# THE HISTOMORPHOLOGICAL-NEPHROTERATOGENIC EFFECTS OF VARIED DOSES OF ENALAPRIL ON THE DEVELOPMENT OF THE FETAL KIDNEYS IN ALBINO RATS (*RATTUS NORVEGICUS*)

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**Abstract:** Though, Enarapril is a widely prescribed antihypertensive medicine in the class of an angiotensin converting enzyme inhibitor, due to its monotherapeutic success and its cost effectiveness, existing literature has however shown that it may interfere with the development of fetal kidneys when exposed *in-utero*. Though this data on its nephroteratogenic effect on the developing fetal kidneys exists there is paucity of data on its histomorphological effects to the cortical and medullary layers in the fetal kidneys when exposed at different window periods and at varied doses.

In carrying out the study, thirty Albino rats' dams weighing between 200-250g were assigned to two broad groups of 3 rats control and 27 rats experimental. To evaluate the nephroteratogenic effects of Enarapril on varied doses the 27 rats in the experimental group were further divided into three subgroups of 9 rats each according to doses of Enarapril applied as follows, 9 rats each for Low that received [LDEG-0.5mg/kg/bw],, 9 rats for medium that received [MDEG-1mg/kg/Bw] and 9 rats high Dose Enalapril Groups [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 dams 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 analysis. This study established that *in utero* enalapril caused significant in reduction in the cortical layer thickness and widened capsular space with glomerular hypertrophy when administered in the second and trimesters. In conclusion, enalapril in doses of 1mg/kg/BW and 2mg/kg/BW in second and third trimesters ($TM_2$) and ($TM_3$) are nephroteratogenic. The most vulnerable period was established to be $TM_2$ at 2mg/kg/BW. The study recommends that maternal enalapril should be avoided in doses of 2mg/kg/BW and 1mg/kg/BW across all trimesters and alternative antihypertensives sought. Further studies are also recommended in higher non-human primates like monkeys and gorillas as they would give finding that are more close to humans.

**Keywords:** Enarapril, Angiotensin converting enzyme inhibitors, kidney teratogenicity, Pregnancy.

## 1.1 Introduction

Enalapril, that is sold under the trade names of Vasotec, Renitec, Enacard among other names in the class of angiotensin converting enzyme inhibitors is widely prescribed first drug of choice in the treatment of hypertension (Oh *et al.*, 2016) in general populations and ameliorates hypertension related complications including nephro protection, diabetic nephropathy and cardiac morbidity. These benefits prompt women of reproductive age to be on Enarapril inadvertently whereas existing literature links it to teratogenicity of fetal viscera including kidneys(Chevalier, 2012)The highly lipophilicity and low molecular weight(376.4467g/mol.) nature of Enarapril countenances traversing of placental barrier interfering with angiotensin enzyme which is vital in renal development(Almeida et al., 2020). The GDNF/c-Ret/Wnt11 signaling pathway is essential for normal renal development; a pathway negatively regulated by Spryl 1 gene. The receptor tyrosine kinase RET and Wnt11, a member of the Wnt superfamily glycoproteins, are specifically expressed in the tips of the branching ureteric

epithelium while glial cell-derived neurotrophic factor (GDNF) is expressed in the metanephric mesenchyme. The outgrowth of the ureteric bud from the nephric duct epithelium and the subsequent invasion of the bud into the metanephric mesenchyme initiate reciprocal cell-cell interactions with formation of renal collecting system i.e. ureters, pelvis, calyces, and collecting ducts from the buds and renal nephrons from mesenchyme.(Menshykau et al., 2019;Shakya et al., 2005)

Angiotensin II Acting via the AT2R (Angiotensin II receptor), upregulates Pax2 that stimulates ureteric bud branching in GDNF/c-Ret/Wnt11 signaling pathway and acting via the AT1R (Angiotensin I receptor), inhibits Spry1 gene(Yosypiv et al., 2008). Angiotensin blockade in Enarapril treatments causes renal atrophy and poor nephron endowment with resultant structurally abnormal kidneys(Chevalier, 2012). The Glomerular filtration through atrophied renal parenchyma causes hyper filtration with resultant hypertrophy of glomerular tuft and expansion of Bowman's space as adaptive mechanisms.

Renal mortality has increased by 31.7% over the last 10 years. Some associated causes remains unclear and existing data reports significant epidemiological shifts in metabolic and cardiovascular risk factors.(Neuen et al., 2017).*In utero.* Enarapril exposure is linked to renal disease but data on its histomorphological teratogenic effects is scarce. Quantification studies in the kidney relate to renal functional reserve and thus some structural renal childhood and adulthood disorders whose causes are unknown could be traced back to foetal life. (Başaran et al., 2013)

This study therefore evaluates the histo-morphological outcomes on the developing fetal Kidneys following *in-utero* exposure to varied doses of Enarapril at different window periods and whether these outcomes are time and dose dependent.

### 1.2 Study Objectives

1. To evaluate the histo-morphological teratogenic effects of Enarapril to the developing fetal Kidneys following *in-utero* exposure to varied doses of Enarapril

2. To establish whether the renal histo-morphological teratogenic effects of Enarapril following in-utero exposure to varied doses of Enarapril are dose dependent.

3. To establish whether the pregnancy outcomes and the histo-morphological effects following in-utero exposure to varied doses of Enarapril are time dependent.

### 1.3 Hypothesis ($H_0$)

*In utero* exposure to Enarapril causes no histo-morphological effects on the developing foetal kidneys.

### 1.4 The study assumptions

Albino rat (*Rattus Norvegicus)* model would replicate the actual teratogenic induction scenario that would occur in humans due to the known close association of this kind of rat species with human biological and functional outcomes when exposed.

## 2.0 Materials and Methods

### 2.1 Study site/Location

All procedures and experiments including breeding, handling, weighing, Enarapril administration and measurements of fetal parameters was done at the Small Animal Facility for Research and Innovation (SAFARI) situated in Jomo Kenyatta University of Agriculture and Technology (JKUAT). Tissue processing was carried out in Histology Lab in JKUAT. Kidney tissues were sectioned into transverse sections of 3-5 microns diameter and routine histological staining using Hematoxylin and Eosin was adopted. Processed tissues were observed using a light microscope at X40 magnification.

### 2.2 Study Design

A static group laboratory based experimental study design was adopted

### 2.3 Description of Albino rats used in the study

Female albino dams used in the study were of the 3rd series breed and weighed between 200-250g.

### 2.4. Acquisition and feeding of the dams

The albino rats were acquired from the Small animal facility for research and innovation (SAFARI) animal house, in Jomo Kenyatta University of Agriculture and Technology (JKUAT) main campus. They were fed on rodent pellets and water *adlibitum* and housed in spacious polycarbonate plastic cages in the animal house as per animal care and use ethics (Levy, 2012)

### 2.5 Sample size calculation

In calculation of the sample size, resource equation by Arifin *et al.*, 2017 was applied to get 30 albino rats as determined by. The formula states that 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 according to this equation. Therefore, a value less than 10 necessitates adding more animals which increases the chance of getting significant results while a value more than 20 has been shown to increase the cost of the study without increasing the significance of the results. Therefore, total number of groups=10 while the total number of animal sis 30. E=Total number of Animals-Total number of groups. E is therefore is 30-10 which is 20

### 2.6 Grouping of animals

Thirty non gravid albino rats' dams' rats weighing between 200-250g were assigned to either control of three dams or treatment groups of twenty-seven dams. 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 ($TM_3$).

### 2.7 Determination of Enarapril doses

A simple guide for conversion of human to animal dosages was used (Nair & Jacob, 2016). The correction factor (Km) is estimated by dividing the average body weight (kg) of species to its body surface area (m2). For example, the average human body weight is 60 kg, and the body surface area is 1.62 m2. Therefore, the Km factor for human is calculated by dividing 60 by 1.62, which is 37. The Km factor values of a rat is used to estimate the HED as: HED mg / kg = Rat dose mg / kg Animal K /Human K Eq. As the Km factor for each species is constant, the Km ratio is used to simplify calculations. Hence, Equation is modified as: HED mg / kg = Animal dose mg / kg K ratio Eq. The Km ratio values are already provided and are obtained by dividing human Km factor by animal Km factor or vice versa. Enarapril administration was done using an oral gavage needle gauge 16.

### 2.8 Administration of Enarapril

All rats in first trimester ($TM_1$) group in Low, Medium and High dose categories received Enarapril doses from gestation day $GD_1$-$GD_{20}$ while the rats in second trimester ($TM_2$) group in Low, Medium and High dose categories received Enarapril from gestation day $GD_7$-$GD_{20}$. Rats in third trimester ($TM_3$) group in Low, Medium and High dose categories received Enarapril from gestation day $GD_{14}$-$GD_{20}$

### 2.9 Harvesting of the fetal kidneys

i. Fetus were mounted onto a dissecting board using mounting pins (dorsal side facing the board)
ii. Using a pair of scissors and forceps the abdominal muscle layers were dissected at the middle to expose the abdominal viscera of the fetus via a midline incision from xiphisternum to pubic symphysis.
iii. The parietal peritoneum of the posterior abdominal was opened in the center along the vertebral column and retracted carefully
iv. Using a magnifying glass, the fetal kidneys were identified and removed.
v. The entire kidneys were excised at the level of the renal pelvis.
vi. The kidneys were then immersed in 10% buffered Formaldehyde for 24 hours.

### 2.10 processing the fetal kidneys for histomorphological analysis

i. The fetal kidneys were fixed in 10% buffered Formaldehyde for 24 hours.
ii. They were dehydrated in an ascending concentration of alcohol (50%, 60%, 70%, 80%, 90%, 95% and 100% (absolute) each for one hour.
iii. They were cleared by immersion in xylene solution for 12 hours and infiltrated with paraplast wax for 12 hours at $56^0$c
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 slides were then dried in an oven at $37^0$ c for 24 hours
x. Blinding was done by coding all the slides by the research assistance in absence of the researcher
xi. They were stained with using Hematoxylin and Eosin (H&E).

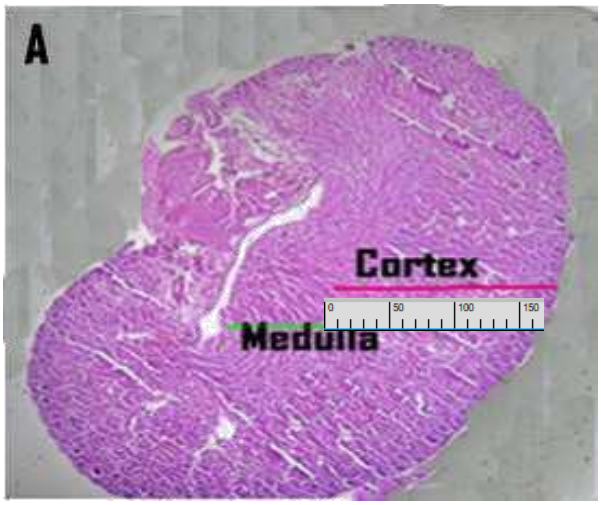
### 2.11 Ethical Approval

All procedures for animal handling, feeding, humane sacrificing and harvesting of organs were performed as per laid down protocols, with approval from Animal Ethics Committee Jomo Kenyatta University of Science and Technology REF: JKU/2/4/896A).

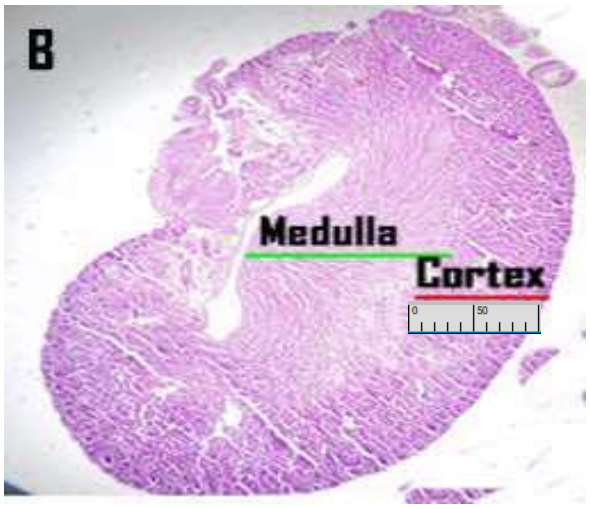
### 3.0 The study findings

### 3.0 Influence of enalapril on the morphological thicknesses of the cortex

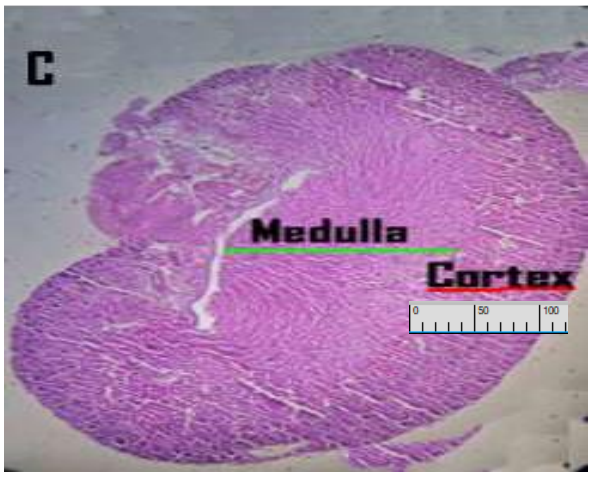
A ruler was used to define visible differences. The cortical thickness was observed to reduce appreciably among all the enalapril treated groups as compared with the control group particularly during $TM_1$ and $TM_3$. The Glomeruli and capsular space increased in all dosages particularly in trimester $TM_2$ and $TM_3$



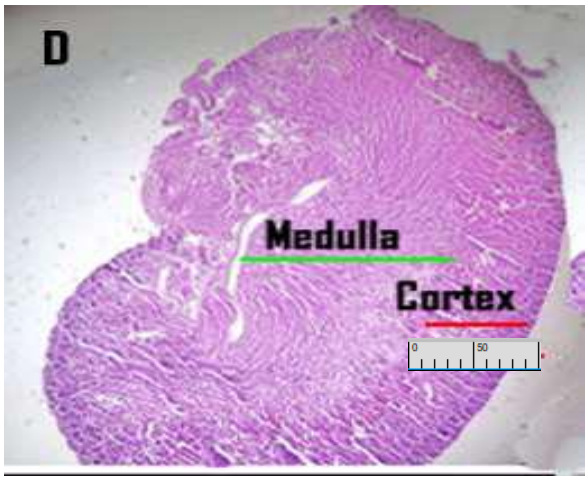

Normal thickness of the fetal kidney cortex in **control** as shown by the red line




Relatively reduced cortex in **LDEG** as indicated by the red line

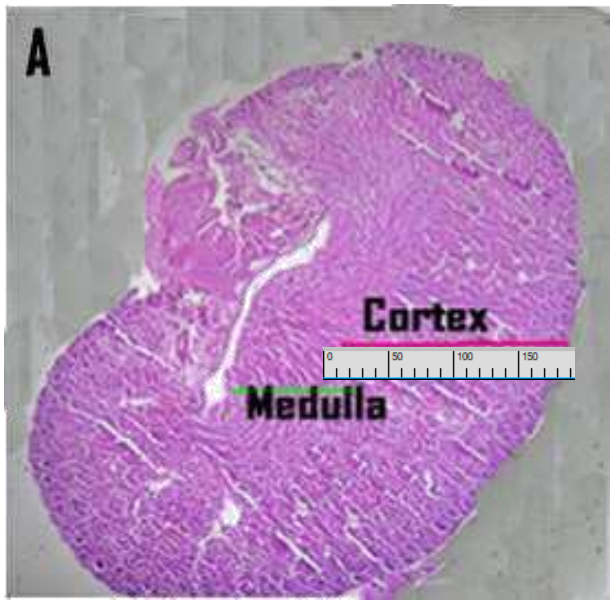



Further reduction of the fetal kidney Cortical in **MDEG** layer as indicated by red line



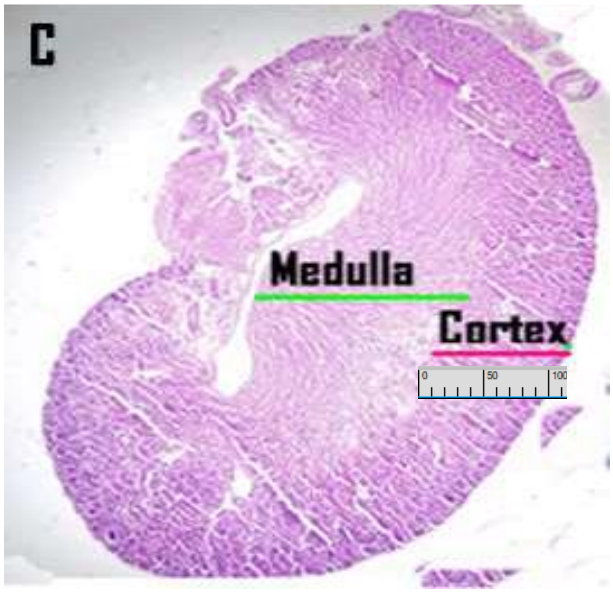

Most reduced fetal kidney cortical Layer in **HDEG** as indicated by the red line

**Comparative thickness of the fetal kidney cortical layer in (a) Control (b) LDEG, (c) MDEG, and (d) HDEG IN $TM_1$ at X 40 magnification**

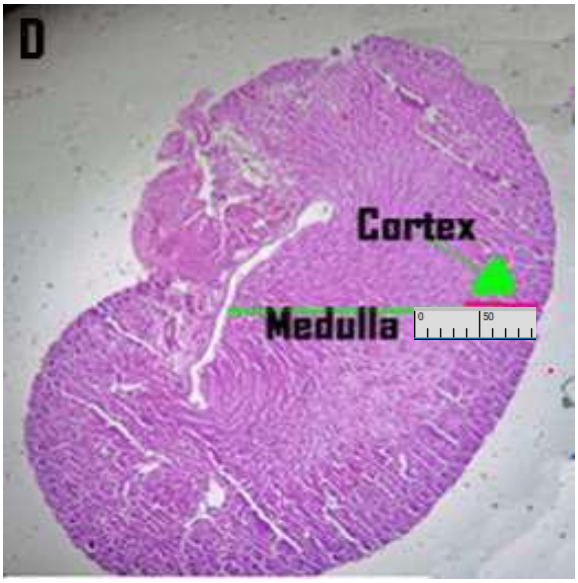



Normal thickness of the fetal kidney cortex in **control** as shown by red line


Relatively reduced cortex in **LDEG** as Indicated by the red line

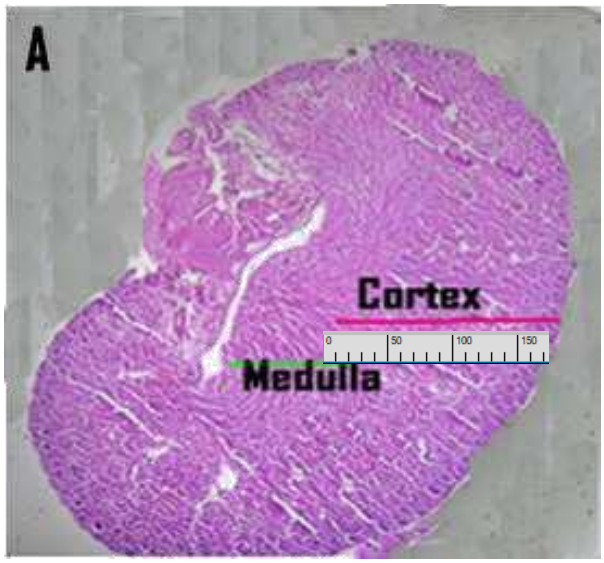



Further reduction of the fetal kidney cortical layer in **MDEG** as indicated by the red line

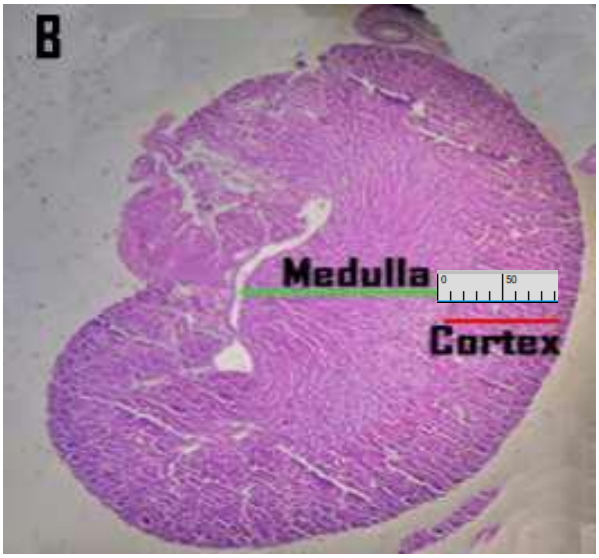



Most reduced fetal kidney cortical Layer in **HDEG** indicated by the red line

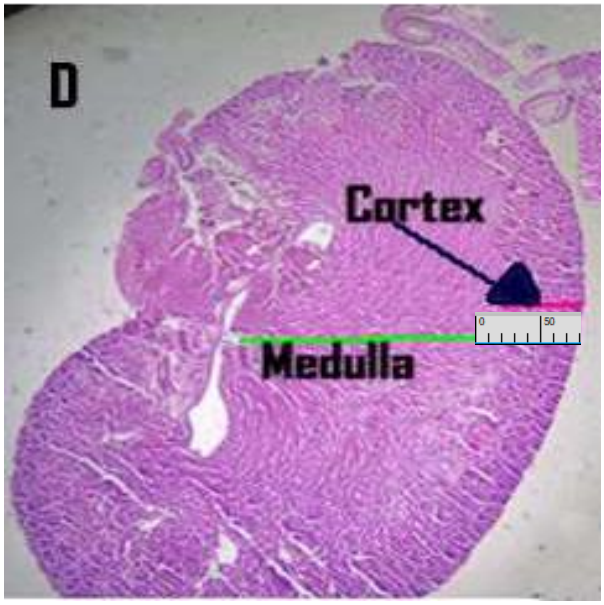
**Comparative thickness of the fetal cortical layer of the kidney in (a) Control b) LDEG, (c) MDEG, and (d) HDEG in $TM_2$ at X 40 magnification**



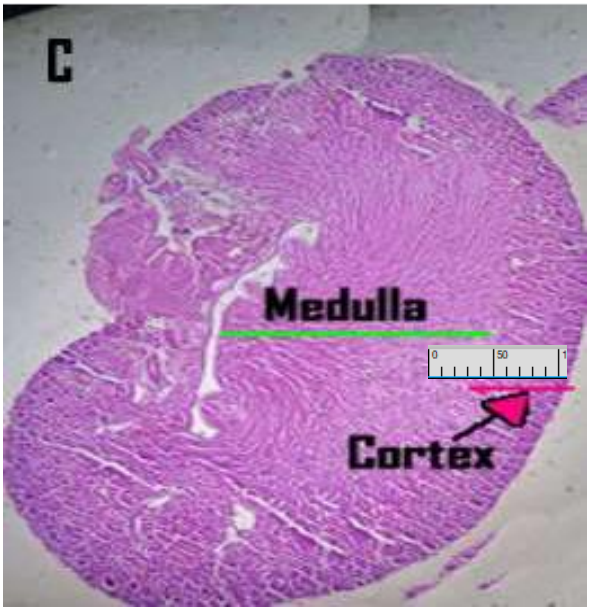

Normal thickness of the Control: fetal kidney cortex as shown by red line




Relatively reduced cortex in LDEG as shown by the red line



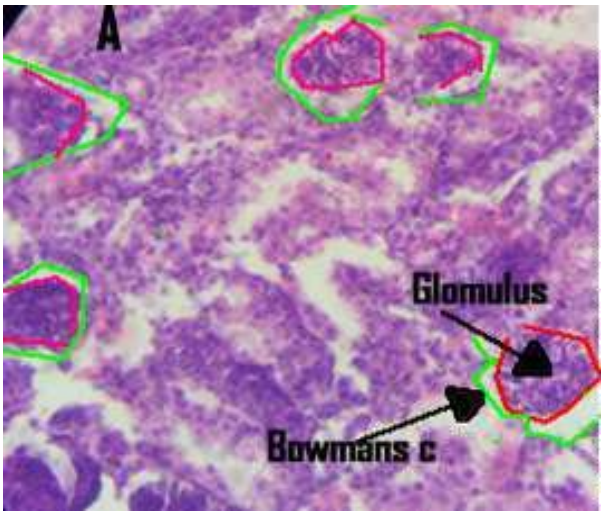

Further reduction of the fetal kidney cortical layer in MDEG as indicated by the red line



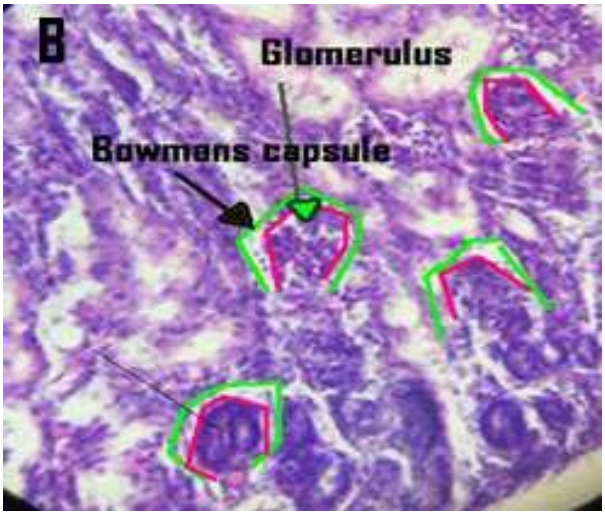

Most reduced fetal kidney cortical Layer in HDEG at $TM_3$ as indicated by the red line

**Comparative thickness of the fetal cortical layer of the kidney in (a) Control (b) LDEG, c) MDEG, and (d) HDEG IN $TM_3$ at X 40 magnification**

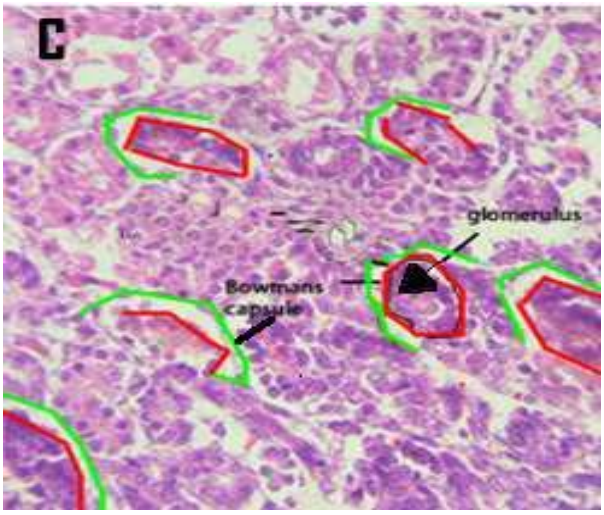
### 3.2 Influence of enalapril on the urinary space and glomeruli size



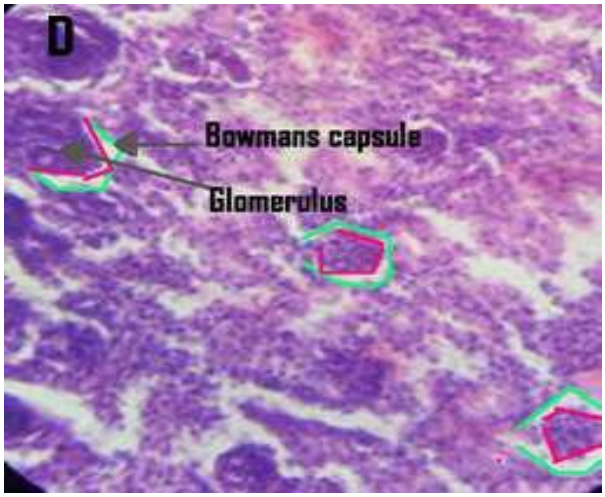

Normal size of the Bowman's capsular space and the glomerulus in **Control** Bowman's capsular space is between the green and red lines while the red lines encloses the glomeruli




Almost the same size of the Bowman's capsular space and size of the glomerulus in the **LDEG** at as compared with the control

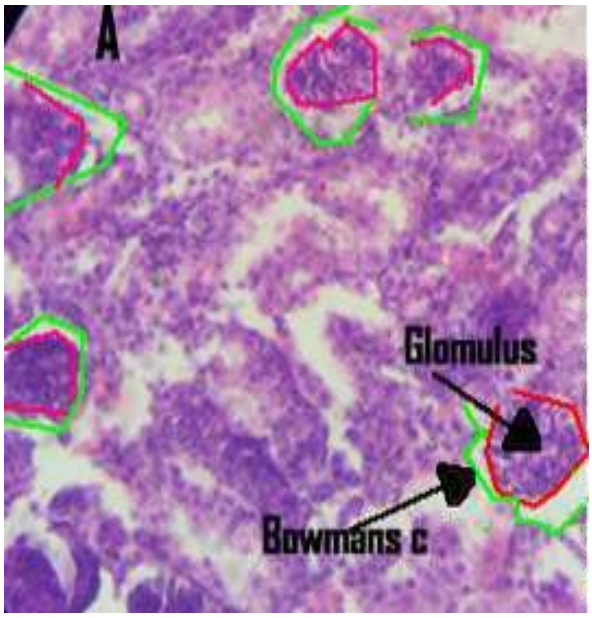



No much difference in Bowman's Capsular space and the size of the glomerulus in the **MDEG** glomeruli

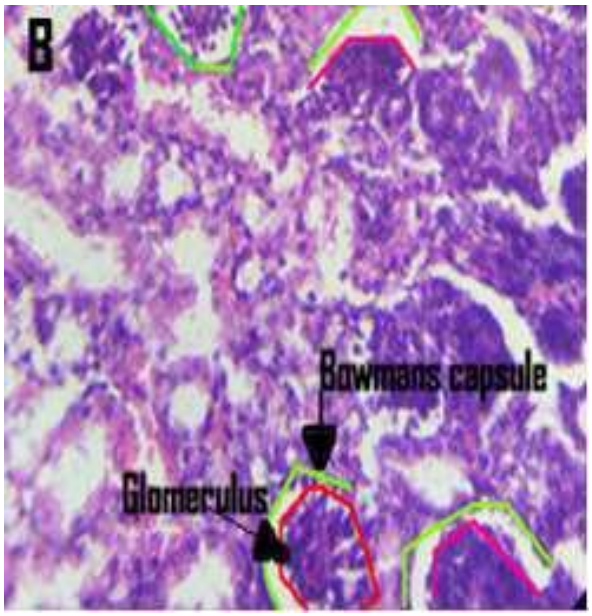



Capsular space and the size of the glomerulus in the **HDEG**

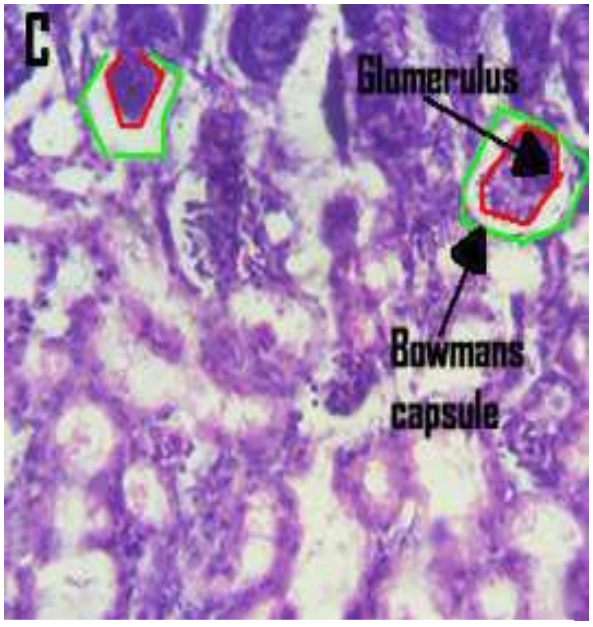
**Comparative sizes of the capsular space and the Glomeruli in (a) Control (b) LDEG, (c) MDEG, and (d) HDEG at $TM_1$ at X 40 magnification**



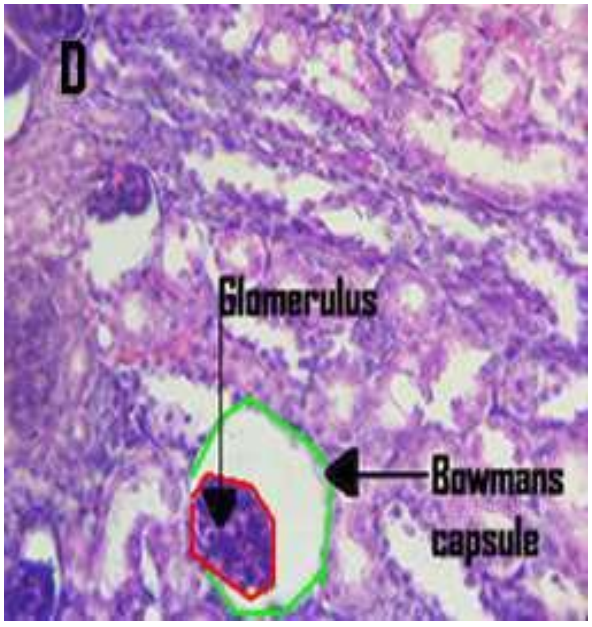

Normal size of the Bowman's capsular space and the glomerulus in **Control** while the red lines encloses the glomeruli




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

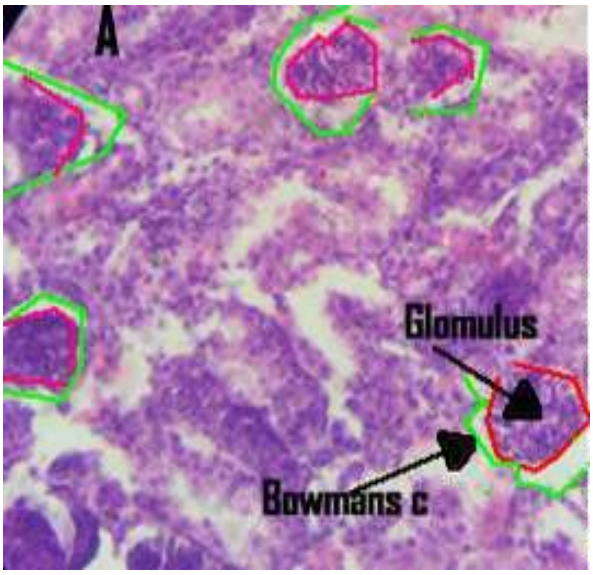



Increased capsular size and glomeruli size at $TM_2$ in **MDEG**

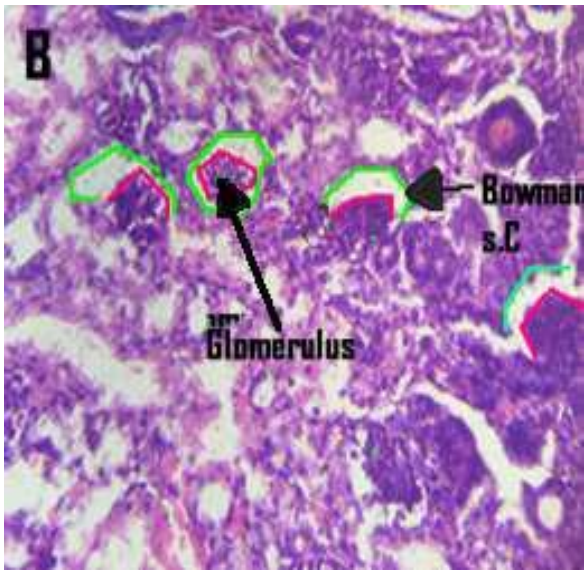



Most increased capsular space and the glomeruli at $TM_2$ in **HDEG**

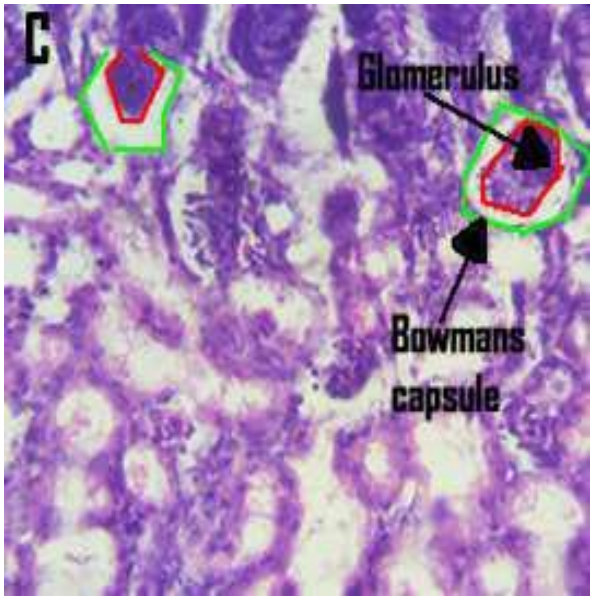
**Comparative sizes of the capsular space and the Glomeruli in (a) Control (b) LDEG, (c) MDEG, and (d) HDEG at $TM_2$ at X 40 magnification**



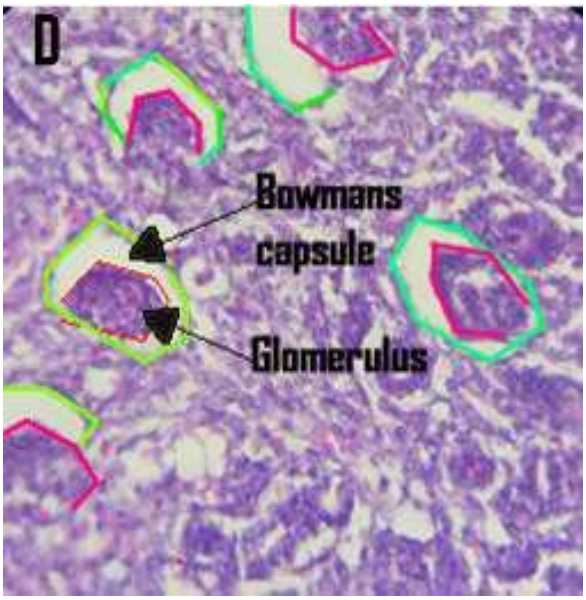

Normal size of the capsular space and the glomerulus in **Control**




Relatively increased size of the capsular space and slightly bigger glomerulus in the **LDEG**




Most increased capsular space and the Glomeruli at $TM_3$ **MDEG**




Increased capsular size and glomeruli size at $TM_3$ in **HDEG**

**Comparative sizes of the capsular space and the Glomeruli in (a) Control (b) LDEG, c) MDEG, and (d) HDEG at TM $_3$ at X 40 magnification**

## 4.0 Discussion

This study has established that enalapril suppresses the mesenchymal differentiation and development of the cortical and medullary layers of kidney cortex. This can be attributed to the Enarapril mode of action that entails inhibiting angiotensin two (II) a rate limiting step in un-inhibition of the spryl gene that shuts down further development to occur.In normal conditions, Angiotensin II Acting via the AT2R (Angiotensin II receptor), upregulates Pax2 that stimulates ureteric bud branching in GDNF/c-Ret/Wnt11 signaling pathway and acting via the AT1R (Angiotensin I receptor), inhibits Spry1 gene(Yosypiv et al., 2008).Angiotensin blockade in Enarapril treatments causes renal atrophy and poor nephron endowment with resultant structurally abnormal kidneys(Chevalier, 2012).The findings in marked reduction in thicknesses of the cortical layers of the fetal kidney cortex 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 (TM2) and trimester three (TM3.) Treatment done at trimester one (TM1), had no significance difference in cortical thicknesses across all groups from the control. 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.

## 5.0. Conclusion

This study 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. Again, 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.

## 6.0 Recommendations

The 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

## Acknowledgements

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