,2016).

Nephrogenesis begins in the fetus and is completed before birth in humans but continues postnatally in the rat up to the 10^{th} postnatal day. (Seely,2017). The formation of nephrons involves tightly controlled genetic and molecular pathways which result in the transformation

of the MM to epithelial lined structures which undergo further configurational changes to form nephrons**,**(Krause *et al.,*2015)**.**

1.4 The histomorphological effects of antihypertensives on developing kidneys

Existing literature has shown that antihypertensive in the same class with enalapril like captopril can interfere with cytodifferentiation of the kidneys that affect the formation of the glomeruli structures leading to reduced glomeruli, hydro-vascularity, with congestion and mesangial cells proliferation (Simeoni *et al.,*2017). Similarly, a study on effects of prenatal exposure to captopril also associated it with cystic and dilated the proximal convoluted tubules, infiltration of inflammatory cells and congestion of blood vessels in the kidney,(Sharma *et al.,*2019)

1.5 Problem Statement.

Globally, the WHO renal case reports show that there is a steady increase in cases of renal failure and other related kidney disease across all age groups (WHO 2018). Though studies have shown that there could be an association between intra-uterine exposures to antihypertensive medicines like enalapril with some renal disorders seen among children born of mothers treated with the drug as shown by Bateman *et al., (*2017), there is no data on the histo-quantitative and histo-morphological effects on the in-utero exposure to enalapril on the development of the fetal kidneys. At the same time, whether or not the observed histoquantitive changes on the fetal kidneys are dose and time dependent is also unclear. This is despite the known fact that enalapril plus its principal metabolites readily cross the maternal placental barrier accumulating in the fetal tissues with potential to induce a wide range of teratogenic injurious effects to the developing fetal kidneys alongside other fetal organs. This study therefore sought to evaluate the histo-morphological and stereological nephroteratogenic effects of enalapril on the development of the fetal kidneys following in utero exposure to varied doses of Enalapril at different gestational periods in albino rats.

1.6 Justification of the study

The availability of nephro-teratogenic data on the intrauterine teratogenic perturbations arising from antihypertensive like enalapril will serve as an important predictor into some of the structural alterations induced into the developing kidneys, that would subsequently inform the causes of increasing cases of renal failure in the adulthood today and whose causes are yet to be established. However, there is paucity of nephro-teratogenic data on the histomorphological and stereological effects of enalapril when it is prenatally exposed in varied doses and at different window periods. The study also sought to unravel the controversy surrounding use or the non-use of enalapril during pregnancy.

Lack of nephro-teratogenic data that elucidates the most critical dose and vulnerable teratogenic periods of enalapril will continue denying mothers the benefits accrued to enalapril and fetus will be at risk of developing structural defects of the kidneys that would lead to the development of some probable renal conditions in adulthood as depicted by the increasing cases of renal diseases both nationally and globally as per the WHO report, 2018. It was therefore imperative to carry out this study out to form baseline data on the rational application of enalapril during pregnancy.

1.7 Significance of the study

Findings from this study would be useful in guiding obstetricians and future researchers on the maternal use of enalapril to confer the maternal benefits in its usage while safe guarding the fetus from any associated nephroteratogenic effects. Again, the observed structural abnormalities in the developing fetal kidneys emanating from in-utero exposure to different doses of enalapril at different window periods would help in extrapolating some of the causes of increasing cases of renal failure among others that are on the rise globally. Data from this study will therefore be useful in redirecting future teratogenic studies on enalapril as well as in redirecting the adjustment of the treatment algorithms in terms of dosages and the appropriate gestational periods in management of some maternal hypertensive conditions during pregnancy.

1.8 Research question, Objectives and hypothesis

1.8.1 Research question

What are the histo-morphological and stereological teratogenic effects of enalapril on the development of the fetal kidneys following in utero exposure to varied doses of enalapril at different gestational periods in albino rats?

1.8.2 Broad objective

To evaluate the histo-morphological and stereological teratogenic effects of *in- utero* exposure to varied doses of enalapril when administered at different gestational periods in albino rats.

1.1.3 Specific objectives

1. To establish the pregnancy outcomes following *in-utero* exposure to varied doses of Enalapril at different window periods.
2. To evaluate the histo-morphological outcomes that occur in the developing fetal Kidney following *in-utero* exposure to varied doses of enalapril at different window periods
3. To evaluate the Histo-stereological changes that occurs in the developing fetal kidneys following *in-utero* exposure to varied doses of enalapril at different window periods.
4. To determine whether the teratogenic histo-stereological effects of enalapril on the developing fetal kidneys structures are time and dose dependent.

1.9 Hypothesis (H_0)

Prenatal exposure to enalapril has no teratogenic effects on the pregnancy outcomes nor nephroteratogenic histomorphological and histostereological effects in the developing fetal kidney in albino rats.

1.10 Study assumptions

In carrying out this study, it was assumed that the albino rats (*Rattus Norvegicus)* model used in animal experimentation would replicate the actual teratogenic induction scenario that would occur to humans due to the known close association of this kind of rat species with human biological and functional outcomes when exposed to the same drug.

1.11 Study limitations

Some of the anticipated study limitations included, failure of some dams to conceive at the same time following the introduction of the males in the cages, death of the animals along the experimental process following mishaps in drug administration while administering enalapril using the gastric gavage needle.

1.12 Study boundaries

To overcome these challenges the following delimitation measures were applied:-

i. Dams that did not conceive on the first day of the experiment were separated from those that conceived, put in a separate cage then a male rat reintroduced overnight again until prove of pregnancy was established. Then their treatment was done separately as they would have different gestational days with the ones that got pregnant the first day.

ii. Dams that fell ill or died in the course of the experimentation in this study were noted as per dosage and the time of exposure. Postmortems were conducted to establish the cause of death then repeat experiments on those that died or fell ill were done after the main experiment was completed.

iii. A pilot study was done to test the study protocol and to minimize causes of errors as much as possible

CHAPTER TWO

LITERATURE REVIEW

2.1. Enalapril class, structure and mode of action

Enalapril, sold under the brand name Vasotec, Renitec, Enacard among others, is a medication used to treat hypertension, symptomatic heart failure, and asymptomatic left ventricular dysfunction. (Turgeon *et al.*,2019). It is in a class of medications called angiotensin-converting enzyme (ACE) inhibitors that decrease certain chemicals that tighten the blood vessels, so blood flows more smoothly and the heart can pump blood more efficiently (Izzedine *et al.*,2015) Its mechanism remains unclear, but enalaprilat is a peptidyl dipeptidase that inhibits the peripheral conversion of angiotensin I in human subjects and animals to angiotensin II (action of angiotensin converting enzyme), hence vasodilation. Decreased production of angiotensin II enhances natriuresis, lowers blood pressure, and prevents remodeling of smooth muscle and cardiac myocytes, (Tai *et al.*,2017).

It has a chemical formula; $C_{20}H_{28}N_2O_5$ with a molecular weight of 376.4467g/mol and is available both in tablet and solution form. Enalapril is a prodrug that is rapidly hydrolyzed in the liver to the active metabolite enalaprilat. Enalapril maleate is 60% absorbed and 40% bioavailable as enalaprilat(Izzedine *et al.*,2015).Both compounds undergo renal excretion without further metabolism. Peak serum concentration of this active form occurs 3–4 h after an oral dose with half-life of accumulation is approximately 11 hours and duration of effect is 12–14 hours (MacFadyen *et al.*,1993). The disposition of enalapril and enalaprilat in patients with renal insufficiency is similar to that in patients with normal renal function until the glomerular filtration rate is 30 mL/min or less (Tai *et al.*,2017)

2.2 The injurious mechanism of enalapril on developing fetal tissues.

The injurious mechanism of enalapril on the developing fetal tissue has well been elucidated by a study done by Yosypiv, (2020)**.**The injurious effects of enalapril to the developing fetal tissues is mainly by blockage of the conversion of angiotensin I to angiotensin II. Angiotensin II receptors are widely expressed in fetal tissues and it is suggested that angiotensin II plays a part in the early development of the brain, heart, and kidney (Wegleiter *et al.*,2018). The ACE inhibitors are competitive inhibitors of angiotensin II. They affect both the angiotensin/aldosterone and bradykinin/prostaglandin systems. In addition, they are associated with reduction in systemic blood pressure and uterine blood flow secondary to the significant vasodilatory effect, possibly caused by a decrease in angiotensin-II production and reduced degradation of bradykinin and prostaglandins mediated by these agents. The reduction in amniotic fluid can also be observed in 30-32 weeks of gestation which might be due to decrease in fetal renal function and urine output. (Al-Maawali, *et al.*,2012).

2.3 The comparative histomorphogenesis of human and rat fetal kidneys

Rats and humans depict similar correlational developmental stages but differ in timing, scale, and global features such as lobe formation and progenitor niche organization. (Seely,2017)**.** In both species, kidneys develop from the intermediate mesoderm, one of the embryonic germ layers is the source of three phases of kidney development; the pronephros, the mesonephros, and the metanephros (Krause *et al.*,2015)**.** Human kidney development commences at around 4 weeks of gestation and ends around 34–37 weeks of gestation Over this period, a reiterative inductive process establishes the nephron complement, while in rats this occurs from embryonic day 15.5 and postnatal day 2. The kidneys then attains morphological and functional maturity in about 11 days post-natally when the total number of nephrons are seen.(Seely,2017)**.**The pronephros and mesonephros are transient structures in mammals which regress allowing the metanephros or metanephric kidney to differentiate into the adult kidney, (Krause *et al.*,015).

The primordial pronephric duct is seen around gestation day twenty two (GD_{22}) in humans and gestation day eleven (GD_{11}) in the rat. The caudal portion of the pronephric duct eventually becomes the Wolffian duct. The branching from the distal portion of mesonephric

duct called the ureteric bud (UB) induces the metanephric mesenchyme (MM) initiating nephrogenesis or the formation of nephrons (Maurya *et al*.,2018).Nephrogenesis is completed before birth in human but continues postnatally in the rat up to the 10^{th} postnatal day. The formation of nephrons involves tightly controlled genetic and molecular pathways which result in the mesenchymal epithelial transformation to form nephrons. (Kobayashi *et al.*,2014.)

2.4 Comparative morphological features of human and rat foetal kidneys

Human and rat kidneys bear some morphological similarity in that it generates approximately 1,000,000 nephrons, (the basic unit of the kidney), while those of a rat are 16,000 nephrons with a 12–13-day post-natal period of active nephrogenesis. (Baldelomar *et al*.,2018). Human glomeruli have similar sizes while rat juxtamedullary glomeruli are larger than glomeruli cortex in rats.(Morya *et al.,* 2018).

2.5 Histo-morphological effects of enalapril on fetal kidney.

Enalapril causes papillary atrophy, interstitial fibrosis and inflammation, tubular atrophy and dilatation, and focal glomerulosclerosis in exposed rat kidneys (Guron *et al.*,1997).In conclusion, neonatal angiotensin-converting enzyme inhibition showed reduced glomeruli and hydro-vascularity, with glomeruli congestion and mesangial cells proliferation,(Paixão *et al.*,2013).It is also associated with cystic and dilated proximal convoluted tubules, infiltration of inflammatory cells and congestion of blood vessels in the kidneys.(Al-Ani *et al.*,2018).

2.6. Histostereological effects of angiotensin converting enzyme inhibitors on fetal kidney

Histo-stereological studies by Boutroy *et al.*,(1984).,Buttar *et al*.,(1997;).,Duminy *et al.*,(1981), noted that quantitative effects to the fetal kidney following angiotensin converting enzyme inhibitors administration showed significant reduction ($P<0.05$) in gross renal volumes of both right and left fetal kidneys and reduction in cortex and medullary layers thickness. Inhibition of the rennin angiotensin aldosterone system culminated into reduction

in the thickness of the cortex, disaggregation of cells in the Bowman's capsule with increase in intra capsular space. (Gubler *et al.*,1981)

2.7.**Patterns of fetal kidney teratogenicity in relation to dose and time of exposure.**

The teratogenic effects of foetal kidney following maternal angiotensin converting enzyme inhibitors is dependent on time of exposure (Arbogast *et al.*,2006). Antihypertensive drugs affects fetal morphogenesis throughout the pregnancy (Bullo *et al.*,2012). A study by Macconi *et al.,* (2009) showed that captopril a drug in the same class with enalapril, caused major structural malformations during the first trimester, a period that corresponds to the embryonic stage during which major organs develops. Other studies by Obic, (2011) and Schreuder *et al.,* (2011) pointed out that antihypertensive drugs issued as monotherapy are safer compared with poly therapy while monotherapy doubles the risk of malformations. Ray *et al*., (2007) noted that poly therapy triples the risk He also reported that the risk of major congenital malformations is influenced not only by type of anti-hypertensive drug, but also by dose and other variables, which should be taken into account in the management of hypertension in women of reproductive age

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study area:

This research was carried out at Small Animal Facility for Research and Innovation (SAFARI) in Jomo Kenyatta University of Agriculture and Technology (JKUAT) for animal experimentation. Processing of specimens for light microscopy and stereology was carried out in histology lab, department of human anatomy

3.2 Study design:

A Laboratory static-group controlled-experimental study design was adopted in carrying out this study

3.3: Study subject:

Thirty nulliparous albino rats' dams weighing between 150-250 grams of the species *Rattus norvegicus* of pure colony were used as the experimental model in this study. The beneficial facts behind using rat model include; (a) similar physiological functions with humans (b)low incidence of spontaneously occurring congenital defects, (b) a relatively short gestational span, (c) a large litter size, (d) low cost of maintaining the animals and, (e) and considerable amount of the reproductive data on the rat is already available (Moran *et al.,*2016). Albino rats have red eyes and white fur. (Cragan,2006). They become sexually mature approximately 4-5 weeks in females and at 45-48 postnatal dates in males. They live for an average of 3 years (Clancy *et al.,*2001). The gestation period in females is roughly estimated at from 21 to 23 days during which the fetuses are viable, has 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 450 grams and male rats weighs (350 to 650 grams), and male rats are usually larger than females in (Kamel *et al.,*2018). In this study, five-teen males were used since every two females were allocated one male.

3.4 Sampling method

The sample size was established at two levels as outlined below

Level one; acquisition of the experimental dams

The resource equation by Arifin, & Zahiruddin, (2017). was adopted and the equation states that E=Total number of Animals-Total number of groups (E=TA-TG). The measured value 'E' which is the degree of freedom of analysis of variance (ANOVA) based on a decided sample size value ('E') should lie between 10 and 20 animals A value less than 10 necessitates adding more animals to increase the chance of getting significant results while a value more than 20 increase the cost of the study without increasing the significance of the results, (Charan & Kantharia, 2013)

Total number of groups=**10** while E =20.

Hence: **20** (Decided sample size) =**X (**total number of animals)-**10** (Total number of groups.

Thus X =20+10

=30

Level two; acquisition of the fetuses for histomorphology and stereology

All 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 making a total sample size of 90 fetuses (i.e. 3 fetuses from each of the 30 study dams equals 90 fetuses). The remaining fetuses were preserved in zenker's solution for use in case of any mishaps.in future in course of the study

3.5 Grouping of dams

The 30 dams used in the study were assigned to either three rats as the control and 27 in the experimental category. To evaluate whether enalapril nephro-teratogenicity is dose dependent, 27 rats in the experimental group were subdivided into three study groups of 9 rats each of Low dose enalapril group [LDEG], medium dose enalapril group [MDEG], and High dose enalapril group[HDEG].To further evaluate whether enalapril nephro-teratogenicity is time dependent, 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 3 rats for trimester one (TM_1), 3 rats for trimester two(TM_2,) and 3 rats for trimester three(TM_3)

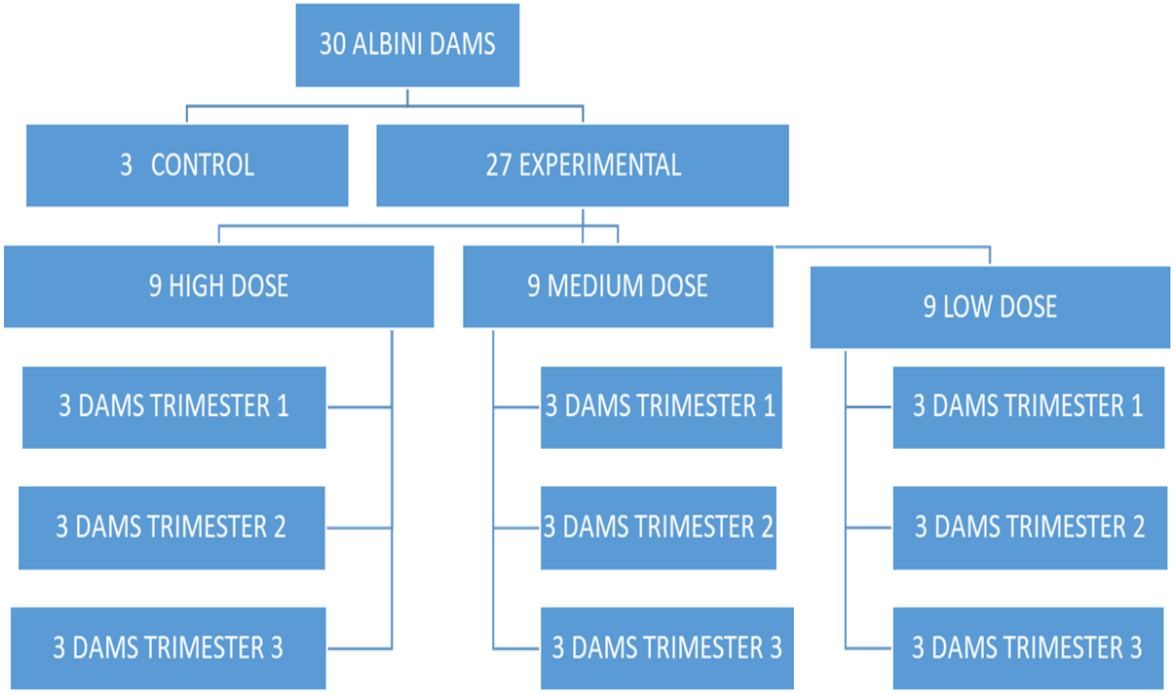


Figure 3. 1: 30 dams in control and experimental groups as LDEG, MDEG and HDEG in TM_1, TM_2 and TM_3.

3.6 Selection and Inclusion criteria

3.6.1 Inclusion criteria

a) The healthy rats
b) Rats that conceived
c) All live fetuses

3.6.2 Exclusion criteria

a) All rats with negative pregnancy result after several overnight exposures to male rats
b) All rats that fell sick in the process of enalapril 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 am. Acclimatization was

allowed for a period of seven days. The animals in the control and in the experimental categories were fed as follows:-

(i). Control sub group;

This 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 from day one(GD_1) to gestation day twenty(.GD_{20}) The mothers were then sacrificed on 20^{th} day of gestation

(ii).Experimental groups:

The animals in the experimental were fed on standard rodent pallets as in the control group and water *ad-libitum* but in addition they received oral enalapril tablets as solutions in distilled water based on their assigned doses of low dose Enarapril group (LDEG,) as 0.5mg/kg bwt, medium dose Enarapril group(MDEG) as 10mg/kg bwt and High dose Enarapril group(HDEG) as 20mg/kg bwt and according to the trimester of exposure in trimester one($TM_{1)}$ trimester two(TM_2) and trimester three (TM_3) The 9 rats in trimester one (TM_1) received enalapril treatment from gestation day one (GD_1) up to day gestation day twenty (GD_{20}); those in trimester two (TM_2) from gestational day seven (GD_7) through gestation day twenty(GD_{20}), while those in trimester three (TM_3) received the tablets from gestational day fourteen (GD_{14}) all through to- gestational day twenty(GD_{20}) i.e. the last day of gestation.(Sengupta,2013)

3.8 Handling of Rats

The rats were handled by principal investigator and a trained research assistant for the purpose of obtaining daily weights between 0800 and 0830am. All procedures were performed according to Guide for the Care and Use of Laboratory Animals (Albus, 2012).

3.9 Breeding of rats and pregnancy determination

One sexually mature male albino rat from a pure colony of age 50 days post natally was introduced into a standard cage with two female rats at 2.30pm (+/- 30 minutes) and after 24 hours. Pregnancy was determined by use of vaginal smears taken from mated dams. Presence of spermatozoon on the smear when observed under light microscope was a confirmation that coitus had taken place while presence of polyhedral epithelial cells on the swab was denoted as the first day of gestation. (GD_1**)** (Hamid & Zakaria, 2013).

a) Materials used in determination of pregnancy

a) Cotton tipped swab
b) 85% phosphate buffered saline
c) Microscope slides
d) Ethanol (95%)
e) Absolute alcohol
f) 10mls blunt tipped disposable pipettes
g) Geimsa stain

b) Procedure followed in the determination of pregnancy

i. The animal was restrained with a gauze holder against the body
ii. 1ml of saline was introduced into the vaginal cavity using a blunt tipped disposable pipette (vaginal wash) and Cotton tipped swab moistened with phosphate buffered saline was then gently inserted into the vaginal cavity
iii. The swab was slightly rolled before withdrawing
iv. The moist swab was withdrawn and rolled onto a clean glass microscope slide
v. The specimen was then spray fixed using 95% ethanol
vi. The slides were subsequently air dried
vii. The slide was then stained with Geimsa stain

viii. Observations of the slide followed, and was done under the BP Olympus microscope

Large polyhedral cornified epithelial cells, many neutrophils on the smear and scattered epithelial cells served as an indicator that fertilization had taken place and this was counted as the first day of pregnancy (GD_1). 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 positive. 99% of the rats tested positive for the pregnancy.

3.10 Determination, calculation and administration of the doses:

The minimum enalapril dose used in this study is 5mg, medium dose is 10mg and maximum dose is 20mg while the average weight of an adult human is approximately 60kg, (Walpole *et al*, 2012)

a) Determination of high dose enalapril group (Nair, 2016)

Highest dose enalapril-20mg

Average weight of a man-60kg

20mg = 60kg

X =1kg

X=1x20/60 =**0.3mg/kg**

AED = HED X Km factor

Therefore, 0.3mg/kg x 6.2 =**2mg/kg**

b) Determination of Medium dose enalapril group

Medium dose enalapril-10mg

Average weight of a man-60kg

10mg = 60kg

X = 1kg

X=1x10/60 =**0.2mg/kg**

AED = HED X Km factor

Therefore 0.02mg/kg x 6.2 = 1**mg/kg**

c) Determination of Low Dose enalapril group

Low dose enalapril -5mg

Average weight of a man-60kg

5mg = 60kg

X = 1kg

X=1x5/60 =**0.08mg/kg**

AED = HED X Km factor

Therefore 0.8mg/kg x 6.2 =**0.5mg/kg**

Dilutions

Dilution was done by use of deionized water.

3.11 Determination of the critical teratogenic dose and the most vulnerable period

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

Animal groupings were done as described in (**figure 3.1*)*** and administration of enalapril in each of the groups was done as follows:

In each of the groups (LDEG, MDEG, HDEG), the 9 dams were sub divided in three sub-groups of 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 gestation day one ($GD_{1)}$ to gestational day seven($GD_{7,}$) while trimester two(TM_2) was between gestation day seven(GD_7) to gestation day fourteen (GD_{14})and third trimester gestation day fourteen(GD_{14}) to gestation day twenty(GD_{20}.)

To determine the vulnerable periods of enalapril teratogenicity on the fetal kidney, it was administered as follows: -

1. All trimester ones **(**TM_1**)** rats in all treatment groups received enalapril dosages from gestation day one(GD_1) to gestation day (GD_{20})
2. All trimester two(TM_2) rats in all treatment groups enalapril from gestation day seven (GD_7) to gestation day twenty(GD_{20})
3. All trimester three (TM_3) rats in all treatment groups received enalapril from gestation day fourteen(GD_{14}) to gestation day twenty(GD_{20})

3.12 Humane sacrificing of the pregnant albino rats

All rats were humanely sacrificed on gestation day twenty (GD_{20}) just before delivery by the use of concentrated carbon dioxide.

a) 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%
vi. Concentration
vii. Mounting board
viii. Mounting pins
ix. A pair of scissors
x. A pair of forceps(toothed)
xi. Scalpel blade
xii. Scalpel blade handle
xiii. Fixatives- 10% Formaldehyde and 5% Zenker's solution
xiv. Drip set 2 in number
xv. Hypodermic needle gauge 20
xvi. Gloves (surgical)
xvii. Magnifying glass
xviii. Ruler
xix. Electronic weighing machine
xx. Specimen collection bottle

b) Procedure for anaesthetizing and perfusing the rats

i. Concentrated carbon dioxide was introduced into a bell jar
ii. A tight fitting lid was then put into the bell jar
iii. The pregnant rat was then put into the bell jar
iv. The rat was waited for 10-15 minutes to be anaesthetized
v. The rat was removed from the bell jar and mounted onto the board using mounting pins with dorsal side on the board
vi. 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

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

viii. The blood was cleared from the rat with physiological saline (200mls of 0.85mol/ltr) through the left ventricle of the heart (saline flew by force of gravity from one of the drip set)

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

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

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

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

xiii. The fetuses were then harvested by opening the abdomen along the linear Alba and the uterine horns opened along the anti-mesometrial borders.

3.13 Harvesting of fetuses

In all cases, the pregnant rats were sacrificed by either inhaled carbon dioxide between 9.30 and 11am at gestational day twenty (GD_{20}). This was done to prevent the mothers from devouring any damaged or congenitally deformed fetus. Twenty minutes after anesthesia with 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 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.

3.1.3.1Procedure 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 enalapril 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
vii. Other fetal growth parameters including fetal lengths, crown-ramp lengths and bi-parietal diameters were taken and recorded

3.14 Harvesting of the fetal kidneys

i. Fetal kidneys histomorphological and stereological analysis were subsequently harvested using the following procedure:-
ii. Fetus were mounted onto the board using mounting pins (dorsal side facing the board)
iii. Using a pair of scissors and forceps the abdominal muscle layers were dissected at the middle to expose the abdominal viscera of the fetus.
iv. Using a magnifying glass the whole fetal kidneys was identified and removed.
v. 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.

vi. The entire kidneys were excised at the level of the renal pelvis. The kidneys were then 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

3.15 Materials used for light microscopy

i. Foetal kidneys
ii. Zenkers solution
iii. DPX moutant
iv. Glass slides and cover slips
v. Haematoxyline and Eosin
vi. Glass staining square jars
vii. Paraffin wax
viii. Microtome knives
ix. Rotary microtome
x. Heater and water bath container
xi. Specimen bottles
xii. Slide holders
xiii. Distilled water
xiv. Formaldehyde 40 % concentration
xv. Xylene
xvi. Isopropyl Enalapril
xvii. Graduated test tubes
xviii. Wood blocs
xix. Beakers
xx. Egg albumin
xxi. Dropper
xxii. Cedar wood oil

3.15.1 Procedure used for processing fetal kidney for light microscopy and stereology

i. The fetal kidneys were fixed in the Bouin's (Zenkers' solution) for 24 hours
ii. They were dehydrated in an ascending concentration of enalapril (50%, 60%, 70%, 80%, 90%, 95% and 100% (absolute) each for one hour.
iii. They were cleared by immersion with cedar wood oil for 12 hours and infiltrated with paraplast© wax for 12 hours at 56^0c
iv. The Kidney tissue was then orientated in the longitudinal axis
v. They were then embedded in paraffin wax on the wooden blocs
vi. Excess wax was trimmed-off till the entire length of the fetal kidney tissue is exposed
vii. 5µm thick longitudinal sections were cut from head to tail regions with sledge rotary microtome
viii. The cut sections were floated in water at 37^0 c to spread the tissue
ix. The sections were stuck onto glass slides using egg albumin, applied as thin film with a micro-dropper.
x. The slides were then dried in an oven at 37^0 c for 24 hours
xi. Blinding was done by coding all the slides by the research assistance in absence of the researcher
xii. 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.

3.16 Stereological analysis

The total kidney volumes and volume densities of the cortex and medulla were calculated as outlined below

3.16.1 Estimation of reference total kidney volume using Water displacement method.

a) After 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 (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.

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

3.16.2 Determination of total fetal kidney volume, volume density of cortex and medulla by cavarieli method.

i. Selection of the spacing for the point probe
ii. Preparation of kidney Cavalieri sections (5µ thick)
iii. The point probe was tossed randomly onto each section
iv. The points that hit the region of interest was counted using stepanizer stereology tool. All sections were processed keeping a tally of counts per section
v. Calculation of the volume
vi. Between twenty to twenty five sections of 5µm thickness were, sampled from each longitudinal kidney section, obtained by systematic uniform random sampling and
vii. Using the microscope's stage Vernier, images were viewed at magnification of 10x.
viii. 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).
ix. The sum of points that hit the structure were estimated using the stepanizer software **(figure 3.2)**
x. 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.
xi. The picture height was ensured that it match the height of the computer monitor, both defined in pixels.
xii. All images captured both for the control and experimental groups were organized appropriately and saved in one folder.

xiii. Point counting using the Cavalieri principle was employed to estimate the total fetal kidney volume using the formula:

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

Where: **estV**= was the estimation of the volume of the kidney,

$\sum^{m}$**i-1P-**= 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 Cortex and medullary structures and points hitting these areas counted at a final magnification of x40. Then, estimates of their volume density, (Vv) in the reference space were obtained using the formula:

Est = 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

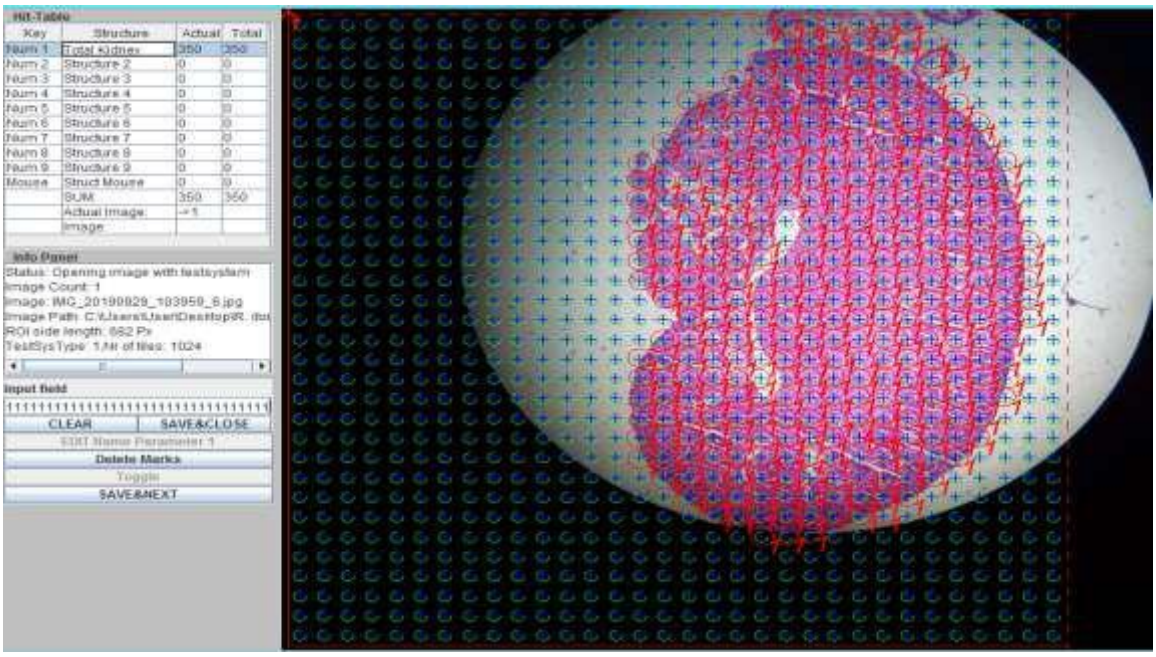


Figure 3.2: Showing a section image from a control slide(x40 H&E) of a fetal kidney superimposed on an equidistant point counting grid

3.16.3 Stereological 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 cavarieli method **(**Tran *et al.*,2015);

$$\text{Shrinkage} = \frac{\text{Volume before immersion - Volume after immersion}}{\text{Volume before immersion}}$$

Where volume before immersion represented volume by water displacement method and volume after immersion represented volume by Cavalieri method

3.17 Equipment and materials for histophotography

3.17.1 equipment and Materials

i. Digital camera (32 megapixels)
ii. BP Olympus microscope
iii. Histological glass slide

3.17.2 Procedure followed in taking photomicrographs

i. Histological slides were mounted on the stage of the microscope
ii. Focus was enhanced by adjusting the microscope
iii. The field was magnified appropriately
iv. Photographs of the regions were taken as they were viewed best under the focus of the microscope and transferred to a laptop
v. The photographs were uploaded in Adobe fireworks programme for labeling

3.18 Statistical analysis

The histomorphological data that included cellular organization, distribution and spatial arrangement of both parenchymal and stromal tissues was analyzed using Histo-photo micrographs at different magnification then exported to Adobe fireworks for qualitative analysis. Histostereological data plus data on pregnancy outcomes was collected using structured checklists and stereological data sheets coded in excel spreadsheets version 23 and analyzed using SPSS programme windows version 23.(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 $P < 0.05$ were considered to be statistically significant.

3.19 Ethical consideration

All procedures were performed with approval from Animal Ethics Committee Jomo Kenyatta University of Science and Technology (JKUAT)

CHAPTER FOUR
RESULTS

4.1: The maternal and fetal pregnancy outcomes

4.1.1: Influence of enalapril on mean daily maternal weight trends from GD_1 to GD_{20}.

Mean maternal weight gain in all the treatment groups across all trimesters depicted an inverse dose response relationship in that, the higher the dose the lower was the trend in the mean maternal weight gain(**figure 4.1),** This parameter also had a direct time dependent relationship, in that when treatment was instituted in trimester two and three (TM_2 &TM_2), there was significant ($p<0.05$) reduction in maternal weight gain in all the treatment groups (LDEG, MDEG, HDEG, unlike in TM_3 where there was no significant difference ($P>0.05$) for the low and medium dose groups compared with the controls (**figure 4.2).**The control group had a trajectory trend in weight gain in comparison to enalapril treated groups(**figure 4.3)**

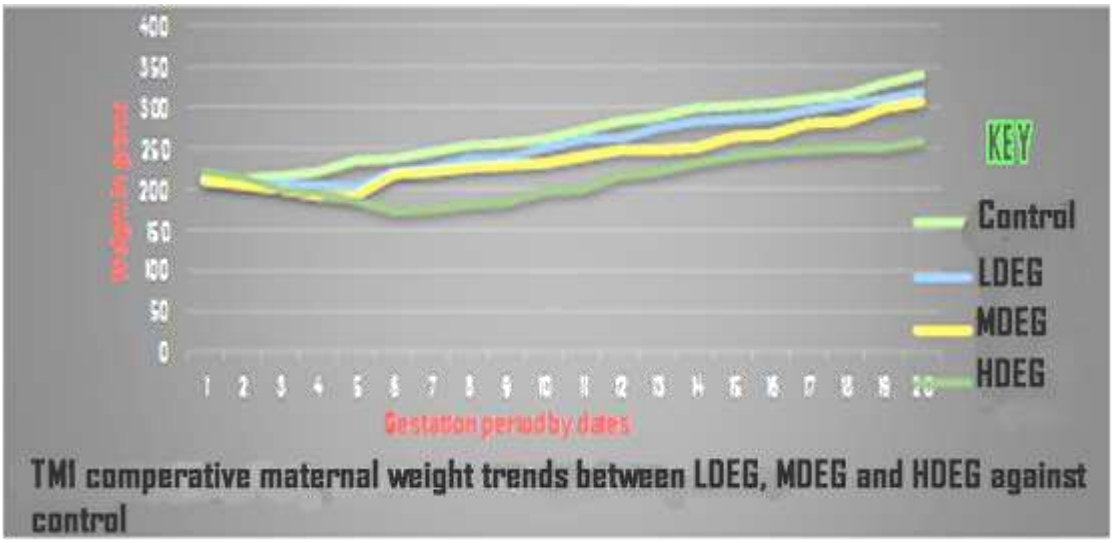


Figure 4. 1: Comparative maternal weight gain trends for the enalapril treated Groups LDEG, MDEG, and HDEG against control group at TM_1.

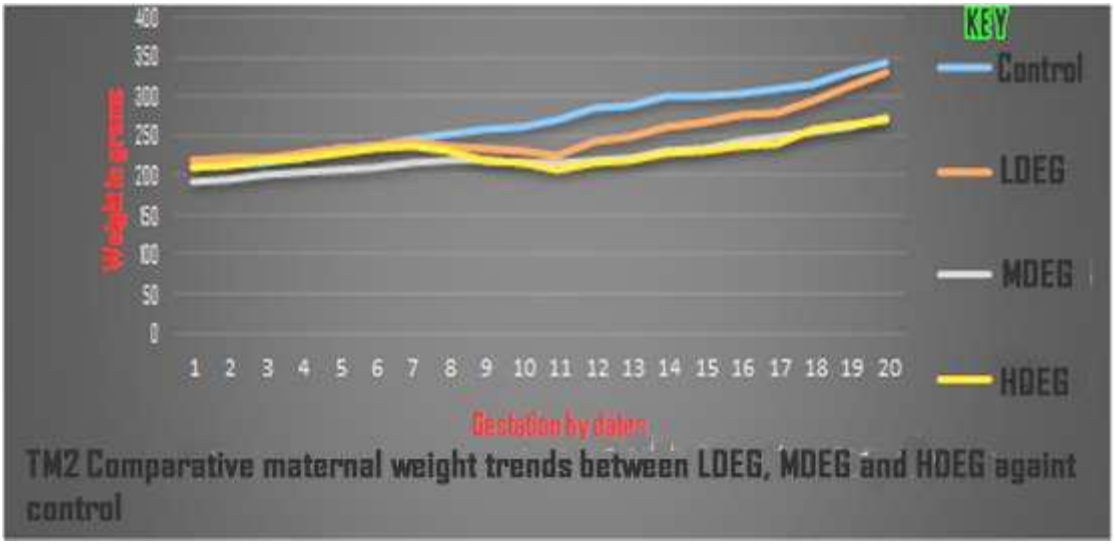


Figure 4. 2: **Comparative maternal weight gain trends between the enalapril treated group (LDEG), (MDEG), and (HDEG) against control TM_2**

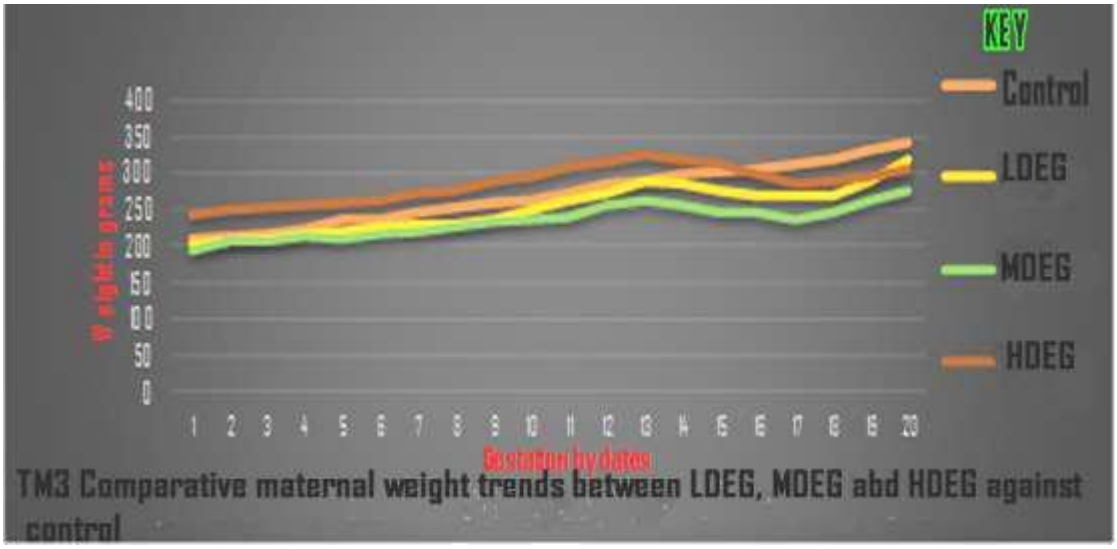


Figure 4. 3: **Comparative mean maternal weight gain trends between the enalapril treated groups LDEG, MDEG, and HDEG against control group at TM_3**

Table 4. 1 The comparative means for maternal weight gain (g) for the LDEG, MDEG and HDEG with the time of exposure (TM_1, TM_2 and TM_3) against the control.

The Study groups	The time of exposure to the enalapril treatment	Mean maternal weight gain + SEM
Control group	none	130.67±0.15.78[a]
Low dose enalapril group (LDEG, 0.15mg/kg)	TM_1	105.7±5.55[a]
	TM_2	112.67±7.42[a]
	TM_3	
Medium dose enalapril group (MDEG, 10mg/kg)	TM_1	88.3±12.1[b]
	TM_2	81.00±3.512[b]
	TM_3	83.33±5.840[b]
High dose enalapril group (HDEG, 20mg/kg)	TM_1	37.7±7.3[b]
	TM_2	60.67±2.67[b]
	TM_3	60.33±5.93[b]

Key: *The means, followed by letter [(a)] in a column are not statistically different from control at (P<0.05) Letters [(b, c and d)] depicts statistical significance difference from control and intragroup statistical Significant difference 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

These parameters depicted an inverse dose response relationship in that as the dose increased the poorer was the clinical outcome and a direct relationship with time particularly when treatment was done in trimester one and trimester two (TM_1, & TM_2) This was found to be statistically significant ($p< 0.05$) when compared with the control (**Table 4.2)**

The mean litter size was lowest in the high dose enalapril group(HDEG) treated at TM_1 with 4.00±0.00 and highest in LDEG treated at TM_1.The mean placental weight, a key indicator of the maternal nutritional exchange with the fetus was lowest in the HDEG when treated at TM_2 and highest in the LDEG treated at TM_1. The number of the resorbed endometrial gland increased with increase in dosages ($p<0.05$) and particularly in TM_2 and TM_3 across all the

enalapril treated groups as compared with the control. Percentage embryo-lethality or mean number of dead fetuses in utero increased with enalapril dose and the time of exposure. However, these changes had no statistical significant difference from control.

Table 4.2: The comparative means litter size, placenta weight, resorbed endometrial glands (g) and percentage embryo-lethality in LDEG, MDEG and HDEG with time of exposure (TM_1, TM_2 and TM_3) against the control group (CG).

Study groups	Period of enalapril treatment	Mean litter size ± SEM	Mean placenta weights ± SEM	Mean resorbed endometrial glands ± SEM	Mean embryo lethality ± SEM
Control group	-none-	13.33±0.882a	5.58±0.021a	0.67±0.67[a]	0.333+0.333[a]
Low dose enalapril group (LDEG, 0.5Mmg/kg)	TM1	11.000±0.577[a]	4.40±0.050[a]	0.33±0.333[a]	0.33±0.333[a]
	TM2	9.00±1.15[a]	3. 53±0.049[c]	0.67±0.667[a]	0.33±0.332[a]
	TM3	10.33±0.667[a]	4.0±0.049[b]	0.67±0.67[a]	0.33±0.333[a]
Medium dose enalapril group (MDEG, 1mg/kg)	TM1	10.00±0.577[a]	4.133±0.0612[c]	0.33±0.33[a]	0.33±0.333[a]
	TM2	6.00±1.53[b]	3.633±0.034[b]	1±0.577[a]	0.67±0.67[a]
	TM3	9.00±0.577[b]	4.03±0.034[b]	0.667±0.333[a]	0.33±0.333[a]
High dose enalapril group (HDEG, 2mg/kg)	TM1	7.67±0.333[b]	3.96±0.030[d]	0.700±0.6506[a]	1.00±0.577[a]
	TM2	4.00±0.00[c]	3.23±0.018[b]	1.3±0.000[b]	1.67±0.882[a]
	TM3	6.00±0.577[b]	3.64±0.0200[c]	0.7±0.53[b]	1.33±0.667[a]

Key: *The means, followed by letter [(a)] in a column are not statistically different from control at (P<0.05) Letters [(b, c and d)] depicts statistical significance difference from control and intragroup statistical significant difference using one way ANOVA with Tukey test on post-hoc t-tests.*

4.1.3 Influence of Enalapril on congenital anomalies

A total of fetuses 40 fetuses among 280 fetuses were found with one type or a combination of a number of congenital anomalies. The most common types of anomalies observed were the musculoskeletal abnormalities. These abnormalities were found to be concentrated in the

high and medium enalapril groups (MDEG and HDEG). This was particularly so when enalapril was administered in the first and the second trimesters (TM_1, TM_2)

Table 4. 3: Types of congenital anomalies observed their numbers and their distribution in LDEG, MDEG & HDEG against the control.

Types of congenital abnormalities	Control	Low enalapril group (0.5mg/kg)	Medium Enalapril group (1mg/kg)	High Enalapril group(2mg/kg)	Total number of fetuses
Spina bifida	1	0	2	4	7
Hypospadias	0	0	1	2	3
Renal dysplasia	0	1	1	2	4
Anencephaly	1	1	2	4	8
Microcephaly	0	1	2	3	6
Meromelia	0	0	1	1	2
Syndactyl	1	0	0	1	2
Oligo dactyl	1	0	1	2	4
Micropthalmia	0	0	1	1	2
Achalasia	0	0	1	1	2
Total	4	4	12	24	40

4.1.4: Correlation analysis on maternal pregnancy outcomes

Table 4. 4: Intra and inter-group correlation comparisons on the various maternal pregnancy outcomes for the LDEG, MDEG and HDEG at TM_1, TM_2 and TM_3 against the control.

		weight gain(gms)	initial weight (gms)	terminal weight (gms)	placental 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	.093							
Terminal weight (gms)	r	.844	.274	1					
	p	.000	.100.15						
Placenta weight (gms)	r	.831	-.116	.768	1				
	p	.000	.0.1500	.000					
Number of resorbed glands	r	-.0.1569	.00.159	-.0.1538	-.649	1			
	p	.000	.733	.001	.000				
litter size	r	.793	-.131	.722	.892	-.668	1		
	p	.000	.447	.000	.000	.000			
Dead fetuses	r	-.0.1517	.00.151	-.490	-.40.150	.482	-.438	1	
	p	.001	.769	.002	.006	.003	.008		
Congenital abnormalities	r	-.313	.072	-.274	-.399*	.447	-.470	.381	1
	p	.063	.677	.107	.016	.006	.004	.022	

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

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

These parameters depicted an inverse dose response relationship and a direct dose response relationship with the time of exposure particularly when the treatment was done in TM_2 and TM_3 across all the enalapril treated groups.

Table 4. 5: Comparative means of the fetal body weight (g) head circumference, (cm) CRL (cm) and bi-parietal diameter (cm) of LDEG, MDEG and the HDEG in (TM_1, TM_2 and TM_3) against the control (C).

Study groups	Period enalapril treatment	Mean fetal body weight + SEM	Mean 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 enalapril group (LDEG, 0.5mg/kg)	(TM_1)	6.66±0.0168a	3.57±0.037a	4.55±0.027a	0.692±0.0026a
	(TM_2)	6.42±0.007b	3.22±0.025b	4.123±0.009b	0.659±0.00073b
	(TM_3)	6.57±0.011c	3.453±0.029	4.5±0.009b	0.686±0.0008a
Medium dose enalapril group (MDEG, 1mg/kg)	(TM_1)	6.53±0.004c	3.52±0.015b	4.44±0.028b	0.69±0.004d
	(TM_2)	6.31±0.046 b	2.95±0.018c	3.865±0.044c	0.6296±0.0033
	(TM_3)	6.42±0.018ab	3.22±0.019c	4.15±0.0029c	0.655±0.0005b
High dose enalapril group (HDEG, 2mg/kg)	(TM_1)	6.21±0.010d	3.41±0.021b	4.34±0.011b	0.68±0.0028d
	(TM_2)	5.42±0.02 b	3.04±0.021d	3.4±0.023d	0.579±0.002b
	(TM_3)	5.92±0.00b	2.35±0.013d	3.85±0.0024d	0.635±0.0011a

Key: *The means, followed by letter (^{a}) in a column are not statistically different from control at ($P<0.05$) Letters ($^{b, c \text{ and } d}$) depicts statistical significance difference from control and intragroup statistical significant difference using one way ANOVA with Tukey test on post-hoc t-tests.*

LDEG did not show statistical significant difference ($P>0.05$) across all the three trimesters when compared with the control in all the four fetal parameters (**Table 4.5)**

4.1.6 Correlation statistics on fetal pregnancy outcomes

The correlation statistics using Pearson correlation comparison procedure shown that there was a strong association between the dose and time of exposure (0.3-0.55) across all the three experimental groups when compared with the control at TM_2, and TM_3. There was however weak association (values of <0.3) in all the fetal pregnancy outcomes (fetal weight, litter size, crown-rump length, head circumference, head length and bi-parietal diameter) at trimester one (TM_1) (**Table 4.6)**.

Table 4. 6: The correlation statistics on the Intra and intergroup comparison on fetal pregnancy outcomes between the enalapril treated groups (LDEG, MDEG and HDEG) at TM_1, TM_2 and TM_3 against control.

	Fetal	weight(g)	litter size	crown lump length	Head Circumferenc e	Bi-parietal diameter	
Fetal weight	r	1					
	p						
Litter Size	r	.836	1				
	p	.000					
Crown Rump length	r	.912	.80.156	1			
	p	.000	.000				
Head Circumferenc e	r	.913	.872	.980.15	1		
	p	.000	.000	.000			
Bi-parietal diameter	r	.910.15	.867	.994	.993	1	
	p	.000	.000	.000	.000		
Head length	r	-.00.159	-.016	-.204	-.120.15	-.177	1
	p	.734	.926	.233	.466	.301	

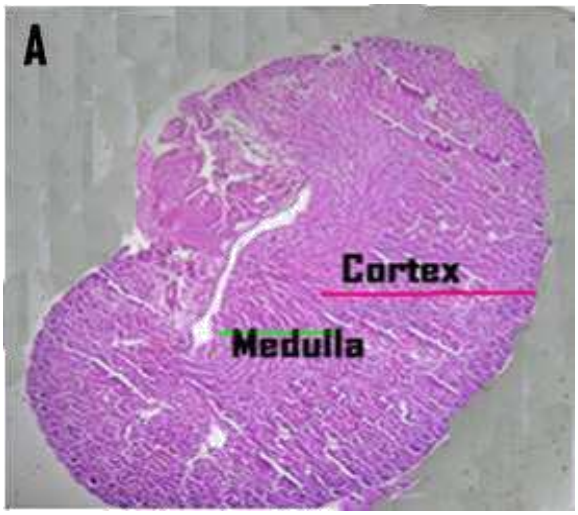
NB: r is the Pearson's correlation coefficient, P is the p-value and indicate significance i.e. p<0.05

4.2: Histomorphological findings on the developing fetal kidneys

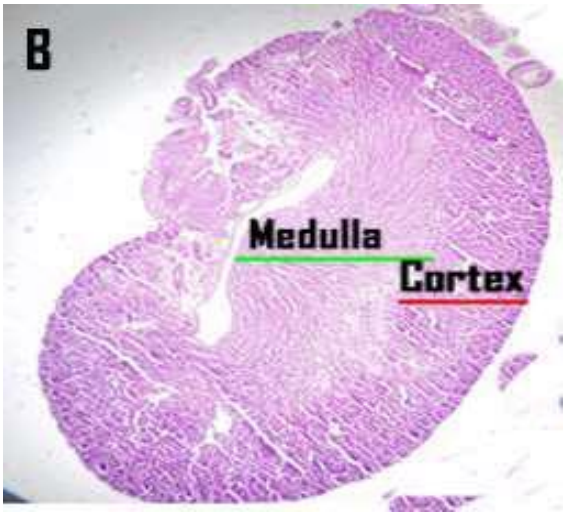
This Qualitative data involved study of the cortical and medullary layers thickness of the kidneys, size of the glomerular and the capsular space

4.2.1 Influence of enalapril on the morphological thicknesses of the cortex

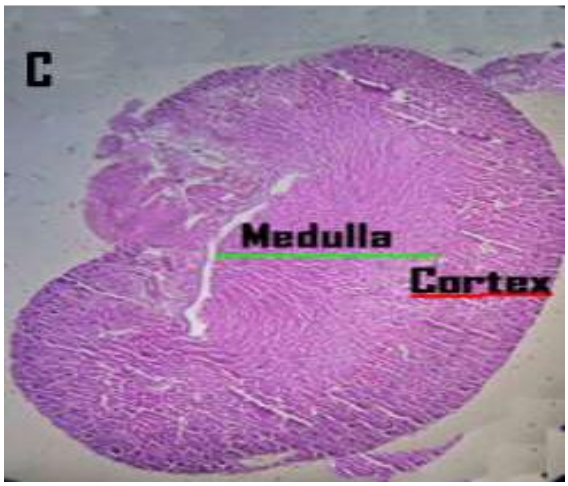
The cortical thickness was observed to reduce appreciably among all the enalapril treated groups as compared with the control group. Most effects were observed during TM_1 and TM_3 The. Glomeruli and capsular space were observed to increase in all dosages particularly when treatment was done in trimester TM_2 and TM_3.



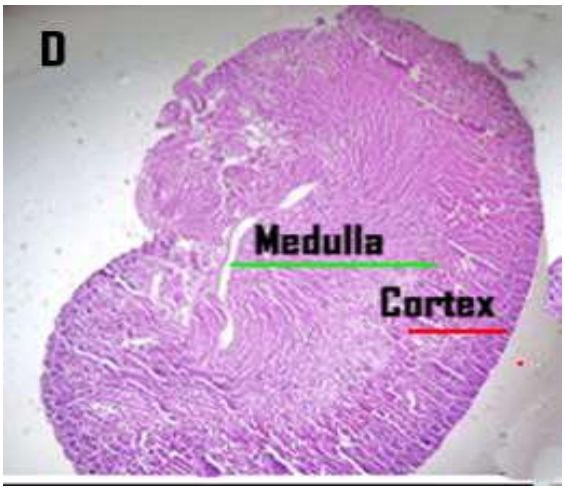
Normal thickness of the fetal kidney cortex treated with enalapril at TM_1 (mag x40 H&E) as shown by the red line



Relatively reduced cortex in LDEG (mag x40 H&E) when enalapril was administered at

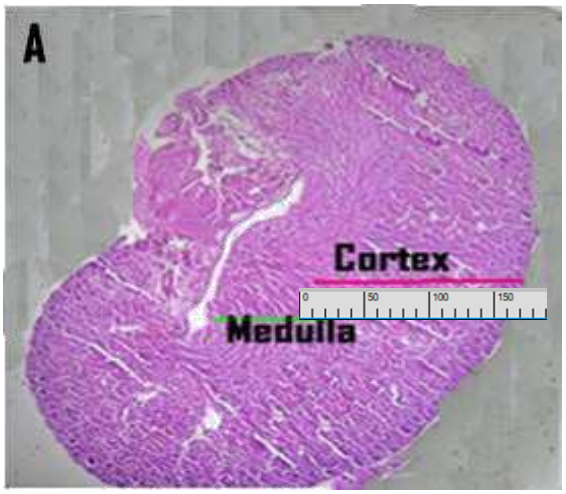


Further reduction of the fetal kidney Cortical layer in MDEG (mag x40 H&E) when enalapril was administered at TM_1 as indicated by red line

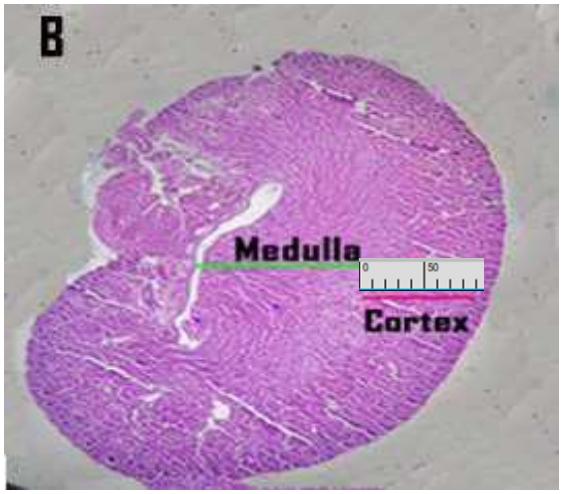


Most reduced fetal kidney cortical Layer in HDEG (mag x40 H&E) when enalapril was administered at TM_1 as indicated by the red line

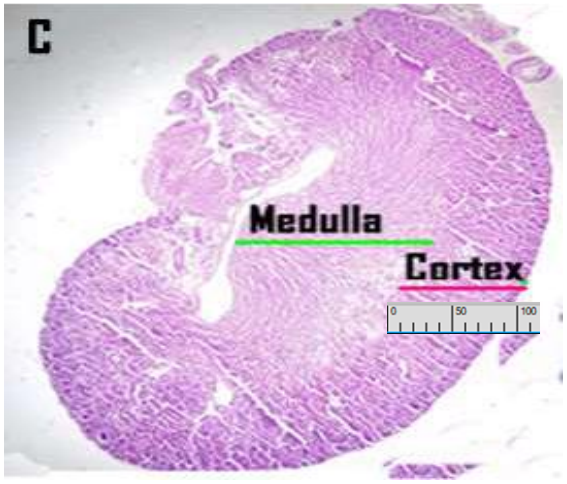
Figure 4. 4: Comparative thickness of the fetal kidney cortical layer in (a) Conrol (b) LDEG, (c) MDEG, and (d) the HDEG IN TM_1



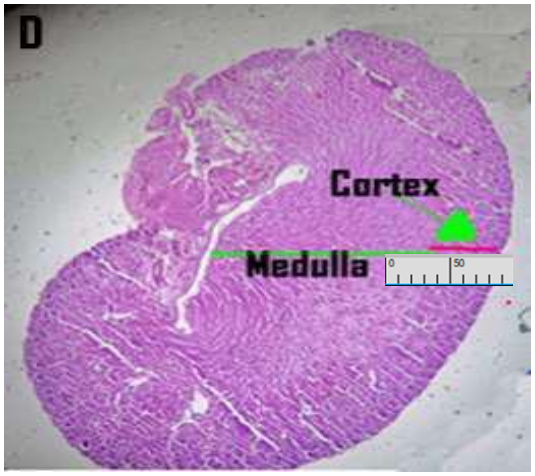
Normal thickness of the Control fetal kidney cortex treated with enalapril at TM_2 (mag x40 H&E) as shown by red line



Relatively reduced cortex in LDEG (mag x40 H&E) when enalapril was administered at TM_2 as Indicated by the red line

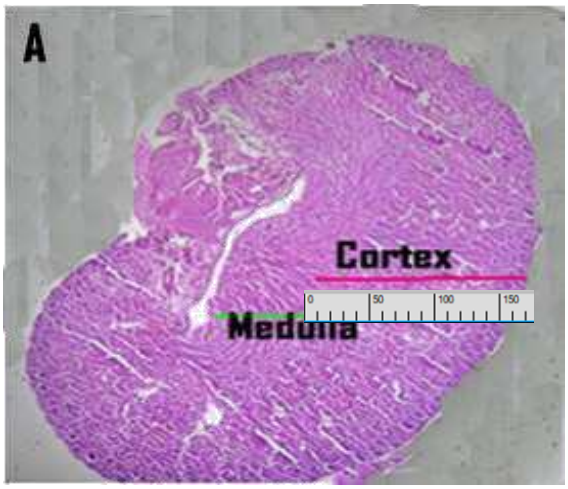


Further reduction of the fetal kidney Cortical layer in MDEG (mag x40 H&E) when enalapril was administered at TM_2 as indicated by the red line



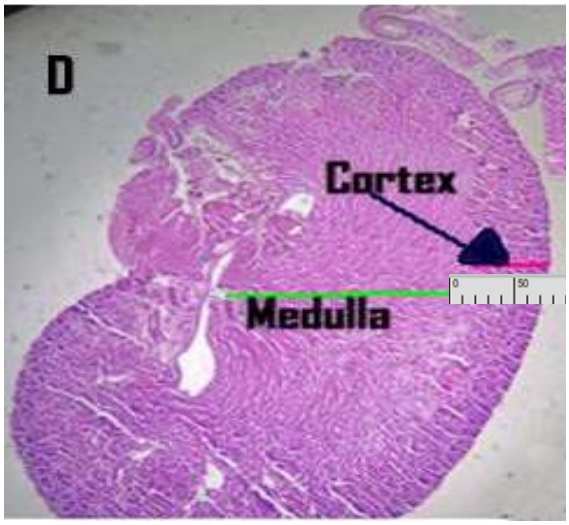
Most reduced fetal kidney cortical Layer in HDEG (mag x40 H&E) when enalapril was administered at TM_2 as indicated by the red line

Figure 4. 5: Comparative thickness of the fetal cortical layer of the kidney in (a) Control b) LDEG, (c) MDEG, and (d) the HDEG in TM_2

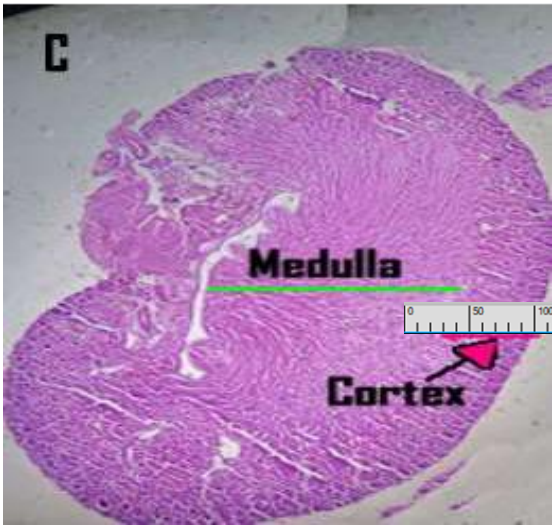


Normal thickness of the Control: fetal kidney cortex treated with enalapril at TM_3 (mag x40 H&E) as shown by red line

Relatively reduced cortex in LDEG (mag x40 H&E) when enalapril was administered at TM_3 as shown by the red line

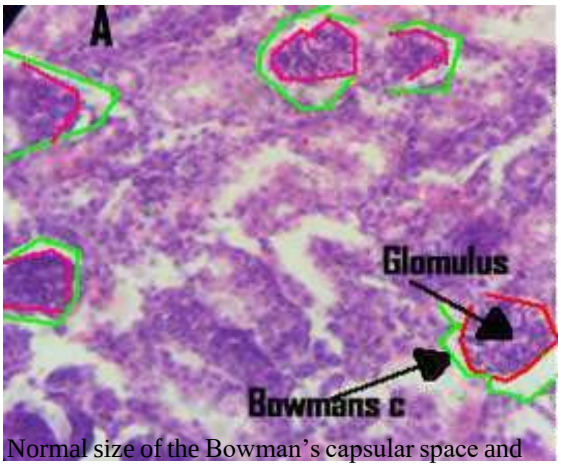


Further reduction of the fetal kidney Cortical layer in MDEG (mag x40 H&E) when enalapril was administered at TM_3 as indicated by the red line

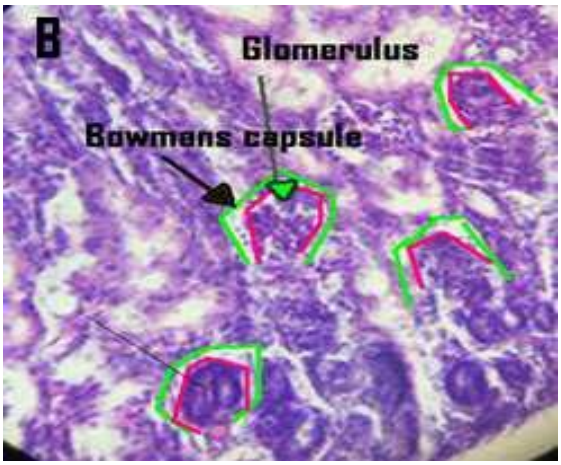


Most reduced fetal kidney cortical Layer in HDEG (mag x40 H&E) when enalapril was administered at TM_3 as indicated by the red line

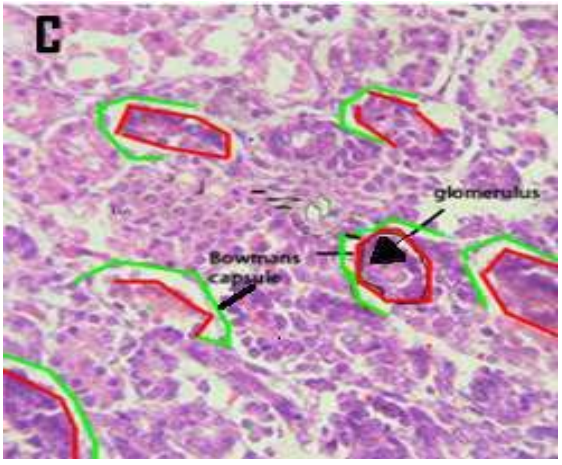
Figure 4. 6: Comparative thickness of the fetal cortical layer of the kidney in (a) Control (b) LDEG, c) MDEG, and (d) the HDEG IN TM_3



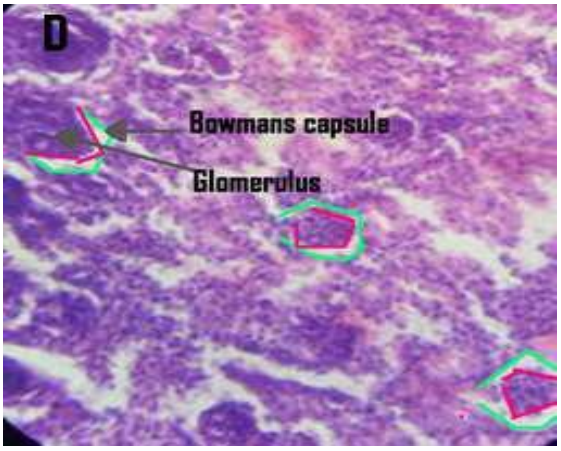
Normal size of the Bowman's capsular space and the glomerulus in Control (mag x40 H&E). Bowman's capsule is shown by green lines while the red lines encloses the glomeruli



Almost the same size of the Bowman's capsular space and size of the glomerulus in the LDEG at TM_1 as compared with the control (mag x40 H&E). Bowman's capsule is shown by green lines while the red lines encloses the glomeruli

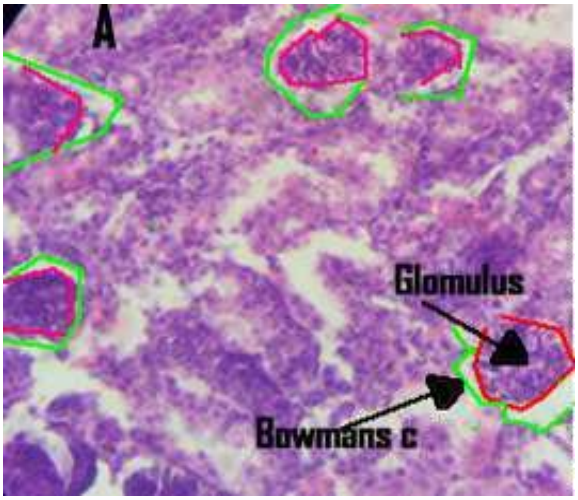


Shows no much difference in Bowman's Capsular space and the size of the glomerulus in the MDEG at TM_1 as compared with the control (mag x40 H&E). Bowman's capsule is shown by green lines while the red lines encloses the

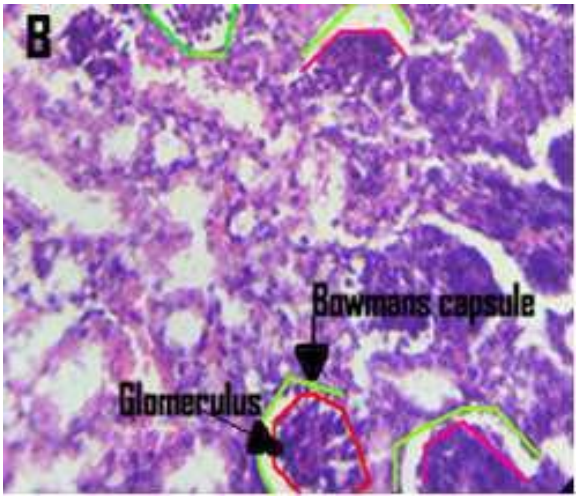


Similarly, there is no much difference in Bowman's capsular space and the size of the glomerulus in the HDEG at TM_1 as compared with the control (mag x40 H&E). Bowman's capsule is shown by green lines while the red lines encloses

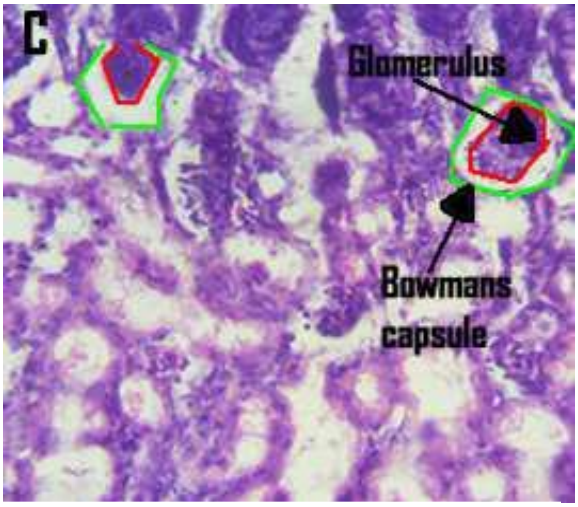
Figure 4. 7: Comparative sizes of the capsular space and the Glomeruli in Control (b) LDEG, (c) MDEG, and (d) the HDEG at TM_1



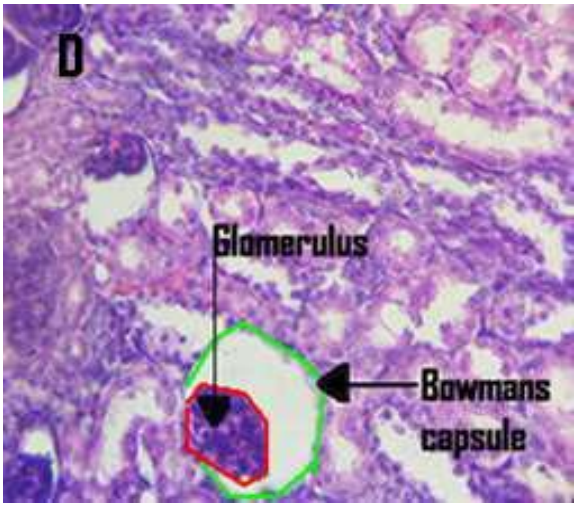
Normal size of the Bowman's capsular space and the glomerulus in Control (mag x40 H&E).



Almost the same size of the Bowman's capsular space and size of the glomerulus in the LEG at TM_2 as compared with the control (mag x40

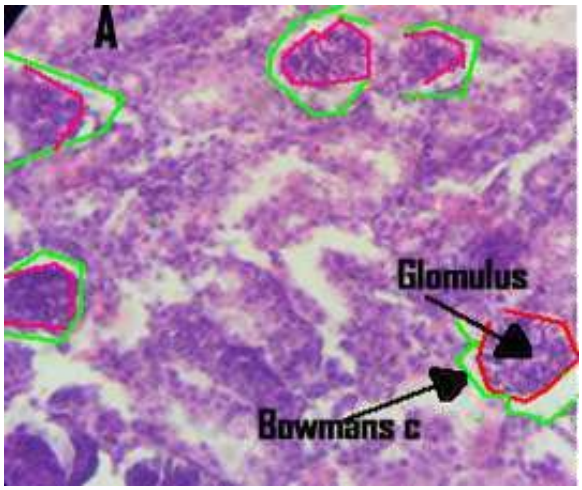


Increased capsular size and glomeruli size at TM_2 (mag x 40 H&E in HDEG

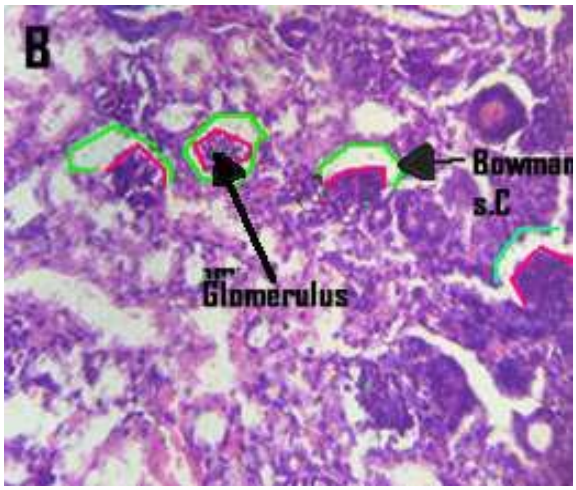


Most increased capsular space and the glomeruli at TM_2 (mag x 40 H&E in HDEG

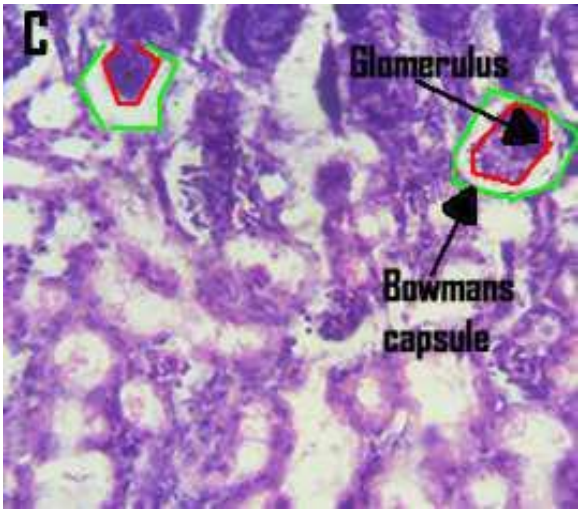
Figure 4. 8: Comparative sizes of the capsular space and the Glomeruli in (a) Control (b) LDEG, (c) MDEG, and (d) the HDEG at TM $_2$



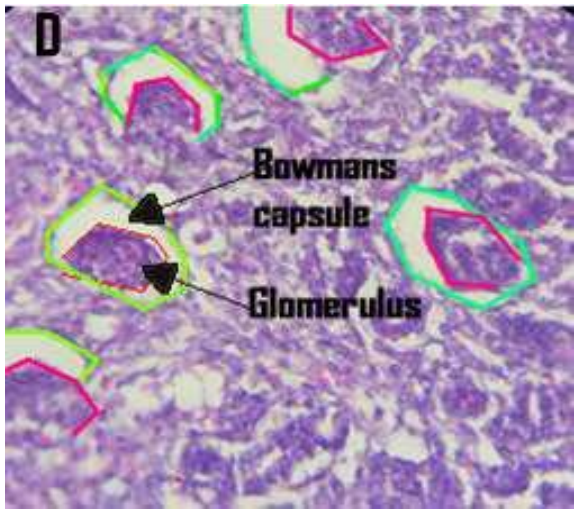
Normal size of the capsular space and the glomerulus in Control TM_3 (mag x40 H&E).Capsular space is enclosed by green lines while the red lines encloses the glomeruli



Relatively increased size of the capsular space and slightly bigger glomerulus in the LDEG at TM_3 (mag x40 H&E). Capsular space is enclose by green lines while the red lines encloses the



Most increased capsular space and the Glomeruli at TM_3 (mag x 40 H&E in HDEG



Increased capsular size and glomeruli size at TM_3 (mag x 40 H&E in HDEG

Figure 4. 9: Comparative sizes of the capsular space and the Glomeruli in (a) Control (b) LDEG, c) MDEG, and (d) the HDEG at TM $_3$

4.3 Stereological Findings

The total fetal kidneys weights, total kidney volume by use of both water displacement method (WDM) and cavarieli 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

Table 4. 7: Comparative means fetal Kidney weight (g) Kidney length, (cm) and width (cm) for LDEG, MDEG and the HDEG treated at TM_1, TM_2 and TM_3 against the control.

Study groups	Period of enalapril treatment	Mean kidney weight + SEM	Mean kidney length + SEM	Mean kidney width + SEM
Control group	--	0.395±0.005[a]	1.295±0.005[a]	1.097±0.003[a]
Low dose enalapril**group** (LDE, 0.5mg/kg)	TM_1	0.394±0.0051[a]	1.304±0.136[a]	1.09±0.009[a]
	TM_2	0.297±0.0033[b]	1.197±0.0033[b]	0.997±0.0033[b]
	TM_3	0.347±0.0059[b]	1.247±0.0033[a]	1.0711±0.0066[b]
Medium dose enalapril group (MDEG, 1g/kg)	TM_1	0.370±0.008[a]	1.31±0.0024[b]	1.073±0.009[a]
	TM_2	0.216±0.008[c]	1.12±0.008[c]	0.916±0.0079[c]
	TM_3	0.300±0.000[c]	1.2000±0.000[a]	1.023±0.012[c]
High dose enalapril**group** (HDEG, 2g/kg)	TM_1	0.33±0.0028[b]	1.287±0.0018[a]	1.02±0.00696[b]
	TM_2	0.12±0.012[d]	1.02±0.012[d]	0.813±0.007[d]
	TM_3	0.247±0.0071[d]	1.285±0.1433[a]	0.948±0.0024[d]

Key: The means, followed by letter [(a)] in a column are not statistically different from control at (P<0.05) Letters [(b, c and d)] depicts statistical significance difference from control and intragroup statistical significant difference using one way ANOVA with Tukey test on post-hoc t-tests.

The fetal kidneys from the enalapril treated groups were observed to be grossly smaller in size with reduced cortical layer when compared with the control. 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 was depicted.

Total kidney weight was found to be lowest in high dose enalapril group (HDEG) at 0.12±0.012gms during trimester two (TM_2) and medium dose enalapril group (MDEG) at trimester two (TM_2) at 0.216±0.008c. In trimester three ($TM_{3,}$) the mean total fetal kidney weight was found to reduce with dosages increase. When treatment was done at trimester one (TM_1) the mean kidney weight was not statistically significant ($p<0.05$) when the comparisons were done across all groups

and when compared with the control. This trend was uniform in all groups across all the trimesters for all the parameters

4.3.2: The influence of enalapril on the total fetal kidney volume and sub component volume densities

Total kidney volume, cortical and medullary volumes depicted an inverse dose response relationship in that increase in enalapril doses, resulted into a corresponding decrease in total kidney volume and its subcomponents and *vice versa*, (**Table 4.8**).

These parameters depicted a direct dose response to the time of exposure in that when enalapril treatment was administered at different trimesters, the kidney volumes decreased directly with the time of exposure.

Total kidney was found to be lowest in high dose enalapril group (HDEG) during trimester two (TM_2**)** and medium dose enalapril group (MDEG) during trimester two (TM_2.)

In trimester three (TM_3,) the volumes reduced as dosages increased. When treatment was done at trimester one (TM_1), kidney volume was not found to be statistically significant different ($p<0.05$) when the comparisons were done across all groups and when compared with the control. This trend was uniform in all groups across all the trimesters for the total kidney volume and subcomponents volumes.

Table 4. 8: A Comparative reference, calculated and percentage shrinkage on total Mean fetal Kidney volume using (WDM) and cavarieli method ($mm^{3)}$ in the LDEG, MDEG and the HDEG treated at TM_1, TM_2 and TM_3 against the control.

Study groups	Period of enalapril treatment	Mean total fetal kidney volume (WIM) + SEM	Mean total fetal kidney volume (Cavarieli method) + SEM	Mean shrinkage + SEM	Mean kidney cortical volume density + SEM	Mean kidney medulla volume density + SEM
Control group	----------	0.248±0.002a	0.244±0.001a	0.017±0.001a	0.073±0.000a	0.171±0.001a
Low dose Enalapril group (LDEG, 0.15mg/kg)	TM_1	0.247±0.002a	0.243±0.001a	0.244±0.0005a	0.073±0.000a	0.171±0.001a
	TM_2	0.233±0.002b	0.231±0.001b	0.010.15±0.005a	0.070±0.000b	0.162±0.001b
	TM_3	0.239±0.001b	0.2305±0.002b	0.016±0.001a	0.071±0.000a	0.162±0.001b
Medium dose Enalapril group (MDEG, 10mg/kg)	TM_1	0.242±0.000b	0.239±0.001c	0.244±0.006a	0.072±0.000b	0.170±0.001b
	TM_2	0.232±0.001c	0.232±0.001c	0.013±0.001a	0.069±0.000b	0.161±0.001b
	TM_3	0.238±0.001c	0.233±0.002c	0.019±0.006a	0.070±0.001b	0.162±0.001b
High dose Enalapril group(HDEG, 20mg/kg)	TM_1	0.242±0.002b	0.236±0.001c	0.244±0.004a	0.071±0.000c	0.167±0.001c
	TM_2	0.222±0.001c	0.220±0.001c	0.009±0.003a	0.066±0.002c	0.054±0.001c
	TM_3	0.233±0.002c	0.230±0.002c	0.244±0.004a	0.069±0.000c	0.054±0.001c

Key: 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.

CHAPTER FIVE
DISCUSSION, CONCLUSION AND RECOMMEDATIONS

5.1 Influence of Enalapril on the Maternal and Fetal Pregnancy Outcomes

The current study established that when enalapril was administered at trimester one(TM_1), there was an inverse dose response relationship with the maternal and fetal pregnancy outcomes in that when the enalapril doses increased there was corresponding decrease in the mean maternal weight gains, mean placental weights, mean fetal weights and the mean litter sizes while the observed percentage embryolethalities and congenital abnormalities showed a dose response relationships when compared with control (Table 4.2**).** These findings collaborated well with study findings by Cooper *et al*,(2006), who established the same dose response relations and pointed out that teratogenesis is a factor of a cascade of events that occur in-*utero* and not a stand-alone event. Hence maternal and fetal pregnancy outcomes observed in this study clearly depicted an association of this kind of perturbations when enalapril was administered in-utero.

This study also established that doses of enalapril that ranged between 1gms/kg/bw and 2mg/kg/bw and administered at trimester two and three (TM_2 and TM_3) respectively) were associated with the most of the intra-uterine growth retardations that resulted into mean reduction in fetal weights, reduced crown lamp lengths (CRL), parietal diameters(BD), Head circumference (HC) among others observed in the fetuses in the enalapril treated groups. These findings were also found to be in congruence with findings from some other two studies by Bullo et *al.*,(2012) and Boutroy *et al,* (1984) who showed that high doses of captopril a drug with similar mode of action to enalapril had adverse effects on corpus luteum of dams. This was further emphasized by another study by Gubler & Antignac, (2010) who established that high doses of enalapril has an associated with low number of corpus luteum and high cases of devoured embryo implantations.

This finding of this study can be explained along the reasoning of a study by Bosco & Diaz, (2012) Musumeci *et al* (2015), who reported that reduced litter size could be due to increased embryo resorption and uterine embryo in prenatal under nutrition due to enalapril use in pregnancy**.** Further, embryonic resorption that was defined by Dorothea & Lidy, (2014) as prenatal death followed by subsequent degeneration of the conceptus that occurs due to abnormal placental villus formation and recognized as a foreign body by maternal immune system hence resorption, was increased in high dose enalapril group (HDEG) in trimester three (TM_3) and trimester one (TM_1) when compared to the control group (CG). The increased frequency of resorption in the first trimester indicates the adverse effects of in-utero enalapril exposure on fetal viability.as has also

been studied by Li & Wright, (2014). This was linked to low placental weights as observed in enalapril treated groups. These findings agrees with findings by Mishra *et al*., (2015), who reported time and dose dependent embryo resorption upon enalapril administration to mouse. Studies by Bosco & Diaz (2012), also showed that intra uterine enalapril exposure reduced placental glucose utilization, caused vascular abnormalities and reduced trophoblastic proliferation hence associated with placental weight deficits.

The mean fetal birth weight and length reduced in all treatment groups across all trimesters in comparison to control. The findings of this study concurs with a study by Musumeci *et al*.,(2015), who correlated fetal enalapril spectrum disorders with level and nature of enalapril exposure and noted that clinical outcome severity following maternal enalapril exposure was time and dose dependent. He also associated teratogenic effects like reduced litter size and reduced foetal birth weight to enalapril use in pregnancy

The means head circumference and bi-parietal diameters deteriorated in all treatment groups across all trimesters in comparison to control. This was particularly in High dose enalapril group (HDEG) in comparison to other treatment groups. These findings are in line with another study done by Sebastiani *et al.,* (2018) who linked enalapril to injurious effects of fetal head circumference. This study has also associated longer treatment duration with higher cases of teratogenic congenital defects. Limb defects, vertebral defects and craniofacial defects which were found to be significantly high $p<0.05$ in the high and medium enalapril groups when compared to the control group (**Table 4.3**.) This resonates studies done by Bosco & Diaz, (2012)**;** and Lui et al., (2014)) who observed that maternal Enarapril exposure prenatally and during early gestation caused defects through several mechanisms; including interference with growth factors, apoptosis of fetal cells, alteration of glucose transport and uptake by the cells.

5.2 Influence of Enalapril on the Histomophology of the Fetal Kidney

Findings from this study has have found out that enalapril suppresses the differentiation and development of the cells by inhibiting angiotensin two(2)a rate limiting step in un-inhibition of the spryl gene that shuts down further development to occur. This has clearly been demonstrated by the marked reduction in thicknesses of the cortical layers of the fetal kidney cortex these findings are in agreement with Alherbi *et al,* (2013), who observed that Cortex and medullary layers of the developing kidney can be suppressed following the suppressive inhibitory effects of antihypertensive. This study observed that the suppression to the thickness of the cortical layers differed variably based on the dose of enalapril exposure as well as with the time of exposure.

This study further established that there was reduced cortical thickness among all the enalapril treated groups particularly when treatment was done in trimester two (TM_2) and trimester three (TM_3.) Treatment done at trimester one (TM_1), had no significance difference in cortical thicknesses across all groups from the control. The findings are in line with a study by Gedzelman & Meador (2012) who reported reduction in medulla and cortex of the Kidney and that early exposure to antihypertensive at TM_2 and TM_3 inhibited mechanisms that result into altered renal development processes leading to dysplasia This study also found out that in all treatment groups and across all trimesters there was significant dilatation of the bowman's space and glomerulus capillaries augmenting findings of Alherbi *et al*,(2013) that histomorphological effects of benazepril, an antihypertensive in the same class with enalapril presented with several features including; less differentiation, fibrin deposition (fibrosis), disorganization of the renal cortex and medulla, dilated and enlarged blood vessels and dilated bowman's space.

5.3 Influence of Enalapril on the Stereology of the fetal Kidney

In the development fetal organs like the kidney.

The mean total kidneys volume and the sub cortical components volume was lower in enalapril treated groups in comparison to control. These findings depicted an inverse relationship with the dose administered and a direct correlation with the time of exposure in that when enalapril treatment was administered at trimester one (TM_1,) the effects were less pronounced in comparison to trimester two (TM_2) and trimester three (TM_3.) The reduction in kidney volumes were seen to decrease directly with the time of exposure in that when enalapril treatment was done at trimester two (TM_2) the total kidney volume was lowest in the high dose enalapril group (HDEG.) These findings augmented a study b**y** Berghuis *et al*, (2017) who found out that Intra and intergroup fetal kidney weights comparisons for the experimental groups, depicted variances in the total gross weights and kidney sizes in relation to the dose and the time of exposure.in mothers exposed to angiotensin converting enzyme inhibitor(ACEi) For example, in treatment groups at TM_2, the mean total kidney weight was found to be lowest in HDEG followed by MDEG. In TM_3, the mean totals fetal kidney weight was lowest in HDEG followed by MDEG. Captopril an angiotensin converting enzyme inhibitor showed a highly significant decrease in kidney weight, alteration of the number and size of nephrons, layers, and massive cell degeneration with cavity formation in the kidney tissue. (Badawy *et al.*, 2019) Perturbations like those impacted by antihypertensives directly impacts negatively to the fetal kidney development including; significant reduction in mean fetal gross kidney weights, total kidney volumes, kidney sizes, reduction in the kidney

cortical thickness, as well as in all the histo-stereological parameters **(**Bateman *et al.*, 2016). In TM_1, the mean kidney weight reduced significantly as the dosages increased (values $p<0.05$) when compared with the control. This corroborates a study by Stapleton *et al*.,(2017) who reported that alterations and the destructive changes especially of kidney tubules during fetal kidney development are dose and time dependent.

5.4 Conclusion

1. This study has established that maternal and pregnancy outcome parameters depicted an inverse dose response relationship in that as the dosages increased the poorer was the clinical outcome and a direct dose response relationship with the time of exposure particularly when the treatment was done in trimester two(TM_2) and trimester three(TM_3) across all the enalapril treated groups.

2. The study has also established that histomorphological effects of enalapril on foetal kidneys included reduced cortical thickness, enlarged glomeruli and enlarged capsular spaces. These effects depicted an inverse dose response relationship in that as the dosages increased the effects were more pronounced and a direct dose response relationship with the time of exposure particularly when the treatment was done in trimester two(TM_2) and trimester three(TM_3) across all the enalapril treated groups.

3. Total kidney volumes and subcomponents volume densities of the cortex and the medulla reduced appreciably as the dosages increased. This was more pronounced during trimester two (TM_2) and trimester three (TM_3) across all the enalapril treated groups.

4. Enalapril in the doses of 2mg/kg/BW and 1mg/kg/BW during pregnancy are teratogenic to the developing fetal kidney particularly when administered during TM_2 and TM_3. Its teratogenic effects to the Kidney when administered at TM_1, has no significant outcomes except when administered in high doses. The most vulnerable period of enalapril teratogenicity was established to be TM_2 while the most critical dose was 2mg/kg/BW.

5.5 Recommendations

This study recommends that;

1. Enalapril use during pregnancy in doses of 0.5mg/kg/bw,1mg/kg/bw and 2mg/kg/BW particularly in trimester TM_2 and TM_3 must be avoided by seeking appropriate alternatives that are safer to the fetus.

2. Expectant mothers on chronic enalapril use can be allowed to continue the drug during TM_1 as safer antihypertensive are introduced during TM_2 and should only be reintroduced postnatally.

3. Other studies need to be carried out in higher non-human primates like gorillas, monkeys among others

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APPENDIX I- PUBLICATION

Growth and Development Effects Following In Utero Exposure to Varied Doses of Enarapril in Albino Rats (*Rattus Norvegicus*)

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Abstract: In utero exposure to Enarapril; a widely prescribed antihypertensive due to its success as a monotherapy, cost effectiveness, relative paucity of side effects, bone, cardio and nephron protective effects in comparison to standard therapy for equal level of blood pressure control has been associated with foetal deleterious effects. These include renal failure, intrauterine growth retardation, hypocalvaria, persistent patent ductus arteriosus and cerebral complications. It blocks Foetal renin- angiotensin- system that comes to play during the second and third trimesters of pregnancy documenting it safe during first trimester. In contrast, other studies reports major deleterious effects during the first trimester including cardiovascular, nervous, and renal and limb defects effects yielding conflicting report on the most critical periods. This has seen Enarapril labelled as class D drug by American FDA, a rationale based on data extrapolated only in single-case reports or in small case series, with no confirmed effects of specific drugs, doses and durations, considerations of severity of hypertension or gene mutations of the RAS System

This experimental study used thirty gravid rats. Three were controls and twenty-seven grouped into three different trimesters of nine animals each. These subgroups divided into three groups of three animals each, received conventional Enarapril dosages converted to animal equivalents of 20mg, 10mg and 0.15mg given daily for twenty one days, fourteen days and seven days for trimesters one, two and three respectively. All animals were sacrificed on day twenty-gestation, three fetuses of median weights from each rat was taken and a sample of ninety fetuses were evaluated for mean weights, kidney weights, and heart weights and head circumference.

This study elucidated that there seems to be little Enarapril specificity in the association between maternal use and an increased risk for foetal deleterious effects the main association being foetal systemic hypotension and foetal circulation disturbances following placental transfers that can equally occur with other classifications of antihypertensives. Notably, these effects were dose but not time dependent.

INTRODUCTION

Enarapril, an antihypertensive drug in the class of angiotensin converting enzyme inhibitors is widely prescribed in management of essential and gestational hypertension due to its success as a monotherapy, cost effectiveness, minimal side effects1, cardio-nephron protective and bone healing effects [2,34] in comparison to standard therapy for equal level of blood pressure control in women of reproductive age. However, its use during the second and third trimesters, has been associated with foetal

Renal failure, intrauterine growth retardation, hypocalvaria, persistent patent ductus arteriosus and cerebral complications [0.1567] by blocking Foetal renin- angiotensin- system that comes to play during the same perinatal period. In contrast, retrospective studies by cooper et al [8] reports major deleterious effects during the first trimester including cardiovascular, nervous, and renal effects conflicting previous literature that Enarapril is safe during first trimester [9–11.] Nephrogenesis in humans commences by gestation day corresponding to embryonic day eight in rats [22,12] and teratogens during this period supports the theory that first trimester exposure is not safe. Enarapril contraindication in pregnancy is un justified considering that the standardizing studies for this rationale was extrapolated only in single-case reports and in small case series, with no confirmed effects of specific drugs, doses, durations and not considering severity of hypertension or gene mutations of the RAS systems, 13,14,depriving women of reproductive age all the benefits conferred by Enarapril.

APPENDIX II - DATA CAPTURE SHEETS

DATA CAPTURE SHEET FOR EXPECTANT ALBINO RATS

ALBINO RAT IDENTITY……

INITIAL WEIGHT……DOSE

CALCULATION……

……

DATE	WEIGHT IN GRAMS	ENARAPRIL 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	F5	F6	F7	F8	F9	F10	F11	F12
Gross appearance												
Fetal wt. (g)												
Fetal crown												
Congenital abnormalities												
Resorptions /devoured fetuses												
Placental weight												
Head circumference												
Bi parietal diameter												
KIDNEY												
Gross appearance												
Congenital anomalies												

Kidney wt.(g)												
Total kidney volume												
Volume density of kidney cortex												
Volume density of kidney medulla												