VOLUMENTERIC ANALYSIS OF THE CORTICAL, MEDULLARY AND TOTAL FETAL KIDNEY VOLUMES IN ALBINO RATS (*RATTUS NORVEGICUS)* WHEN PRENATALY EXPOSED TO VARIED DOSES OF ENALAPRIL

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Abstract: Though Enarapril nephroteratogenicity when exposed *in utero* is documented in literature there is paucity of data on its teratogenic volumetric histo-quantitativeeffects on the cortical, medullary and its total volumes when exposed at differing window period and at different doses. This study therefore evaluates thetotal, cortical and medullary layers histo-Quantitive changes on the developing fetal Kidneys following *in-utero* exposure to varied doses of Enarapril at different window periods.

In carrying out the study thirty gravid rats of 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). 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 histologically processed for histo-Quantitive analysis. The kidneys' total volume was measured by both Archimedean Principle and Cavalieri method to adopt a less than 10% shrinkage from the two methods and the volumes of the cortical and medullary layers were estimated by Cavalieri method.

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 tables. This study elucidated that prenatal exposure to enalapril results to altered structural organization of the renal parenchyma including significant reduction in gross kidney volumes, reduction in cortical and medullary layer thickness. In conclusion, Enalapril in the doses of 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). 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.

Keywords: Enarapril, kidney teratogenicity, Pregnancy, stereology

1.1 Introduction

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) in general

populations and ameliorates hypertension related complications including renal pathology, 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 key determinant of a healthy urological system is the volume estimates of the renal parenchyma and in combination with any prevailing stereological changes dictates the renal functional reserve.(Michael et al., 2007) This yields valuable data on the status of the organ(Lenger & Akosman, 2013)

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 signalling 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).

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 histostereological 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)

1.2 Study Objectives

1. To evaluate the cortical, medullary and the total kidney volumetric quantitativeeffects of Enaraprilon the developing fetal Kidneys following *in-utero* exposure to varied doses of Enarapril at different window periods

2. To establish whether thevolumetrichisto-stereologicalchanges on the total kidney volume, cortical and medullary layers of fetal kidneys are doses dependent.

3. To examine whether theobserved volumetric histo-stereological effects on the total, cortical and medullary layers of fetal kidneys are time dependent.

1.3 Hypothesis (H_0)

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

1.4 The study assumptions

Albino rat (*Rattus Norvegicus)* model would simulate the 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 to chemical agents

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

2.2 Study Design

A static group laboratory based experimental study design was adopted

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)

2.5Sample size calculation

In calculation of the sample size, resource equation was applied to get 30 albino rats as determined by (Arifin & Zahiruddin, 2017). 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 gravid rats of 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 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 kidneys for light microscopy

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 paraplastwax 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 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 Slides selections and histostereological analysis

Tissue processing was carried out in Histology Lab in JKUAT. Kidney tissues were sectioned into transverse sections of 5 microns diameter and routine histological staining using Hematoxylin and Eosin was adopted. The kidneys' total volume was estimated by both Archimedean Principle and Cavalieri method while the volumes of the cortical and medullary layers were estimated by Cavalieri method

Twenty five sections of 5µm thickness of the total 150 sections were picked using systematic uniform random sampling from each kidney, and using the microscope's stage Vernier, images were viewed at magnification of 10x. Points that hit the inclusion grid were summed up from the 25 sections and multiplied by 6 to get all points and total volume estimated using the formula:

Est V = ∑i-1P. a/ p. t

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

∑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, (0.25 µ X0.25 µ))

t = was the distance between sections and the results multiplied by 6 to account for the whole kidney volume
Volume density, (Vv) in the reference space were obtained using the formula:

Est Vv = P (part)/P (ref),

Where P (part) and P (ref) were the number of test points falling in reference space and all structure profiles respectively

2.10 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 Results

Stereological Findings

The following parameters were estimated. The total fetal kidneys weights, total kidney volume by use of both water displacement method (WDM) and cavarieli method of point counting and volume densities of both cortex and medulla of the fetal kidney structures.

The gross fetal kidney size and weight

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 enalapril treatment	of Mean kidney weight + SEM	Meankidney 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}	$1.0711\pm0.0066_{b}$
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 significant 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 found 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 (TM3,) the mean total fetal kidney weight was found to reduce with dosages increase. When treatment was done at trimester one (TM1) the meankidney 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

The total fetal kidney volume and sub component volume densities

Comparative reference, calculated and percentage shrinkage on total Mean fetal Kidney volume using (WDM) and cavarieli method (mm3) in the LDEG, MDEG and the HDEG treated at TM1, TM2 and TM3 against the control

Study groups	Period of enalapril treatment	Mean total kidney fetal 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) Letters [b, c and d])depicts statistical significant difference from control and intragroup statistical significant difference using one way ANOVA with Tukey test on post-hoc t-tests.

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*, as shown in the table above.

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 (TM3,) the volumes reduced as dosages increased. When treatment was done at trimester one (TM1), 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.

3.0 Discussion

Renal Histoquantitive changesfollowing Enarapril exposure*in utero*

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 (TM2) the total kidney volume was lowest in the high dose enalapril group (HDEG.) These findings augmented a study by (Amann et al., 1993)who found out that the number of glomeruli per kidney was reduced significantly in the enalapril-treated groups. 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 TM2**,** the mean total kidney weight was found to be lowest in HDEG followed by MDEG. In TM3,the mean totals fetal kidney weight was lowest in HDEG followed by MDEG. Angiotensin converting enzyme inhibitor causes reduced blood flow to the kidneys presenting with decrease in kidney weight, alteration of the number and size of nephrons, layers, and massive cell degeneration with cavity formation in the kidney tissue.(Martin et al., 1992)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., 2017)In TM1**,** the mean kidney weight reduced significantly as the dosages increased (values $p<0.05$) when compared with the control. This corroborates a study by(Bullo et al., 2012) who reported that alterations and the destructive changes especially of kidney tubules during fetal kidney development are time dependent.

5.0. Conclusion

This study has established that 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.

Enalapril in the doses of 2mg/kg/BW and1mg/kg/BWduring pregnancy are teratogenic to the developing fetal kidney particularlywhen 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

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References

1. Amann, K., Irzyniec, T., Mall, G., & Ritz, E. (1993). The effect of enalapril on glomerular growth and glomerular lesions after subtotal nephrectomy in the rat: A stereological analysis. *Journal of Hypertension*, *11*(9), 969–975. https://doi.org/10.1097/00004872-199309000-00011

2. Arifin, W. N., & Zahiruddin, W. M. (2017). Sample Size Calculation in Animal Studies Using Resource Equation Approach. *The Malaysian Journal of Medical Sciences: MJMS*, *24*(5), 101–105. https://doi.org/10.21315/mjms2017.24.5.11
3. Başaran, E., Akosman, M. S., & DemİrkanA. Ç. (2013). Volumetric analysis of the total and subcomponents of the chinchilla kidneys. *Eurasian Journal of Veterinary Sciences*, *29*(4), 171–175. https://www.cabdirect.org/cabdirect/abstract/20133426513
4. Bateman, B. T., Patorno, E., Desai, R. J., Seely, E. W., Mogun, H., Dejene, S. Z., Fischer, M. A., Friedman, A. M., Hernandez-Diaz, S., & Huybrechts, K. F. (2017). Angiotensin-Converting Enzyme Inhibitors and the Risk of Congenital Malformations. *Obstetrics and Gynaecology*, *129*(1), 174–184. https://doi.org/10.1097/AOG.0000000000001775
5. Bullo, M., Tschumi, S., Bucher, B. S., Bianchetti, M. G., & Simonetti, G. D. (2012). Pregnancy outcome following exposure to angiotensin-converting enzyme inhibitors or angiotensin receptor antagonists: A systematic review. *Hypertension*, *60*(2), 444–450.
6. Chevalier, R. L. (2012). Mechanisms of fetal and neonatal renal impairment by pharmacologic inhibition of angiotensin. *Current Medicinal Chemistry*, *19*(27), 4572–4580. https://doi.org/10.2174/092986712803306411
7. Lenger, O. F., & Akosman, M. S. (2013). A urological model: Volume estimation of the bovine renal parenchyma. *Bulgarian Journal of Agricultural Science*, *19*(4), 848–853.
8. Levy, N. (2012). The use of animal as models: Ethical considerations. *International Journal of Stroke: Official Journal of the International Stroke Society*, *7*(5), 440–442. https://doi.org/10.1111/j.1747-4949.2012.00772.x
9. Martin, R. A., Jones, K. L., Mendoza, A., Barr, M., & Benirschke, K. (1992). Effect of ACE inhibition on the fetal kidney: Decreased renal blood flow. *Teratology*, *46*(4), 317–321. https://doi.org/10.1002/tera.1420460402
10. Menshykau, D., Michos, O., Lang, C., Conrad, L., McMahon, A. P., & Iber, D. (2019). Image-based modelling of kidney branching morphogenesis reveals GDNF-RET based Turing-type mechanism and pattern-modulating WNT11 feedback. *Nature Communications*, *10*(1), 239. https://doi.org/10.1038/s41467-018-08212-8
11. Michael, B., Yano, B., Sellers, R. S., Perry, R., Morton, D., Roome, N., Johnson, J. K., & Schafer, K. (2007). Evaluation of organ weights for rodent and non-rodent toxicity studies: A review of regulatory guidelines and a survey of current practices. *Toxicological Pathology*, *35*(5), 742–750.
12. Nair, A. B., & Jacob, S. (2016). A simple practice guide for dose conversion between animals and human. *Journal of Basic and Clinical Pharmacy*, *7*(2), 27–31. https://doi.org/10.4103/0976-0105.177703
13. Neuen, B. L., Chadban, S. J., Demaio, A. R., Johnson, D. W., & Perkovic, V. (2017). Chronic kidney disease and the global NCDs agenda. *BMJ Global Health*, *2*(2), e000380. https://doi.org/10.1136/bmjgh-2017-000380
14. Oh, P. C., Sakuma, I., Hayashi, T., & Koh, K. K. (2016). Angiotensin converting enzyme inhibitors remain the first treatment of choice. *The Korean Journal of Internal Medicine*, *31*(2), 237–241. https://doi.org/10.3904/kjim.2016.026
15. Shakya, R., Jho, E., Kotka, P., Wu, Z., Kholodilov, N., Burke, R., D'Agati, V., & Costantini, F. (2005). The role of GDNF in patterning the excretory system. *Developmental Biology*, *283*(1), 70–84. https://doi.org/10.1016/j.ydbio.2005.04.008
16. Yosypiv, I. V., Boh, M. K., Spera, M. A., & El-Dahr, S. S. (2008). Downregulation of Spry-1, an inhibitor of GDNF/Ret, causes angiotensin II-induced ureteric bud branching. *Kidney International*, *74*(10), 1287–1293. https://doi.org/10.1038/ki.2008.378