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Histosterelogical Effects of Curcuma Longa on Sildenafil Induced Nephrotoxicity among Male Albino Rats

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ABSTRACT
Kidneys are vital organs of the body involved in ultrafiltration of toxins. Each kidney has two layers namely cortex and medulla. Nephrotoxicity occurs when there is damage to different kidney parts like glomerulus, bowman's capsule and proximal convoluted tubules that enhance the function of kidneys. This damage causes accumulation of toxins leading to altered glomerular structure and increased bowman`s capsule size. The aim of this study was to assess the histostereological effects of Curcuma longa on sildenafil induced nephrotoxicity among male albino rats. The key focus was on glomerulus volume and volume of epithelial cells. A total of 25 male albino rats was used having been calculated using modified resource formula. The animals were systematically simple randomly sampled having been grouped into control and experimental groups. The animals were humanely sacrificed and kidneys harvested for stereological studies. The absolute glomerulus volume of positive control (sildenafil 1µg/gmbwt/day) group reduced significantly (P=0.037) as compared to negative control group while that of medium and high Curcuma longa dose group was statistically significantly different (P=0.001) and (P=0.016) respectively as compared to positive control (sildenafil 1µg/gmbwt/day) group. The mean number weighted volume fraction of proximal tubular cells in positive control (sildenafil 1µg/gmbwt/day) group reduced (P=0.001) as compared to negative control group while that of medium and high Curcuma longa dose groups was statistically significantly different (P=0.017) and (P=0.032) respectively (Table 1). It was therefore, concluded that Curcuma longa has histostereological effects on sildenafil induced nephrotoxicity among male albino rats
Key words: Bowman`s capsule, Glomerulus, Nephrotoxicity and Sildenafil.

I. INTRODUCTION

Curcuma longa commonly known as turmeric, is a popular herbal plant mostly found in India and Southeast Asia, Kenya and all regions abundantly. Curcumin is the active compound of *Curcuma longa* with multisystemic benefits at 70%, ar-tumerovel 20.50%, beta sesquiphellandrene 5.2 % and curcumerol at 5.11% (Lestari & Indrayanto, 2014). It has mild side effects that includes headache, stomach ache and flushing. It is yellow in color due to Curcumin and is used to enhance extra flavor of food. Sildenafil is a prescription drug that is manufactured in two forms, a suspension and a tablet. It is a first line oral therapeutic agent phosphodiesterase type 5 inhibitor used in treatment of erectile dysfunction and pulmonary hypertension (Hsu et al., 2015; Mulhall et al., 2020). The drug works by prolonging the signaling action of nitric oxide that is in corpus cavernosum thereby increasing cyclic guanosine monophosphate levels that leads to pooling of blood effect in the penis thus causing erection (Mohammed & Khudair, 2020).

Drug induced nephrotoxicity is increasingly the major factor that contributes to development of kidney diseases as this type of nephrotoxicity has a wide range of damage on nephron. Sildenafil affects different parts of kidney namely; cell membrane, glomerulus, bow man capsule, medulla, cortex, medullary tubule, cortical tubules, proximal convoluted tubule, renal vessels and glomerular capillaries. It releases oxidative chemicals that can cause stress thereby damaging cell membrane (Cadirci et al., 2011; Ebrahimi et al., 2009). It causes accumulation of lactate in kidney tubules causing damage to cytoplasm leading to elevation in osmotic pressure that cause water intracellular influx (Medeiros et al., 2017). It causes disturbances in renal function which leads to accumulation of calcium that causes calcification in glomeruli and reduced renal perfusion (Küçük et al., 2012). The aim of this study was therefore, to assess the histostereological effects of Curcuma longa on sildenafil induced nephrotoxicity among male albino rats.

II. MATERIALS AND METHODS

This study was a posttest only true experimental design in which a total of twenty-five male albino rats were used. The rats were group into control and experimental groups. They were selected into this study using systematic simple random sampling method. The rats in control group were fed on standard rodent pellets and received water ad libitum. All the rats in experimental groups received standard rodent pellets, water ad libitum, sildenafil and later *Curcuma longa* was introduced at low, medium and high dose. Each group had five rats. The sample size of 25 male albino rats that were used in this study was calculated using modified resource formula (Arifin & Zahiruddin, 2017). These animals were then treated with Sildenafil 1µg/gmbwt/day for 15 days to induce nephrotoxicity then *Curcuma longa* was introduced at varying doses for 7 days to try and restore the damage caused. During the care of animals, high hygiene standards were observed and animals were handled carefully as per the care and use of animals in laboratory setting (Leary et al., 2013).

On the last day of experiment, the animals were humanely sacrificed and necessary organs were harvested. Upon harvesting the kidneys, gross morphological parameters were determined then they were put in 10% formalin and later prepared for stereological examination.

Steps used when determining Absolute glomerulus volume and mean number weighted fractional volume of epithelial cells.

The absolute volume of glomeruli and number weighted volume fraction of PCT cells had to be determined independently to assess the morphological changes that occur on rat kidney among the experimental group. To achieve this, each slide was bordered by lines drawn using a board marker to form rectangle around the tissue. Microscope stage was moved from one corner to the other on the X -axis at 3 and 5 microscope stage unit intervals on the X and Y axis respectively where snapshots of renal corpuscles and tubules were taken. This was done until the whole area of tissue was covered. Using the image J photoshop software, a stereological grid consisting of uniformly spaced points 1cm x 1cm and 0.5cm x 0.5 cm were superimposed over each micrograph of the glomerulus and PCT respectively to count the number of points which intersected the glomerulus and epithelial cells of PCT. The following equations were used to determine the volume of glomerulus and PCTs all of which are components of cavarieli principle.

V=∑P x (a/p) x t

Where V=volume, ∑p= sum of all test points encountered, a/p= area per point of stereological grid, t= thickness of section and M= linear magnification.

Number weighted volume fraction of PCT per cortex

Ps=P/A x 100%

Where p= total number of test points encountered with PCT, A= total points per stereological grid used and given by ∑ap, a/p is the area per point of stereological grid.

Mean Ps per group=∑ P/A/n x 100

Where n= number per samples.

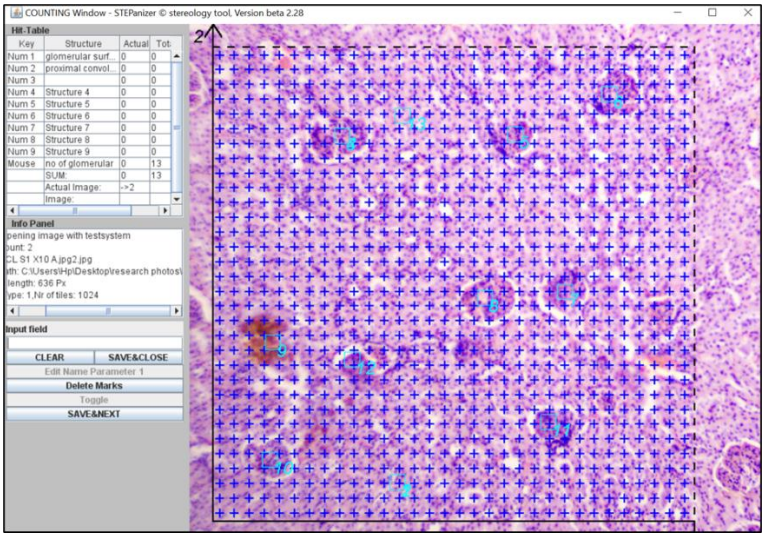

Figure 1: illustration of stereological analysis

The ethical approval to carry out the study was sought from Baraton University of Eastern Africa (UEAB/ISERC/08/01/2023) and NACOSTI (NACOSTI/P/2023/23374). Once numerical values were obtained, they were entered into SPSS version 26 and analyzed. Here one way ANOVA was used to obtain inter group

significance. The values were then subjected to post hoc Bonferroni to obtain mean significance. A P value of ≤ 0.05 was considered significant.

III. RESULTS

This part focused on assessment of stereological parameters namely; absolute glomerular volume and number weighted volume fraction of proximal tubular cells. Glomerulus and proximal convoluted tubule were considered as this are parts largely affected by toxins.

Comparative mean absolute glomerular volume and number- weighted volume fraction of proximal tubular cells among the restorative and control groups.

The absolute glomerulus volume of positive control (sildenafil 1µg/gmbwt/day) group reduced significantly (*P*=0.037) as compared to negative control group while that of medium and high *Curcuma longa* dose group was statistically significantly different (*P*=0.001) and (*P*=0.016) respectively as compared to positive control (sildenafil 1µg/gmbwt/day) group. The mean number weighted volume fraction of proximal tubular cells in positive control (sildenafil 1µg/gmbwt/day) group reduced (*P*=0.001) as compared to negative control group while that of medium and high *Curcuma longa* dose groups was statistically significantly different (*P*=0.017) and (*P*=0.032) respectively (Table 1).

Table 1: Comparative means of absolute glomerulus volume and number weighted volume fraction of epithelial cells in control groups and experimental groups.

Control and experimental groups.					
Stereological measurements	**Control (feeds + water ad libitum)**	**SIN (1µg/gmbwt/day sildenafil)**	**Low C*urcuma longa* 38.75mg/kg/day**	**Medium *Curcuma longa* 77.5mg/kg/day**	**High *Curcuma longa* 155mg/kg/day**
Absolute glomerulus volume(×10^3µm 3)	29508±.86	22844±.79	24288±.53	26191±.11	28392±.96
Number-weighted volume fraction of proximal tubular cells (epithelial cells)	1.879±0.09	0.3086±0.07	0.7078±0.26	0.9228±0.19	1.234±0.17

KEY: *All values are expressed as the mean, ± is the standard error of the mean (SEM). The test of significance was performed in rows. Values are expressed as mean ± standard error of mean (n=5), SIN- sildenafil induced nephrotoxicity, LCL-Low curcuma Longa, MCL-Medium Curcuma Longa, HCL-High Curcuma Longa*

IV. DISCUSSION

Stereology is a method of obtaining quantitative data from microscopic images utilizing a special software that uses a three-dimensional method. Normally the software used, analyses images of tissues that are histologically prepared to generate accurate and precise data. Glomerulus is one of the kidney structures that is prone to nephrotoxic injuries due to drug metabolism and excretion. Any slight injury to glomerulus can alter the general pathway of drug excretion leading to accumulation of drugs within kidney tubules. This is characterized by reduced kidney functions namely; decreased glomerular filtration rate, increased levels of creatinine and urea and stereologically evidenced by decreased glomerulus volume.

It was observed that there was a significant reduction of mean glomerular volume and mean volume of epithelial cells in Sildenafil Induced Nephrotoxicity group as compared to control group. This significant reduction is pertinent evidence of nephrotoxicity that occurs secondary to treatment with Sildenafil. This drug is known to cause toxicity by altering glomerulus histoarchitecture, blocks glomerulus vessels which leads to reduced filtration and excretion. These findings are in tandem with (Yadegari et al., 2024) who recorded a significant reduction of glomerulus volume on administration of acrylamide. Contrary to this, (Nemati et al., 2020) noted a p value ≤0.02 increase of glomerulus volume while studying the effect of cerium oxide. Similarly, (Mansouri et al., 2016) observed P value of ≤0.05 increase on evaluation of pomegranate on glomerulus volume.

In the current study, it was observed that there was increase in glomerular volume and mean volume of epithelial cells in experimental groups (Table 1). The increase in volume might have been due to restoration of glomerulus, proximal convoluted tubule and epithelial cells to normal. (Ragab et al., 2022; Russo et al., 2018)

noted similar improvements in glomerulus and epithelial cell`s structure and densities while using *Curcuma longa* as a protective agent in diclofenac-sodium and doxorubicin induced kidney injuries. However, a study done in Brazil (Melchioretto et al., 2020) reported a decrease in epithelial cell volume among the experimental groups when assessing the stereological changes that occur on renal aging. This reduced volume might have been due to physiological changes that occur on kidney with aging such as reduced cells genesis, glomerular atrophy and tubular degeneration.

V. CONCLUSION

The study found that *Curcuma longa* has histostereological restoration properties to the kidneys and most critical dose is *Curcuma longa* 77.5mg and 155mg. Therefore, with further studies on ascertain its pharmacokinetics and dynamics this drug can be adopted into the market as it is cheap and readily available locally.

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REFERENCES

[1]. 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.

[2]. Cadirci, E., Halici, Z., Odabasoglu, F., Albayrak, A., Karakus, E., Unal, D., Atalay, F., Ferah, I., & Unal, B. (2011). Sildenafil treatment attenuates lung and kidney injury due to overproduction of oxidant activity in a rat model of sepsis: a biochemical and histopathological study. Clinical & Experimental Immunology, 166(3), 374-384.

[3]. Ebrahimi, F., Shafaroodi, H., Asadi, S., Nezami, B. G., Ghasemi, M., Rahimpour, S., Hashemi, M., Doostar, Y., & Dehpour, A. R. (2009). Sildenafil decreased cardiac cell apoptosis in diabetic mice: reduction of oxidative stress as a possible mechanism. Canadian journal of physiology and pharmacology, 87(7), 556-564.

[4]. Hsu, J., Tang, D., & Lu, C. (2015). Risk–benefit assessment of oral phosphodiesterase type 5 inhibitors for treatment of erectile dysfunction: a multiple criteria decision analysis. International Journal of Clinical Practice, 69(4), 436-443.

[5]. Küçük, A., Yucel, M., Erkasap, N., Tosun, M., Koken, T., Ozkurt, M., & Erkasap, S. (2012). The effects of PDE5 inhibitory drugs on renal ischemia/reperfusion injury in rats. Molecular biology reports, 39, 9775-9782.

[6]. Leary, S. L., Underwood, W., Anthony, R., Cartner, S., Corey, D., Grandin, T., Greenacre, C., Gwaltney-Brant, S., McCrackin, M., & Meyer, R. (2013). AVMA guidelines for the euthanasia of animals: 2013 edition.

[7]. Lestari, M. L., & Indrayanto, G. (2014). Curcumin. Profiles of drug substances, excipients and related methodology, 39, 113-204.

[8]. Mansouri, E., Basgen, J., & Saremy, S. (2016). The effects of pomegranate extract on normal adult rat kidney: A stereological study. Veterinary Research Forum,

[9]. Medeiros, V. d. F. L. P., Azevedo, Í. M., Carvalho, M. D. F., Oliveira, C. N., Egito, E. S. T. d., & Medeiros, A. C. (2017). The renoprotective effect of oral Tadalafil pretreatment on ischemia/reperfusion injury in rats1. Acta Cirurgica Brasileira, 32, 90-97.

[10]. Melchioretto, E. F., Zeni, M., Veronez, D. A. d. L., Filipak Neto, F., Digner, I. d. S., & Fraga, R. d. (2020). Stereological study and analysis of oxidative stress during renal aging in rats. Acta Cirurgica Brasileira, 35.

[11]. Mohammed, Y. J., & Khudair, Z. W. (2020). Study the Histopathological Effects of Sildenafil Treatment on the Kidney of Adult Male Rats. مجلة بار الانبار لعلوم الطبية لـ.

[12]. Mulhall, J. P., Chopra, I., Patel, D., Hassan, T. A., & Tang, W. Y. (2020). phosphodiesterase type-5 inhibitor prescription patterns in the united states among men with erectile dysfunction: An update. The journal of sexual medicine, 17(5), 941-948.

[13]. Nemati, A., Assadollahi, V., Peluso, I., Abbaszadeh, A., Beigi-boroujeni, M., Khanipur, Z., & Gholami, M. (2020). Research Article A Stereological Study of the Toxic Effects of Cerium Oxide during Pregnancy on Kidney Tissues in Neonatal NMRI Mice.

[14]. Ragab, S. M., Abd-Elkareem, M., Abou Khalil, N. S., & Atia, M. M. (2022). Protective effect of curcumin on the kidney of diclofenac sodium-challenged mice: apoptotic, redox potential and histopathological outcomes. The Journal of Basic and Applied Zoology, 83(1), 1-16.

[15]. Russo, E. R., Facincani, I., Nakazato, K. C., Coimbra, T. M., Crevelin, E. J., Pereira, A. M. S., & Carmona, F. (2018). Oral administration of powdered dried rhizomes of Curcuma longa L.(turmeric, Zingiberaceae)

is effective in the treatment of doxorubicin- induced kidney injury in rats. Phytotherapy Research, 32(12), 2408-2416.

[16]. Yadegari, M., Zare Mehrjerdi, F., Rezvani, M. E., Dortaj, H., & Pourentezari Zarch, M. (2024). Effect of vitamin C on histomorphometric changes of testis, epididymis, prostate, and seminal vesicle induced by acrylamide in rat. Naunyn-Schmiedeberg's Archives of Pharmacology, 397(1), 549-557.